NONINVASIVE HEMODYNAMIC STUDY OF THE PULMONARY CIRCULATION

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NONINVASIVE HEMODYNAMIC STUDY OF THE PULMONARY CIRCULATION (Abstract): Cardiac catheterization is the reference method for the hemodynamic study of the pulmonary circulation. However, cardiac catheterization is an expensive and invasive method. Echocardiography is a reliable noninvasive alternative method. The purpose of our study was to determine the correlation between echocardiographic and haemodynamic data obtained by cardiac catheterization in patients with pulmonary hypertension. **Material and methods:** We conducted a prospective study on 55 subjects diagnosed with pulmonary hypertension. They were examined by echocardiography and cardiac catheterization. The following parameters were measured: systolic, mean and diastolic pulmonary artery pressure and pulmonary vascular resistance. We determined the coefficient of linear correlation (Pearson) between the two methods. **Results:** The mean pulmonary artery pressure obtained by cardiac catheterization was 48.82 ± 18.4 mm Hg; the systolic pulmonary artery pressure at cardiac catheterization was 67.86 ± 22.31 mm Hg; the diastolic pulmonary artery pressure was 32.85± 15.49 mm Hg; the pulmonary resistance was 10.4 ± 5.14 Wood units. We obtained very good correlation between the two methods for systolic pulmonary artery pressure (r 0.847, p <0.0001), mean pulmonary artery pressure (r 0.7, p <0.001), diastolic pulmonary artery pressure (r 0.54, p 0.01) and pulmonary vascular resistance (r 0.82, p <0.001). **Conclusions:** Echocardiography is an excellent method for the hemodynamic study of the pulmonary circulation. **Key words:** PULMONARY HYPERTENSION, ECHOCARDIOGRAPHY, CARDIAC CATHETERIZATION, HEMODYNAMICS

Pulmonary circulation is a vascular system subject to certain pressure and volume conditions different from the systemic circulation. It is a system of low pressures and resistances, which functions under increased flow conditions and which creates a resistance by ten times lower than the systemic resistance. The pulmonary resistance is quantified according to Ohm's law, by the ratio between the difference of pressure of the pulmonary artery (PAP) and the pulmonary capillary pressure (PCP) and the cardiac output (CO). Its value can be expressed in mmHg/l/min (or Wood units) or converted by multiplication with 80 in order to express the result in dyne.sec.cm$^{-5.}$

RVP = (PAP – PCP)/ (1)

Even under effort conditions, where the

cardiac flow can increase by 5-6 times, the mean PAP does not exceed 20 mm Hg, because of certain adaptive mechanisms which include a great distensibility of the pulmonary artery tree, the low active vasomotricity and the recruitment of new vascular territories, usually closed (process called passive vasodilatation) (1).

The right cardiac catheterization is the reference method for determining the degree of hemodynamic affectation and for testing the pulmonary circulation vasoreactivity. However, the cardiac catheterization is an invasive, expensive method, with potential risks (including vital risks), which is performed only in reference centers. This aspect caused the interest for developing certain alternative techniques of studying the pulmonary hemodynamic parameters. The echocardiography occupies an important role in this research direction. Our study aims at determining the correlation between echocardiographic data and haemodynamic parameters obtained by cardiac catheterization in patients with pulmonary hypertension.

MATERIAL AND METHODS

We conducted a prospective study on 55 patients diagnosed with pulmonary hypertension (PH) over the period 1 November 2008 – 1 November 2011 in "Prof. Dr. George Georgescu" Institute of Cardiovascular Diseases of Iaşi. The pulmonary hypertension was defined as an increase of the mean pressure of the pulmonary artery at 25 mm Hg or more at rest (2). The pulmonary hypertension clinical classification was performed according to Dana Point 2008 classification (2). The echocardiographic examination was performed in maximum 24 hours as compared to the cardiac catheterization, using a Vivid E9 (GE Healthcare) or Acuson Sequoia C512 (Siemens) echocardiograph. All the patients signed an informed consent for the performance of the cardiac catheterization. The echocardiographic examination included the determination of the following parameters:

1. The estimation of the systolic pressure in the pulmonary artery based on the tricuspid regurgitation velocity. In the patients without pulmonary stenosis, the systolic pressure from the pulmonary artery (sPAP) is equal with the systolic pressure from the right ventricle (SRVP). sPAP is determined by calculating the gradient right ventricle – right atrium using Bernoulli simplified equation, plus the pressure from the right atrium (RAP). The right atrium pressure was assessed based on the dimension of the right atrium, on the severity of the tricuspid regurgitation and on the diameter and collapse degree of the inferior vena cava.

$$sPAP = SRVP = 4 \max v\ TR^2 + RAP \quad (2)$$

max v TR = maximum velocity of the tricuspid regurgitation jet

2. The diastolic pressure in the pulmonary artery (dPAP) was assessed using the pulmonary regurgitation.

$$dPAP = 4Vd^2 + RAP \quad (3)$$

Vd = end-diastolic velocity of the pulmonary regurgitation

RAP = right atrium pressure

3. The mean pressure in the pulmonary artery (mPAP) was assessed based on the maximum pressure gradient between the pulmonary artery and the right ventricle at the beginning of diastole.

$$PAPm = 4Ved^2 \quad (4)$$

Ved = early-diastolic velocity of the pulmonary regurgitation

4. The echocardiographic assessment of the pulmonary vascular resistances (PVR) was performed by three different methods:

4.1. Abbas formula is the ratio between the maximum speed of the tricuspid regurgitation flow (max v TR) and velocity – time integral in the ejection tract of the right ventricle (VTI_{RVOT})

$$PVR = \text{max v TR}/ VTI_{RVOT} \quad (5)$$

4.2. Another formula employed, derived from the previous one, is:

$$PVR = \text{max v TR}/ VTI_{RVOT} \times 10 + 0,16 \quad (6)$$

4.3. The third method employed is the ratio between sPAP and the product between VTI_{RVOT} and heart rate (HR):

$$PVR = sPAP/ (VTI_{RVOT} \times HR) \quad (7)$$

The statistic analysis was performed using MedCalc version 12.1.3 (MedCalc Software, Mariakerke, Belgium). The continuous variables were presented as mean ± DS. We calculated Pearson linear correlation coefficient between the results obtained by the two methods.

RESULTS

Our study included 8 patients with idiopathic pulmonary artery hypertension (class 1.1), 1 patient with pulmonary artery hypertension secondary to connective tissue disease (class 1.4.1), 28 patients with congenital cardiopathies (class 1.4.4), 7 patients with PH due to the left heart diseases (class 2) and 11 patients with chronic thromboembolic pulmonary hypertension (class 4). The average age was 48.72 ± 17.67 years old. The sample comprised 30 female patients (54.54%) and 25 male patients (45.45%) (tab. I).

TABLE I

The comparative study of the pulmonary pressures

Parameter	Echocardiography	Catheterization	r	p
sPAP, mm Hg	66.34 ± 24.47	67.86 ± 22.3	0.847	< 0.0001
mPAP, mm Hg	46.24 ± 17.03	48.82 ± 18.4	0.7	<0.001
dPAP, mm Hg	31 ± 14.22	32.85 ± 15.49	0.54	0.01

We compared sPAP calculated on the echocardiography with sPAP obtained at cardiac catheterization. We obtained a strong positive correlation between the two methods (r 0.847, p < 0.0001). We defined measurements' accuracy as a difference smaller than 10 mm Hg between the echocardiography and the cardiac catheterization. We obtained this concordance in 23 patients (45%). The number of underestimations and overestimations was equal (9 patients, 17.64%). The average underestimation degree was 17.77 mm Hg, and the overestimation was of 17.33 mm Hg (p 0.3). Overestimations of more than 20 mm Hg were recorded in 4 patients (7.84%), and underestimations of more than 20 mm Hg in 3 patients (4.22%). sPAP echocardiographic determination allowed the correct framing in severity classes in 49 cases (96%) (fig. 1).

For pulmonary resistance estimation we obtained excellent correlations with the cardiac catheterization, regardless of the method employed (fig. 2, 3, 4). PVR average value at cardiac catheterization was 10.4 ± 5.14 Wood units.

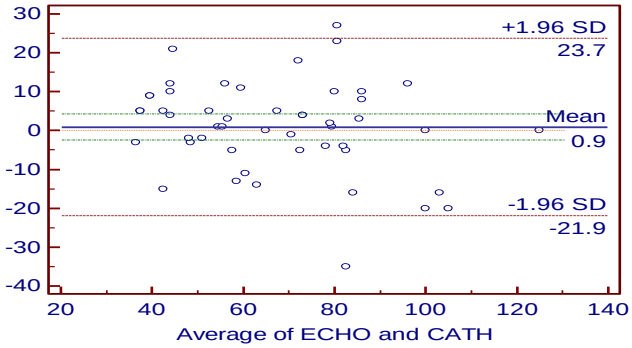

Fig. 1. Bland - Altman analysis for sPAP assessment by echocardiography and cardiac catheterization

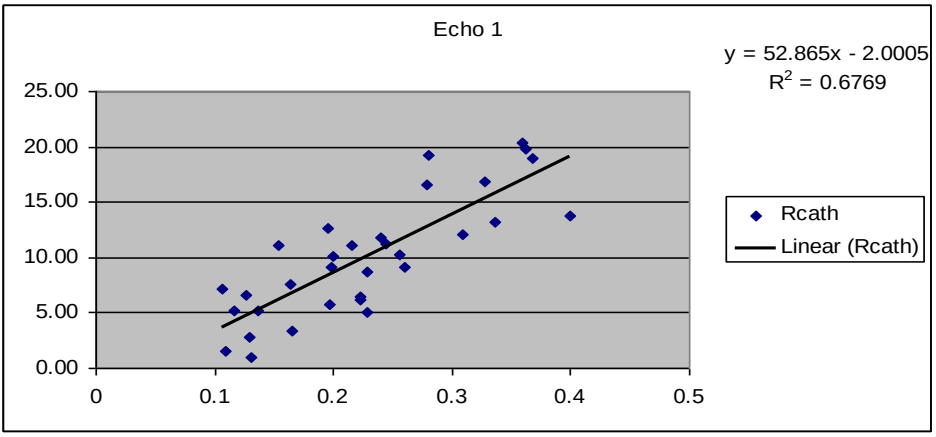

Fig. 2. Correlations cardiac catheterization - echocardiography (according to equation 5)

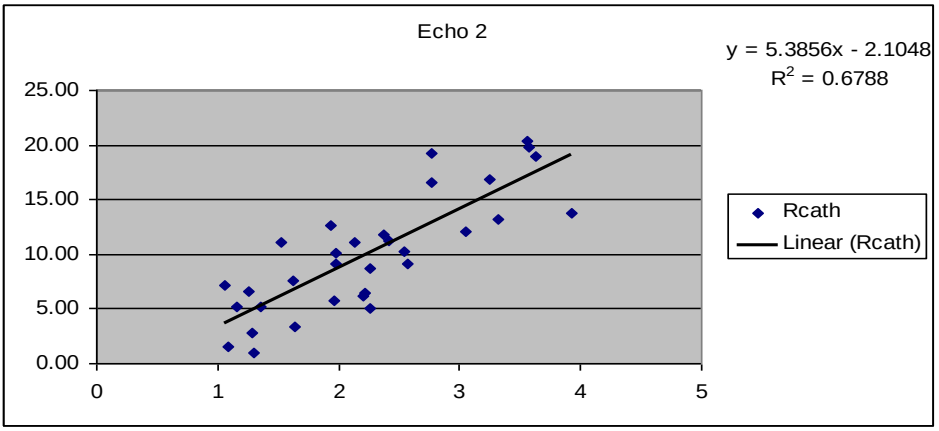

Fig. 3. Correlations cardiac catheterization - echocardiography (according to equation 6)

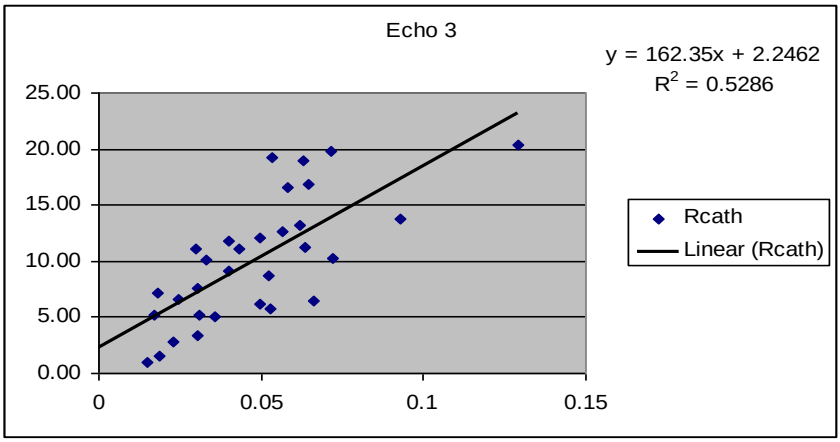

Fig. 4. Correlations cardiac catheterization - echocardiography (According to equation 7**)**

DISCUSSION

The hemodynamic study of the pulmonary circulation has a crucial importance, the pulmonary hypertension diagnostic being established, according to the guides in force, based on a hemodynamic parameter (mPAP). Determining the pulmonary pressures and resistances, besides diagnosis identification, allows the framing in the clinical classes, the selection of the optimal therapeutic scheme and the supervision of the therapeutic response. The exclusive achievement of these desiderata by invasive investigations supposes high costs and the risk of complications. Our study, together with the other recent articles, proves that the echocardiography can represent a method of determining both the pulmonary pressures and resistances. Other studies found very good correlations between sPAP value assessed on the echocardiography and the one measured invasively (r 0.98) (5). sPAP underestimation can be produced by the wrong alignment of the cursor with the jet direction, in patients with severe tricuspid regurgitation (because of the modified geometry of the tricuspid orifice) or very low regurgitation or caused by the precarious echogenicity in patients with chronic pulmonary diseases. In the last two situations, the use of a contrast agent can improve the Doppler signal quality and can increase measurements accuracy (6). sPAP overestimation is possible too. The main explanation is connected to the fact that the echocardiography measures the maximum instantaneous gradient, which is by definition higher than peak to peak gradient measured at the cardiac catheterization. Very recent studies obtained very good results for the mean PAP value deducted from the systolic PAP value based on certain mathematic formulae (7, 8). Using this formula, there was identified a threshold value of 36 - 38 mm Hg of sPAP for the PH diagnosis, corresponding to an mPAP > 25 mm Hg (7). The sensitivity and specificity of the method were excellent (97.9%, respectively 98.6%).

The same excellent correlations were obtained for the pulmonary vascular resistance values; from this point of view, the results of our study frame in the modern tendency of echocardiographic estimation

of the pulmonary resistance (9, 10). The formula that we selected for our study is fast and consistent. The normal value of the ratio max v TR/ VTI_{RVOT} is ≤ 0.15 (11). The ratio max v TR/ $VTI_{RVOT} > 0.2$ identifies with a good sensitivity and specificity a pulmonary vascular resistance higher than 2 Wood units and integrally the pulmonary vascular resistances higher than 4 Wood units (12). A ratio max v TR/ $VTI_{RVOT} > 7.6$ allows the identification of the patients with negative prognostic (11).

CONCLUSIONS

The echocardiography is a reliable method for the study of pulmonary circulation hemodynamics. Even though the echocardiography cannot substitute yet the invasive measurement of the pulmonary vascular resistance especially when it determines a therapeutic decision, the method is very useful in the case of the pulmonary hypertension caused by increased flow (with normal pulmonary resistances) or in advanced stages of the disease, where the systolic pulmonary artery pressure decreases by a reduced cardiac flow, but the pulmonary resistances are high, as for the serial examinations performed during the supervision of the therapeutic response.

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HIPERTENSIUNEA ARTERIALĂ ESENTIALĂ LA TINERI – AMBULATOR *vs.* SPITAL –

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ESSENTIAL HYPERTENSION AT YOUNG PEOPLE – AMBULATOR VERSUS HOSPITAL (Abstract): Cardiovascular disease is the leading cause of death in our country. The number of young people with hypertension grow up quickly, so a good control of dyslipidemia and blood pressure (BP) is essential in prevention of cardiovascular disease. **Aim**: To investigate the prevalence of HTA at young people and established the corelation with another risk factors like smoke, colesterol, obesity and heredity, few data are available on the blood pressure characteristics of young patients. **Material and method**: It has been investigate 366 young people between 19-25 years old, in ambulatory system and 350 younger with the same age, in hospital. Blood pressure was measured according to standard procedures, and was considered well-controlled if it was <140/90 mm Hg. **Results**: From our ambulatory patients were 198 women (54,1%), 168 men (45,9%) and from hospital were 178 women (50,9%) and 172 men (41,9%). HTA was present at 37 patients (10,1%) in ambulatory system and 50 patients (14,3%) in hospital. Between the intensity of smoke, the number of cigarette and the prevalence of HTA is a direct relation. The heredity factor is very important, too. The prevalence grow more than 2,5 at this patients. The incidence of HTA is 1,9 bigger at women with big values of colesterol and 2,1 at men with big colesterol. The relation between HTA – obesity is proven in our study, the incidence of HTA is 2,6 bigger at the obeses patients. These arguments should also promote further research in primary care on the control and the therapeutic behavior of the physicians. **Key words**: HYPERTENSION, OBESITY, SMOKE, HEREDITY.

Hipertensiunea arterială esențială (HTA), factor de risc cardiovascular independent, este tot mai frecvent diagnosticată la tineri. Prezența unor valori crescute ale tensiunii arteriale (TA) în copilărie şi adolescență reprezintă un risc pentru HTA a adultului.

Relația pozitivă dintre TA sistolică, TA diastolică şi riscul cardiovascular este recunoscută de multă vreme; această relație este puternică, continuă, graduală, consistentă, independentă şi predictivă atât pentru prezența cât şi în absența cardiopatiei ischemice manifeste (1, 2). În plus, prevalența mare în populație face ca HTA să fie o problemă majoră de sănătate publică (3, 4). La adult valorile crescute ale TA sunt corelate cu riscul apariției infarctului miocardic, accidentului vascular cerebral (stroke), bolilor renale, HTA fiind un factor de risc cardiovascular independent, extrem de im-

portant (5, 6).

În ultima perioadă asistăm la progrese importante legate de diagnosticul şi evaluarea HTA la copil, adolescent şi tânăr. Sub aspectul etiologiei, timp îndelungat s-a afirmat, că la aceste categorii de vârstă domină forma secundară a afecţiunii, dar s-a constatat că HTA esenţială devine gradat etiologia dominantă (7). Prezenţa unor valori crescute ale TA la copil şi adolescent constituie un factor de risc pentru apariţia HTA la adult; numeroase studii relevă fenomenul de tracking, de persistenţă a HTA din adolescenţă la maturitate (8, 9).

Pe lângă prevalenţa mare a HTA, tinerii asociază şi diferite comorbidităţi - dintre acestea pe primul loc se află obezitatea, afecţiune ce are un caracter cu adevărat epidemic la această vârstă (10, 11).

La tineri HTA esenţială îmbracă o formă uşoară sau de gradul I, subiecţii fiind adesea supraponderali sau obezi, ori având antecedente heredocolaterale pozitive pentru HTA sau boala cardiovasculară. Apariţia HTA la vârste tinere are două consecinţe majore: creşterea riscului de boală cardiovasculară şi modificarea stării de sănătate a subiectului în sens negativ. Ca urmare devine importantă evaluarea cât mai corectă şi completă a unui asemenea subiect, dar şi instituirea măsurilor de tratament (12, 13).

Pornind de la cele câteva premise menţionate scopul acestui studiu a fost pe de o parte de a stabili prevalenţa HTA esenţiale la tineri şi pe de altă parte de a evalua corelaţia existentă între HTA şi alţi factori de risc cardiovascular: fumat, hipercolesterolemie, antecedente heredo-colaterale şi obezitate.

MATERIAL ŞI METODĂ

Scopul acestui studiu a fost de a evalua prevalenţa HTA la un lot de 366 tineri cu vârsta cuprinsă între 19-25 ani, ce s-au adresat medicului în ambulator versus 350 tineri spitalizaţi, precum şi de a stabili corelaţiile existente cu alţi factori de risc. Factorii de risc cardiovascular care influenţează puternic prevalenţa HTA la tineri sunt: fumatul, valoarea colesterolului, obezitatea, precum şi prezenţa afecţiunii la părinţi.

Măsurarea tensiunii arteriale a fost efectuată cu tensiometrul cu mercur în spital, respectiv tensiometru de braţ în ambulator, verificat în prealabil. În ambulator, pacientul a fost lăsat minim 15 minute în repaus şi su fost efectuate 3 măsurători, la distanţă de câteva minute. S-a notat valorea medie a determinărilor. Metoda de determinare a valorilor tensionale a fost cea cunoscută; manşeta tensiometrului a fost poziţionată la nivelul braţului, după stabilirea pulsului la nivelul arterei brahiale.

Pacienţii din ambulator au fost împărţiţi în funcţie de sex în 54,1% femei şi 45,9% bărbaţi, iar cei din spital, incluşi în studiu, din aceeaşi categorie de vârstă, au putut fi scindaţi în 50,9% femei şi 49,1% bărbaţi. Nu s-a constatat o diferenţă semnificativă statistic între cele două loturi. Tuturor li s-au determinat parametrii antropometrici (înălţime, greutate, IMC), valorile TA, colesterolul. De asemenea, s-a stabilit statusul de fumător sau nefumător, consumul de alcool, activitatea fizică şi au fost consemnate antecedentele heredocolaterale.

Definirea HTA s-a făcut folosind criteriile indicate în cel de-al IV-lea raport privind diagnosticul, evaluarea şi tratamentul HTA la adolescent şi tineri (14).

REZULTATE

Incidenţa prehipertensiunii şi HTA la lotul studiat este de 13,7% şi respectiv 10,1% la pacienţii din ambulator. Raportul

se inversează în cazul pacienților spitalizați, incidența pacienților cu HTA (14.3%) fiind mai ridicată decât cea a pacienților prehipertensivi (12.6%) (fig. 1).

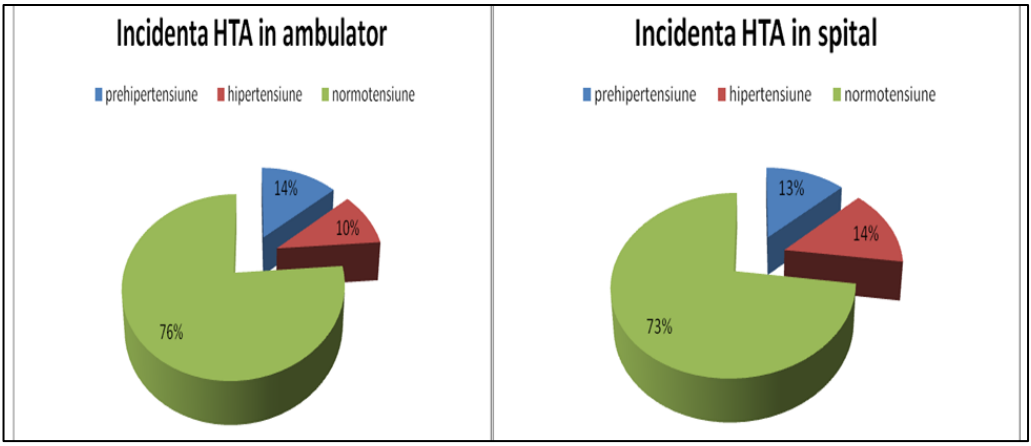

Fig. 1. Incidența prehipertensiunii şi HTA la tineri în ambulator şi spital

În ceea ce privește incidența HTA pe sexe se remarcă o uşoară predominanță a acesteia la sexul masculin 11,2% în ambulator, 10,8% în spital, față de sexul feminin 9,2% ambulator şi 10,6% în spital. Deci, indiferent de modalitate de prezentare la medic numărul pacienților de sex masculin este mai mare decât cel al pacienților de sex feminin, dar aceasta diferență nu este semnificativă statistic.

Rolul fumatului ca factor de risc direct, independent pentru HTA este incomplet elucidat, rezultatele diverselor studii fiind contradictorii (15, 16). La tinerii luați în studiu statusul de fumător sau nefumător nu influențează semnificativ prevalența HTA. În schimb între intensitatea fumatului, exprimată prin numărul de țigarete fumate zilnic şi prevalența HTA există o relație de directă proporționalitate (fig. 2).

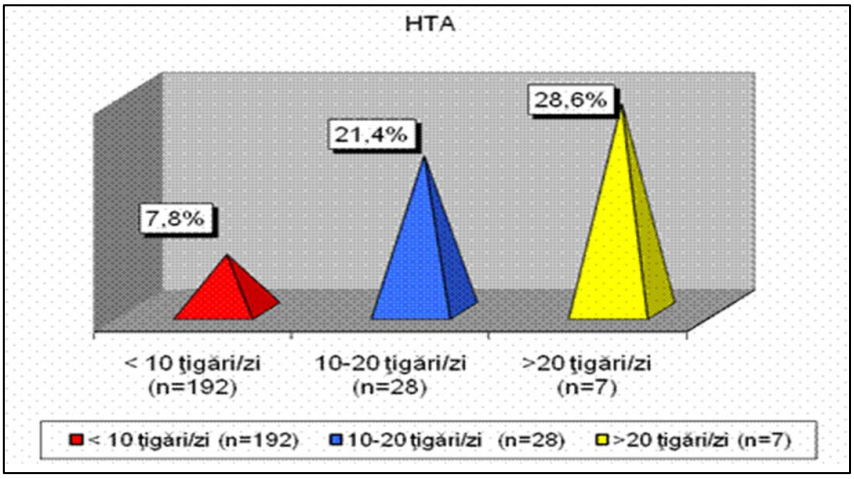

Fig. 2. Incidența HTA în funcție de intensitatea fumatului

Antecedentele heredocolaterale pentru HTA influenţează puternic prevalenţa acesteia la descendenţi. Prevalenţa HTA este de 2-2,5 ori mai mare dacă unul dintre părinţi este hipertensiv şi se triplează dacă ambii părinţi au afecţiunea (fig. 3).

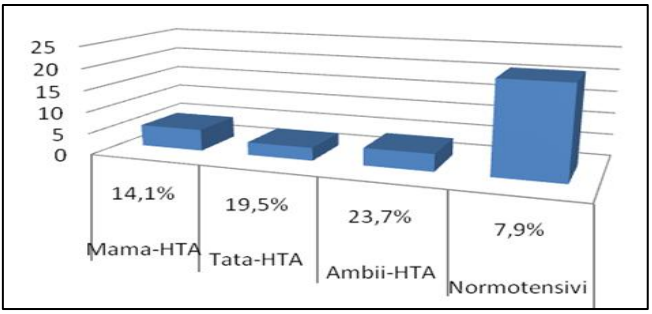

Fig. 3. Incidenţa HTA în funcţie de AHC de boală hipertensivă

O corelaţie cu totul particulară s-a constatat în cadrul relaţiei colesterol - HTA. Incidenţa HTA este de 1,9 ori mai mare la pacientele de sex feminin, cu hipercolesterolemie comparativ cu cele cu valori normale ale colesterolului, iar la sexul masculin incidenţa HTA este de 2,1 ori mai mare la pacienţii cu valori ridicate ale colesterolului (tab. I). Nu s-a constatat o diferenţă semnificativă statistic între cele două sexe. Rezultă deci că hipercolesterolemia este un puternic factor de risc pentru apariţia HTA.

TABELUL I.

Incidenţa HTA în funcţie de valorile colesterolului

	Femei		Bărbaţi		Total	
	Nr.	**% HTA**	**Nr.**	**% HTA**	**Nr.**	**% HTA**
Colesterol total < 180 mg/dl	15	8,4	14	9,6	28	9,0
Colesterol total >180 mg/dl	4	15,6	5	20,0	9	17,9

Relaţia HTA - obezitate este dovedită şi în studiul nostru, incidenţa HTA fiind mult mai mare la hipertensivi faţă de normotensivi – respectiv de 2,6 ori (tab. II). Această diferenţă este semnificativ statistic ($p<0.01$) şi se constată în ambele categorii de pacienţi studiaţi: ambulator sau spital.

TABELUL II.

Prevalenţa HTA în funcţie de statusul ponderal

	Femei			Bărbaţi		
	Nr.	Pondere	Prevalenţă	Nr.	Pondere	Prevalenţă
Normoponderali						
Normotensivi	291	84,3	57,7	213	75,3	42,3
HTA	54	15,7	43,5	70	24,7	56,5
Supraponderali+obezi						
Normotensivi	22	48,9	48,9	23	47,9	51,1
HTA	23	51,1	47,9	25	52,1	52,1

DISCUȚII

Datele din literatură ce fac referire la prevalența HTA la adolescent şi tânăr oferă cifre cuprinse între 1-13%, iar după unii autori procentul poate ajunge şi la 17% (18, 17). Aceste variații foarte mari sunt determinate de criteriile adoptate pentru a defini valorile normale ale TA, numărul determinărilor valorilor TA în cursul unei examinări, numărul examinărilor în timp precum şi de durata urmăririi subiecților în timp. Prevalența HTA a fost de 10,1%, cu o uşoară predominanță la sexul masculin. Este foarte probabil ca rezultatul obținut de noi să ofere o supraevaluare a prevalenței HTA la lotul studiat deoarece nu au fost excluşi subiecții ce ar fi putut prezenta HTA de halat alb (frecvent întâlnită la consultațiile în ambulator) şi nu au fost înlăturate posibilele cauze de HTA secundară.

Se observă prevalența mare a prehipertensiunii echivalent al categoriei high normal la adult şi a cărei depistare constituie indicație de schimbare a stilului de viață (19).

Uşoara prevalență a HTA la sexul masculin a fost explicată prin faptul că la lotul nostru aceştia au fost mai frecvent obezi şi fumători.

Faptul ca incidența HTA este cvasiegală, indiferent de statusul de fumător sau nefumător s-a explicat prin faptul că vechimea acestui obicei nu e foarte mare în rândul tinerilor şi ca urmare efectele sale pe termen lung (disfuncția endotelială, creşterea rezistenței vasculare periferice, stimularea proliferării celulelor musculare netede şi creşterea nivelului de endotelină) nu sunt manifeste. Celălalt aspect constatat şi anume creşterea prevalenței HTA cu numărul de țigări fumate zilnic s-ar putea explica prin efectele acute (în principal stimularea simpatică) care domină la adolescenți şi tineri. Rezultate similare între prevalența HTA şi intensitatea fumatului au fost obținute şi în alte studii (20).

Influența antecedentelor heredocolaterale în apariția afecțiunii la descendenți este o certitudine, fiind relevată în numeroase studii. Se apreciază că în apariția HTA esențiale predispoziția genetică are o contribuție medie între 20 şi 60-70% (21).

Relația dintre nivelul colesterolului şi incidența HTA reprezintă o corelație cu totul particulară pe care am observat-o şi vine să sublinieze necesitatea determinării valorii acestuia ori de câte ori tânărul este găsit ca având valori crescute ale TA, lucru subliniat de altfel în ghidul de evaluare a dislipidemiilor, indiferent de vârstă (23, 22).

Relația HTA-obezitate este dovedită de numeroase studii, HTA fiind de altfel componentă a sindromului metabolic din ce în ce mai frecvent diagnosticat la tinerii obezi, hiperinsulinismul şi insulinorezistența fiind una dintre căile patogenetice care realizează legătura dintre cele două afecțiuni (24, 25).

CONCLUZII

1. Prevalența HTA esențiale este în continuă creştere la tineri, devenind etiologia dominantă.

2. Prevalența este puternic influențată de modul de definire a valorilor normale, tehnica şi durata urmării în timp a subiecților.

3. La tineri HTA esențială îmbracă o formă uşoară sau de gradul I.

4. Numărul de țigarete fumate zilnic influențează valorile tensionale.

5. Hipercolesterolemia este un puternic factor de risc pentru apariția HTA.

6. Obezitatea este un alt factor de risc dovedit în apariția şi creşterea valorilor tensionale.

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NOUTĂȚI

EFECTELE ANTI-RETROVIRALE ALE AGONIŞTILOR CB2

Drogurile canabinoide au efecte terapeutice la pacienţii aflaţi în stadiul SIDA, „marijuana medicală" fiind utilizată în tratamentul durerii şi caşexiei la aceşti pacienţi. Receptorii canabinoizi CB2, dar nu şi CB1, de pe suprafaţa celulelor imune, pot influenţa răspândirea HIV-1, având în plus, efecte antivirale. Agoniştii canabinoizi CB2 modulează funcţia co-receptorului CXCR4 şi blochează procesul de semnalizare responsabil de pătrunderea virusului în celule, împiedicând infectarea unor noi limfocite T CD4+. Utilizarea agoniştilor CB2-selectivi ca tratament adjuvant în SIDA poate ameliora simptomatologia pacientului şi preveni răspândirea virusului, fără efectele nedorite determinate de administrarea marijuanei şi asociate stimulării receptorilor CB1. (Costantino CM, Gupta A, Yewdall AW, Dale BM, Devi LA, et al. Cannabinoid Receptor 2-Mediated Attenuation of CXCR4-Tropic HIV Infection in Primary CD4+ T Cells. *PLoS ONE* 2012;7(3): e33961. doi:10.1371/journal.pone.0033961).

Teodora Vremera

ADVERSE EFFECTS OF CHOLINESTERASE INHIBITORS IN THE ELDERLY PATIENT WITH MYASTHENIA GRAVIS. CASE REPORTS

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ADVERSE EFFECTS OF CHOLINESTERASE INHIBITORS IN THE ELDERLY PATIENT WITH MYASTHENIA GRAVIS. CASE REPORTS (Abstract). Myasthenia gravis (MG) is an autoimmune neuromuscular disease caused by the blockade of acetylcholine receptors at the post-synaptic neuromuscular junction. The clinical signs of MG are obvious in the young patient, but they may be misinterpreted in an elderly person with comorbidities either as manifestations of sarcopenia, as the onset of a minor stroke or "equivalents" of acute coronary syndrome or heart failure. Anticholinesterases (Pyridostigmine, Neostigmine) increase acetylcholine concentration at the neuromuscular junction and are the standard agents for symptomatic treatment; in elderly patients, their side-effects can easily be attributed to other age-related problems, such as bradycardia, arrhythmia or epigastric pain and nausea. We present two cases that illustrate the particularities of diagnosing and treating elderly patients with comorbidities and MG. We conclude that it is necessary to periodically monitor the elderly patient with MG for the early identification of adverse effects. It is also useful to remember that in the elderly patient with comorbidities and MG the risk of cholinesterase inhibitor overdose is increased, partly due to numerous concomitant drugs and partly due to low therapy adherence. Both statins and associated metabolic diseases are possible causes of disease progression, therefore caution is required in prescribing medication to the patient with myasthenia gravis. **Keywords:** CHOLINESTERASE INHIBITORS SIDE EFFECTS, MYASTHENIA GRAVIS, ELDERLY.

Myasthenia gravis (MG) is an autoimmune neuromuscular disease caused by the blockade of acetylcholine receptors at the post-synaptic neuromuscular junction, inhibiting the effects of acetylcholine on nicotinic receptors. Clinical manifestations of MG are varying degrees of weakness of skeletal muscle and/or muscles that control eye movements, facial expression, swallowing (1). Life expectancy of these patients is like that of the general population, and, as the population ages, estimates indicate that the number of elderly patients with MG will significantly increase. Anticholinesterases (Pyridostigmine, Neostigmine) increase acetylcholine concentration at the neuromuscular junction and are the standard agents for symptomatic treatment (2, 3).

The clinical signs of MG are obvious in the young patient: drooping eyelid, double vision, chewing and swallowing difficulties,

decrease in limb muscle strength. However, the same symptoms occurring in an elderly person with comorbidities may be misinterpreted by the physician either as manifestations of the aging phenomenon (reduced muscle strength is the cardinal feature in sarcopenia), or as the onset of other conditions that are more frequent in the elderly (transient ischemic attack, minor stroke) or "equivalents" of concomitant chronic diseases (acute coronary syndrome, heart failure) (4, 5). If the elderly patient was diagnosed with MG years ago and was properly treated, there is a greater probability that the side effects of medication will interfere with the progression of some concomitant conditions, especially cardiovascular, thus accounting for the symptoms and changes reported by the patient for which we either do not find an explanation, or the explanation we find is deeply erroneous. For this reason, minimal medication and careful and sustained monitoring of treatment is recommended in elderly MG patients (3,6).

We report two cases of elderly patients with MG which illustrate the adverse effects of treatment with neostigmine at therapeutic doses and the consequences of neostigmine overdose at the time of diagnosis and treatment initiation.

CASE REPORTS

Case 1. A 64-year-old female patient diagnosed with ocular MG 26 years ago, for which she followed a symptomatic treatment with cholinesterase inhibitor neostigmine at the therapeutic dose of 15mg x 2/day. She associated a cardiovascular condition, namely grade 3 essential hypertension, for which she received treatment with angiotensin converting enzyme inhibitor and thiazide-like diuretic. The patient also had a history of operated right ear mastoiditis with secondary hearing loss and peripheral vertiginous syndrome, being on treatment with betahistine.

Under the afore-mentioned treatment, the patient, although asymptomatic, detected by self-monitoring low heart rates for about one month before presentation to her attending physician. Resting electrocardiogram revealed sinus bradycardia 50/min, and 24-hour Holter EKG monitoring revealed a mean heart rate of 57/min with a minimum value of 37/min and a maximum of 108/min, with no sinus pause > 2,500 msec. Of note, the patient was not taking drugs inducing bradycardia. The biological profile revealed normal thyroid status, and bilateral reduced muscle strength in the upper limbs. Following the assessment of the recently occurred bradycardia, we considered the possible occurrence of side effects to chronic neostigmine treatment, and the patient was referred to a neurologist for the reassessment of therapeutic options. Ideally, this re-evaluation should be done in the presence of both physicians for optimal approach.

Case 2. A 74-year-old male patient known with important comorbidities (grade 3 essential hypertension, paroxysmal atrial fibrillation, chronic ischemic cardiomyopathy, associated with type 2 diabetes and amiodarone-induced hypothyroidism), for which he received multiple drugs (antiarrhythmic, chronic oral anticoagulation, antihypertensive, antidiabetic, hormone replacement, coronary dilation, and statin - rosuvastatin 5mg/day - therapy), presented in the out-patient geriatric clinic for epigastric pain and nausea. In the last two months he was diagnosed with late-onset seropositive MG (high anti-acetylcholine receptor antibody levels) that presented with important myalgia and muscle fatigue; the clinical examination revealed right palpebral ptosis. The patient was assessed by a neurologist and treatment with Pyridostigmine 60 mg x 3/day was initiated. Later,

another cholinesterase inhibitor, namely Neostigmine 15 mg x 3/day was recommended as an alternative. For reasons of noncompliance, the patient continued the treatment with both these drugs for 10 days and presented to the internist for overdose phenomena, represented by epigastralgia and nausea. A new neurological consultation was recommended, with the readjustment of therapeutic regimen to Neostigmine 15mg x 3/day and corticotherapy.

DISCUSSION

As to the age of onset of MG there is a bimodal distribution, with a predominance for women in decades 3-4 of life and for men in decades 4-8, respectively. At advanced ages (diagnosis after age of 50), MG is more common in males, and diagnostic confusions are also more frequent. The here-reported cases illustrate both situations and fall within the epidemiological characteristics cited in the literature (1, 7, 8).

MG requires long-term treatment with cholinesterase inhibitors, which can cause bradycardia and even asystole (9). The symptomatic benefit of both Pyridostigmine and Neostigmine is due to the increase in acetylcholine concentration at the neuromuscular junction through the nicotinic effect at this level. However, cholinesterase inhibitors have muscarinic side effects at the cardiac level, most frequently bradycardia induced by M2 cardiac receptor overload (10). In Case 1 bradycardia occurred after a long period of Neostigmine treatment. Although the patient may be asymptomatic, bradycardia may progress to atrioventricular blocks of varying degrees, so monitoring for treatment efficacy and adverse effects in case of long-term administration is required. There are literature data on Pyridostigmine-induced high-grade atrioventricular blocks which required pacemaker insertion (9). Of the cholinesterase inhibitors, Pyridostigmine is the safest due to the reduced rate of muscarinic adverse effects compared to Neostigmine (11), but its availability in Romania is reduced. The most effective alternatives are corticotherapy and immunosuppressive agents (2, 12).

Age, and not the duration of treatment with cholinesterase inhibitors, correlates with the incidence of cholinergic side effects. Among the most common muscarinic side effects of cholinesterase inhibitors are gastrointestinal disorders, including cramps or abdominal pain, as expression of autonomic dysfunction (3, 11). Overdosage induces a moderate cholinergic crisis (expressed as muscle weakness and exacerbation of gastrointestinal adverse effects), this being the case of the second patient we presented (13). For reasons of noncompliance, possibly due to polymedication, the patient had self-administered supratherapeutic doses. Frequent monitoring for adverse effects is required because the cholinergic crisis is difficult to differentiate from worsening of myasthenia gravis (14).

Statins are also mentioned in the literature as possible triggers of disease exacerbation in the elderly. Thus, Gale (15) reports a rare side effect of statins, namely the induction or worsening of autoimmune diseases, myasthenia gravis included. Statins are drugs often prescribed because of their important role in cardiovascular disease prevention. On one hand, statin treatment may aggravate the MG symptoms due to statin-induced myopathy, but on the other hand there are documented cases of seropositive MG secondary to statin therapy. Also, the metabolic disorders are cited among the factors that precipitate the worsening of MG symptoms (5). In Case 2, the patient followed a treatment with Rosuvastatin, was diabetic and was on hormone replacement therapy for amiodarone-induced hypothyroidism. All these factors

are elements that can aggravate symptoms, but also cause management difficulties due to comorbidities (15).

CONCLUSIONS

With these cases we underline the necessity of periodical monitoring of patients with cholinesterase inhibitors treatment for early identification of adverse effects. Cholinesterase inhibitors are the major symptomatic treatment of MG. However, long-term administration in therapeutic doses may cause adverse effects in vulnerable population groups.

Also, we draw attention to the fact that in the elderly patient with comorbidities the risk of cholinesterase inhibitor overdose is increased. Polymedication favors reduced compliance, which can lead to a cholinergic crisis. This situation is more delicate as the worsening of MG symptoms can mimic a cholinergic crisis.

Both statins and associated metabolic diseases are possible causes of disease progression, therefore caution is required in prescribing medication to the patient with myasthenia gravis and a correct treatment of associated diseases.

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TERAPIA CU ANTIBIOTICE ÎN INFECȚIILE PICIORULUI DIABETIC

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ANTIBIOTIC THERAPY IN THE INFECTED DIABETIC FOOT (Abstract): Foot infections in patients with diabetes cause substantial morbidity and may lead to amputation of a lower extremity. Management of diabetic foot infections involves evaluating and determining the severity of infection as the basis for selecting the appropriate approach to antibiotic treatment. **Aim:** The purpose of our study is to analyse the etiologic agents commonly involved in diabetic foot infections and to appreciate the outcome of these infections with antibiotic therapy. **Material and methods:** We included 90 patiens with infection of the diabetic foot, admitted in the Diabetes Clinic, „Sf. Spiridon" Hospital; wound swabs were collected from 79 infected ulcers. The seriousness of the infection was assesed using the IDSA clinical classification. **Results:** 45% of the infections were monomicrobial (typically with gram-positive cocci), but we also found gram-negative rods and enterococci. Initial therapy was usually empiric, while definitive therapy is based on the results of culture and sensitivity tests, as well as the clinical response to empiric therapy. **Conclusions:** The effectiveness of the antibiotic therapy, based on the clinical evolution and the absence of surgical intervention is related to the presence or absence of ischaemia and other diabetes-related complications rather than to the microorganism itself; antibiotics are a crucially important adjunct in the complex therapy of the diabetic foot. **Key words:** DIABETIC FOOT ULCERS, INFECTION, ANTIBIOTICS

Ulcerele piciorului diabetic sunt o serioasă problemă de sănătate. Statisticile arată că 15% din populația diabetică are pe parcursul vieții ulcerații la nivelul picioarelor (1). Ulcerele sunt rezultatul neuropatiei sau ischemiei. Neuropatia periferică se caracterizează prin scăderea senzațiilor protective si modificări biomecanice care produc ulcerații în zonele de presiune. Neuropatia autonomă determină scăderea secreției sudorale şi modificări vaso-motorii care vulnerabilizează tegumentele la injurii. Ischemia este datorată bolii arteriale periferice. Tulburările circulatorii în zona ulcerativă sunt asociate cu reducerea oxigenării şi nutriției şi scăderea şanselor de vindecare a ulcerației. Țesuturile necrotice sunt un bun mediu de creştere a bacteriilor şi au mecanisme de aparare la infectii scăzute. Infecțiile care de obicei complică ulcerele piciorului diabetic sunt de cele mai multe ori consecința şi nu cauza ulceraților. Complicațiile acestor ulcere sunt principalele cauze de spitalizare si amputații la pacientii diabetici (2). Tratamentul antibiotic sistemic este totdeauna indicat la pacienții cu ulceratii infectate. Obiectivul lucrării de față este de a analiza patogenii cei mai frecvent implicați în infecțiile piciorului

diabetic, severitatea acestor infecţii, corelaţia cu factorii de risc prezenţi (cel mai frecvent complicaţii asociate diabetului) şi evoluţia infecţiilor piciorului diabetic la tratament antibiotic

MATERIAL ŞI METODE

Lotul studiat a fost format din pacienţii internaţi în Clinica de Diabet, Nutriţie şi Boli Metabolice Iaşi în perioada 1.01.2010 – 31.12.2010 cu diagnostice în relaţie cu "piciorul diabetic". Am realizat un studiu retrospectiv, realizând o bază de date cu următoarele informaţii despre pacienţii studiati: date generale (sex, vârstă, mediul de proveniență, durata spitalizării), informaţii referitoare la nivelul de şcolarizare, indici antropometrici [greutatea(G), înălţimea(T) şi circumferinta abdominală (CA); s-a calculat indicele de masă corporală [(IMC) = $G(kg)/T^2(m^2)$]

Informaţii despre diabetul zaharat (tipul de diabet zaharat, tratamentul efectuat, controlul diabetului zaharat (valoarea HbA1c la internare) prezenţa complicaţiilor cronice specifice.

Informaţii despre ulceraţia prezentă : tipul leziunii (ulceraţie, gangrenă, altele), localizarea leziunii (la nivelul degetelor, la nivel plantar, altele), clasificare (Texas, PEDIS, IDSA)

Informaţii despre prezenţa infecţiei confirmată prin culturi pozitive şi/sau prin semne locale de infecţie (celulită, osteoliză pe radiografie) şi/sau prin semne generale de infecţie (febră, leucocitoză).

Informaţii despre tratamentul antibiotic sistemic si evoluţia ulceraţiei Am luat in evidenţa tipul şi durata tratamentului antibiotic şi de asemenea, evoluţia spre amputaţie, evidenţiată prin necesitatea transferului într-o clinică chirurgicală.

Datele au fost colectate în programul Windows Office Excel 2007, în care s-a realizat şi prelucrarea statistică.

REZULTATE ŞI DISCUŢII

În Clinica de Diabet zaharat, Nutriţie şi Boli Metabolice (DNBM) au fost 90 internări în anul 2010 (4,5% din total internări) pentru diagnostice legate de "piciorul diabetic". Dintre acestea, 68 au fost bărbaţi (79,1%), iar 22 au fost femei (20,9%). Media vârstei la bărbaţi a fost 60,68 ani; media vârstei la femei a fost 63,64 ani. Din mediul rural proveneau 49 pacienţi (54,4%). În ceea ce privesc studiile pacienţilor: un pacient (1,1%) a fost fără studii; 11 pacienţi (12,2%) au declarat 4 clase; 35 de pacienţi (38,8%) aveau gimnaziul; 10 pacienţi (11,1%) aveau şcoală profesională; 23 pacienţi (25,5%) aveau liceul; 1 pacient (1,1%) avea şcoală post-liceală; 4 (4,4%) aveau studii superioare; la 5 pacienţi nu s-a specificat nivelul de instruire

În funcţie de IMC, s-a constatat că: un pacient (1,1%) era subponderal; 16 pacienţi (17,7%) erau normoponderali; 28 de pacienti (31,1%) erau supraponderali, iar 31 de pacienţi (34,4%) erau obezi; la 14 pacienţi (15,5%) nu s-au măsurat indicii antropometrici. 9 pacienţi (10%) aveau CA normală, 81 de pacienţi (90%) aveau CA groasă (>94 cm la barbati şi > 80cm la femei). Durata medie a spitalizării în Clinica DNBM a fost de 14,72 zile, cu un maximum de 55 zile şi un minimum de o zi. Mai puţin de 7 zile de spitalizare au avut 14 pacienţi (15,5%); între 7-13 zile de spitalizare au avut 34 pacienţi (37,7%); 23 pacienţi (25,5%) au stat internaţi între 14-20 zile; 10 pacienţi (11,1%) au stat internaţi între 21-27 zile; 9 pacienţi (10%) au stat internaţi peste 28 zile.

În ceea ce priveşte tipul de diabet zaharat, 7 pacienţi (7,7%) aveau DZ tip 1, 82 de pacienţi (91,2%) aveau diagnostic de DZ tip 2 şi un pacient (1,1%) avea diagnostic

de DZ secundar pancreatic. 63 dintre pacienți (70%) urmau tratament cu insulină la momentul internării. Doar 5 pacienți (6,8%) au avut $HbA_{1c} \leq 7\%$, media HbA_{1c} pe intregul lot fiind de 9,68%. Luând în considerare complicațiile cronice specifice diabetului zaharat cu răsunet asupra piciorului, mai exact polineuropatia periferică senzitivo-motorie (PNPSM) şi arteriopatia obliterantă a membrelor inferioare (AOMI), s-a constatat că: 3 pacienți (3,3%) nu prezentau nici una dintre aceste complicații; 3 pacienți (3,3%) prezentau numai AOMI (std. IV); 84 de pacienți (93,3%) aveau PNPSM, dintre aceştia 29 (32,2%) aveau şi AOMI, (25 pacienți prezentau chiar AOMI std. IV, adică 27,7%). Complicațiile cronice microvasculare specifice diabetului zaharat au fost prezente după cum urmează: 54 de pacienți (60%) prezentau retinopatie diabetică (RD), dintre care 16 cu formă proliferativă (17,7%). Dintre cei cu RD, 22 aveau şi nefropatie diabetică (ND), adică 40,7% din cei cu RD. S-a mai întâlnit ND la 11 pacienți fără RD. În total, ND a fost prezentă la 33 pacienți (36,7%).

Leziunile/ulcerele s-au situat: 35 la nivelul degetelor, 7 la nivelul degetelor şi plantar, iar 46 la nivel plantar. Prezentau celulită 36 dintre pacienti (40%). Secrețiile s-au recoltat în marea majoritate a cazurilor cu un tampon steril şi doar la cazuri selectate s-a dat spre cultură țesut debridat, aspirat purulent sau material bioptic. Secrții s-au putut recolta de la 79 dintre pacienți: 7 au fost negative, restul au fost pozitive (72 de cazuri). Totuşi, între cele 18 cazuri în care nu s-a evidențiat prezența infecției în cultură, în 16 s-a pus diagnosticul de infecție pe criterii clinice, locale (prezența celulitei) şi generale (febră şi/sau leucocitoză). În doar 2 dintre cele 90 de cazuri internate nu a existat infecție, fiind vorba de o ulcerație deget şi un mal perforant plantar neinfectate. 27 de pacienți (30%) mai avuseseră amputații în antecedente.

S-a izolat un singur microorganism în 60 dintre culturi, 2 microorganisme în 10 probe recoltate din plagă şi trei microorganisme într-un singur caz. Dintre cocii gram pozitivi: *S. aureus* a fost identificat drept agent unic în 32 cazuri(45 %); în 6 cazuri (8 %) a făcut parte din agenții patogeni ce cauzau o infecție polimicrobiană. In 27 de culturi s-au izolat stafilococ auriu meticilino-rezistent (SAMR). Stafilococi coagulazo-negativi au fost identificați în 2 cazuri. Streptococi β-hemolitici au fost identificați drept agent unic în 2 cazuri (grup A & G) şi în asociere cu alte microorganisme într-un caz (grup B). Enterococii au fost prezenți în 8 plăgi (11,26%); toate speciile izolate prezentau sensibilitate păstrată la vancomicină. Bacili gram-negativ au fost prezenți după cum urmează: Enterobacteriacee*: Klebsiella pneumoniae* – 10 cazuri, *Proteus* spp. – 6 cazuri, *Escherichia coli* – 3 cazuri, *Morganella* spp.– 2 cazuri, *Serratia* spp – 2 cazuri, *Enterobacter* spp – 3 cazuri, *Citrobacter* – un caz. *Pseudomonas aeruginosa* a fost prezent în 4 cazuri.

Clasificarea severității infecției a arătat că 88 din pacienții din lotul de studiu prezentau semne clinice de infecție, uşoară în 11% dintre cazuri, moderată în 40 % dintre cazuri şi severă în 49 % dintre cazuri. Numai 15 pacienți au avut febra (16,6%). La 20 de pacienți s-au recoltat hemoculturi(HC), la toți cei febrili, dar nu numai, la 5 pacienti HC fiind pozitive (3 dintre acestea la pacienți care nu erau febrili!). Doar 49 pacienți (54,4%) au avut leucocitoză (GA>10000/mmc), 56 (62,2%) au avut neutrofilie. Conform clasificării Texas: 15 sunt BI (16,6%), 32 sunt BII (35,5%), 8 sunt BIII (8,9%), unul este CI (1,1%), 8 sunt DI (8,9%), 16 sunt DII (17,7%), 8 sunt DIII (8,9%), la 2 nu s-a calculat (2,2%).

După clasificarea PEDIS, 16 pacienți (17,7%) sunt clasa *2*, ("*uşoară*" în IDSA), 47 (52,2) sunt *3*, ("*moderată*" în IDSA), 25 (27,7%) sunt *4*, ("*severă*" în IDSA).

Numărul mediu al zilelor de tratament antibiotic injectabil a fost de 12,6 zile. În principiu, numărul de zile de spitalizare se corelează cu numărul de zile în care a fost necesar tratament antibiotic injectabil. 37 de pacienți (42%) au primit un singur antibiotic, restul au primit, alternativ sau concomitent: 2 antibiotice – 30 de pacienți (34%), 3 antibiotice – 11 pacienți, 4 antibiotice – 9 pacienți. 25 pacienți (27,7%) au fost transferați în clinici chirurgicale pentru amputație limitată, 3 pacienți la chirurgie plastică pentru grefă de piele, 3 la chirurgie vasculară pentru revascularizare, 4 în alte clinici pentru probleme grave apărute pe durata spitalizării (cardiologie, neurologie, boli infecțioase).

Am comparat sublotul de pacienți care prezenta numai PNPSM (fără AOMI), cu cel al pacienților care prezenta şi AOMI. Proporția de bărbați a fost mai mare în grupul cu AOMI: 78,1% bărbați în grupul cu AOMI *vs.* 74,1% bărbați în grupul fără AOMI. Media de vârstă a celor din grupul cu AOMI a fost mai mare decât a celor din grupul fără AOMI: la bărbați 63,7 ani în grupul cu AOMI *vs.* 59,1 ani în grupul fără AOMI, iar la femei 65,5 ani la cele cu AOMI *vs.* 63,1 ani în grupul fără AOMI. Ponderea pacienților cu diabet zaharat tip 2 a fost mai mare în grupul cu AOMI (93,8%) față de cei fără AOMI (89,7%).

Au existat diferențe semnificative şi în ceea ce priveşte localizarea leziunilor. Astfel, leziunile la nivelul degetelor au fost mult mai frecvente în rândul celor care prezentau AOMI (65,7%), față de 36,2% la cei care prezentau numai PNPSM. În schimb, leziunile plantare au fost mult mai frecvente la cei cu PNPSM (63,8%), față de 46,9% la cei cu AOMI. Deci, se poate confirma că pacienții cu AOMI dezvoltă mai ales leziuni distale, spre deosebire de cei care prezintă numai PNPSM, care dezvoltă mai ales leziuni plantare.(3) şi cu spectru microbiologic diferit. (4). Astfel, cel mai frecvent patogen întâlnit la cei cu PNPSM a fost SAMR (38,3%), în timp ce la cei cu AOMI au existat 2 patogeni aproape la fel de des întâlniți, şi anume SAMS (21,9%) şi *Klebsiella pneumoniae* (18,8%). Numărul zilelor de tratament antibiotic intravenos a fost uşor mai mare în cazul celor cu PNPSM (13,5 zile) față de cei cu AOMI (11,1 zile), dar numărul de antibiotice folosit a fost acelaşi în cazul celor cu PNPSM şi a celor cu AOMI (în medie 1,9, respectiv 1,8 antibiotice). În concordanța cu datele din literatură, cel mai frecvent au fost implicați cocii gram-pozitivi aerobi; dintre aceştia, *S. aureus* a fost identificat drept agent unic în 45 % din cazuri, iar mai mult de 2/3 dintre tulpini fiind meticilino-rezistente. Deşi este uneori dificil de stabilit dacă microorganismele identificate, în acest caz stafilococii, sunt agenții etiologi sau doar colonizanți ai plăgii (neexistând nicio metodă care să poată indica agentul etiologic sau agenții colonizanți într-o cultură în care s-au obținut mai multe specii), prezența semnelor clinice şi paraclinice de infecție, uneori neidentificarea altor microorganisme şi chiar pozitivarea în 2 cazuri a hemoculturilor duce la considerarea stafilococilor agenți etiologici ai infecției piciorului diabetic în aceste cazuri. Identificarea S. aureus în plagă, fie ca agent unic, fie ca parte a unei flore mixte, implică în primul rând luarea unei atitudini terapeutice imediate, respectiv inițierea de urgență a antibioterapiei. În afară de managementul infecției piciorului diabetic, trebuie evaluată atitudinea față de portajul cronic de SAMR (5).

La majoritatea pacienților s-a inițiat o terapie empirică, a cărei alegere a fost influențată de mai mulți factori: estimarea

complianţei pacientului la un anumit tratament (mod de aministrare, numărul dozelor parţiale etc), istoricul de reacţii alergice la anumite antibiotice sau clase de antibiotice, efectele secundare şi reacţiile adverse (în special în administrarea orală), siguranţa administrării şi posibilitatea unei externări mai rapide (în special la administrarea parenterală), posibilitatea apariţiei rezistenţei, în special riscul de colonizare cu SAMR, riscul apariţiei diareei cu *C. difficile*, costurile asociate antibioterapiei. De asemeni s-a luat în considerare şi disponibilitatea anumitor antibiotice, care poate fi restricţionată prin lipsă sau urmare a dificultăţilor în administrare (6).

Nevoia frecventă de a iniţia tratament empiric în infecţiile piciorului diabetic poate determina alegerea unui regim cu spectru fie prea îngust ca să fie eficient, fie inutil de larg, care ar putea contribui la selecţia unor microorganisme rezistente. Monoterapia pare să fie mai avantajoasă decât asocierile de antibiotice: este mai uşor de administrat, are costuri mai reduse şi scade riscul apariţiilor reacţiilor adverse (7). Pe de altă parte, asocierile de antibiotice pot acoperi o gamă mai larga de microorganisme (având moduri de acţiune diferite, posibil efect sinergic); sunt indicate în infecţiile cu germeni rezistenţi, cum ar fi *P. aeruginosa.* Asocierea antibioticelor reduce deasemenea riscul de apariţie a rezistenţei. Nu exista date suficiente care să recomande asocierea de rutină a antibioticelor pentru a preveni emergenţa rezistenţei, dar această abordare este indicată la iniţierea antibioterapiei empirice la pacienţii cu afecţiuni severe, care au un risc crescut de a avea infecţii cu microorganisme multidrog-rezistente. Când au fost disponibile rezultatele investigaţiilor de laborator, inclusiv a culturii şi antibiogramei, s-a reevaluat terapia antibiotică. Este de preferat alegerea unor agenţi ţintiţi, dacă este posibil cu spectru redus; dar trebuie luat în considerare şi răspunsul clinic la tratamentul iniţial, empiric. Dacă s-a observat regresia semnelor clinice de infecţie, pacientul a tolerat terapia şi plaga a evoluat spre vindecare, nu a mai fost nevoie de remaniere, chiar dacă unele sau toate microorganismele izolate sunt rezistenţi la antibioticul prescris iniţial. Dacă infecţia nu a avut o evoluţie favorabilă, tratamentul a necesitat reevaluare, astfel încât să se obţină un spectru care să acopere toate microorganismele izolate. Dacă infecţia a continuat să evolueze nefavorabil, în ciuda sensibilităţii bacteriilor izolate la antibioticele prescrise, s-a considerat oportună intervenţia chirurgicală sau/şi posibilitatea existenţei unor bacterii ce necesită medii speciale de cultivare. În cazul infecţiilor polimicrobiene, cu evoluţie severă, s-a instituit de la început antibioterapia cu spectru larg cu administrare intravenoasă (8). Antibioticul singular sau în asociere a fost ales să fie activ atât pentru agenţii Gram pozitv, cât şi pentru Gram negativ (*Enterobacteriaceae, P aeruginosa*) şi pe organismele strict anaerobe (peptococi, peptostreptococi, *Bacteroides*). În infecţiile moderate, s-au administrat fluorochinolone în asociere cu clindamicină, iar în infecţiile severe s-a administrat piperacilină-tazobactam sau imipinem-cilastatin. Există studii care au arătat eficienţa ertapenemului *vs* piperacilină tazobactam (9), la fel, unele studii au demonstrat că linezolidul este mai puţin eficient decăt unele βlactamine asociate cu inhibitori de βlactamază, cu spectru mai larg (10). La orice grad de severitate a infecţiei, s-a evaluat riscul implicării SAMR, pentru a lua în considerare fie folosirea agenţilor eficienţi pentru această infecţie (linezolid, vancomicina, daptomicină) fie folosirea unora care nu au fost primit această indicaţie, dar a caror utilizare s-a dovedit în mod repetat eficientă (teicoplanin, acid fusidic, doxiciclină sau trimetoprim-sulfametoxazol), posibil în combinaţie sau asociaţi cu rifampicina

(care nu trebuie niciodată folosită ca monoterapie). În ciuda inițierii precoce a terapiei cu administrare de antibiotice parenteral, uneori folosind asocierile, 25 de pacienți (27,7 %) din lotul cercetat au necesitat transfer într-o secție chirurgicală în vederea amputației; procentul destul de ridicat este probabil influențat şi de gradul de severitate al infecției, rezultate concordante cu datele din literatură, care arată că pacienții diabetici care dezvoltă o infecție la nivelul piciorului au o probabilitate de a necesita amputație de aproximativ 30 de ori mai crescută (11) Terapia cu antibiotice a fost aleasă inițial empiric, în unele cazuri existând o remaniere ulterioară, calea de administrare fiind cea injectabilă. Fluorochinolonele (ciprofloxacina) a fost administrată în terapie unică la 20 (22,2 %) dintre pacienți, alți 46 (51,1 %) primind fluorochinolone în asociere cu cefalosporine de generația a 3–a, metronidazol, glicopeptide, linezolid.

CONCLUZII

Toți pacienții din lotul studiat aveau infectii ale piciorului de severitate medie sau grava care au necesitat spitalizare între 1 şi 55 zile (media 14,72 zile) S-a izolat un singur microorganism în 60 dintre culturi, 2 microorganisme în 10 probe recoltate din plagă şi trei microorganisme într-un singur caz. La pacienții cu ulcerații pe teren neuropat, cel mai frecvent patogen, a fost SAMR, iar la infecțiile pe picior diabetic arteriopat au fost izolați SAMS şi *Klebsiella pneumoniae*. Toti pacienții au primit tratament antibiotic inițial cu spectru larg, ulterior după rezultatele antibiogramei. Din experiența noastră cel mai util antibiotic s-a arătat a fi ciprofloxacina dat inițial empiric, dar care a fost în 72% din cazuri recomandat şi de sensibilitatea aratată de antibiogramă.

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RELEVANŢA CANALELOR DE CALCIU DE TIP L PENTRU EFECTELE NON-GENOMICE ALE ESTRADIOLULUI ŞI ALDOSTERONULUI ASUPRA ACTIVITĂŢII CONTRACTILE ÎN ARTERE IZOLATE

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RELEVANCE OF L-TYPE CALCIUM CHANNELS FOR THE NON-GENOMIC EFFECTS OF ESTRADIOL AND ALDOSTERONE UPON CONTRACTILE ACTIVITY IN ISOLATED ARTERIES (Abstract): Non-genomic vascular effects of steroids are incompletely understood, despite progress made regarding some aspects, such as the mechanism of endothelium-dependent relaxation by estrogens. **Aim**: To investigate the involvement of certain mechanisms in the rapid, non-genomic effects of estradiol (EST) and aldosterone (ALD) on endothelium-dependent and -independent vasomotor responses. Material and methods: Isometric myography of rings from aorta, mesenteric arch, and first order mesenteric branches isolated from male Wistar rats was used. **Results**: We found that L-type calcium channels (Cav1.2) are important for endothelium-independent relaxation induced by EST, while ALD reduces the involvement of Cav1.2 in phenylephrine-induced contraction and potentates both NO- and EDHF-mediated endothelium-dependent relaxation. To further examine the relevance of Cav1.2 for the vascular effects of EST and ALD, we were using rings with and without functional endothelium, precontracted by direct activation of Cav1.2 (Bay K 8644), high extracellular K+, phenylephrine, and under complete Cav1.2 block (nifedipine). Data suggest that EST, which directly inhibits Cav1.2 in transfected HEK cells, uses mainly this path to induce endothelium-independent relaxation, and that ALD may induce a rightward shift in the voltage-dependence of Cav1.2. **Key words**: SMOOTH MUSCLE, L-TYPE CALCIUM CHANNELS, ESTRADIOL, ALDOSTERONE.

Prezente în diferite tipuri de celule, canalele de calciu de tip L (Ca_L) contribuie la variatele semnale citosolice de calciu ce asigură controlul multor procese celulare fundamentale, cum ar fi contracţia, secreţia, neurotransmisia, expresia genică, etc. Influxul prin Ca_L reprezintă sursa majoră a semnalului contractil de Ca^{2+} în miocitele netede din artere de rezistenţă şi de conducere. Varietatea $Ca_v1.2$, prezentă în muşchiul neted vascular (MNV), are rol esenţial în contracţie şi în consecinţă în reglarea diametrului arterial, perfuziei tisulare şi a presiunii arteriale.

Estradiolul şi aldosteronul prezintă, în teritoriul vascular, pe langă efectele clasice, genomice, o serie de efecte rapide non-genomice. Aceste efecte ale estradiolului

(EST), mediate de receptori specifici şi parţial explicabile prin inhibiţia $Ca_v1.2$ din MNV, sunt ceva mai intens studiate datorită interesului pentru terapia hormonală de substituţie (1, 2). Deşi există o varietate a efectelor observate (3), în general acţiunea vasculară rapidă a ALD (mediată de receptorul clasic pentru mineralocorticoizi, MR) se traduce prin atenuarea efectului agenţilor vasoconstrictori, datorită activării NO-sintazei endoteliale (eNOS) precum şi prin potenţarea vasoconstricţiei în absenţa endoteliului, efecte comune cu cele ale cortizolului şi EST (4).

Am investigat importanţa $Ca_v1.2$ în efectele vasculare endotelio-independente ale EST şi ALD, precum şi implicarea unor mecanisme endotelio-dependente în efectele vasculare non-genomice ale acestor steroizi.

MATERIAL ŞI METODĂ

Am utilizat miografia izometrică a fragmentelor arteriale (~2 mm) din arcada mezenterică şi respectiv ramurile sale de ordin I de la şobolani Wistar masculi (~200 g). Preparatele au fost montate pe fire de oţel inoxidabil în migraful orizontal (JP Trading), tensionate treptat, până la o valoare a forţei pasive de 1 g şi echilibrate 90' (soluţie tampon HCO_3^-; 37° C; pH=7.2-7.4). Toate substanţele bioactive utilizate au fost din categoria pentru cercetare (Sigma, US) şi au fost administrate direct în baia de organ (volum util 5 ml) sub formă de volume mici de soluţii stoc; 50 µl; diluţie 1:100.

Prelucrarea datelor s-a efectuat off-line (Origin, Microcal Software, US). Pentru toate seriile experimentale rezultatele sunt exprimate ca medie a efectului respectiv (± ESM; n = 6). Diferenţele observate sunt testate din punct de vedere statistic prin testul Student pentru date grupate şi considerate semnificative pentru un coeficient de încredere mai mare de 99% ($p < 0,01$). Toate procedurile experimentale folosite sunt în concordanţă cu reglementările internaţionale în vigoare privind utilizarea animalelor în cercetarea ştiinţifică (Directiva 609 din 24 Noiembrie 1986, a Consiliului Comunităţii Europene).

REZULTATE ŞI DISCUŢII

Canalele de tip L din muşchiului neted vascular şi EST

EST relaxează arterele mezenterice de rezistenţă de la şobolan în absenţa endoteliului, independent de mecanismul precontracţiei (fig. 1), dar numai dacă acesta presupune influx de calciu prin Ca_L. Anterior am arătat că efectul relaxant al EST în aorta de şobolan în absenţa endoteliului nu se poate produce atunci când Ca_L sunt deja inhibate cu D600, un derivat de verapamil (5). Mai mult, în ramuri de ordin I ale arterei mezenterice, D600 inhibă efectul relaxant al EST în preparatul fără endoteliu funcţional (fig. 2).

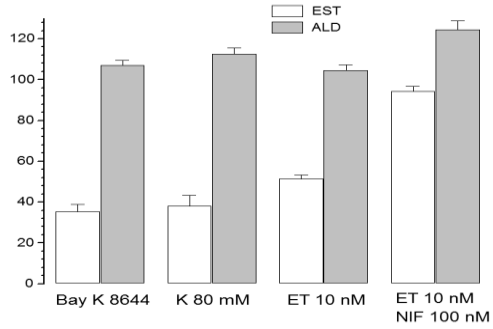

Fig. 1. Efectul relaxant al estradiolului (EST, 1µM) şi efectul contractant al aldosteronului (ALD, 1 µM) în inele din ramuri de ordin I ale arterei mezenterice de şobolan precontractate submaximal după cum urmează: prin activarea directă a $Ca_V1.2$ de către compusul Bay K 8644, prin depolarizare de către K^+ crescut extracelular (K 80 mM, prin activarea receptorilor ET_A de către endotelin (ET 10 nM) şi prin stimulare cu endotelin în condiţii de blocare completă a $Ca_V1.2$ de către nifedipină (NIF 100 nM).

Datele noastre completează observaţiile altor autori, care au arătat foarte recent că EST relaxează parţial arterele coronare porcine precontractate cu endotelin, iar nifedipina nu are efect suplimentar (6). EST inhibă contracţiile spontane şi pe cele induse de $CaCl_2$ şi KCl în duodenul de şoarece, prin activarea unor canale de K^+ şi inhibarea Ca_L (7). Cu toate acestea, EST are efect neuroprotector prin activarea Ca_L, creşterea calciului citosolic (Ca_c) ducând la activarea cascadei Src/ERK/CREB/Bcl-2 (8). Receptorul membranar de tip α pentru estrogeni (ERα) este capabil să asigure activarea Ca_L de către EST în celule pituitare, semnalul de Ca_c rezultat fiind implicat în stimularea de către EST a secreţiei de prolactină (9). Efectul inhibitor direct al EST asupra subtipului $Ca_V1.2$ de Ca_L a fost recent dovedit (10) prin studii de patch-clamp asupra acestor canale exprimate în celule de tip epitelial, din care lipsesc în mod normal.

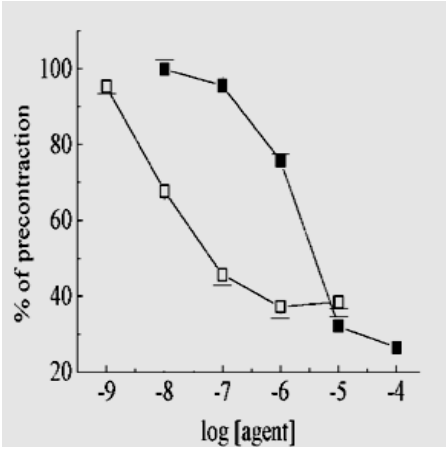

Fig. 2. Concentraţia corespunzătoare IC50 pentru D600 $5x10^{-7}$ M, induce o deplasare spre dreapta (pătrate goale) a curbei doză-relaxare pentru estradiol (pătrate pline) în artere mezenterice de rezistenţă în absenţa endoteliului.

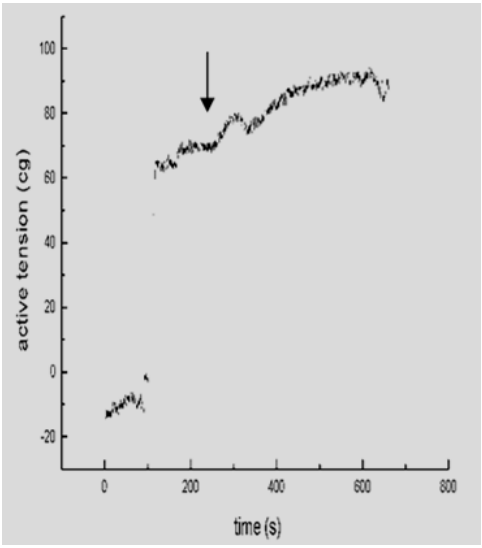

Fig. 3. Supracontracţia indusă de 1 μM aldosteron în ram din artera mezenterică precontractat cu fenilefrină 10 μM în absenţa endoteliului.

Canalele de tip L din muşchiului neted vascular şi ALD

ALD (10^{-9}-10^{-6} M) nu induce contracţie, nu influenţează contracţia indusă de K 40 mM sau fenilefrină 10^{-5} M, iar la concentraţii mari (10^{-6} M) determină o uşoară supracontracţie a ramurilor din artera mezenterică precontractate cu fenilefrină (fig. 3). În toate tipurile de vase studiate ALD reduce sensibilitatea contracţiei induse de fenilefrină faţă de acţiunea relaxantă a unui blocant Ca_L (metoxi-verapamil; D600, 10^{-7}-10^{-5} M).

Surprinzător, ALD are un efect asemănător, dar evident mai slab, chiar în cazul contracţiei induse de K^+ crescut, esenţialmente dependentă de Ca_L (5). S-a arătat de către alţi autori (11) că în artera mezenterică de şobolan ALD (10 nM) determină vasoconstricţie rapidă şi potenţează efectul vasoconstrictor al fenilefrinei, crescând rapid pH-ul intracelular şi Ca_c şi că activa-

rea MR în MNV de şobolan potenţează efectul contractil al fenilefrinei şi angiotensinei II, pe calea ERK1/2 (c-Jun, p38, Src) (12). În ramurile arterei mezenterice de la şobolan noi nu am observat un efect contractil indus de ALD şi nici efect potenţator asupra altor efecte contractile, spre deosebire de aortă, unde am observat potenţarea efectului fenilefrinei şi reducerea contracţiei induse de angiotensina II (5). Diferenţele menţionate pot fi explicate prin liniile diferite de animale folosite în aceste studii. Totuşi, supracontracţia indusă de ALD în artere mezenterice de rezistenţă precontractate cu fenilefrină (fig. 3) ar putea indica o potenţare a efectului fenilefrinei. Ulterior am identificat abilitatea ALD de a potenţa efectul contractil al endotelinului, dar numai când Ca_L sunt complet blocate (fig. 1).

Deşi nu sunt disponibile date de electrofiziologie în acest sens, reducerea sensibilităţii la D600 a efectului contractil indus de fenilefrină şi chiar de K^+ extracelular crescut (5), coroborată cu evidenţierea efectului potenţator al contracţiei indus de ALD (fig. 1), ne permite să speculăm privind posibilitatea ca ALD să deplaseze spre dreapta dependenţa de voltaj a Ca_L.

Implicarea unor mecanisme endotelio-dependente în efectele EST

În continuarea cercetărilor noastre asupra efectelor vasculare non-genomice ale EST (5), am examinat efectul rapid al EST asupra relaxării endotelio-dependente (RED) globale şi asupra componentei de tip EDHF, în cazul RED induse prin stimularea receptorilor muscarinici endoteliali în preparate vasculare precontractate prin stimularea alfa-1 adrenoceptorilor miocitari.

Am observant că EST facilitează RED, care devine mai puţin sensibilă la efectul inhibitor al blocării canalelor de potasiu dependente de calciu cu conductanţă mare (BK_{Ca}). În condiţiile în care raportul dintre componentele mediate de EDHF şi NO nu pare să fie modificat, motivul pentru care implicarea BK_{Ca} este diminuată şi mecanismul de potenţare a RED rămân să fie investigate; datele preliminare indică o eliberare crescută de oxid nitric (NO) şi o hiperpolarizare diminuată. Deşi mecanismele disponibile pentru activarea eNOS de către EST sunt relativ bine cunoscute, iar creşterea eliberării de NO este clar implicată în RED indusă de EST şi în potenţarea de către EST a RED indusă de alţi agenţi (13, 14) reducerea sensibilităţii RED la blocarea BK_{Ca} presupune o acţiune simultană a EST asupra MNV, cum ar fi inhibarea Ca_L.

Implicarea unor mecanisme endotelio-dependente în efectele ALD

Am examinat efectul rapid al ALD asupra RED globale şi asupra componentei de tip EDHF, în cazul RED induse prin stimularea receptorilor muscarinici endoteliali în preparate vasculare precontractate prin stimularea alfa-1 adrenoceptorilor miocitari. În arcada mezenterică de şobolan şi mai ales în ramurile sale de ordin I, aldosteronul exercită următoarele efecte în fragmente vasculare cu endoteliu intact: induce contracţie dacă este inhibată eNOS, inhibă în manieră NOS-dependentă contracţiile induse de fenilefrină şi de K^+ extracelular crescut şi potenţează RED indusă de carbachol, atât prin stimularea eNOS, cât şi prin amplificarea componentei EDHF evidenţiată în condiţiile inhibării eNOS (fig. 4). Efectele apar în general la concentraţii mai mari decât cele fiziologice şi nu sunt marcante ca în cazul teritoriului renal (15), ci relativ asemănătoare cu cele din sectorul brahial

uman (16). În mod evident aceste efecte se opun efectelor procontractile ale ALD pe care le-am discutat anterior şi este posibil ca disfuncţia endotelială (17) să încline balanţa în favoarea celor din urmă, în special în teritorii cheie, cum este cel renal.

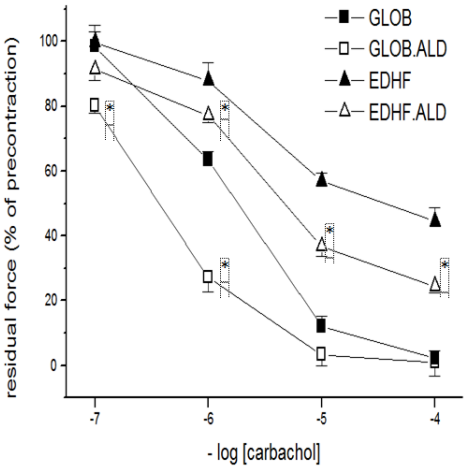

Fig. 4. Efectul relaxant endotelio-dependent indus de carbachol (pătrate pline) în artere mezenterice de rezistenţă de la şobolan este potenţat (*p<0.01) în prezenţă de 1 μM aldosteron (pătrate goale), cu participarea unei componente EDHF (triunghiuri pline) amplificate (*p<0.01) de aldosteron (triunghiuri goale).

CONCLUZII

Prin experimente de miografie izometrică a fragmentelor arteriale am identificat următoarele posibilităţi privind mecanismele care stau la baza efectelor vasculare non-genomice, rapide, ale EST şi ALD:

1. Ca_L sunt esenţiale pentru efectul relaxant al EST în aorta de şobolan şi sunt implicate în acest efect în artera mezenterică şi ramurile sale. Datele sugerează că EST, capabil să inhibe direct $Ca_v1.2$ transferate în celule renale embrionare (HEK), utilizează mai ales această cale pentru a induce RED .

2. ALD anulează participarea Ca_L la influxul de calciu activator al contracţiei în aorta de şobolan şi o reduce în artera mezenterică şi ramurile sale, posibil datorită unei deplasări spre dreapta a dependenţei de voltaj a Ca_L.

3. Chiar dacă nu produce RED în arterele mezenterice de la şobolan, EST este capabil să potenţeze RED indusă de carbachol, care devine mai puţin sensibilă la efectul inhibitor al blocării BK_{Ca}, iar ALD potenţează ambele mecanisme ale RED, mediate de NO, respectiv de EDHF, fapt ce contrabalansează efectele sale procontractile directe asupra MNV.

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ABORDAREA LAPAROSCOPICĂ A TUMORILOR CHISTICE DE OVAR

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LAPAROSCOPIC APPROACH OF THE OVARIAN CYST TUMORS (Abstract): Given the continuous technical upgrade and increased surgical expertise, laparoscopy is replacing laparotomy in day-to-day gynecological surgical practice. Laparoscopic management offers many advantages for the patient: accurate diagnosis, minimal bleeding, reduced need for analgesia, less adhesion formation, fast recovery, better cosmetic results, shorter hospital stays (one day surgery in selected cases), and reduced costs for the patient and hospital. **Key words:** LAPAROSCOPY, OVARIAN CYST

În prezent tratamentul laparoscopic al tumorilor chistice de ovar a devenit o practică standard cu condiția unui diagnostic precis la momentul intervenției. Majoritatea tumorilor chistice ovariene sunt benigne (chisturi funcționale, dermoide sau endometriozice), tumorile maligne având o incidență de 7-13% în premenopauză şi 8-45% în postmenopauză (1), iar cele borderline de 9,2-16% (2). Riscul de a descoperi un cancer ovarian la momentul laparoscopiei este redus, 0.4% după Nezahat (3). Posibilitatea efectuării examenului anatomopatologic extemporaneu este obligatorie, deoarece o astfel de constatare obligă la conversie pentru stadializare şi tratament. Pentru a evita astfel de situații pacientele trebuie evaluate preoperator cât mai cuprinzător.

Evaluarea preoperatorie începe cu anamneza şi examenul clinic atent. Palparea unei formațiuni tumorale fixe, neregulate, însoțită de ascită este sugestivă pentru cancer. Examenul ecografic transvaginal este metoda principală de evaluare a maselor anexiale. Tumorile vor fi caracterizate din punct de vedere al mărimii, localizării, conturului, conținutului, loculației, grosimii septurilor, prezenței vegetațiilor şi a lichidului liber intraperitoneal. Predictibilitatea ultrasonografiei endovaginale este de aproximativ 96% pentru tumorile benigne (4). Utilizarea ecografiei Doppler transvaginale poate facilita diferențierea tumorilor benigne de cele maligne, acestea fiind caracterizate de impedanță scăzută a fluxului sanguin (IP<1). Dozarea preoperatorie a markerilor tumorali, în mod particular a CA125, este utilă în special la pacientele aflate în postmenopauză. Valori peste 65 UI/ml au o specificitate de 97% cu o sensibilitate de 78% pentru tumorile maligne (5).

Dacă la finalul evaluării suspiciunea de malignitate este ridicată pacienta va fi evaluată în continuare prin examen CT sau RMN, urmând a fi supusă laparotomiei de stadializare. În caz contrar se va planifica intervenția laparoscopică (6).

Pregătirea preoperatorie

Pacienta va fi consiliată despre posibilele

descoperiri intraoperatorii, despre tehnica chirurgicală, despre riscul de conversie şi se va obţine consimţământul informat. Se va pregăti mecanic intestinul, se va monta o sondă Foley şi un manipulator uterin după pregătire vaginală prealabilă. Se va dezinfecta întreaga suprafaţă a abdomenului datorită riscului de conversie (7).

Tehnica chirurgicală

Intervenţiile se vor desfăşura sub anestezie generală. Instrumentarul laparoscopic constă in 1 trocar de 12 mm, 2-3 trocare de 5 mm, telescop de 10 mm, cu unghi de vizualizare de 0°, ac Veress, canula de irigaţie-aspiraţie, electrocauter bipolar sau monopolar, pense, foarfeci, ac laparoscopic pentru aspirarea conţinutului chistului. Se începe cu o incizie subombilicală de 2-3 mm necesară introducerii acului Veress. În cazul abdomenului cicatriceal poate fi necesară utilizarea unei tehnici de laparoscopie deschisă. După verificarea localizării intraperitoneale a acului Veress şi realizarea pneumoperitoneului se introduce subombilical trocarul de 12 mm. Trocarele de 5 mm vor fi introduse, unul la 3-4 cm suprasimfizar, iar celelalte două la nivelul crestelor iliace, extern de muşchii drepţi abdominali şi vasele epigastrice inferioare. Inspecţia cavităţii peritoneale şi recoltarea de lichid peritoneal pentru examen citologic este urmată de intervenţia propriu-zisă. În cazul tumorilor chistice de ovar 3 tehnici chirurgicale sunt utilizate în prezent: chistectomia, ovarectomia şi anexectomia (8).

Chistectomia

Indicaţii: vârstă sub 45 ani, chist benign.

Procedeul constă în îndepărtarea în totalitate a peretelui chistului în scopul evitării recidivei şi realizării unui examen anatomopatologic cât mai complet (9). Chistectomia ideală presupune îndepărtarea chistului intact. Dacă acest lucru nu este posibil se recurge la puncţia chistului, aspirarea conţinutului şi excizia peretelui. Ovarul este expus complet prin tracţiunea ligamentului utero-ovarian cu o pensă atraumatică pentru a putea aprecia dimensiunea corectă a chistului (10). Cu ajutorul foarfecii se efectuează o incizie la nivelul cortexului ovarian la limita dintre chist şi ţesutul ovarian normal. Incizia serveşte creării unui plan de clivaj între capsula ovarului şi peretele chistului. Ea trebuie să fie paralelă cu axul lung al ovarului şi nu trebuie să producă ruptura chistului. Utilizând hidrodisecţia (soluţie Ringer, vasopresină diluată) sau disecţia boantă, se avansează treptat in planul de clivaj pentru enuclearea chistului. Metoda este preferată atunci când există un chist dermoid sau unul mucinos, deoarece contaminarea peritoneului cu astfel de conţinut poate fi însoţită de peritonită granulomatoasă, în primul caz, sau apariţia pseudomixomei peritonei, în cel de al doilea caz (8).

Îndepărtarea intactă a chisturilor mai mari de 12 cm se poate dovedi dificilă pe cale laparoscopică. În această situaţie este necesară o altă abordare care constă în puncţionarea iniţială şi aspirarea conţinutului. Acest lucru poate fi realizat prin intermediul unui ac laparoscopic de 18 gauge introdus prin canula de aspiraţie-irigaţie sau cu ajutorul unui trocar de 5 mm. Trocarul este introdus în interiorul chistului împreună cu teaca sa, iar după puncţie trocarul este extras şi în teacă este introdusă canula de aspiraţie (6). Conţinutul este trimis pentru examen citologic. După lavajul suprafeţei interne a chistului se practică chistoscopia prin introducerea laparoscopului în interiorul chistului. Dacă se constată prezenţa vegetaţiilor intrachistice, acestea vor fi biopsiate şi examinate anatomopatologic extemporaneu. Următorul timp operator constă în evacuarea peretelui chistului prin separarea acestuia de cortexul

ovarian. Cele două structuri sunt prinse cu pense atraumatice şi prin tracțiune divergentă se realizează separarea lor. Pot exista dificultăți în identificarea peretelui chistic. În această situație marginile orificiului de puncție vor fi lărgite cu foarfeca rezultând margini clare uşor de identificat. Dacă prin aceste manevre cămaşa chistului nu poate fi îndepărtată ea va fi apucată şi tracționată din interior concomitent efectuându-se contratracțiune pe ovar (6).

După îndepărtarea chistului, ovarul restant va fi irigat abundent cu ser fiziologic sau soluție Ringer. Hemostaza se realizează frecvent spontan, în caz contrar va fi utilizat electrocauterul bipolar. Dacă marginile capsulei ovariene se suprapun, ovarul este lăsat să se vindece singur. Dacă nu se suprapun pot fi necesare câteva suturi cu fir subțire monofilament, rezorbabil.

Ovarectomia este indicată în cazul chisturilor ovariene recidivante, atunci când chistul a distrus în totalitate parenchimul ovarian, sau în cazul unor mase ovariene persistente în postmenopauză.

Principiile ovarectomiei clasice se aplică şi în cazul ovarectomiei laparoscopice. Inițial este importantă identificarea traiectului ureterelor, iar apoi identificarea şi izolarea vaselor ovariene la nivelul ligamentului lombo-ovarian (11). Acest lucru se realizează prin incizia peritoneului ligamentului larg între ligamentul rotund şi trompa uterină. Fiind un spațiu avascular, accesul între foițele anterioară şi posterioară a ligamentului larg este facilă, iar scheletizarea ligamentului lombo-ovarian se realizează cu uşurință. Eliberarea ovarului de pediculii săi poate fi realizată prin mai multe metode. Fie se cauterizează ligamentul lombo-ovarian, scheletizat în prealabil şi apoi ligamentul utero-ovarian, fie se utilizează ligaturile preformate (Endoloop) sau clipurile metalice.

Anexectomia se efectuează pacientelor cu leziuni benigne care nu doresc o nouă sarcină.

Tehnica este similară ovarectomiei cu mențiunea că este necesară disecția bazei anexei la nivelul ligamentului larg şi coagularea extremității proximale a trompei (8).

Țesuturile vor fi extrase din abdomen utilizând unul din următoarele procedee:

1. Endobag – mici saci din material plastic introduşi în cavitatea peritoneală prin intermediul trocarului de 10 mm. Țesutul este introdus in sac, acesta este tensionat şi după îndepărtarea tecii trocarului, sacul este extras prin incizia de la nivelul peretelui abdominal. Folosirea endobag-ului necesită aspirarea conținutului tumorilor chistice sau morselarea celor solide in interiorul sacului. Nu este recomandată evacuarea intactă a tumorilor deoarece există riscul de rupere a sacului şi de contaminare a cavității peritoneale şi a peretelui abdominal.

2. Colpotomie posterioară – utilizată în cazul tumorilor solide de dimensiuni mai mari. Colpotomia va fi închisă pe cale vaginală sau laparoscopică. Studiile au arătat că acest procedeu nu este însoțit de aderențe postoperatorii semnificative (12).

3. Extragerea prin peretele abdominal. Unul din trocarele de 5 mm este înlocuit cu cel de 10 mm, iar piesa operatorie este extrasă odată cu teaca trocarului.

Este importantă evitarea contaminării cavității peritoneale sau a peretelui abdominal cu țesuturi tumorale – au fost raportate metastaze la nivelul peretelui în caz de tumoră malignă (13, 14). Dacă acest lucru se întâmplă, este necesară spălarea peritoneului cu cantități mari de soluție Ringer.

Complicații

- ruptura intraperitoneală a chistului – semnificativă în cazul chistului mucinos, dermoid sau endometriozic. Se va spăla

abundent peritoneul până la clarificarea lichidului de lavaj.

• hemoragia – dacă nu poate fi controlată prin electrocauterizare poate duce la conversia intervenţiei.

• insuficienţă ovariană – în caz de coagulare în exces a parenchimului ovarian.

• leziuni ale ureterului – se vor rezolva prin conversie.

• recurenţa – în caz de excizie incompletă.

• formarea de aderenţe postoperatorii – mai frecventă după chisturile endometriozice (15). Se vor evita suturile tranşei ovariene. La sfârşitul intervenţiei se vor lăsa în pelvis aproximativ 1000 ml soluţie Ringer sau se vor folosi dispozitive care impiedică apariţia aderenţelor (Interceed).

CONCLUZII

1. Managmentul laparoscopic al tumorilor ovariene chistice este în continuă creştere datorită avantajelor în ceea ce priveşte morbiditatea, recuperarea şi implicaţiile economice comparativ cu chirurgia clasică.

2. Pacientele trebuie atent selectate prin evaluare preoperatorie cât mai completă.

3. Examenul anatomopatologic extemporaneu trebuie să poată fi efectuat imediat dacă se tentează evacuarea tumorii prin laparoscopie.

4. Dacă este detectat un proces malign este indicată conversia la laparotomie de stadializare.

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SYSTEMATIC REVIEW REGARDING PSYCHOLOGICAL AND PHYSIOLOGICAL PROCESS ASPECTS ABOUT THE METHODS OF INDUCING LABOR

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SYSTEMATIC REVIEW REGARDING PSYCHOLOGICAL AND PHYSIOLOGICAL PROCESS ASPECTS ABOUT THE METHODS OF INDUCING LABOR (Abstract**): Aim** review article aims to synthesize the mechanisms involved in triggering labor, focusing on the cervical phenomena resulting in maturation, cervical dilation and fetal expulsion. Furthermore, this article highlights the ways in which labor may be induced. **Material and methods:** The data collected by accessing *Medline; Medscape; PubMed* and *ISI Web of Knowledge* using the following keywords: labor induction, oxytocin, cervix ripening, prostaglandins, uterine contraction, amniotomy, membrane stripping, misoprostol, Foley catheter. Review articles, original articles, as well as, case reports were taken into consideration when the search was performed. We identified more than 1,000 abstracts and reviewed over 150 full text articles using the keywords listed above. We preferentially included high quality systematic reviews or large randomized trials. **Results:** After reviewing the literature we included 28 full text articles in our analysis. A series of factors should be taken into consideration by the obstetrician before considering inducing labor. There are studies that compare the use of oxytocin versus prostaglandins for labor induction and the results are in favor of prostaglandins with a lower rate of number of pregnancies completed by segmental transversal cesarean. However, administration of oxytocin may increase the risk of termination of pregnancy through segmental transversal cesareans. In contrast to, oxytocin and prostaglandins, the mechanical methods of inducing labor may increase the risk of both maternal and neonatal infectious. **Conclusions:** The decision to trigger labor requires the obstetrician to carefully analyze the timing of induction of birth and the risks and benefits, considering, for each patient, the most effective method of triggering. Thus, we emphasize on the necessity of developing protocols to guide obstetricians, since there is no consensus on the doses that should be administered. The cost-effectiveness of the treatment should be considered. **Keywords**: PSYCHOLOGICAL, PHYSIOLOGICAL, BIRTH, TRIGGER, INDUCING LABOR, OXYTOCIN, CERVIX RIPENING.

Birth is an ensemble of phenomena culminating with the expulsion of the fetus and its annexes from the maternal body. Myometrial and cervical cell interactions

are decisive in triggering labor. There are certain situations where expectation is not a solution, thus labor must be induced. This review article aims to synthesize the mechanisms involved in triggering labor, focusing on the cervical phenomena resulting in maturation, cervical dilation and fetal expulsion. Furthermore, this article highlights some of the ways in which labor may be induced. Existing literature suggests that labor should be triggered under certain conditions, calculating very carefully the risks and benefits. The goal of the induced labor is to give birth, in a safely manner, to a healthy newborn (1).

MATERIAL AND METHODS

Throughout its evolution, consciously or mimetic, everyone chooses a model to build his labor (2, 3). We choose for this study the data collected by accessing MEDLINE; MEDSCAPE; PUBMED and ISI WEB OF KNOWLEDGE using the following keywords: labor induction, oxytocin, cervix ripening, prostaglandins, uterine contraction, amniotomy, membrane stripping, misoprostol, Foley catheter. Review articles, original articles, as well as, case reports were taken into consideration when the search was performed. We identified more than 1,000 abstracts and reviewed over 150 full text articles using the keywords listed above. We preferentially included high quality systematic reviews or large randomized trials.

BACKGROUND

The physiological process of birth occurs on average after 280 days (40 weeks or 10 obstetric months of 28 days each) from fertilization, during which the fetus becomes viable in extrauterine conditions, and is the result of completing a fetal-placental development process, which induces numerous endocrine and neurohormonal regulation systems in the maternal body (4). The mechanisms involved in triggering labor are multiple, varied and are regulated by maternal, fetal and placental factors (the concept of maternal-feto-placental unity), which may be classified as initiation factors such as oxytocin, prostaglandins and gap junctions, and complementary factors (adjuvants) (Humphrey *et al.*, 2009).

The uterine muscle is intrinsic inactive, and according to Caldeyro Barcia's theory, the most important factor in birth initiation is oxytocin (5), a hormone secreted in the posterior pituitary gland that enables the sensitization of the myometrial fibers allowing the propagation of the wave contraction between adjacent muscle fibers, and further on the transmission of the contraction in the myometrium (6).

Physiological mechanism of labor

During pregnancy, the fluctuations of circulating oxytocin levels may not correspond to changes in uterine activity, however, the onset of birth, because of prostaglandins action and lower uterine distention in the inferior part (Ferguson reflex), the blood level of this hormone is significantly increased (7).

A fundamental factor in labor, prostaglandins (PG) influence both the cervical maturation (PG E2) as well as the myometrial contractility. In contrast to PG F2α and PG E2, which stimulate uterine contraction, PG I2 induces relaxing action on the musculature in the vascular wall and myometrium (8). The main sources of prostaglandin production are in the fetal membranes, PG E2 at the level of the chorion and amnius, deciduous (PG E2 and PG

F2α) and in the myometrium (PG I2).

As the birth term approaches, the amniotic and deciduous levels of prostaglandin grow sensitively until a certain time when more intense, coordinated, active-propagated uterine contractions are induced, either directly by the action of prostaglandins or indirectly after the myometrium has been sensitized by its own oxytocin (9) or, perhaps, of by oxytocin of fetal origin.

The interaction between OXT and PG determines the stimulation of the myometrial contractility. The latter mentioned potentiate the oxytocin-induced uterine contractions, leading to cervical wiping and the establishment of uterine activity (10). In order for the labor to progress to the active phase, the deciduous prostaglandin levels must increase.

The gap-junctions, also called nexus, represent a type of intercellular junctions, virtually absent during gestation, which allow the electrical, ionic and molecular coupling between two myocytes, favoring their coordination and the propagation of the uterine contraction at the level of the entire myometrium (11).

Unlike the His beam, responsible for the synchronization of the cord contractions, no such structures have been identified in the uterus. The period preceding labor is characterized by an increase in the number of junctions, as well as an amplification of their length. Meanwhile, JP is also involved in the dilation of the cervix (12).

Methods of inducing labor

Among the adjuvant factors of labor initiation, it is worth mentioning the steroid hormones, immunological and mechanical factors and prolactin, which through its antagonistic action on the uterus compared to relaxin, can stimulate uterine contractility (the urotropine/uterotonine complex signal theory).

Steroid hormones (estrogen and progesterone) play a fundamental role in the labor mechanism (13), having on one hand a modulating action on PG and oxytocin and, on the other hand, regulating the membrane potential of the myometrial cells and actin-myosin interaction.

Urotropines are the substances that determine the synthesis of the functional elements that prepare the myometrium for labor: steroid hormones, growth factors (TGFβ, activin A), PGF2α, PTH-rP (14). Just it is clear that muscle involvement in chronic inflammatory disease represents a well-recognized manifestation, multiple factors contributing to the development of muscle disease: pro-inflammatory cytokines such as TNF-α and IL-1 acting in muscle environment with subsequent damage of muscle and abnormal muscle metabolism (15, 16, 17).

Uterotonins are substances that cause the contraction of smooth uterine muscle fibers by increasing intracytoplasmic calcium concentration: oxytocin, endothelin-1 (18).

Labor induction is an intervention method that aims to initiate contractions before their spontaneous triggering (19). The decision to triggering labor is influenced by many factors, which should be should consider by the obstetrician: gestational age, fetal pulmonary maturity, maternal and fetal state severity, and predictive maternal factors for triggering success, such as the Bishop score or parity (20).

According to the ACOG Committee on Practice Bulletins - Obstetrics in 2009 (1), birth is induced in 1 of 5 pregnancies due to maternal factors (preeclampsia, re-

nal/cardiac), fetal causes (intrauterine growth restriction with Doppler indices on fetal mean cerebral artery and on the umbilical artery), or maternal-fetal causes such as uncontrolled gestational diabetes, premature spontaneous rupture of membranes, or post-mature pregnancies (when the pregnancy exceeds 40s + 6z, up to 41s + 6z) (21).

Notwithstanding, these pathologies are associated with increased maternal and fetal morbidity and mortality. In cases where natural birth is contraindicated, or if the physician is in the presence of an obstetric emergency (e.g. hemorrhage in central placenta previa or fetal acute distress), cesarean operation must be performed. The contraindications of induced birth may be absolute or relative.

Some of the absolute contraindications are: cephalo-pelvic disproportion (tight birth canal/macrosomic fetus), dystocic pelvis, central placenta previa, transverse fetal presentation, umbilical cord prolapses, obstructive mass at the level of the female genital tract, invasive cervical cancer. The relative contraindication is: previous c-sectioned uterus, bridge presentation, multiple pregnancies, maternal cardiopathic, low biophysical score, previous myomectomy, neonatal trauma at previous birth.

Over time, the role of the cervix in contending the product of conception, its implication in labor and parturition were considered key elements for the successful completion of a pregnancy. Cervical maturation refers to changes in the cervix that are required for triggering labor, such as cervical dilation and vaginal expulsion of the fetus.

The uterine cervix is composed of smooth muscle fibers (10%) and connective tissue (90%), in which the fibroblasts ensure the synthesis of most of extracellular matrix consisting of collagen (type I and type III) and representing 80% of the proteins cervical: glycoproteins and proteoglycans. Collagen fibers are closely linked and solidarized by glycoproteins and proteoglycans.

Collagen fibers are closely linked and solidarized by glycoproteins and proteoglycans. Proteoglycans are macromolecules consisting of a protein base, branched into polyacid structures, glycosaminoglycans (GAGs). Hyaluronic acid and hydrogenated proteoglycan represent 15% of GAG and play a major role in tissue hydration. The cervical maturation phenomena take place in the last trimester of gestation. Clinically, these modifications may be described as a rambling of the cervix and diminished resistance to dilation, because of collagen network total organization, which is responsible for changes in the mechanical properties of the cervix (22).

During pregnancy, several morpho-functional changes occur in the cervix, leading to a cellular infiltration (23). Fibroblasts become more numerous and mature. They represent a phagocytosis activity through local histiocytes as well as other cells. Additionally, there are other cellular infiltrations (polynuclear, mast cells) present. Ultrastructural studies demonstrated that the complete modification of the collagen network, which spreads, becomes hydrated.

At term, the amount of intermolecular collagen binding decreases, subsequently, the collagen becomes more easily dispersible and more hydrophilic (24). The collagenolytic capacity of the cervix increases as the birth approaches, favoring collagen destruction and cervical rupture. Thus, there is a decrease in collagen concentra-

tion and a destruction of the tropocollagen.

With the onset of labor, under the influence of uterine contractions that create increased intrauterine pressure, the uterine cervix relieves progressively, the cervical canal dilates, and the gelatinous plug is removed from the cervix area. Because of the gradual opening of the canal, the canal takes the form of a funnel that is embedded in the lower segment. During the opening, the cervical canal is shortened, completely wiped out, leaving only the external opening closed. The edges of the neck are thin, elastic, and the external opening begin to open.

With each contraction, the degree of opening of the external orifice increases until it is fully opened. Assessment of cervical condition is the most important factor in the decision of triggering labor. In the long run, cervical status is quantified using the Bishop score (25), which involves examining five factors: cervical dilation (cm), neck length/wiping, consistency, position, position of the fetal pole (ratio of the lower pole of the fetal head to interspines ischia line). In the past, at a score of ≥ 6, triggering of labor was achieved by performing amniotomy (26).

Amniotomy increases the synthesis and local elaboration of prostaglandins, which, in turn, contributes at triggering birth. Currently, due to complications such as umbilical cord prolapses and/or compression, increased risk for maternal/neonatal infections, fetal heart rate deceleration, early membrane rupture is no longer recommended to be used as first intended technique for labor induction. In such cases, intravenous infusion of oxytocin is administered while the fetal heart beats are closely monitored.

Studies have shown that induction of labor by oxytocin decreases the number of days of hospitalization and the number of newborns requiring neonatal care in intensive care units but increases the risk of termination of pregnancy through segmental transversal cesareans (27). Nevertheless, the complications of oxytocin administration may be:

1. increased uterine tone - In addition, there are many contradictions in this area of research regarding the levels of some oxidative stress markers, as they have been shown to either increase, decrease or remain unchanged after labor. That labor-induced oxidative stress might be prevented by some antioxidant interventions, there is still insufficient knowledge about the possible protective effects of antioxidants against oxidative damage (28).
2. impaired fetal heart rhythm,
3. uterine rupture on the uterine scar,
4. when the total oxytocin dose exceeds 60 IU, it may cause a psychomotor retardation in 3-5-year-old children.

Parity, cervical dilation and gestational age are predictive factors for a favorable response to oxytocin administration.

A Bishop score less than 6 involves a cervix that is appropriate for triggering the birth. In such cases, pharmacological or mechanical methods for cervical maturation may be used.

Pharmacological methods of inducing labor

Prostaglandins (PGE1 and PGE2) are the most commonly used cervical maturation drugs. The synthetic analogue of PGE1 is Misoprostol, which is found under the form of tablets and is a gastric cytoprotector used predominantly in the treatment and prophylaxis of gastric ulcer. At the level of the cervix, it increases the concentration of prostaglandins, increases the activity of

collagenase, which in turn increases the concentration of elastase, glycosaminoglycans, dermatan sulfate and hyaluronic acid, resulting in rambling and dilation of the cervix.

Misoprostol may be administered intravaginally (25 μg/6 hrs.) (either in the vaginal posterior fornix or intracervical) or orally (25 μg/2 hrs.), and studies have shown that Misoprostol administered orally has superior benefits as compared to intravaginally administration (29). Furthermore, a meta-analysis conducted by Alfirevic (30), revealed that the low dose administration of Misoprostol may be more cost-effective than other combinations used for the induction of labor.

The synthetic analogue of PGE2 is called Dinoprostone and is marketed in various forms: gel, suppositories, tablets, intravaginal dose-dispensing device while may be administered intracervical or intravaginal. There are studies that compare the use of oxytocin versus prostaglandins for labor induction and the results are in favor of prostaglandins with a lower rate of number of pregnancies completed by segmental transversal cesarean (31).

Mechanical methods of inducing labor

The mechanical methods used to induce labor have the same principle of action, local pressure, which induces the local secretion of endogenous prostaglandins. For this purpose, are used Foley catheter with / without extra amniotic saline infusion and osmotic dilators (32).

The main principle of action of membrane stripping is like mechanical dilation of the cervix. By manual detachment of membranes, a chain of successive reactions is triggered by activation of phospholipase A2 and prostaglandin F2α, leading to cervical maturation (33). This method of triggering labor is not recommended for patients in which was identified a pathogen in the culture of vaginal secretions prelevated previously. In these cases, labor induction will be attempted only after the patient is completely treated with antibiotic and the culture of the vaginal secretion will present only the saprophytic flora. Recent studies have shown that the use of mechanical methods increases the risk of endometrial infections and neonatal sepsis, hemorrhages, premature rupture of the amniotic sac or normal insertion of the placenta.

Artificial triggering of labor may be accompanied by complications such as hyperstimulation, defined as uterine contraction that lasts >60 seconds, or more than 4 contractions in 10 minutes, tachysystole (6 or more uterine contractions in 10 minutes), hypersistolia (a contraction lasting at least 2 minutes), uterine rupture, amniotic embolism, postpartum uterine hypotonia, and fetal heart rate deceleration (34).

CONCLUSIONS

The purpose of meta-analysis is to help clinicians to make the appropriate decision based on solid information. Worldwide, the rate of labor induction has increased constantly. Although many methods for inducing labor have been tested, prostaglandins remain the method of choice in many hospitals and clinics, misoprostol tablets being administered either orally or vaginally. However, in terms of cesarean section risk, the oral administration is safer.

The decision to trigger labor requires the obstetrician (the results are important for obstetrics physicians to explain the level of satisfaction related to their personality) (35), which must carefully analyze the timing of induction of birth, the risks

and benefits, considering, for each patient, the most effective method of triggering (Leventhal's Self-Regulatory Model-SRM) (36, 37).

Thus, we emphasize on the necessity of developing protocols to guide obstetricians, since there is no consensus on the doses that should be administered. The cost-effectiveness of the treatment should be considered.

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PARATIROIDECTOMIA TOTALĂ FĂRĂ AUTOTRANSPLANT GLANDULAR ÎN TRATAMENTUL HIPERPARATIROIDISMULUI RENAL „REFRACTAR"

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TOTAL PARATHYROIDECTOMY WITHOUT AUTOTRANSPLANTATION IN THE MANAGEMENT OF „REFRACTORY" RENAL HYPERPARATHYROIDISM (Abstract)**.** The optimal surgical treatment in patients with refractory renal hyperparathy-roidism (RHP) on hemodialysis for end stage renal disease is still a point of controversy. The high percentage of recurrences after standard surgical procedures i.e. subtotal parathyroidec-tomy (SPTx) and total parathyroidectomy with autotransplantation (TPTx + At) reactualised the practice of total parathyroidectomy (TPTx). **Material and method :** Fortythree patients with RHP underwent surgery between 1994-2009. There were 24 SPTx and 6 TPTx + At, both procedures determining 20,7 % (6 cases) recurren-ces so in the last five years TPTx was performed in a series of 13 cases (7 men and 6 women, with median age 43,6 years ; range 22 – 65 years and median dialysis time before PTx 8,2 years ; range 3 -12 years. Parameters studies included demographics, preoperative and follow-up laboratory tests, surgical techniques, pathology results and postoperative immediate and medium term results. **Results :** Main indications for PTx were severe bone disease, soft tissue calciphycations, neuro muscular phenomena, grossly elevated iPTH and sometimes hypercalcemia. TPTx was done in 12 patients, the 13th one suffering a completion PTx one year after outward exeresis of only two glands. Postoperatively the majority of symptoms markedly improved and the values of calcaemia, phosphatemia and alkaline phosphatasis normalised together with low or no measu-rable level of iPTH. One patient required a re-exploration for a cervical hematoma but no one presented permanent hypocalcemia or recurrent hyperparathyroidism.Pathology revealed nodu-lar hyperplasia in all the cases, a parathyroid carcinoma but also an incidental thyroid papillary microcarcinoma in a complementary thyroidectomy. **Conclusions :** TPTx alone proves to be an equally safe and successful as another techniques currently used in management of RHP eliminating the hyperparathyroid status but being supe-rior with regard to aparition of recurrences. The procedure is indicated especially in cases with aggressive, refractory forms of RHP without the prospect of renal transplantation. **Key words** : PARATHYROIDECTOMY, AUTOTRANSPLANTATION, RENAL HYPERPARATHYROIDISM

Hiperparatiroidismul renal (secundar/terţiar) – HPR condiţie care virtual interesează totalitatea bolnavilor uremici supuşi hemodializei cronice este redefinit în prezent într-un concept integrativ sub acronimul CKD-MBD – boala metabolică osoasă din insuficienţa renală cronică, caracterizat prin prezenţa izolată sau combinată a trei componente :

- anomalii ale metabolismului calciului, fosfaţilor, PTH şi vitaminei D datorită

hiperfuncţiei paratiroidiene ;
- anomalii ale turnoverului, mineralizării, volumului, creşterii sau rezistenţei osoase (mai vechea osteodistrofie osoasă) ;
- calcificări vasculare, cardiace şi ale ţesuturilor moi şi calcifilaxie, toate realizând în timp o morbi-mortalitate crescută.

Creşterea constantă a numărului bolnavilor cu insuficienţă renală cronică implicit a celor cu HPR a condus la dezvoltarea eficientă a programelor de hemodializă, completată de administrarea de vitamine active din grupul D sau omologi, bifosfonaţi şi preparate calcimimetice care în absenţa tratamentului ideal – transplantul renal – controlează eficient pe perioade îndelungate, evoluţia clinică şi biologică a majorităţii acestor bolnavi.

Rămâne însă un procentaj de 15% dintre cazuri de aşa zis HPR „refractar" (care poate atinge chiar 38% după 20 ani de hemodializă) în care transformarea ireversibilă a hiperplaziei difuze în hiperplazie nodulară, alături de evoluţia severă, invalidantă a sindromului clinic şi a constantelor biologice nu mai poate fi influenţată de tratamentul medical şi este indicată o soluţie chirurgicală.

Procedurile operatorii reducţionale iniţiate în urmă cu jumătate de secol au recurs preferenţial fie la paratiroidectomia subtotală - PTxsT - fie la paratiroidectomia totală cu autotransplant glandular în diverse zone anatomice – PTxT+At -, considerate în mod tradiţional tehnicile standard pentru tratamentul acestor condiţii, au fost marcate negativ în timp de procentajul ridicat de recidive – 5-80%. Propusă în 1967 de către Ogg paratiroidectomia totală – PTxT – a fost rapid abandonată datorită riscului de osteopatie adinamică şi hipoparatiroidism permanent este reconsiderată în ultimele două decenii de numeroşi specialişti datorită anulării acestui risc şi rezultatelor favorabile imediate şi pe termen mediu comparabile cu celelalte două metode.

În experienţa noastră – însumând 43 observaţii de HPR operate între 1994-2009 – am practicat toate cele trei tehnici. Iniţial şi prioritar am preferat PTxsT – 24 cazuri şi PTxT+At – 6 cazuri, dupa ambele proceduri înregistrând 20,7% (n – 6) recidive, astfel că în ultimii cinci ani ne-am orientat pentru anumite cazuri catre PTxT, executată în 13 cazuri.

PACIENŢI ŞI METODĂ

Seria noastra cuprinde 13 observaţii de HPR „refractar" – 7 bărbaţi şi 6 femei, cu vârste cuprinse între 22 – 65 (medie 43,5) ani, aflaţi de 3 – 12 (medie 8,2) ani în regim de hemodializă.

În 12 cazuri a fost vorba de intervenţii primare, într-un singur caz reexplorarea chirurgicală fiind practicată unui tânăr de 22 ani căruia în cadrul unei exereze „ţintite" efectuate în alt serviciu i-au fost identificate şi îndepărtate doar paratiroidele superioare (!).

În etiologia HPR am notat glomerulo-(pielo)nefrita cronică – 9 cazuri, urolitiaza – 2 cazuri, rinichi polichistic şi nefroangioscleroză în câte un caz. Doi bolnavi prezentau în antecedente şi un transplant renal eşuat.

Criteriile de includere în varietatea „refractară" a HPR au cuprins evoluţia prelungită a afecţiunii cât şi a hemodializei, severitatea deosebită a unor manifestări clinice şi complicaţiilor acestora, modificările semnificative ale unor date de laborator dar mai ales lipsa de răspuns (mai mult decât de complianţă) la măsurile medicale şi mai ales a oricarei perspective de efectuare a unui transplant renal.

Astfel sindromul clinic, componenta implicită a indicaţiei chirurgicale a cuprins (tab. I).

Valorile principalelor constante biologice şi a produsului Ca/P au fost de asemenea semnificativ modificate (tab. II).

Ultrasonografia practicată de rutină, respectiv scintigrafia cu sestamibi sau tetrofosmin accesibila sporadic nu au fost

TABELUL I

Frecvenţa principalelor manifestări clinice în HPR „refractar"

Manifestări clinice	Nr. cazuri	%
osteoartralgii, osteoporoza, fracturi	13	100
prurit rebel	12	92
insomnie, sindrom astenocomportamental	10	78
(mi)astenie, anemie, hipoproteinemie, malnutriţie	10	78
HTA, cardiopatie ischemică,miocardoscleroză	8	61
calcifilaxie, calcificări ectopice, ochi roşii "uremici"	6	46
manifestări dispeptice	4	30
urolitiază, colici renale	2	15

TABELUL II

Valorile medii ale principalelor teste de laborator în HPR „refractar"

iPTH	1620 + 320 ng/l
Calciu seric	8,70 + 1,00 mg/dl
Calciu ionic	4,45 + 0,38 mg/dl
PO4 seric	4,60 + 0,55 mg/dl
Fosfataza alcalină	280 + 38 ui/l
Hb	11,3 + 2,3 mg/dl
Proteine	49,5 + 0,9 g/dl
Uree	140 + 4o mg/dl
Creatinina	1,8 + 0,3 mg/dl

relevante pentru diagnosticul observaţiilor noastre, utilitatea lor rezumându-se la identificarea a 1 – 3 glande „mai mari" – peste 8 – 10 mm O - dar mai ales a leziunilor tiroidiene concomitente. Prezenţa a cel puţin unei paratiroide cu aceste dimensiuni s-a adăugat însă anamnezei, sindromului clinic sever şi valorilor exagerate ale iPTH – toate consfinţind eşecul tratamentului medical - şi mai ales considerentelor sociale cu lipsa perspectivei unui eventual transplant renal, toate constituind argumentele care au formulat indicaţia tratamentului chirurgical în aceste cazuri.

Constatările intraoperatorii au fost însă elementul precumpănitor care au decis opţiunea pentru practicarea acestui tip de exereză glandulară. Astfel, cel puţin două, dacă nu toate paratiroidele au apărut globuloase, pseudoadenomatoase dar mai ales mult mărite de volum depăşind copios dimensiunile obişnuite fiind astfel mult mai uşor de identificat intraoperator, biopsia extemporanee căpătând un caracter formal. Au fost excizate în total 51 glande cu dimensiuni între 0,8 – 2,8 cm (trei paratiroide din care una supranumerară situată retrosternal la bolnavul cu reintervenţie). Masa totală a parenchimului glandular extirpat a depăşit în unele situaţii de 10 ori greutatea normală atingând chiar 1500 mg.

În toate aceste cazuri prin dimensiuni şi conformaţie, aspectul macroscopic a obligat practic la îndepărtarea succesiva a tuturor celor patru paratiroide, caracterul global patologic al ţesutului glandular neoferind nici chiar după secţiune porţiuni aparent normale care să poată fi conservate sau transplantate.

De altfel în toate specimenele examinarea microscopică a identificat hiperplazia nodulară sub formă de microformaţiuni alcătuite din celule principale şi mai puţin celule clare sau oxifile dispuse acinar sau trabecular care alături de aspecte degenerative, scleroza, necroza, zone chistice sau încărcate cu hemosiderina interesa practic toata glanda fără „insule" de parenchim normal.

Într-una din primele observaţii de HPR „refractar" tipic care asocia sindromului

clinic şi biologic sever un nodul tiroidian „banal" de 3 cm O, intraoperator au fost relativ usor identificate şi excizate trei paratiroide anormale dar nu şi cea de a patra. Deoarece examenul histopatologic al nodulului menţionat a fost incert s-a practicat o lobistmectomie stângă iar cercetarea la parafină a precizat diagnosticul de carcinom paratiroidian cu celule principale, exereza glandulară devenind astfel totală. Cazul – reprezentând a 26-a observaţie de asociere a HPR în regim de hemodializă cu un carcinom paratiroidian din literatură – a avut o evoluţie postoperatorie favorabilă atât imediată cu ameliorare clinică şi biologică dar şi la 5 ani după paratiroidectomie continuându-si programul de hemodializă fără semne de recidivă sau depozite secundare.

În trei situaţii s-au efectuat şi exereze tiroidiene asociate, două hemitiroidectomii pentru leziuni nodulare (una fiind cea menţionată mai sus) şi o operaţie Dunhill pentru prezenţa unei formaţiuni de 0,5 cm dure descoperite intraoperator care s-a dovedit a fi un microcarcinom papilar tiroidian „ocult".

Excizia ţesutului celulogrăsos retromanubrial nu a fost practicată sistematic, în cazurile în care a fost totuşi executată fiind identificată paratiroida supranumerară (singura din experienţa proprie), un timom benign şi resturi timice involuate la alţi trei pacienţi.

Evoluţia postoperatorie imediată şi pe termen mediu (6 – 60 luni) a fost în general favorabilă.

S-a înregistrat un hematom postchirurgical care a impus reintervenţie şi drenaj iar trei bolnavi au prezentat hipocalcemie pasageră (5,2 – 8,4 mg/dl), doi fiind simptomatici. Toti operaţii au primit solutii calcice şi preparate de vitamina D, ultimele fiind recomandate şi la domiciliu.

În timp, evoluţia clinică a fost spectaculoasă, evidenţiată mai ales prin dispariţia rapidă a pruritului şi atenuarea fenomenelor dureroase, mai lentă şi incompletă fiind influenţa benefică a paratiroidectomiei asupra asteniei, stării generale şi manifestărilor psihonevrotice.

Valorile calcemiei, fosfatemiei şi FA s-au normalizat sau redus semnificativ comparativ cu cifrele preoperatorii. Nu am avut posibilitatea monitorizării iPTH decât la 6 pacienţi şi la intervale diferite – valorile fiind communicate de centre de dializa situate la distanţă de Iaşi – reducerile fiind însă semnificative.

Cel mai important element în redusa noastră experienţă este faptul că nici unul dintre bolnavi nu a prezentat recidivă clinică sau biologică, fenomene de hipoparatiroidism permanent sau boală adinamică osoasă, toţi continuându-şi programul de hemodializă cu o calitate a vieţii substanţial ameliorată.

DISCUŢII

Tehnicile chirurgicale recomandate actualmente în chirurgia hiperparatiroidismului renal – secundar sau terţiar – redefinit ca boală metabolică osoasă din insuficienţa renală cronică sunt comparate şi dezbătute perrmanent, diverşi specialişti sau echipe străduindu-se să argumenteze avantajele şi superioritatea rezultatelor fiecareia dintre ele.

În mod tradiţional, paratiroidectomia subtotală – prin aparenta expeditivitate – şi respectiv paratiroidectomia totală cu autotransplant – metoda preferată de şcoala japoneză care exclude teoretic posibilitatea reinterventţiilor cervicale - sunt alternativele recomandate majoritar.

Obiectivul principal al ambelor proceduri este reducerea – relativ aproximată – a parenchimului glandular hipersecretor. Inadecvarea cantităţii şi calităţii ţesutului paratiroidian conservat conducând în timp la apariţia în 5 – 80% din cazuri a unor persistenţe sau recidive – dificil de evaluat şi tratat, mai ales chirurgical, au stimulat în ultimele două decenii reconsiderarea în cazuri bine selecţionate a exerezei parati-

roidiene totale.

Experienţa este limitată la câteva studii însumând sub 200 observaţii în care s-au obţinut rezultate încurajatoare şi stabile în timp, fără boală adinamică osoasa ori hipocalcemie simptomatică, întrutotul comparabile, dacă nu superioare, celorlalte metode.

Pentru unii autori, eliminarea totală a ţesutului paratiroidian – definită prin anularea sau valoarea postchirurgicala sub 1 ng/l a iPTH – apare ca imposibilă, deoarece în dezvoltarea embriologică a acestor glande de-a lungul traiectului de migrare în regiunea cervicală şi mediastin persistă unele „cuiburi" de ţesut glandular funcţional supus aceloraşi factori de stimulare ca şi cel care alcătuieşte paratiroidele propriu zise eu sau ectopice. În condiţiile unei îndepărtări chirurgicale totale a glandelor patologice, aceste resturi pot asigura o secreţie hormonală convenabilă (ceea ce nu se întâmplă după exereza inadvertentă a tuturor paratiroidelor normale întâlnită cateodată în chirurgia acestui teritoriu).

Din punct de vedere tehnic, hipertrofia impresionantă în unele observaţii – depăşind 1 – 2 cm O, facilitează evidenţierea şi chiar disecţia şi exereza acestor glande, fapt observat şi în cateva din cazurile noastre. Oricum dificultăţile date de vechimea, dimensiunile, raporturile sau eventualele anomalii topografice sunt incomparabil inferioare celor din reintervenţii.

În sfarsit, în cadrul monitorizarii postchirurgicale complianţa bolnavilor autohtoni la evaluare periodică, compensare calcică sau administrare de vitamina D pare favorizată de prezentarea prelungită în timp la şedintele de hemodaliză aşadar superioară comparativ cu mulţi dintre pacienţii cărora li s-a practicat o tiroidectomie totală care evoluează inexorabil către hipotiroidism definitiv.

Ambele suferinţe întră în cadrul patologiei sociale, definitorie şi pentru una din principalele indicaţii ale paratiroidectomiei totale în HPR şi anume lipsa posibilităţilor şi/sau perspectivei de a efectua un transplant renal (uneori chiar şi eşecul acestuia ca în cele două observaţii ale noastre.

Acest criteriu, împreună cu celelalte de ordin anamnestic, clinic-evolutiv şi lezional pot constitui elementele unei selecţii riguroase a bolnavilor, în care se recomandă a aplica această tehnică, singurele care pot asigura rezultatele postoperatorii favorabile preconizate pe termen scurt şi mediu.

Desigur rămâne obligatorie o urmărire prelungită în timp a acestor cazuri, completată de o ajustare medicamentoasă calcică şi vitaminică adecvată, pentru a certifica paratiroidectomia totală ca un procedeu rezonabil şi a o aşeza alături de celelalte tehnici chirurgicale folosite actualmente pentru această patologie.

În concluzie, alături de posibilitatile tratamentului medical, cele trei tehnici de paratiroidectomie îşi dispută indicaţiile, avantajele şi succesele în managementul hiperparatiroidismului renal. Reconsiderarea paratiroidectomiei totale în cazuri bine selectate de hiperfunctie paratiroidiana severă, refractară la orice medicaţie şi care nu au perspectiva practică a unui transplant renal, oferă în condiţiile unei morbidităţi inferioare celorlalte proceduri – în special anularea riscului de recidivă – efecte simptomatice evidente, cu absenţa surprinzătoare a hipoparatiroidismului postoperator, stabile în timp.

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EXPECTATION FROM COCHLEAR IMPLANTATION IN PATIENTS WITH LONG-TERM PRELINGUAL DEAFNESS

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EXPECTATION FROM COCHLEAR IMPLANTATION IN PATIENTS WITH LONG-TERM PRELINGUAL DEAFNESS (Abstract): Cochlear implantation is a well-established method of treating bilateral profound cochlear deafness. One should be aware that the medical literature is quite poor, in regard to the outcomes of the implantation of adolescents or adults with prelingual deafness. The aim of this study is to evaluate the audio-verbal outcome in late implanted adolescents and adults with congenital deafness. **Material and methods**: the study included 8 late implanted patients with bilateral profound deafness 4 of them were implanted as adolescents and the other 4 as adults; all of them were implanted at the Clinical Rehabilitation Hospital from Iasi. The patients' hearing abilities were tested using 2 scales: Speech Perception Rating Scale and Speech Intelligibility Rating Scale. **Results**: all of the patients have benefited from the implant when their hearing performances were compared to those before implantation. **Conclusions:** the topic remains open as long as the studies had only a limited number of subjects, but even so it can be affirmed that, with no doubt, all the subjects have had significant improvements after implantation. **Keywords:** LATE COCHLEAR IMPLANTATION, PRELINGUAL DEAFNESS.

Cochlear implantation is a well-established method of treating bilateral profound cochlear deafness. In the beginnings of the cochlear implant era, the cochlear implants were used to treat only the post linguistically deaf adults. Later on, due to the success encountered with the adults, the cochlear implant teams were stimulated to go further, so they started to implant small children with congenital hearing loss, as well. After half a century of cochlear implantation, plenty of scientific papers regarding this subject matter have been written, especially on the results obtained with small children with congenital deafness and with adults with post linguistic hearing loss (1-4). The results of the cochlear implantation depend on numerous variables such as: the age of onset of deafness, the presence of residual hearing, the duration of hearing loss, the communication mode before implantation, the age at implantation, the cause of deafness, and so on. All authors

agree now that the most important of these predictive factors are the duration of the deafness for the adults with prelingual hearing loss and the age of implantation for the children with congenital deafness (5-7).

The issue is, however, more complex because, even among small children with apparently same characteristics (congenital hearing loss, small age at implantation), there are significant differences in the audio-verbal gain after implantation.

Things are much more complex when it comes to the borderline candidate: the teenagers and adults with prelingual deafness, the patients with residual hearing, the patients with single side deafness or with chronic otitis media, and so on. These patients were not included among those who had received traditional recommendations for cochlear implantation.

Regarding the outcomes of the implantation of adolescents or adults with prelingual deafness, the medical literature is quite poor. Even so, it seems that there are two different trends of opinions: some of the authors advocate that, in spite of a poor auditory-verbal improvement the patients will still benefit from the late implantation mainly because of the psycho-social implications while the other category of authors asserts that the implantation of such patients is useless (8).

The aim of this study is to evaluate the audio-verbal outcome in late implanted adolescents and adults with congenital deafness.

MATERIAL AND METHODS

The longitudinal study included 8 late implanted patients with bilateral profound deafness: 4 of them were implanted as adolescents and the other 4 as adults.

All of them were implanted at the Clinical Rehabilitation Hospital from Iasi. The preoperative evaluation was performed using the standard protocol for the candidates to cochlear implantation (history of illness, clinical, genetic and functional evaluation of the ear, CT and RMN of the temporal bone) (9). The approach was through the posterior tympanotomy and cochleostomy; the electrode array was fully inserted in all cases (10). There were no accidents or complications during or after surgery. The first fitting was performed 4 to 6 weeks after the surgery. After implantation, all patients were included into an intensive audio-verbal rehabilitation program.

The patients were evaluated at 1, 3, 6, 12 months after the first fitting and, after that, yearly, up to 7 years. Two tests were used: Speech Perception Rating Scale and Speech Intelligibility Rating Scale.

The Speech Perception Rating Scale (tab. I) includes 7 items from 0 to 7 which allowed estimation of the progress of each patient after the cochlear implantation. To evaluate speech perception abilities, we assumed that hearing improvements are made in a hierarchical fashion starting from simple sound detection to comprehension of spoken words. Testing followed this hierarchy and patients were required to reach criteria scores for each level before moving to the upper level.

The Intelligibility Rating Scale (tab. II) includes 5 items intended to estimate, from 1 to 5, the evolution of speech production of the patient. We obtained scores either from familiar or from unfamiliar listeners.

Both tests asses the improvements of each recipient and compare the results obtained for all 8 patients.

TABLE I
Speech Perception Rating Scale

0	No detection of environment sounds
1	Speech detection
2	Discrimination between speech and
3	Closed-set word identification based
4	Closed-set word identification based
5	Open-set word recognition without
6	Sentence recognition with a family
7	Can use phone with a family member

TABLE II
Intelligibility Rating Scale

1	Imitation of examiner's spoken model
2	Elicit picture naming (isolated words)
3	Sentences recognized by familiar listeners
4	Sentences could be understand easy by unfamiliar
5	Is easy understand by unfamiliar listener in daily living

RESULTS

As the result of the evaluation before implantation, the patients were diagnosed with bilateral profound congenital cochlear deafness. The age at implantation ranged between 9 and 35 years. The duration of implant use was between 1 and 7 years, with a median of 3 years and an average of 3 years and 3 months; 6 of the patients were females and 2 of them were males.

All patients were fitted with conventional hearing aids prior to implantation with no benefit. The IQ and psychological development of all patients was normal (11).

Five of the patients had no communication mode prior to implantation, two patients were sign language and lip-reading users, and one patient used oral communication and lip-reading.

TABLE III
Results of cochlear implanted patients
("pre" means the patient had the ability before implantation)

No. of case	Age at CI (years)	Speech Perception rating scale								Speech Intelligibility rating scale					IC use (in years)
		0	1	2	3	4	5	6	7	1	2	3	4	5	
1	12	pre	1	3	6	12	36	42	42	3	9	12	36	36	9
2	10	pre	1	3	6	12				3	9	24			7
3	12	pre	1	3	6	12				3	6	12			1
4	9	pre	1	3	12					3	12				1
5	18	pre	1	3	9	12				3	Pre	pre	pre		3
6	19	pre	1	3	9					3	Pre	pre			6
7	30	pre	1	2	5					3	Pre	pre	pre		1
8	35	pre	1	3	9					3	9	12			6 y

The results obtained with the cochlear implants are presented in the table III. The numbers from the first row represent the scores for the two tests used for evaluation, from 0 to 7 for the Speech Perception Rating Scale and from 1 to 5 for the Speech

Intelligibility Rating Scale. The first four cases from the table are the adolescents and the last are the adults. The period the implant was used, necessary to reach each level, is expressed in months passed from the first fitting. The results show that all the patients included in the study have benefited from the implant when comparing their current hearing abilities with their hearing performances before implantation. They wear their implant daily.

DISCUSSION

Even as early as 1987, in a paper published during that period, G. Clark had drawn the conclusion that the age of deafness onset, the age at implantation, and the motivation of the patient play a major role in the outcome of cochlear implanted persons (12).

In congenitally deaf children, if the implantation is done very early, the outcomes are comparable with the normal hearing child. A matter that is often overlooked is that actually, in a group of children with the same characteristics, implanted very early, after 3-4 years of implant use the results would not be the same for each of them.

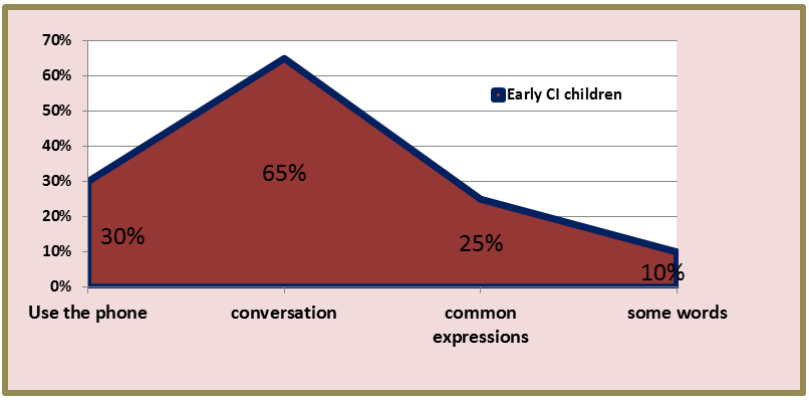

Fig.1. Results after cochlear implantation

In a study published in 2005, B. Krahulcova shows that only 65% of these children are able to understand a conversation without lip-reading and 30% of them are able to use the phone (13); 25% can understand simple sentences and the remaining 10% can understand isolated words (fig. 1). It is possible to presume that, to a certain extent, the same happens with late implanted patients with congenital deafness: some of them may have better outcomes than others. We do not have yet any way of knowing preoperatively who the best candidates from this group are. The debate regarding these patients is once more justified as long as it is not possible to quantify the risk/benefit ratio in these subjects8. At last, but not at least, it is important not to overlook the financial aspects.

Regarding our patients, the individual variability regarding to the age of implantation, the length of implant use (14), the communication mode prior to implantation etc. are reflected in the outcomes of audio-verbal rehabilitation post implant (15). These differences determine us to assess separately each subject.

When assessing the audio-verbal improvements from the day of the first fitting until today, we found that even for these

subjects the age at implantation makes a great difference concerning the outcomes. The patients implanted as adolescents score higher either at Speech Perception Rating Scale or Speech Intelligibility Rating Scale than those implanted as adults. The progress made by adolescents is gradual thus, in the most successful case, after 4 years of implant use (case number 1) with a sustained rehabilitative program and with a consistent familial support, one of the adolescents, implanted at 12 year of age, is able to speak on the phone and can be understood by an unfamiliar listener. Some authors argue that the poor outcomes of this group are not due to the absence of the auditory stimulus but to the lack of knowledge about the acoustic structure of the oral language (16). So, they affirm that subjects that are educated in an oral manner prior to implantation may be capable, after the implantation, to integrate the sounds they perceive (17, 18). In our study, although the patients had been using sign language before implantation, the outcomes, after 4 years of implantation, are quite remarkable. Yet, this success may have some other, unknown yet, explanation.

The expectations regarding the outcomes of our patient who had the implantation when she was in her '30s and benefited from oral communication prior to this procedure should be high if we believe the theory that argues that oral communication facilitates the outcome in the late implanted subjects. Taking into consideration that only one year has passed since the implantation, we can presume that better results are to be expected. At the same time, in a paper published in 2004 by Teoh it is shown that for prelingual deaf adults the maximum benefit is reached at the end of the first year following the implantation with no further improvement and the performance achieved by this group of patients is significantly below the levels of hearing achieved by post lingual patients (19, 20).

This was the case for the other three adult patients who had used their implants for more than 3 years and whose progress, compared to that of the prelingual deaf adolescents with implants, ceased at a lower level after the first 9 to 12 months following the implantation.

The results of our study indicate that prelingual deaf persons implanted in adolescence do better when compared to those implanted as adults. Only one exception was found, a patient who has had limited benefits from the implant, so far, in comparison with the others from the adolescent group, even though she was implanted at a younger age (case 4). The explanation for this is the presence of a malformation of the cochlea – common cavity (21).

CONCLUSIONS

The topic remains open as long as the studies had only a limited number of subjects, but even so it can be affirmed that, there is no doubt that all the subjects included in this study have had significant improvements after implantation.

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CHIST ARAHNOIDIAN ASOCIAT CU STENOZA PILORICĂ LA UN COPIL DE VÂRSTĂ MICĂ

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ARACHNOID CYST ASSOCIATED WITH PYLORIC STENOSIS IN A YOUNG BOY (Abstract): An unusual association between an arachnoid cyst and a decompensated pyloric stenosis in a three years-old boy is presented. The little patient was admitted into hospital with haematemesis, melena, influenced generally condition and acute posthemorrhagic anaemia following aspirin intake for hypertermia. Specific intensive care was successful and the little patient was discharged but without an upper digestive endoscopy(parents refusal, technical reasons). After one week he returned with progressive worsening vomitings and an intracranial hypertension was suspected. CT documented an arachnoid cyst in the right middle cranial fossa and the patient is directed to the Neurosurgical Clinic where a cyst fenestration was done. Subsequent to operation the vomitings reinstaled with severe dehydration and an upper GI series showed a decompensated pyloric stenosis. He was operated on underwenting an antrectomy. Finally the child recovered with good short and long-term evolution. The coincidental presence of an intracranial congenital mass and a complicated aspirin-induced peptic ulcer in this young patient, misleaded us and in the lack of an early endoscopy an intempestive neurosurgical operation was initially done. **Key words**: ARACHNOID CYST, ASPIRIN, ULCER, PYLORIC STENOSIS, ENDOSCOPY

În practica pediatrică vărsăturile constituie un simptom frecvent întâlnit, atât în afecţiuni digestive cât şi extradigestive. Diagnosticul diferenţial al acestora este deseori dificil, mai ales în situaţii în care asocierea unor entităţi patologice are ca expresie clinică sindromul dispeptic. Masele cerebrale intra sau extraparenchimatoase pot avea drept consecinţă apariţia sindromului de hipertensiune intracraniană, semnalat la copii mici de cefalee şi vărsături alături de modificări ale fundului de ochi. Chisturile arahnoidiene sunt formaţiuni benigne care apar în axul cerebrospinal la nivelul arahnoidei şi care nu comunică cu sistemul ventricular. Ele reprezintă 1% din totalul maselor intracraniene iar marea majoritate sunt anomalii congenitale. În general leziunile devin simptomatice în prima copilărie astfel că între 60 şi 90% dintre pacienţi sunt copii (1). În marea majoritate a cazurilor diagnosticul este stabilit incidental în cursul unor investigaţii imagistice recomandate pentru alte afecţiuni. Ulcerul secundar la vârsta pediatrică poate apare în contextul administrării unei medicaţii cu efect gastrotoxic. Leziunea debutează în general printr-o complicaţie de tip hemoragie sau perforaţie dar se poate

manifesta şi prin sindrom funcţional digestiv ce asociază vărsături şi dureri abdominale (2). Prezentăm în continuare un caz de asociere între ulcer secundar la aspirina complicat cu stenoză pilorică şi chist arahnoidian temporal drept la un copil de 3 ani.

PREZENTAREA CAZULUI

Pacientul M.I. în vârstă de 3 ani de sex masculin, din mediul rural, normal dezvoltat staturo-ponderal (T = 101 cm, G = 17 kg) se internează în urgenţă în Secţia de Terapie Intensivă a Spitalului Clinic de Urgenţă pentru Copii „Sf. Maria" pentru hematemeză şi melenă apărute la 48 de ore dupa administrarea de către părinţi a unei jumătăţi de tabletă de aspirină în scop antitermic. La internare copilul prezenta stare generală influenţată, paloare, faringe intens congestiv, amigdale hipertrofice, tahicardie (AV = 130/min), suflu sistolic gr. II/6 în toate focarele, epigastralgii intense, vărsături în zaţ de cafea, melenă. Investigaţiile paraclinice au relevat anemie posthemoragică acută (Hb cu valori ce au variat între 9,9 şi 6,5 mg/dL), leucocitoză cu neutrofilie (GA = 15600/mm^3; PMN = 79%), hipoglicemie (65 mg/dL), hiponatremie (128 mmol/L), hipopotasemie (2,8 mmol/L); uree cu valoare crescută (50 mg/dL), coagulograma normală (TQ = 14,5 sec, APPT = 36 sec, INR = 1).

Radiografia toraco-abdominală a fost de asemenea normală. Sub tratament cu antiacide, antisecretorii i.v, hemostatice, transfuzii de masă eritrocitară, antibioterapie cu spectru larg pentru infecţia respiratorie intercurentă, starea generală se ameliorează, vărsăturile şi durerile epigastrice dispar, tranzitul intestinal se normalizează. Menţionăm că, deşi indicaţia ar fi fost certă, nu s-a efectuat endoscopie digestivă superioară datorita refuzului părinţilor şi unor motive tehnice. Pacientul se externează ameliorat recomandandu-i-se terapie marţială pentru corectarea anemiei dar revine peste o săptămână cu sindrom de deshidratare acută prin vărsături. Examenul clinic evidenţiază stare generală influenţată, scădere ponderală de 2 kg, G = 15 kg, facies încercănat, paloare, semne de deshidratare şi vărsaturi incoercibile alimentare şi bilioase, lipsa tranzitului intestinal pentru materii fecale de 48 de ore, absenţa semnelor de iritaţie meningeană.

Paraclinic: anemie hipocromă microcitară (Hb = 9,3 mg/dL, VEM = 69 μ^3, MCHC = 28 g/dL, MCH = 22 pg), leucocitoză cu neutrofilie (GA = 17600/mm^3, PMN = 81%), hipopotasemie (K = 2,4 mmol/L), hiponatremie (Na = 121 mmol/L), hipocloremie (Cl = 90 mmol/L), alcaloză metabolică (RA = 30mEq/L).

Ecografia, radiografia abdominală pe gol şi examenul chirurgical infirmă un sindrom ocluziv.

Cu tot tratamentul energic de reechilibrare hidroelectrolitică şi acido-bazică vărsăturile alimentare şi bilioase persistă. Examenul neurologic şi fundul de ochi nu arată modificări patologice dar pentru a exclude un eventual sindrom de hipertensiune intracraniană se efectuează tomografie computerizată care evidenţiază un chist arahnoidian voluminos (4,5/4,5 cm) dez-

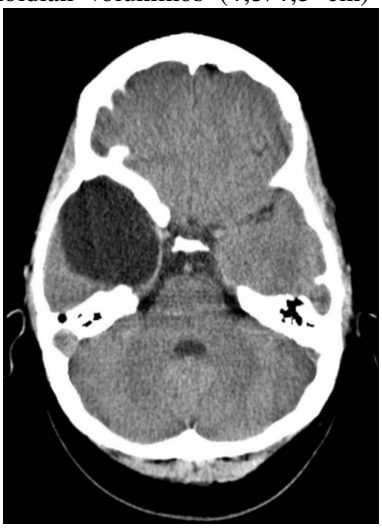

Fig. 1. CT craniocerebral (secţiune axială): chist arahnoidian drept (M.I, 3a)

Fig. 2. Tranzit baritat : Stenoză pilorică (M.I, 3a)

voltat în valea sylviană şi temporo-polar drept, neînsoţit de hidrocefalie activă ; structurile liniei mediane în poziţie normală şi mega cisterna magna (fig. 1). Pacientul este transferat la Spitalul de Neurochirurgie Iaşi unde se practică intervenţia chirurgicală (fenestrarea chistului şi liza aderenţelor prin craniotomie frontotemporală dreaptă). Este readmis în serviciul nostru unde, după o evoluţie postoperatorie favorabilă de doar două zile, reapar vărsăturile incoercibile şi modificările hidroelectrolitice consecutive. Tranzitul baritat indică un aspect caracteristic de stenoză pilorică (fig. 2).

Laparotomia efectuată în Clinica de Chirurgie Pediatrică evidenţiază un stomac dilatat cu pilor de aspect pseudotumoral, mult mărit de volum, cu leziune ulceroasă şi edem important al mucoasei (fig. 3). Se practică rezecţia zonei (antrectomie) şi refacerea continuităţii digestive prin gastroduodenostomie Pean-Bilroth I. Examenul anatomopatologic al piesei de rezecţie confirmă diagnosticul de leziune ulceroasă. Ulterior, evoluţie favorabilă cu drenaj minim nazogastric şi abdominal, cu reluarea tranzitului pentru materii fecale şi gaze a patra zi postoperator când se suprimă şi drenurile. Externat a 7-a

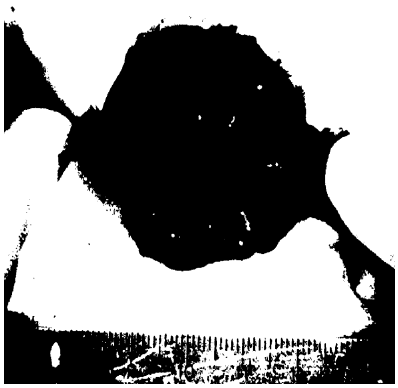

Fig. 3. Piesa operatorie

zi postoperator pacientul revine la control după 6 săptămâni, când se efectuează nou tranzit baritat ce indică un aspect normal ; controalele anuale ulterioare arată o evoluţie favorabilă cu curbă ponderală ascendentă şi parametri biologici în limite normale.

DISCUŢII

Descrise pentru prima data de Bright în 1831, chisturile arahnoidiene sunt leziuni congenitale sau pot apărea în asociere cu tumorile sau cu aderenţele determinate de meningite, hemoragii sau intervenţii chirurgicale (3). Cam 50-60% apar în fosa craniană mijlocie. Afecţiunea interesează mai frecvent sexul masculin (2-3/1) şi se localizează predominant pe partea stânga, leziunile bilaterale sau multiple fiind rar semnalate (1,3). Au fost raportate cazuri cu agregare familială, precum şi asocieri între chisturi arahnoidiene bilaterale la nivelul scizurii sylviene şi acidurie glutarică tip I, sindrom Marfan, neurofibromatoză sau boala rinichiului polichistic (4,5). Simptomatologia include cefalee, greţuri, vărsături, somnolenţă, întârziere în dezvoltarea psiho-motorie, semne de focar, convulsii ; chisturile supraselare mai pot avea ca expresie clinică hidrocefalia, pubertatea precoce, alterări ale acuităţii vizuale şi „head bobbing”

(mişcari de flexie şi extensie ale capului). Ultrasonografia este utilă în perioada fetală şi pe parcursul primului an de viaţă, fiind neinvazivă şi având un randament apreciabil în detectarea maselor intracraniene (1,3). Radiografia de craniu are o sensibilitate redusă; la copii poate arăta craniomegalie, mărirea fontanelei anterioare şi subţierea oaselor bolţii craniene, modificări care pot fi însă variante ale normalului (1,3). Tomografia computerizată relevă structuri unilaculare sau septate, cu margini nete, conţinut izodens cu LCR şi modificări minime ale ţesutului cerebral subiacent; rar apar calcificări. Utilizarea CT poate duce la erori de diagnostic, mai ales în cazul chisturilor hemoragice, al căror conţinut are o densitate diferită faţa de LCR. De asemenea, CT nu poate face diferenţierea faţă de chisturile epidermoide. Investigaţia imagistică de elecţie rămâne RMN care determină cu precizie locaţia, extinderea şi raportul cu ţesutul cerebral sau coloana vertebrală în cazul chisturilor spinale. Se poate practica RMN folosind secvenţa de difuzie si secvenţa FLAIR (fluid-attenuated inversion recovery), dar cea mai sensibilă rămâne PC-RMN (cine phase-contrast magnetic resonance imaging) (6,7). Angiografia este rar practicată deoarece metodele non-invazive ca angio CT sau angio RMN au aceeaşi acurateţe diagnostică. În ceea ce priveşte tratamentul opiniile sunt împărţite între tendinţa de a trata doar chisturile clinic manifeste şi cea de a practica decompresia leziunilor asimptomatice pentru a evita viitoare complicaţii. Opţiunile terapeutice sunt controversate şi includ fenestrarea deschisă sau endoscopică, plasarea unui şunt chistoperitoneal sub ghidaj ultrasonografic sau aspiraţia stereotaxică. Reacumularea postoperatorie de lichid a fost descrisă în până la 25 % dintre cazuri (3,8).

Utilizarea aspirinei la copii este limitată în prezent la pacienţii cu reumatism articular acut, artrită reumatoidă juvenilă sau boala Kawasaki mai ales datorită riscului de apariţie a sindromului Reye dar şi al altor entităţi patologice ca ulcerele peptice secundare. Aspirina este formal contraindicată în combaterea febrei la vârsta pediatrică iar utilizarea pe scara largă a ibuprofenului în scop antitermic a facut ca acesta să devină principalul factor implicat în lezarea mucoasei gastroduodenale la copil (2). Efectele gastrotoxice ale AINS sunt atât directe cât şi sistemice (inhibarea sintezei de prostaglandine). Administrarea de AINS este urmată de apariţia unor hemoragii gastrice subepiteliale şi ulterior de apariţia eroziunilor mucoasei dacă expunerea continuă (9). AINS inhibă ciclooxigenaza COX-1 implicată în producerea prostaglandinelor cu efect protector care reglează integritatea mucoasei gastrice, homeostazia plachetară şi funcţia renală. Scăderea agregării plachetare şi prelungirea timpului de sângerare cresc riscul de hemoragie digestivă superioară. Aspirina şi cele mai multe AINS scad proprietăţile hidrofobe ale stratului de mucus, secreţia de bicarbonat şi sunt asociate cu efecte iritante topice (9,10). Ulcerul produs de aspirină debutează prin accident hemoragic la copiii mici iar localizarea este predominant gastrică. Arsenalul terapeutic include prostaglandinele de sinteză, inhibitorii H2 şi IPP. În general, are prognostic bun dar se descrie posibilitatea formării unui diafragm antral prepiloric într-un interval cuprins între o lună şi 2 ani (11).

În observaţia noastră, deşi elementul anamnestic şi simptomatologia clinică iniţială (hemoragia digestivă superioară) orientau către diagnosticul de ulcer secundar, neefectuarea în urgenţă a endoscopiei digestive superioare alături de evoluţia iniţial favorabilă sub tratament antisecretor au condus la ignorarea leziunii ulceroase care s-a complicat rapid cu o stenoză pilorică. În momentul reinternării ne-am aflat în faţa unui sindrom dispeptic cu semne de deshidratare

acută ; aspectul vărsăturilor nefiind caracteristic s-a impus un diagnostic diferenţial al etiologiei acestora care a inclus şi sindromul de hipertensiune intracraniană. Coincidenţa asocierii cu leziunea malformativă a condus la intervenţia neurochirurgicală intempestivă, a carei indicaţie rămâne discutabilă având în vedere că evoluţia ulterioară şi evidenţierea prin tranzit baritat a stenozei pilorice au stabilit cauza reală a dispepsiei şi au dus la rezolvarea chirurgicală adecvată. Asocierea chistului cu mega cisterna magna şi evoluţia rapid stenozantă a ulcerului particularizează suplimentar acest caz de coexistenţă a patologiei tumorale intracraniene cu cea digestivă.

CONCLUZII

Cazul prezentat ilustrează posibilele consecinţe ale administrării intempestive de către aparţinători a unei medicaţii agresive pentru mucoasa gastroduodenală, a cărei utilizare la copil trebuie atent supravegheată. Considerăm că explorarea endoscopică se impune ca gest de primă-intenţie în hemoragiile gastrointestinale, imediat ce statusul bolnavului o permite. Nu în ultimul rând, originea extradigestivă a unui sindrom dispeptic va fi luată în discuţie după eliminarea sistematică a tuturor cauzelor digestive.

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COMPARISON BETWEEN DIGITAL MAMMOGRAPHY AND DIGITAL BREAST TOMOSYNTHESIS IN THE DIAGNOSIS OF BREAST CANCER

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COMPARISON BETWEEN DIGITAL MAMMOGRAPHY AND DIGITAL BREAST TOMOSYNTHESIS IN THE DIAGNOSIS OF BREAST CANCER (Abstract): The purpose of this study was to evaluate the impact of adding digital breast tomosynthesis (DBT) to digital mammography (DM) on the detection and characterization of breast lesions and to compare the diagnostic performance of DM and DBT in order to determine whether or not simultaneously viewing is more informative in detection of breast cancers. **Material and methods:** We retrospectively reviewed the records of 58 female patients (age 38 to 79 years old), who had pathologically proved breast cancers and had undergone both DM and DBT during a period of eight month (between February and September 2018). The diagnostic performance of each modality was evaluated. **Results:** The sensitivity of DM was 73% and its specificity was 47%, respectively the sensitivity of DBT was 98% and specificity 73%. The positive predictive value was 0.81 for DBT, while DM had a positive predictive value of 0.58. The negative predictive value was 0.98 in DBT and 0.62 in DM. The margins of the mass lesions and inhomogeneity of the focal asymmetric densities were better evaluated on DBT. Distributions and morphology of microcalcifications were better characterized on digital mammography than on tomosynthesis. **Conclusions:** In our study, DBT significantly enhances the detection and characterization of tumors in the context of mass lesions and focal asymmetric densities. **Keywords:** MAMMOGRAPHY, TOMOSYNTHESIS, BREAST CANCER.

Breast cancer remains one of the leading causes of death in women over the age of 40 years. Mammography is an effective imaging tool for the detection of early-stage breast cancer. The sensitivity of screening mammography for breast cancer had been reported to be 80-90% in adipose breast but may be as low as 48% in extremely dense breast because of overlapping dense fibro glandular breast tissue, which poses a significant obstacle to interpretation by reducing the conspicuity of breast lesions (1, 2, 3). Digital breast tomosynthesis (DBT) is a new tool which eliminates tissue overlap due to its ability to acquire 3D images of the breast and is considered one of the most challenging and promising up to date imaging technology (4, 5). Several studies have shown that tomosynthesis may offer superior diagnos-

tic accuracy when compared with digital mammography (DM), not only in the routine diagnostic practice, but also in breast cancer screening, for the evaluation of breast lesions (6, 7). It was considered that 3D breast tomosynthesis using the cranio-caudal (CC) and medio-lateral oblique (MLO) views diminish false-positive findings, minimize the masking effect of overlying tissue and enable improved cancer detection while (8, 9, 10).

The purpose of this study is to evaluate the impact of adding DBT to DM for the detection and characterization of breast lesions and to compare the diagnostic performance of DM and DBT in order to determine whether or not simultaneously viewing is more informative in the detection of breast cancer.

MATERIAL AND METHODS

We retrospectively reviewed 69 pathologically proven breast cancers found at 58 female patients who had undergone both DM and DBT during a period of 8 month (between February and September 2018). The mammographic findings included the density of breast, the type of malignant features (focal asymmetric density) and breast imaging reporting and data system (BIRADS) score. A combined DM and DBT examination was performed with a SenoClaire GE mammography unit in 2 views: CC and MLO; the system attains a "Combo-mode" imaging technique (2D + 3D imaging) that acquires a digital mammogram and a tomosynthesis during a single compression. The tomosynthesis consist of nine low-dose images of the breast are acquired at different angles while the X-ray tube moves in a 25º arc across the breast. These images are then used to produce a series of one-millimeter thick images (from 60 to 90 slices) that can be reconstructed to a three-dimensional (3D) image of the breast. Images were displayed on a dedicated diagnostic mammography workstation with special capabilities that are tailored for breast imaging. The reconstructed tomosynthesis images can be viewed slices, slabs or in a cine loop manner.

We identified breast lesions with DM and DBT and the following parameters were characterized: dimension, number, morphology and distribution of the calcifications in order to classification them in benign, intermediate or malignant; intensity; homogeneity and margins (convex or concave) of the mass lesions.

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of either modality were calculated with histology as the gold standard. When evaluating imaging modalities, these results were classified into two categories: a positive test result is any of BI-RADS assessment categories 0, 4 or 5 and a negative test result is any of BI-RADS assessment categories 1, 2, or 3. A true positive is a positive imaging finding that is followed by a diagnosis of breast cancer within 12 months. A false positive is a positive imaging result that is not followed by a cancer diagnosis within 12 months. True negatives are the negative test results that remain negative during follow-up. False negatives are the negative test results with cancer detected at histopathological examination. The sensitivity is defined as the number of positive tests in breast cancer patients divided by the total number of women, and was reported as a percentage. The specificity is defined as the number of true negative cases divided by the true negatives and false positives. The

positive PPV is the number of true positives divided by the total number of positive tests. The NPV is the number of true negatives divided by the total number of negative tests. All statistical tests were performed with MedCalc for Windows (MedCalc Software, Mariakerke, Belgium). Imaging findings were tabulated and categorical results of DM and DBT were included. Both modalities were compared with the histopathology report in the context of detection and diagnosis, each was individually assessed, using the Pearson Chi Square tests. P value <0.05 is considered to be significant.

RESULTS

All patients were female. The average age of patients was 48 years (ranging from 38-79 years). Among the 69 malignant breast lesions identified, we discovered 18 (26.08%) clusters of microcalcifications (three clusters of more than 15 amorphous microcalcifications, BIRADS 4c, and 15 cases with more than 20 pleomorphic microcalcifications with segmental orientation BIRADS 5, 45 (65.21%) mass lesions and 6 (8.69%) focal asymmetric densities which were classified BI-RADS 4c and 5 according to Breast Imaging Reporting and Data System atlas (11) (fig.1).

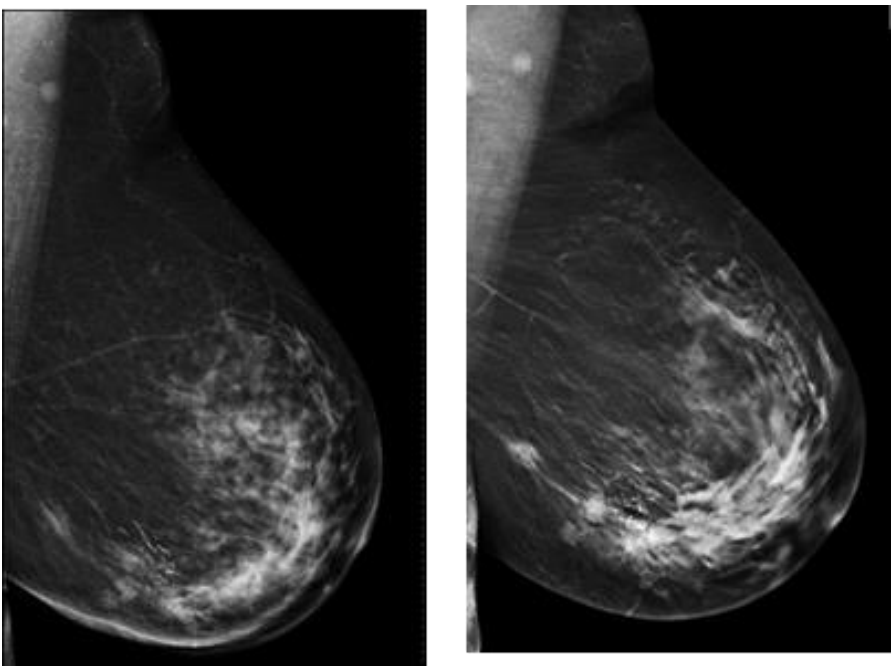

Fig. 1. Cluster of BI-RADS 5 microcalcifications on digital mammography (a) and breast tomosynthesis (b)

The malignant lesions were correctly diagnosed by DM in 58 cases (84%) (18 out of 18-100% clusters microcalcifications, 40-88.89% out of 45 mass lesions); the estimation became more accurate when DBT was included in the evaluation of the mass lesions (all 45 cases were classified BIRADS 4c and 5) and the focal asymmetric densities (5 out of 6 lesions were classified BIRADS 4c). Tomosynthesis elimi-

nates tissue overlap and the margins of the masses were better depicted on DBT than on DM (39 spiculated and 6 micro lobulated margins were very well seen); DM couldn't evaluate correctly the margins of the masses in 9 (20%) cases, which appeared to have indistinct margins. DM and DBT evaluated correctly the intensity of all 45 masses (36 high intensity and 9 medium intensity) (fig. 2).

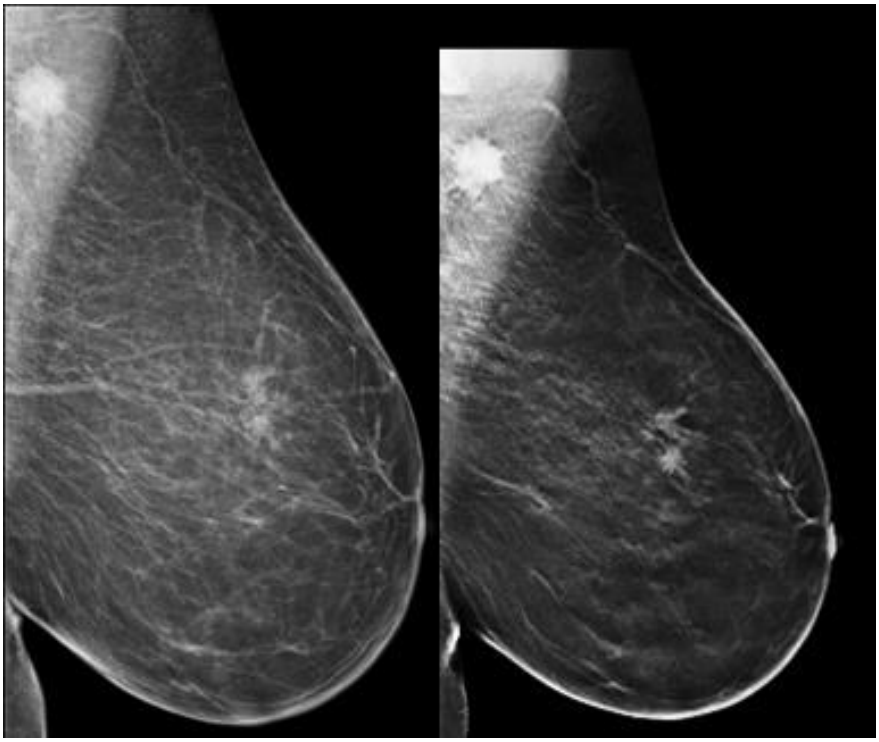

Fig. 2. Two malignant breast tumors and axillary lymphadenopathy – the margins of the masses are better evaluated on breast tomosynthesis (b) than on digital mammography (a)

In 5 cases the presence of convex margins, which indicates a possible malignancy were better seen on DBT (fig. 3).

A significant difference in diagnostic performance ($p \leq 0.05$) between DM and DBT was found. The sensitivity of DM was 73% and its specificity was 47%, respectively the sensitivity of DBT was 98%, and specificity 73% ($p = 0.04$, Pearson's chi-squared test). The positive predictive value was 0.81 for DBT, while DM had a positive predictive value of 0.58. The negative predictive value was 0.98 in breast tomosynthesis and 0.62 in DM ($p = 0.05$, Pearson's chi-squared test). The inhomogeneity of all focal asymmetric densities was better evaluated on DBT and the higher intensity which was seen inside suggested a malignant lesion; the presence of convex margins, which indicates a possible malignancy were better seen on DBT in 5 cases (fig. 3).

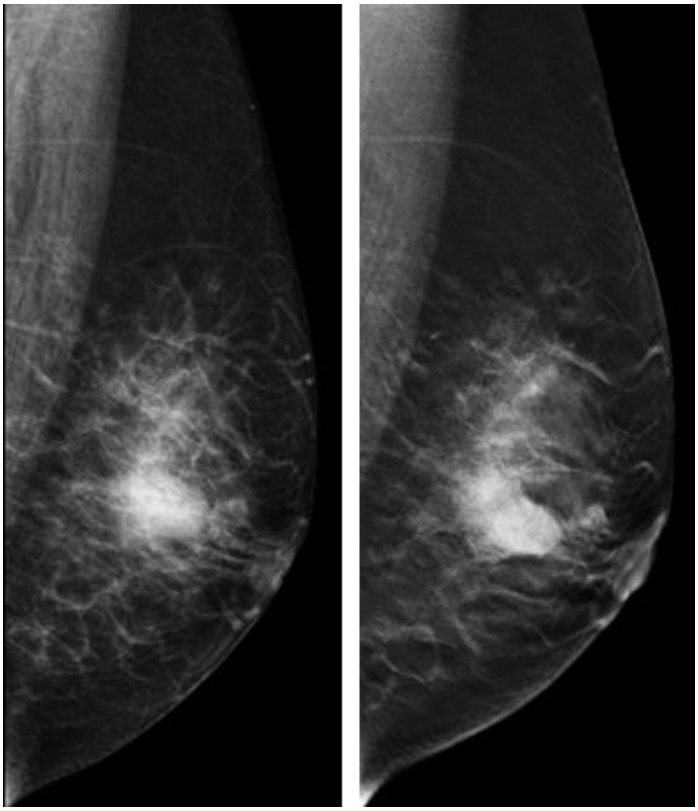

Fig. 3. Focal asymmetric density (BIRADS 4c) visible on digital mammography (a), better depicted on breast tomosynthesis (b)

DISCUSSION

The objective of this study was to evaluate the potential impact of breast tomosynthesis and digital mammography in the detection and diagnosis of breast lesions.

Our results showed an increased sensitivity of 25% (from 73% to 98%) with use of combined DBT and DM, which is consistent with the result of prior studies. Rafferty demonstrated an increased sensitivity from 65.5% for DM alone to 76.2% with the addition of DBT (13). Michell *et al.* in a study of 738 women found an increase in sensitivity of 18% (from 40% to 58%) when DBT with DM was compared with DM alone (14). Teertstra *et al.* examined 513 cases of which 109 were cancers, reported similar sensitivity for DBT and DM (92.9%). It should be noted that the authors did not investigate the use of DBT in combination with DM. A borderline significant improvement ($p = 0.07$) in sensitivity with the addition of DBT to DM (DM 87%; DBT and DM 89%) was found by Gilbert *et al.* (16,17). The investigators included 7060 subjects who underwent combined two-view DM and DBT recruited from six centers (women recalled following breast screening, and women below 50 years of age, with a family history of breast cancer).

In our study, specificity was shown to

be improved by 26% with the addition of DBT. The impact of DBT/DM on specificity as compared with DM alone was also demonstrated by Michell *et al.* which showed an improvement in specificity from 51% to 74% in a study of 738 women (14). Skaane *et al.*, in the much larger study of 24,301 cases of the Oslo Tomosynthesis Screening Trial (OTST), found that specificity was significantly higher ($p<0.001$) for DBT/DM (97.5%) compared with DM alone (96.4%) (19).

The detection and characterization of microcalcifications was better with DM. Breast tomosynthesis does not save the day in case of pathologies which have the microcalcifications as only image. The number of microcalcifications seems to be smaller on the slices; the distribution pattern and the microcalcifications morphology were characterized with difficulty on DBT and better depicted on DM. Previously published work by Tagliafico *et al.* regarding the performance of DM and DBT in characterization of microcalcification clusters using BIRADS scoring, found that sensitivity was 100% for DM and 91.1% for DBT, whereas specificity was 94.6% for DM and 100% for DBT. The authors concluded that DBT may miss a small number of cancers (20).

CONCLUSIONS

In our study, DBT significantly enhances the detection and characterization tumors in the context of mass lesions and focal asymmetric densities; the margins of the mass lesions and the inhomogeneity of the focal asymmetric densities were better evaluated on DBT then on digital mammography. Tomosynthesis isn't helpful to count or characterize the morphology and distribution of the calcifications. The microcalcifications were better characterized on digital mammography than on tomosynthesis.

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STUDIUL PREVALENŢEI SUPRAPONDERII ŞI OBEZITĂŢII ÎNTR-O COMUNITATE RURALĂ DIN NORD-ESTUL ROMÂNIEI

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PREVALENCE OF OVERWEIGHT AND OBESITY IN A RURAL POPULATION (Abstract): The main objective of this study is to estimate the prevalence of overweight and obesity in a rural population, from the north-east part of Romania. **Material and method**: The study population included 3248 adult subjects (1341 men, 1907 women), aged 18 or over, registered with their general practitioner. Anthropometric parameters were assessed by medical staff and obesity and overweight were defined according to World Health Organization's (WHO) criteria. Abdominal obesity was described according to International Diabetes Federation's (IDF) criteria. **Results**: The prevalence of overweight and obesity is 33.9% and 12.2% in men and respectively 35% and 21.3% in women. The majority of overweight and obese subjects had an increased waist circumference. There can be observed the predominance of the women in the overweight and obese group, as well as the increase of the obesity prevalence with age. **Conclusion**: Our results show that the weight excess could become a public health problem in Romania, therefore national screening programs are needed along with public information campaigns regarding effectual prevention and treatment possibilities. **Key words**: OVERWEIGHT, OBESITY, WAIST CIRCUMFERENCE

Încă din 1977, Organizaţia Mondială a Sănătăţii (OMS) a atras atenţia asupra evoluţiei statusului ponderal al populaţiei, declarând obezitatea o afecţiune cu evoluţie epidemică în întreaga lume (1). Dacă până la mijlocul anilor '70, creşterea ponderală a fost asociată cu ameliorarea stării de sănătate şi a longevităţii, în ultimele decenii se observă o schimbare a raportului risc-beneficiu, astfel încât obezitatea este recunoscută ca fiind responsabilă de creşterea morbidităţii, respectiv a mortalităţii generale şi cardiovasculare (2,3,4,5,6), devenind prioritate a politicilor de sănătate publică în întreaga lume. OMS atrage atenţia asupra necesităţii cunoaşterii dimensiunilor reale ale problemei, în condiţiile în care doar jumătate din ţările europene deţin date valide cu privire la indici antropometrici (7). În România, în absenţa unor studii epidemiologice la nivel naţional, prevalenţa supraponderii şi obezităţii a fost estimată pornind de la rezultatele unor studii efectuate în diverse teritorii. La ora actual, nu există o statistică recentă în ceea ce priveşte situaţia excesului ponderal în regiunea Moldovei (aflată în nord-estul României), ceea ce motivează realizarea acestui studiu ce are drept obiectiv principal aflarea prevalenţei supraponderii şi obezităţii într-o comunitate rurală din judeţul Iaşi.

MATERIAL ŞI METODĂ

Au fost incluse în studiu 3248 de per-

soane cu domiciliu stabil în localitatea Deleni, judeţul Iaşi, în perioada iulie 2007 - iulie 2008, cu vârsta >18 ani (reprezintă 46,07% din populaţia adultă a localităţii). S-a stabilit diagnosticul de supraponderе sau obezitate conform criteriilor OMS care apreciază statusul ponderal în funcţie de valoarea IMC (raportul dintre greutate în kg şi pătratul înălţimii în metri). Măsurarea greutăţii s-a realizat în condiţii de îmbrăcăminte uşoară şi pe nemâncate, utilizând un cântar calibrat, standardizat şi etalonat conform normelor internaţionale. Măsurarea înălţimii s-a făcut în poziţie verticală, cu subiectul descălţat, cu ajutorul unui stadiometru portabil, standardizat şi calibrat, etalonat conform normelor internaţionale. Stabilirea statusului ponderal a fost efectuată astfel : subponderal pentru IMC < 18,5 ; normoponderal pentru 18,5 ≤ IMC ≤ 24,9 ; supraponderal pentru 25 ≤ IMC ≤ 29,9 ; obezitate grad 1 pentru 30 ≤ IMC ≤ 34,9 ; obezitate grad 2 pentru 35 ≤ IMC ≤ 39,9 ; obezitate grad 3 pentru IMC ≥ 40. Circumferinţa abdominală (CA, talia) a fost măsurată la jumătatea distanţei dintre rebordul costal şi creasta iliacă, cu o bandă gradată neextensibilă. Obezitatea abdominală a fost diagnosticată la valori ale CA ≥ 80 cm la femei, şi ≥ 94 cm la bărbaţi, conform criteriilor IDF. Analiza statistică a datelor s-a efectuat cu softul *Statistical Package for Social Sciences* (*SPSS*).

REZULTATE

În populaţia studiată, doar 45% erau normoponderali, 2,9% erau subponderali iar restul de 52,1% erau supraponderali sau obezi (IMC ≥ 25 kg/m^2 conform criteriilor OMS) (tab. I).

S-a observat o prevalenţă a supraponderii de 34,5% şi a obezităţii de 17,5% (fig. 1), predominând sexul feminin în ambele grupe (prevalenţa supraponderii în rândul femeilor versus bărbaţi de 35%, respectiv 33,5%, prevalenţa obezităţii în rândul femeilor versus bărbaţi de 21,3%, respectiv 12,2%,).

În ceea ce priveşte analiza pe grupe de vârstă, s-a observat o creştere a prevalenţei excesului ponderal odată cu înaintarea în vârstă, prevalenţa maximă a supraponderii fiind pentru grupa 65-69 ani iar a obezităţii în grupa 55-59 ani (fig. 2). Grupele de

TABELUL I
Descrierea lotului în funcţie de valoarea IMC

	Subponderali	Normoponderali	Supraponderali	Obezi
Bărbaţi (1341)	1,6% (22)	52,3% (702)	33,9% (454)	12,2% (163)
Femei (1907)	3,8% (73)	39,9% (760)	35% (667)	21,3% (407)
Total (3248)	2,9% (95)	45% (1462)	34,5% (1121)	17,5% (570)

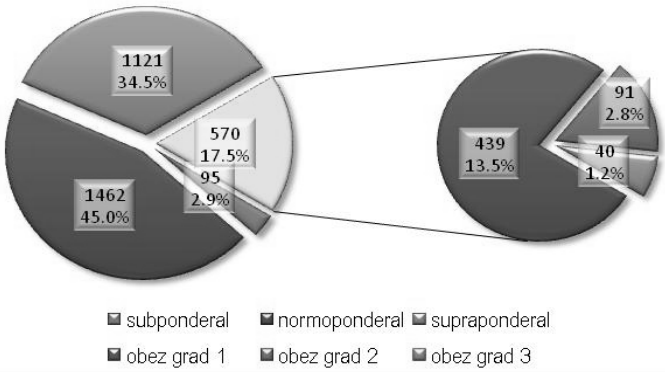

Fig. 1. Prevalenţa supraponderii şi a diferitelor grade de obezitate în populaţia studiată.

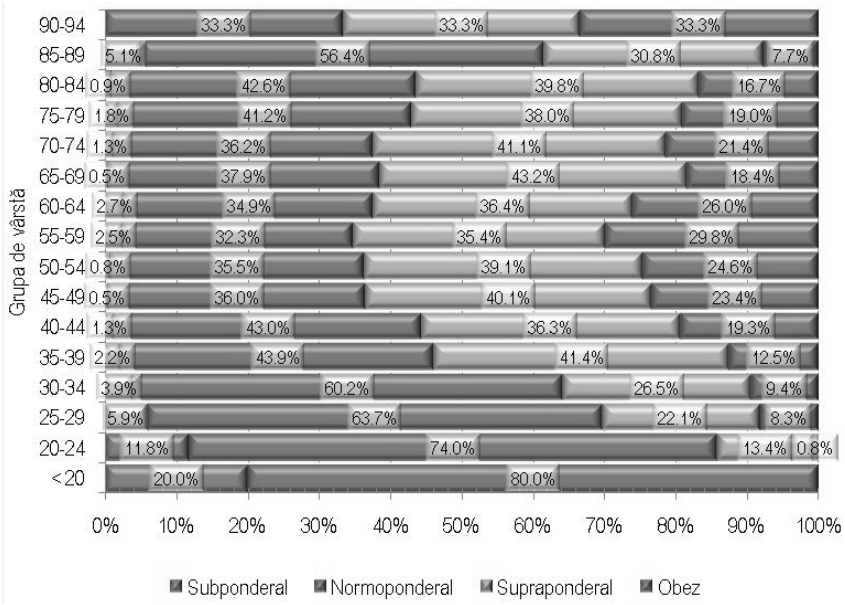

Fig. 2. Prevalenţa supraponderii şi obezităţii pe grupe de vârstă

vârstă extreme, <20 şi ≥90 ani, nu au reprezentativitate statistică deoarece sunt prea puţini pacienţi, de aceea rezultatele nu respectă trendul populaţiei.

S-a observat pentru întregul lot existenţa unei relaţii direct proporţionale între vârstă şi IMC, 5,2% din variaţia acestuia fiind explicată de variaţia vârstei ($p < 0,005$). La o creştere în vârstă cu 10 ani, media creşterilor IMC-ului este de 0,6 ($p < 0,005$) (fig. 3).

Majoritatea persoanelor cu supraponderе sau obezitate aveau şi talia (CA) crescută (tab. II, fig. 4) iar în grupul normoponderalilor (definit pe baza valorii IMC) se observă o prevalenţă crescută a prezenţei taliei mari la femei versus bărbaţi ($p < 0,001$). Acest subgrup de persoane cu IMC normal dar cu obezitate abdominală ar putea reprezenta o categorie cu risc crescut pentru afecţiuni cardio-metabolice, ştiut fiind faptul că CA reprezintă un indicator al excesului de ţesut adipos visceral.

S-a observat că 9,39% din variaţia CA (creşterea CA cu vârsta) este determinată de modificarea (creşterea) vârstei ($p < 0,001$). Putem aprecia că, în medie, la o creştere în vârstă cu 10 ani se ajunge la o creştere a circumferinţei abdominale cu 2,15 cm. Femeile prezintă o dependenţă mai puternică, în 10 ani creşterea CA este de 2,72 cm comparativ cu bărbaţii unde există o creştere a CA de 1,316 cm ($p < 0,001$) (fig. 5).

Dependenţa dintre valoarea IMC-ului şi CA este puternică, 58.3% din variaţia CA fiind explicată de variaţia IMC ($p < 0,001$) (fig. 6).

DISCUŢII

Statisticile actuale arată o creştere îngrijorătoare a prevalenţei supraponderii şi obe-

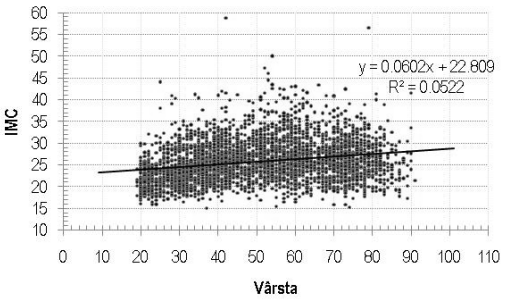

Fig. 3. Variaţia IMC în funcţie de vârstă.

TABELUL II

Caracteristici antropometrice ale populaţiei cu IMC ≥ 30 kg/m²

M – media, cu Min (minim), Max (maxim) şi ± STDEV – standard deviation

Circumferinţa abdominală		M-STDEV	Max	Min	M+STDEV
M	obez grad 1	103,3	135	95	118,7
	obez grad 2	111,1	133	101	128,7
	obez grad 3	106,8	153	90	148,5
	Total	**102,9**	**153**	**90**	**122,4**
F	obez grad 1	100,5	140	56	118,3
	obez grad 2	107,9	137	97	126,3
	obez grad 3	105,5	160	70	138,7
	Total	**101,2**	**160**	**56**	**122,4**
Total	obez grad 1	101,3	140	56	118,5
	obez grad 2	108,4	137	97	126,7
	obez grad 3	105,8	160	70	140,6
	Total	**101,7**	**160**	**56**	**122,4**

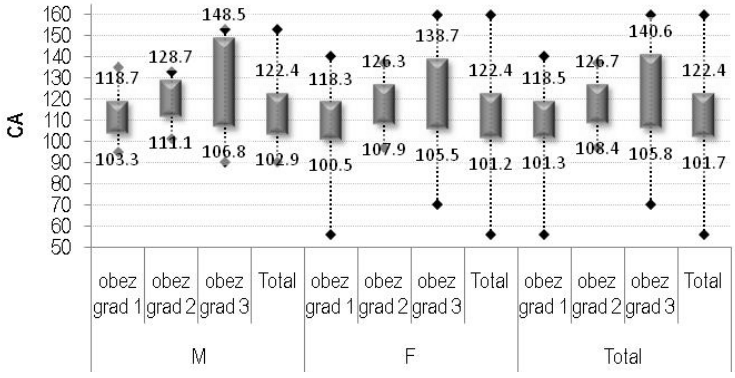

Fig. 4. Caracteristici antropometrice ale populaţiei cu IMC peste 30 kg/m²

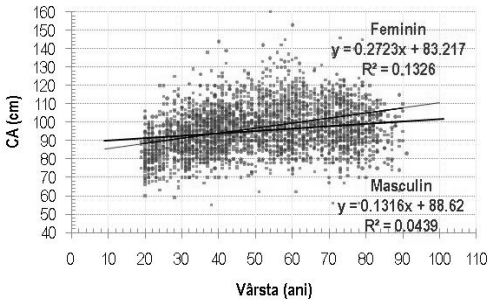

Fig. 5. Variaţia circumferinţei abdominale cu vârsta, la ambele sexe

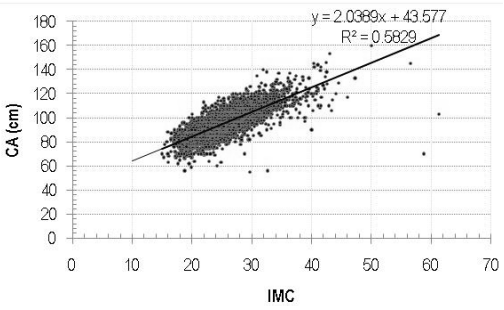

Fig. 6. Variaţia circumferinţei abdominale în funcţie de IMC

zităţii în lume. Comparaţii clare în ceea ce priveşte prevalenţa excesului ponderal în populaţii diferite sunt greu de efectuat datorită diferenţelor în ceea ce priveşte alcătuirea loturilor, criteriile de definire utilizate şi, nu în ultimul rând, metodologiei de studiu. Cu toate acestea, rezultatele studiilor şi statisticilor publicate de organizaţiile în domeniu arată că obezitatea este cel mai frecvent întâlnită în Statele Unite şi Canada, cea mai scăzută prevalenţă fiind înregistrată în China şi Japonia. În cadrul Europei, pre-

valenţa este cea mai scăzută în ţările scandinave şi cea mai crescută în ţări precum Albania, Marea Britanie, Israel (8). Rezultatele studiului nostru sunt incluse în intervalele de prevalenţă declarate de OMS în 2007 pentru Europa (7). Conform acestora, prevalenţa obezităţii este între 5,4 - 22,8% în rândul bărbaţilor (12,2% în studiul nostru) şi între 7,1 - 35,6% în rândul femeilor (21,3% în studiul nostru).

Comparativ cu datele raportate de România în 2000, observăm o prevalenţă mai mică a supraponderii în rândul bărbaţilor în studiul nostru (33,9% faţă de 38,1%) dar mai mare în rândul femeilor (35% faţă de 28,6%) (7). În ceea ce priveşte obezitatea, rezultatele noastre arată o prevalenţă mai mare atât în rândul bărbaţilor (12,2% faţă de 7,7%) cât şi în rândul femeilor (21,3% faţă de 9,5%). Aceste discrepanţe pot fi explicate, pe de o parte, de faptul că statistica României este efectuată pe o populaţie cu vârsta ≥15 ani. Pe de altă parte, studiul nostru a inclus date antropometrice măsurate de către cadrele medicale, iar datele României la OMS sunt bazate pe autoestimarea greutăţii şi taliei, importantă sursă de eroare dacă ţinem cont de faptul că adeseori supraponderalii au tendinţa de a subestima propria greutate iar înălţimea este frecvent supraestimată, indiferent de statusul ponderal (9). Rezultatele studiului nostru sunt similare cu cele obţinute în diverse localităţi urbane din România, în care prevalenţa supraponderii şi obezităţii a fost în limite destul de variabile, între 29% şi 34,6%, şi respectiv 14,6% şi 26% (10). Studiul SEPHAR efectuat în România în 2005 a arătat o prevalenţă a obezităţii în populaţia adultă de 37%, fiind mai mare în rândul femeilor, la toate categoriile de vârstă (11).

În Europa, datele cele mai apropiate de ale noastre privind prevalenţa obezităţii sunt cele raportate de Serbia şi Muntenegru în 2000 (14,3% la bărbaţi, 20,1% la femei), iar rezultate asemănătoare observăm pentru Polonia (15,7% la bărbaţi, 19,9% la femei), Bosnia şi Herţegovina (16,5% la bărbaţi, 25,2% la femei) (7). Comparând datele noastre cu alte date provenite din mediul rural, observăm o prevalenţă mai mică a obezităţii decât valoarea de 24% raportată de ţara noastră în 2003 (12).

Obezitatea (IMC ≥ 30 kg/m^2) se asociază cu o creştere a riscului cardiovascular de ~ 45% la bărbaţi şi de 30% la femei (13). Rezultatele noastre arată că la o creştere în vârstă cu 10 ani, media creşterilor IMC-ului este de 0,6 ($p < 0,005$). Studiile publicate arată că pentru fiecare creştere a IMC-ului cu 2 kg/m^2, are loc o creştere a riscului coronarian cu 23% şi a riscului de accident vascular cerebral cu 13% (14). Importantă este dispoziţia excesului de ţesut adipos, fiind demonstrată implicarea adipozităţii centrale în creşterea riscului cardiometabolic (15) dar la nivel mondial există puţine date cu privire la prevalenţa obezităţii abdominale. Grecia a raportat o prevalenţă de 54,3% în rândul bărbaţilor şi de 56,5% în rândul femeilor iar Anglia o prevalenţă de 31,1% la bărbaţi faţă de 41,1% la femei, cu specificaţia că în acest caz s-au folosit alte limite ale CA (≥ 102 cm la bărbaţi şi ≥ 88 cm la femei), şi deci datele nu pot fi comparate (7). Datele unui studiu efectuat pe o populaţie din centrul României arată o prevalenţă a obezităţii abdominale de 62,7% (52,4% în rândul femeilor şi 70% în rândul bărbaţilor) (16). În studiul nostru s-a observat un trend ascendent al valorii CA odată cu înaintarea în vârstă şi, mai mult decât atât, o relaţie de directă proporţionalitate între valorile IMC şi CA, sugerând faptul că excesul de ţesut adipos acumulat va fi depus la nivel visceral, crescând astfel riscul cardiometabolic al acestor persoane.

CONCLUZII

Prevalenţa supraponderii şi obezităţii în mediul rural din nord-estul României este

de 34,5% şi respectiv 17,5%, predominând obezitatea de gradul 1 (13,5%). Se observă o predominanţă a sexului feminin printre cei cu exces ponderal, precum şi creşterea prevalenţei obezităţii cu înaintarea în vârstă. Există o relaţie de directă proporţionalitate între indicele de masă corporală şi circumferinţa abdominală, sugerând predispoziţia depozitării excesului adipos la nivel visceral. Aceste rezultate reprezintă argumente în favoarea necesităţii dezvoltării şi implementării de programe naţionale cu scopul de a efectua screening-ul obezităţii şi tratamentul corespunzător al acesteia, de a informa populaţia cu privire la consecinţele nefaste ale excesului ponderal, alegerile alimentare sănătoase şi beneficiile activităţii fizice ca metode eficiente de prevenţie şi tratament.

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THE ROLE OF OXIDATIVE STRESS ON PERINATAL PATHOLOGY

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THE ROLE OF OXIDATIVE STRESS ON PERINATAL PATHOLOGY (Abstract): Normally, oxygen is a vital gas that allows the body's cells to function by producing mitochondrial ATP. Despite its essential physiological role, molecular oxygen is a toxic potential in the body. In extreme cases, breathing with high oxygen concentrations leads to hyperoxia, which causes CNS, lung, renal, hepatic and ocular toxicity due to membrane and other cellular disruption. Effects on cells are supposed to be associated with oxidative stress and are mediated by ROS. In placental mammals, the placenta is a transient organ, with a very important function - to manage the nutrient, oxygen and waste exchanges between the mother and the fetus. An additional level of complexity is that in humans, a hypoxic state must be maintained in the first trimester, while later, the oxygen pressure rises to that of normal tissue conditions. This implies that a very strict control of oxygen pressures must be maintained in the placenta, and this process, involving thousands of genes and numerous steps, allows for multiple possibilities of error. These dysfunctions become either the causes or consequences of placental diseases such as IUGR, spontaneous premature birth, gestational diabetes and, most importantly, preeclampsia. In the cascade of events leading to eclampsia, HELLP syndrome, premature birth or other consequences on mother and child, placental oxidative stress is placed in a very high position, even under placental ischemia due to deficient spiral artery remodeling. A deeper understanding of the mechanisms governing oxidative stress in both placenta and maternal vascular endothelium is likely to provide new opportunities for the development of innovative therapeutic approaches to prevent IUGR, improving the future quality of life for newborns. **Keywords:** OXIDATIVE STRESS, PERINATAL PATHOLOGY, INTRAUTERINE GROWTH RESTRICTION.

Oxygen has both benefits and adverse effects that are potentially harmful for biological systems. The main role of this element is to participate in the generation of adenosine-5-triphosphate (ATP), thus sustaining the energy and life of complex, multicellular organisms (1).

Faced with a constant oxidative attack from reactive oxygen species (ROS) the human body has developed a complex antioxidant defense system that generally maintains oxidation in balance. However, occasionally, this balance can be disturbed, resulting in oxidative stress. Due to the multiple and various effects that oxygen toxicity can has on cells, oxidative stress is best defined in general terms as a change in the pro-oxidant-antioxidant balance in favor of the former. Oxidative stress is now recognized to play a central role in the pathophysiology of many disorders, including complications of pregnancy (2).

The concept of pro-oxidant-antioxidant balance is essential for an understanding of oxidative stress for several reasons. Firstly, it underlines the fact that disturbance can be caused by changes on both sides of the balance (e.g. abnormally high ROS generation or antioxidant defense defects). Secondly, it highlights the homeostatic concentrations of ROS. Although ROS have first come into the attention of biologists as potentially harmful byproducts of aerobic metabolism, it is now recognized that they play an important role as secondary messengers in many intracellular signaling pathways (3). Finally, the concept of balance draws attention to the fact that there will be a gradual response to oxidative stress. Therefore, minor balancing disorders can lead to homeostatic adaptations in response to changes in the environment, while major perturbations can lead to irremediable lesions and cell death (2).

The developing embryo and fetus are very susceptible to the adverse effects of the oxidative stress, both initiated by the endogenous processes and given by xenobiotics that increase ROS formation. Susceptibility is given on the one hand by the relatively high levels of embryonic and fetal enzymes involved in ROS formation and xenobiotic bioactivation to free radical intermediates and, on the other hand, by low levels of protective antioxidant enzymes. Effects on the embryo/fetus are due in part to the high rates of cell division and differentiation and the complex processes involved in the formation of organs. Changes in these processes may result in in utero or perinatal death or structural and functional birth defects, also known as teratogenesis. The instability of ROS, especially of hydroxyl radicals means the formation, first of all, of ROS in the product of conception side, rather than on the mother's side; this plays a critical role in teratogenesis.

Oxidative stress

1. Overview

Oxidative stress (OS) is defined as "an imbalance between oxidants and antioxidants, leading to an interruption of signaling and redox control and/or molecular deterioration"(4). OS involves ROS, the most frequent ones being superoxide ($O2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (•HO).

A parallel process is known as nitrosative stress (NS), which is defined as an imbalance in the ratio of nitrosant agents with antioxidants. NS mainly involves the nitrogen oxide (•NO) and peroxynitrite ($ONOO^-$) species of reactive nitrogen (RNS).

In aerobic organisms, controlled yield of ROS and RNS is a physiological phenomenon that plays a fundamental role in the metabolism and cascade of cellular signaling. OS and NS occur when there is an imbalance between the formation of oxidizing substances and the antioxidant molecules that realize their detoxification. Because of their highly reactive properties, ROS and RNS can cause structural and physiological lesions of DNA, RNA, proteins and lipids, including cell membrane-bound lipids (5).

ROS and RNS are produced in different cellular compartments or metabolic pathways (6):

- Mitochondria, endoplasmic reticulum (ER) and nuclear membrane produce superoxide anions due to autooxidation of the components of the electron transport chain.
- ROS are also produced as a consequence of acidic metabolism and cytochrome P_{450}.
- Nicotine-amide dinucleotide phosphate oxidases are still an important source of ROS. They generate superoxide anion by transferring electrons inside the cell through the membrane and by reducing molecular oxygen.
- NO synthase can generate superoxide

and hydrogen peroxide, especially when concentrations of its substrate, l-arginine or its cofactor, tetrahydrobiopterin are low.

2. Reactive oxygen species

As mentioned above, there are several reactive oxygen species (ROS). This term applies to both free radicals and their non-radical intermediates. Free radicals are defined as species containing one or more non-participant electrons, and this incomplete electronic coating gives them their high reactivity. Free radicals can be generated from several elements, but the most important biological systems are those that involve oxygen and nitrogen.

In physiological conditions, the most frequently generated free radical is the superoxide anion, and the mitochondria are considered the main source for it (7).

In addition, superoxide anion can also be generated by leaking electrons from the shorter electron transport chain within the endoplasmic reticulum (8). Formation of disulfide bonds during protein folding is an oxidizing process and approximately 25% of superoxide is formed in this way in the endoplasmic reticulum. This process increases in cells with high secretion and also under stress of the endoplasmic reticulum when repeated folding attempts of wrong folded proteins can occur.

Other sources of superoxide under physiological conditions include nicotinamide-adenine-dinucleotide phosphate (NADPH) oxidase enzymes (which generate substantial amounts during pregnancy but especially at the beginning of pregnancy) (9), cytochrome P_{450} and other oxido-reductases. Therefore, various growth factors, drugs and toxins may increase the generation of ROS (2).

Superoxide is detoxified by superoxide-dismutase enzyme, that converts it into hydrogen peroxide. Hydrogen peroxide is not a free radical, so it is less reactive than superoxide. However, it falls under the term ROS as it is inherently involved in the generation and detoxification of free radicals. Because it is nonpolarized, it is capable of diffusing through cellular and organelle membranes and therefore acts widely as a secondary messenger in signal transduction pathways. Hydrogen peroxide is, in turn, detoxified to water by the enzymes catalase and glutathione-peroxidase.

It is important that antioxidant enzymes act in a conjoined manner, because an imbalance in superoxide and hydrogen peroxide concentrations can lead to the formation of the more dangerous hydroxyl ion. This reaction is catalyzed by free ferrous ions during the Fenton reaction. The hydroxyl ion has an estimated life span of 10^{-9} seconds and reacts with any biological molecule in its immediate vicinity in a diffusion-limited manner. Because it is so strongly reactive, there is no known hydroxyl ion capture agent.

Increased superoxide generation can also lead to interactions with nitric oxide to form peroxynitrite. Peroxynitrite is a potent pro-oxidant and, because it is capable of diffusing up to 5 μm, can affect neighboring cells (6).

3. Antioxidant factors

The oxidant effect is inhibited by enzymatic and non-enzymatic protection factors.

a. The enzymatic pathway

Enzymatic protection factors are based on a transition metal capable of taking on different valences in electron transfer during the detoxification process. Two isoforms of superoxide dismutase convert superoxide to hydrogen peroxide. This is then decomposed into water through the action of catalase or glutathione peroxidase, a tetrameric selenoprotein. Glutathione is the major redox buffer in cells and is synthesized in cytosol from 1-glutamate, 1-cysteine and glycine (10).

b. The non-enzymatic pathway

Non-enzymatic oxidoreduction systems

include ascorbic acid (vitamin C) and α-tocopherol (vitamin E). They act in conjunction, the ascorbate being necessary for the regeneration of reduced α-tocopherol. In addition, thiol compounds, such as thioredoxin, are capable of detoxifying hydrogen peroxide, but in turn require conversion back into reduced form by thioredoxin reductase.

Ceruloplasmin and transferrin also play important roles by sequestering free iron ions and thus inhibiting Fenton's reaction and hydroxyl radical production (2).

Polymorphisms of antioxidant enzymes (10, 11) or dietary restrictions of micronutrients such as selenium can thus play an important role in the predisposition to oxidative stress and complications of pregnancy (12).

Biologic effects of reactive oxygen species

1. Activation of transcription factors sensitive to the redox reaction

Activation of redox-sensitive transcription factors, such as AP-1, p53 and NF-κB, regulates the expression of proinflammatory cytokines and other cytokines, cell differentiation and apoptosis. Under normal conditions, NF-κB is kept inactive by binding to the IκB inhibitory subunit. However, under stress conditions, IκB becomes phosphorylated and dissociates from NF-κB, which then translocates to the nucleus and activates expression of proinflammatory cytokines and other cytokines (2).

2. Activation of protein-kinases

By activation of protein kinases, cells respond to a variety of extracellular signals and stress through a family of mitogen-activated protein kinases (MAPK). From this family, ROS-induced activation of extracellular regulated kinases generally promotes cell survival and proliferation, while p38-MAPK stimulation and SAPK-JNK kinases induce apoptosis. p38 and SAPK-JNK are activated by phosphorylation by an upstream kinase, apoptosis 1 kinase signal (ASK1). Under normal conditions, ASK1 is kept inactive by binding to thioredoxin, but superoxide is able to oxidize thiol groups in the latter, leading to a conformational change and its release (2).

3. Opening of ionic channels

ROS imbalances lead to the loss of intracellular calcium homeostasis, with the release of calcium ions from the endoplasmic reticulum and other forms of storage. Calcium concentration in the lumen of the endoplasmic reticulum is much higher than in the cytosol. ROS are able to activate the calcium-releasing channels in the endoplasmic reticulum membrane, including the inositol-1,4,5 triphosphate receptor (IP3R) and the ryanodine receptor (13). The result is calcium release from the endoplasmic reticulum, which will activate various calcium-sensitive processes inside the cell including many of the above-mentioned signaling pathways (14). The increase in concentration of calcium ions in the cytosol will adversely affect mitochondrial function, with high ROS production within the cell. As a result, the potential of the mitochondrial membrane and ATP synthesis collapse. If mitochondria are affected throughout the whole cell, ATP concentrations decrease, ionic homeostasis is lost, and cell necrosis occurs (2).

4. Lipid peroxidation

Hydroxyl radicals are able to cause lipid peroxidation in the cell membrane or any organelle containing large amounts of polyunsaturated fatty acid side chains. By extracting hydrogen from the hydrocarbon side chain of a fatty acid, these radicals create a carbon-based radical. If oxygen is present, it can react to form a peroxyl radical, which in turn is able to absorb hydrogen from an adjacent fatty acid, thus propagating the

reaction.

Because vitamin E is liposoluble and possesses a hydrophobic tail, it tends to accumulate inside the lipid membrane. Here, it is the most important breaker of the above-mentioned chain reaction, because it reacts with the lipid peroxidic radicals about four times faster than they can react with the adjacent fatty acid side chains (2).

5. Protein modifications

Amino acids, both free and in proteins, are a target for oxidative lesions. Direct oxidation of the side chains leads to the formation of carbonyl groups (aldehydes and ketones), and proline, arginine, lysine and threonine are particularly vulnerable to attack (15).

At physiological levels, it is believed that protein nitration is a selective and reversible process that leads to activation in a manner similar to phosphorylation, but at higher levels it can be detrimental. Nitration in the placenta may have different effects, both with gain and loss of function (16).

6. DNA oxidation

DNA is primarily attacked by hydroxyl radicals and a variety of products can be generated by reactions with either DNA bases, or with deoxyribose.

Attacks on sugars can cause chain breakage, while those on histone proteins can lead to cross-links that interfere with chromatin folding, DNA repair, and transcription. Consequently, the aberrant mutation or expression of genes may occur.

Mitochondrial DNA is extremely vulnerable to the attack of ROS due to its proximity to the site of generation of superoxide in the electron transport chain, lack of the protection of histones and minimum repair mechanisms that exist at this level. Consequently, mitochondrial DNA damage is important even under normal conditions, and mutations occur at a frequency of five to ten times greater than that observed in the nucleus (17). Since mitochondrial DNA encodes several proteins, including electron transport enzymes, mutations can lead to decreased energy production and the risk of additional electron loss, exacerbating initial stress.

Effects of oxidative stress on the maternal-fetal interface

Oxidative stress occurs at the maternal-fetal interface at the beginning of pregnancy. It plays a role both in the normal development of the placenta and in the pathophysiology of complications such as spontaneous abortion, preeclampsia, intrauterine growth restriction (IUGR) and premature rupture of membranes (18).

1. Types of placental cells and the origin of oxidative stress at this level

The human placenta is composed of numerous cell types, as shown recently by the sequencing performed by Tsang *et al.*, which showed twelve major cell groups identified through their expressed profile (19). Among these, the most abundant are stromal cells, endothelial cells, the villous cytotrophoblast, and extravillous trophoblast. In addition, syncytiotrophoblast resulting from fusion of syncytiotrophoblastic cells was clearly separated from other trophoblastic cell lines.

Oxidative stress can be induced by a low oxygen partial pressure, hyperoxia, or alternation of hypoxia and reoxygenation, as seen in various very vascularized tissues such as brain or eye (20). In addition, there have been identified immune cell groups (Macrophages, dendritic cells, T cells), which may also be responsible for generating oxidative stress.

In normal placenta, hypoxia is a physiological element, detectable in the first trimester, as demonstrated through in vivo methods (21). This naturally occurring phenomenon renders unclear the distinction

between common physiological phenomenon and the possible harmful effects of hypoxia at certain stages of placental development.

In addition to trophoblastic cells, oxidative stress can come from endothelial cells present in placental tissue, stromal cells of cells, or immune cells (Hofbauer cells). Generally, trophoblastic cells undergo changes in gene expression due to oxidative stress. This event, occurring in the placenta, generates extracellular vesicles that are released into the maternal circulation and will influence gene expression in both maternal immune and endothelial cells. These extracellular vesicles are exosomes, measuring 30-100 nm and micro-vesicles of 100-1,000 nm. Also, larger cellular debris is released from the placenta, which includes nuclear aggregates and apoptotic bodies (20-500 μm and 1-4 μm, respectively) (5).

2. Oxidative stress and placental remodeling

The human placenta is unique because the villi are initially formed on the entire surface of the chorionic sac. However, from with the end of the first trimester, villi on the superficial side regress to leave the definitive, discoid placenta. Oxidative stress is currently recognized to play a central role in this process, thus this regression, occurring in all pregnancies, can be considered physiological.

The general perception of the intrauterine environment in early pregnancy has undergone significant changes over the past two decades. It is now accepted that placental development occurs in a relatively low concentration of oxygen, maintained by secretions in the endometrial glands, rather than by the maternal circulation (22). It has been speculated that this environment protects the embryo during the development from the teratogenesis mediated by free oxygen radicals (23). The maternal arterial blood flow is prevented from entering the placental intervillous space by extracellular cytotrophoblast cell plugs that invade the uterine spiral arteries, as part of the physiological transformation process (24). Intraplacental maternal circulation is fully established by the end of the first trimester, when the abovementioned plugs are displaced by a mechanism that is currently unknown. The ultrasound evidence has shown that the circulation begins preferentially at the periphery of the placenta, where the trophoblastic invasion is the least extensive and progressively extends to the central region (25).

The onset of placental circulation is associated with a threefold increase in placental oxygen concentration (26). This will mean increasing ROS generation rates, especially in the syncytiotrophoblastic critical layer, which contains antioxidant enzyme defense factors, copper or zinc superoxide dismutase and catalase (27, 28). As a result, specimens collected from the peripheral region of the placenta contain high levels of chaperon HSP70 protein, nitrotirozine residues indicating peroxynitrite formation and morphological evidence of degenerative changes in syncytiotrophoblast compared to those in the central region (25). Molecular evidence confirms the activation of the apoptotic cascade in peripheral villi and, over an extended period, this would be sufficient to lead to their regression.

3. Oxidative stress in intrauterine growth restriction

Intrauterine growth restriction (IUGR) may have various causes, such as maternal malnutrition, excessive alcohol consumption, smoking, and also glucocorticoid treatment. This is often observed with comorbidities such as preeclampsia and gestational diabetes. In humans, IUGR is associated with increased oxidative stress in the placenta (29, 30).

Although IUGR can have many causes, most cases that have no genetic or infectious cause are thought to occur by compromising maternal placental circulation. This conclusion is based on the common association with high-strength uterine artery waves, but also on previous morphological studies of the spiral arteries. Studies have reported a poor physiological conversion of the arteries as in preeclampsia, but to a lesser extent, especially in the intramiometric segment (31). Of course, some studies note a positive correlation between birth weight and conversion rate (32).

Unlike preeclampsia, surprisingly little attention was paid to the role of placental oxidative stress in standalone IUGR cases. The low available evidence indicates that the level of oxidative stress is either similar to normal or intermediate between a normal control and an early-onset pre-eclamptic placenta (33). These findings are consistent with the vascular insults presented above.

However, morphological and molecular evidence of endoplasmic reticulum stress is observed in the placenta of these patients (34). The level of stress is lower than in preeclampsia without any evidence of apoptotic cascade activation, but it is sufficient to induce phosphorylation of IF2α and suppression of protein synthesis with a reduction in cyclin D1 level.

Consequently, it is speculated that the low chronic stress of the endoplasmic reticulum may occur from the onset of maternal circulation and lead to a lower growth of the placenta. This is consistent with the low development of the placenta observed with serial ultrasound scans in these cases (35).

In rodent models, the different etiologies of IUGR led to oxidative stress in the placenta, confirming that oxidative stress is highly relevant in the pathophysiology of this disease. In mice, when IUGR is induced by ischemia-reperfusion cycles of the placenta (temporary occlusion of uterine arteries), the oxidative stress-induced DNA lesion is present (36). In a dexamethasone induced IUGR model, no difference was detected in the classical oxidative stress pathways, but a strong increase in antioxidant enzyme activity was observed, meaning that the placenta adapted to its pro-oxidant conditions (34).

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NEWS

COLON MICROBIOTA AND CARCINOGENESIS

A study published by Bundgaard-Nielsen C, *et al.* investigated the association between colon microbiota and the outcome and carcinogenesis of colorectal cancer. qPCR technique was used to detect the presence and load of bacteria directly in colon tissue samples from patients diagnosed with colorectal cancer, adenomas, or diverticular disease. qPCR was set to detect *Streptococcus gallolyticus, Fusobacterium nucleatum,* and *Bacteroides fragilis*. The bacterial microbiota was then compared to prognosis. The results did not support the role of these bacteria during colorectal beginning, since they showed the absence of *S. gallolyticus* from all tested tissue samples, while *F. nucleatum and B. fragilis* were equally distributed in tumors and normal tissue. However, *F. nucleatum* and *B. fragilis* were less present in adenomas. The study also revealed that *F. nucleatum and B. fragilis* status did not influence the five-year prognosis of the patients. When 16S rRNA gene sequencing was performed, it found an association between *Prevotella* and the presence of tumors, as well as an association between *Acinetobacter* and adenomas or diverticula (Bundgaard-Nielsen C, Baandrup UT, Nielsen LP, Sørensen S. The presence of bacteria varies between colorectal adenocarcinomas, precursor lesions and non-malignant tissue. *BMC Cancer* 2019; 19(1): 399. doi: 10.1186/s12885-019-5571-y.).

EPIDERMOID CYST OF THE SPLEEN

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EPIDERMOID CYST OF THE SPLEEN (Abstract): Epidermoid cyst of the spleen are rare benign formations within the splenic tissue. They are mostly discovered incidentally because they have minimal symptomatology to none. This kind of cyst is difficult to diagnose despite the advanced imaging investigations and only the histological examination can determine a epidermoid cyst. We present the case of a 21-year-old man who presented with vague left lumbar discomfort after intense physical effort and was found to have a large splenic mass with cystic components. Patient underwent a classical splenectomy and had an uneventful recovery with histopathology revealing an epidermoid splenic cyst. **Conclusions:** Epidermoid splenic cysts are difficult to diagnose and differentiate with imaging alone. They have a variable presentation and can present like as a cystic mass. It is important to include them in the differential diagnosis of splenic masses since histopathology is the final determinant of the diagnosis. **Keywords:** EPIDERMOID, CYST, SPLEEN.

Epidermoid cyst of the spleen is a very rare form of splenic mass. They are classified into true cyst (primary-composed of fibrous tissues, 25%) and pseudocyst (secondary, 75%), according to the presence of an epithelial cellular lining inside the cyst. Splenic epidermoid cyst, a true cyst, constitutes approximately 10% of benign, nonparasitic cysts and is more commonly encountered in individuals under 40 years old and in females (1). The most common symptoms are nonspecific such as left upper abdominal mass, followed by left upper abdominal pain and epigastric pain. Few patients may present with complication, such as infection, rupture, and bleeding (2). A surgical treatment is recommended for symptomatic cysts larger than 5 cm (3).

CASE PRESENTATION

A 21-year-old male presented in our department with complaints of dull pain and discomfort in the left lumbar area, which had been present in the last month. Pain was intermittent in nature, which had increased in intensity since past 15 days. There was no history of fever, trauma and there was no association with food intake. On physical examination, a firm, smooth, slightly tender mass was seen in left hypochondrium, which was palpable.

Ultrasonography of abdomen was done,

and it showed enlarged spleen 150/93 mm, with well-defined inhomogeneous cystic mass, which measured 84/74/82 mm in size, 65 cm^2 with internal calcifications (fig.1).

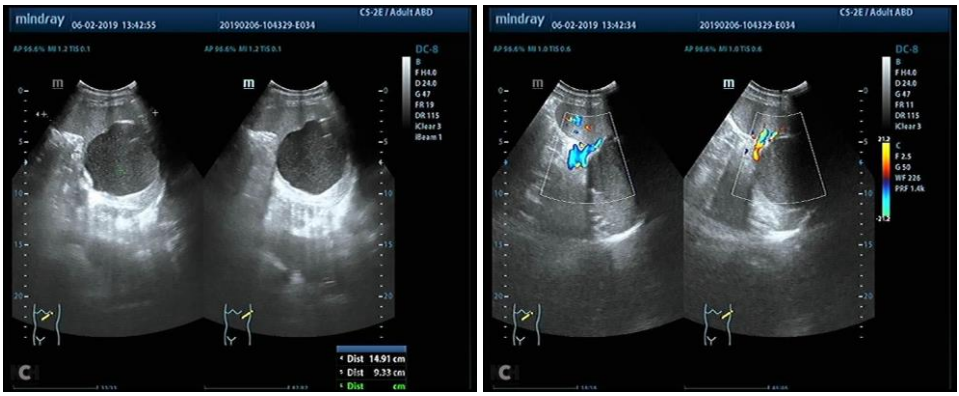

Fig. 1. Ultrasonography of abdomen

Computed Tomography of abdomen was performed after administration of intravenous contrast. The scan showed enlarged spleen, with the bipolar diameter about 130 mm, and a large well-defined cystic mass in the medial segment, intra-parenchymal, which was 91/73/63 mm in size, (fig. 2), with thin septum in the downside and calcifications.

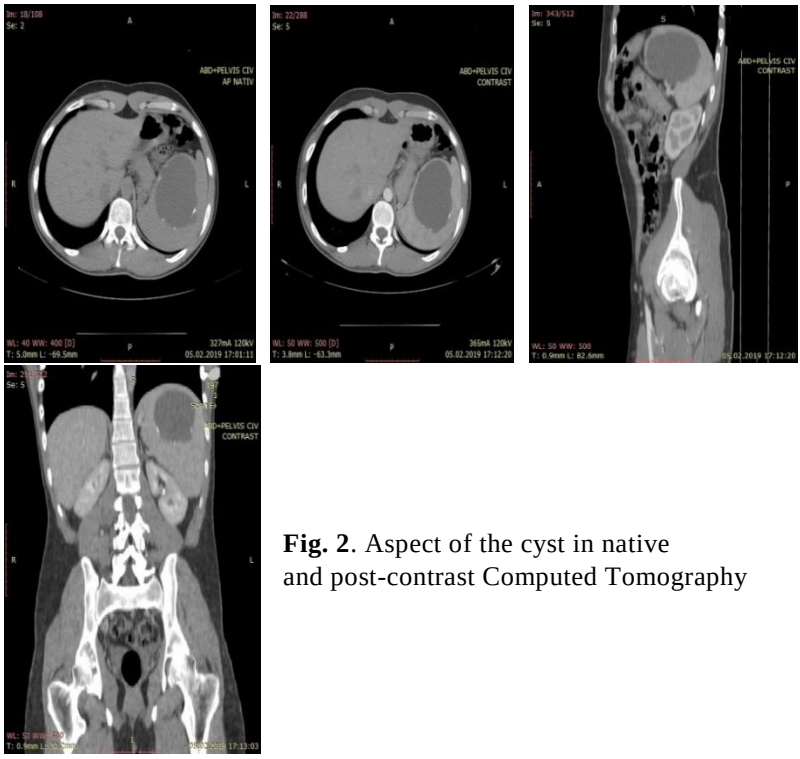

Fig. 2. Aspect of the cyst in native and post-contrast Computed Tomography

Elective laparotomy was performed, and a large splenic cyst was found, which was located at the middle part of the spleen. Due to location and size of cyst, preservation of the spleen was considered as impossible and splenectomy was done. Grossly, large sized cystic lesion which was approximately 100 of maximal diameter was discovered, with almost total replacement of splenic tissue at one side (fig. 3).

Fig. 3. The spleen of 12.5/11.0/6.5 cm with the cyst 7.5 / 4.7 cm

After the section of the spleen, the macroscopic examination revealed two cystic formation was found of 8.4/7.0/4.8 cm and 2.1/1.6/1.4 cm with irregular internal conformation, and calcifications .

Histopathological, microscopic examination revealed that the wall was formed on the internal side from squamous epithelium without keratin function , with large areas of ulcerations and macrophage adherence. Underneath the epithelium was sclerahyalinosis and calcifications (fig. 4). The diagnosis of splenic epidermoid cysts was confirmed. The post-operative clinical course of the patient was satisfactory and uneventful.

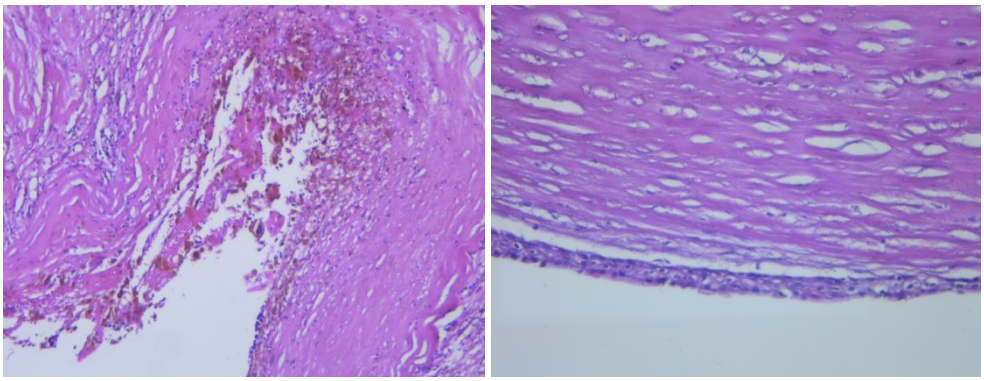

Fig. 4. Histopathological aspect of the epithelium.

DISCUSSION

The first case of epidermoid cyst was reported by Andral, in 1829, that was found at autopsy (4). In 1867 J. Pean performed

the first successful splenectomy for a splenic cyst (5). After that 12 isolated case were reported in the literature. In 1940 Fowler published a collective review of 137 cases of splenic cysts and made an update in 1953 to include 265 cases of nonparasitic splenic cysts (6, 7).

Since the first case was reported in 1829 by Andral many pathophysiological hypotheses have been proposed, the main one being enfolding or entrapment of peritoneal mesothelial cells in the splenic parenchyma during embryogenesis in the intrauterine life (mesothelial invagination theory, endodermal inclusion theory) (8). Splenic cysts are classified worldwide according to Martin's criteria: type 1 are true cysts with a lining epithelium, while type 2 are false cysts without a defined lining epithelium. The first group includes both nonparasitic and parasitic cysts, the latter ones being most frequently caused by *Echinococcus granulosus*. Type 1 nonparasitic cysts have been further divided into congenital (epidermoid, dermoid) and neoplasic (hemangiomas, lymphangiomas) cysts, among which hemangiomas and dermoid cysts are the most and least common, respectively. The second group includes the so-called pseudo-cysts which are usually related to trauma, hemorrhage, abscess, and infarction, and occur much more frequently than epithelium-lined true cyst (9).

Epidermoid cysts are the rare, only 10% of benign, nonparasitic cysts of the spleen(10). True cysts and false cysts cannot be differentiated on imaging explorations; false cysts have thicker fibrous walls and calcifications, along with internal debris. On ultrasonography epidermoid cysts are described as well-defined, with a thin wall, anechoic cystic lesions. There can be increased echogenicity caused by intracystic hemorrhage and cholesterol crystals and inflammatory necrotic debris which are same as those which are seen in false cysts. Although peripheral calcifications occur more frequently in post-traumatic or false cysts, in epidermoid cysts with occasional internal septations, cystic walls may show curvilinear or plaque like calcifications. In addition, computed tomography may give clearer picture regarding intracystic fluid, internal septations or calcifications (11).

Although partial splenectomy offers a definite solution in case of splenic cysts (< 5 cm), conservative treatment like aspiration alone, incision and drainage can also be considered. But total splenectomy is the treatment of choice in case of larger cysts (> 5cm), as it prevents serious complications like rupture, hemorrhage, infection and sometimes, the recurrence which is attributed to coexistence of invisible tiny cysts that had remained in the splenic tissue after the dissection of the major cyst. In the cases where hilum is involved, splenectomy remains a relatively safe procedure (12).

Since it was showed before that the response to this disorder can vary depending on multiple factors (13), described above, we can also connect the present aspects with the previous works of our group (14-17) in the multifactorial approach (e.g. related to inflammation and oxidative stress, social influence, pain perception or drug development substrate) for the management and treatment of this specific epidermoid cyst of the spleen.

Radiologically distinguishing between true and false splenic cysts does not seem to be possible preoperatively and the final diagnosis relies on histopathological examinations of wall of the cyst. However, total splenectomy (open or laparoscopic) is primarily recommended for the prevention of

complications which are seen as infections, hemorrhage and rupture.

CONCLUSIONS

Patients with splenic cysts are usually asymptomatic, who present occasionally with vague symptomatic presentations. Discovery of cystic lesions of spleen is an easy task which can be achieved by doing imaging studies, but determination of true nature and pathology of the cyst is not possible all the times preoperatively. Although, nowadays emphasis is being laid on spleen preserving surgeries, it has been observed that conservative treatment is not possible in the cases where cysts are of large sizes and where the chances of surgical complications are higher than the rare risk of overwhelming post-splenectomy sepsis. The ultimate diagnosis, however, depends upon histopathological findings.

Generally, the tumor size correlates with clinical presentation and small cysts are asymptomatic, but large lesions of the spleen tend to present symptoms related to bulky size, such as left upper abdominal pain, upper abdominal mass, and epigastric pain and fullness.

In our case, the patient presented the most common clinical findings left lumbar pain, and hematological and biochemical investigations were within normal limits. Accurate clinical diagnosis of primary epithelial cysts is difficult. The differential diagnoses for a cyst in the spleen include parasitic echinococcal disease, congenital cyst, posttraumatic pseudocysts, infraction, infection, pyogenic splenic abscess, metastatic disease, and cystic neoplasm. The instrumental investigations are important to complete the diagnosis. It is possible to employ ultrasonography, magnetic resonance, and computerized tomography. Ultrasonography could differentiate solid and cystic lesions, identify calcifications, and recognize some intra-cystic septa or irregular walls. We used CT scan to complete the diagnosis and to have information in regard to the morphology of the cyst, the nature of fluid and the exact location and its relationship with adjacent structures. At the same time, we affirm that radiologically distinguishing between true and false splenic cysts do not seem to be certain preoperatively and the final diagnosis relies on the histopathological examinations.

Because the spleen had a large size, we preferred to perform a classic laparotomy and the size and position in the middle of the spleen dictated the total splenectomy.

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NEWS

AUTOPHAGY IN ALZHEIMER'S DISEASE AND PROMISING MODULATORY EFFECTS OF HERBAL MEDICINE

Alzheimer's disease (AD) is a progressive and unremitting neurodegenerative disorder characterized by memory loss and cognitive impairment. It affects the quality of life of victims severely. The prevalence of AD has been increasing in recent years. Therefore, it is of great importance to elucidate the pathogenesis of AD and find out effective therapeutic approaches. Autophagy, a primary intracellular way of degrading aggregated proteins and damaged organelles, has been discovered to be involved in the pathological changes of AD. In the last few years, much progress has been made in finding autophagy regulators from natural products, providing new insights to develop treatment strategy for AD by targeting autophagy. In the present review, we provided an overview of the recent research progress regarding the function role of autophagy in AD, the regulation mechanisms of autophagy-lysosomal pathway as well as therapeutic potential of herbal medicine on AD by targeting autophagy. Modern pharmacological research uncovers that some active components from herbal medicine exert curative effect against AD through various mechanisms including cholinesterase inhibition activity, neuroprotective effect via anti-oxidation, anti-apoptosis and anti-neuroinflammation, neurogenic activity, as well as modulation of Aβ and p-tau metabolism by targeting autophagy. Recently, more and more natural products are attracting attention to identify autophagy regulators. A large number of studies confirm that the active compounds regulating autophagy process contain saponins, alkaloids, flavonoids, polyphenols and other structures, which provide alternative therapies for neurodegenerative diseases in vitro and in vivo (Qiang Zenga *et al*. Autophagy in Alzheimer's disease and promising modulatory effects of herbal medicine. *Experimental Gerontology* 2019; 119: 100-110).

DRUG RESISTANT EPILEPSY DUE TO BILATERAL PERIVENTRICULAR NODULAR HETEROTOPIA. CASE REPORT

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DRUG RESISTANT EPILEPSY DUE TO BILATERAL PERIVENTRICULAR NODULAR HETEROTOPIA. CASE REPORT (Abstract): Periventricular nodular heterotopia (PNH) is among the most frequent malformations of cortical development due to disturbance in neuronal migration and is associated with intractable epilepsy. Neuronal migration disorders cause severe syndromes, including refractory epilepsy and major psychomotor development disorders. It has been demonstrating that approximately 80% of familial cases of PNH are related to FLNA mutation, gene located on the long arm of the X-chromosome inherited in a dominant fashion. A constellation of syndromes, including heterotopic nodules with intractable epilepsy, microcephaly, and severe develop-mental delay is seen in ARFGEF2 mutations, a gene located in chromosome 20 with an autosomal recessive inheritance pattern. We present a case of 18-years old patient, right-handed, without remarkable findings in personal history of childhood, with normal social and psychiatric development. At the age of 15 she was admitted in the department of Psychiatry for depression and behavioral disorder (irritability and aggressive behavior) and she was treated with selective serotonin reuptake inhibitors (this happens frequently showed a small study which revealed that EEG can be abnormal in patients with neurotic disorders. **Keywords:** PERIVENTRICULAR NODULAR HETEROTOPIA, EPILEPSY, DRUG.

Periventricular nodular heterotopia (PNH) is among the most frequent malformation of cortical development, due to disturbance in neuronal migration and is associated with intractable epilepsy.

Neuronal migration disorders cause severe syndromes, including refractory epilepsy and major psychomotor development disorders. Several of these disorders are caused by mutations in genes encoding cytoskeleton proteins.

PNH correspond to a groups of neuronal cells that didn't migrate, remaining in the subependymal region (periventricular heterotopia) or have stopped from migration, in their way towards the cortex surface (subcortical heterotopia). During first trimester of gestation, post mitotic neurons migrate along the scaffold of radial glia, to form the multi-layered cortex (1).

The prevalence of PNH in the general population is unknown, although some reports suggest that it represents 15-20% of the various forms of cortical malformations. It has been demonstrating that approximately 80% of familial cases of PNH are related to FLNA mutation, gene located on the long arm of the X-chromosome, inherited in a

dominant fashion. Heterozygous mutation in females with bilateral and symmetrical PNH is usually associated with normal intellect, with or without epilepsy. Mutations in males are thought to be lethal, usually with intrauterine death (2).

FLNA is a cytoskeleton molecule, that plays an important role in the initiation and progression of neuronal movement, in the process of migration, possibly by maintaining these cells attached to supporting cells, until they receive the signal for locomotion (2).

A constellation of syndromes, including heterotopic nodules with intractable epilepsy, microcephaly and severe developmental delay, is found in ARFGEF2 mutations, a gene located on chromosome 20, with an autosomal recessive inheritance pattern, that encodes a protein BIG2, localized along the Golgi system and recycling endosomes (3).

Other genes implicated in this disease are C6orf70 (6q27), that encodes ERMARD protein and is responsible for bilateral PNH, FAT4 (4q28.1), which codes FAT atypical cadherin 4 and DCHS1 (11p15.4) with Dachsous cadherin-related 1 protein, responsible for posterior PNH (3).

PNH has also been associated with duplication 5p15, deletion 6q26-q27 or 7q11.33, fragile X syndrome, Williams syndrome, 22q11 microdeletion syndrome and 6q terminal deletion syndrome, demonstrating that PNH is a genetically heterogeneous disorder (2).

There are complex interactions between PNH and allo- or neocortex , which shows that PNH is part of a network, implicated in epileptogenesis and higher cortical functions. Interictal spiking activity was found in ectopic grey matter, in the cortex overlying the nodules and in the mesial temporal structures (4, 5, 6).

Tractography and functional MRI (fMRI) studies have suggested anatomic and functional connections between heterotopic nodules and overlying cortex, as well as connections between nodules and regions of contralateral cortex, other heterotopic nodules and ipsilateral cortex (3).

CASE REPORT

We present a case of 18-years old patient, right-handed, without remarkable findings in personal history of childhood, with normal social and psychiatric development. At the age of 15, she was admitted in the department of Psychiatry for depression and behavioral disorder (irritability and aggressive behavior) and she was treated with selective serotonin reuptake inhibitors. A year later, she was admitted in the department of Neurology for recurrent seizures, manifested with rising epigastric sensation, visual hallucinations, associated with cognitive seizures such as déjà vu and oral automatisms. Despite the association of two antiepileptic drugs (Levetiracetam 2,000 mg/day and Carbamazepine 800 mg/day), the frequency of seizures remained high (1-2 seizures/ week).

The MRI with high resolution revealed hyperintense T2 and hypointense T1 bilateral bands located adjacent to the temporo-occipital horn of the lateral ventricle, with the same signal as the cortex. PET-CT scan showed left mesial temporal lobe hypometabolism (fig. 1, 2).

The patient underwent prolonged video-EEG monitoring, that detected interictal epileptiform discharges, such as sharp waves, spike-wave complexes and polispike-waves in left posterior temporal and basal-temporal region, with phase reversing over T6. There have also been recorded multiple seizures, manifested clinically with epigastric aura, speech arrest, oral automatisms, hyper motor behavior, with a duration of approximately three minutes. During the seizure, she was unresponsive, unable to carry out simple com-

mands and she had no recollection of the seizure. EEG showed a teta rhythm of 5 Hz, with low amplitude, started initially in FB1, then in T3 and T5, followed by ictal rhythm observed in FB1 and T3, with rapidly bilateral propagation (fig. 3).

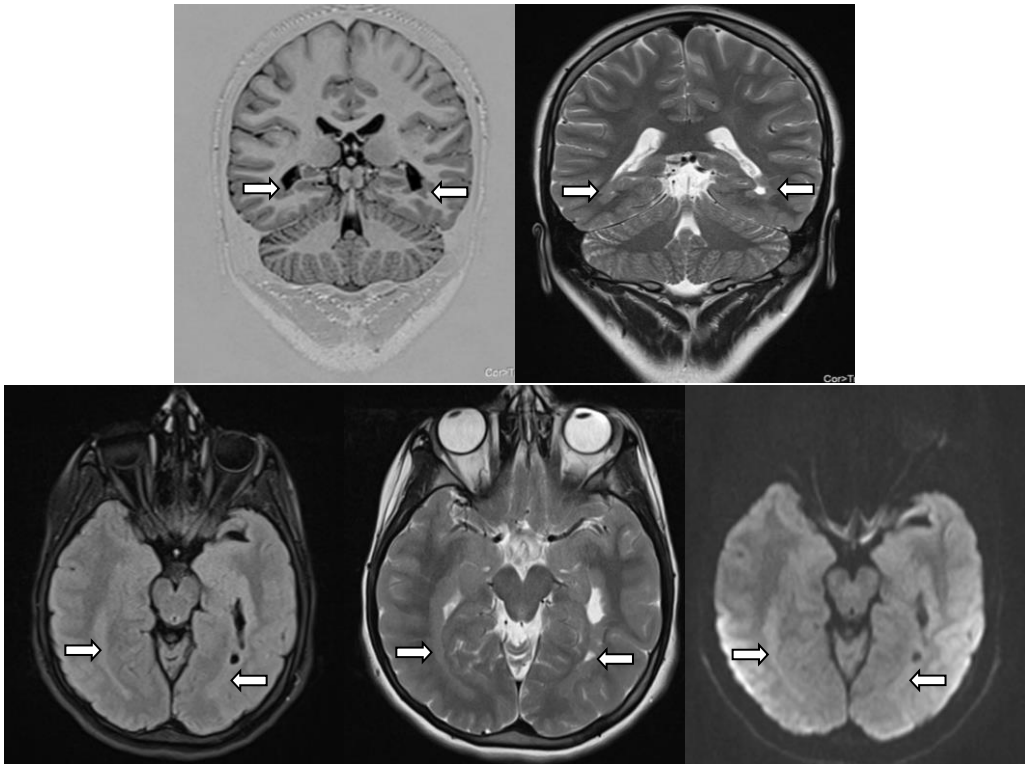

Fig. 1. MRI scan shows bilateral symmetrical bands of ectopic cortex adjacent the temporal-occipital horn of lateral ventricle

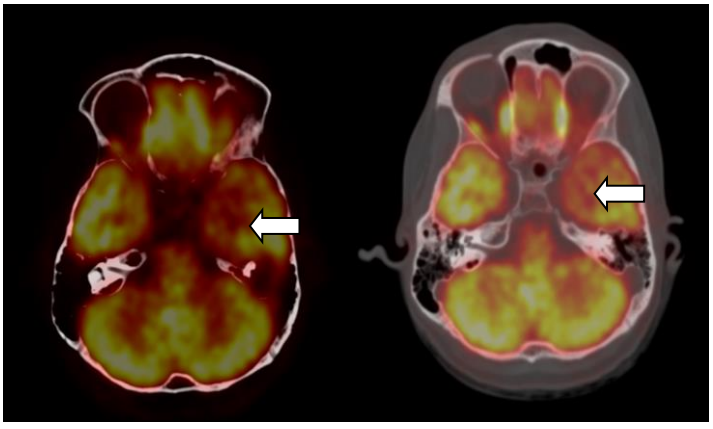

Fig. 2. PET-CT scan shows mesial temporal lobe hypometabolism;

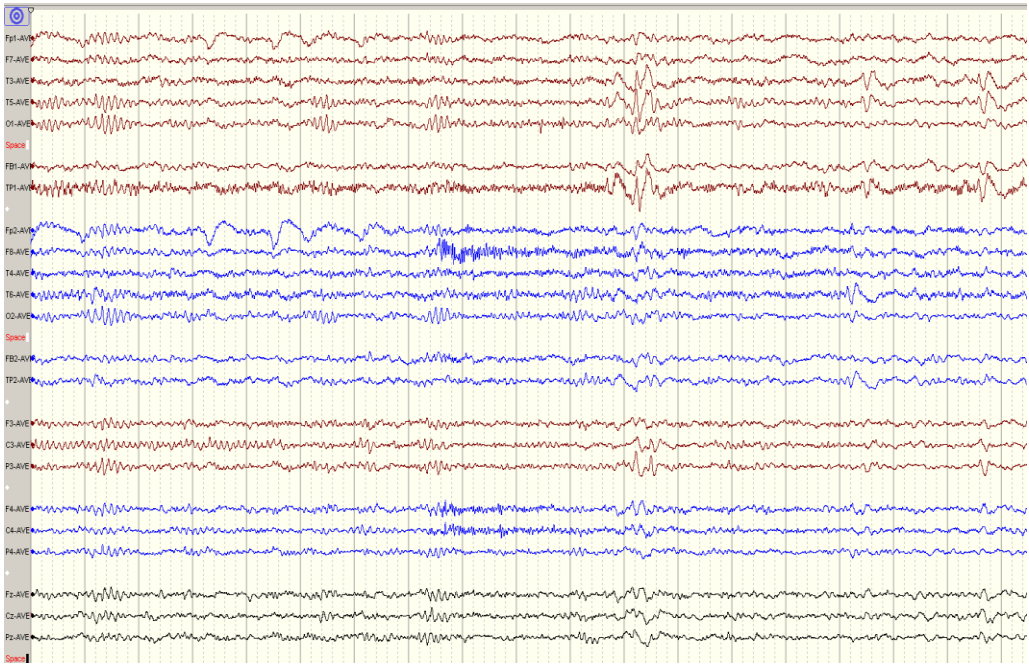

Fig. 3. Interictal EEG: sharp-waves with maximum electronegativity in T3, T5, FB1 and TP1

The patient was referred to an epilepsy center, in order to establish the opportunity for epilepsy surgery. Because of the large and bilateral lesions and the hypometabolism in the left mesial temporal lobe, a conservatory treatment was chosen.

The diagnosis in this case was especially imagistic by highlighting the presence of bilateral band of heterotopia adjacent to the temporo-occipital horn of the lateral ventricle as a marker of neuronal migration disorders.

DISCUSSION

Periventricular nodular heterotopia is a malformation of cortical development, associated with intractable epilepsy. Pharmacoresistant focal epilepsies due to periventricular nodular heterotopia pose a diagnostic and therapeutic challenge, because of the need of invasive presurgical exploration of the brain and the selection of an optimal surgical approach. Invasive electrophysiological exploration in the recent studies, showed that focal epileptic activity can be correlated predominantly, either with one of the nodular heterotopia or with neocortical epileptogenic areas, distant to the periventricular nodules. Thus, using stereo – electro – encephalography (SEEG) methods, it has been showed that both heterotopic and underlying cortex are responsible for ictal onset and interictal discharges (8, 9, 11). The connectivity between the nodular heterotopia and underlying cortex was observed in almost all patients included in studies, using functional imaging studies (PET-CT scans) and diffusion tensor tractography (7).

F-fluorodeoxyglucose (FDG) positron emission tomography (PET) / computed tomography (CT) has been suggested in

patients with PNHs-related epilepsy, to assess the functional activity of ectopic neurons and to identify the epileptogenic foci. Hypometabolic regions in the brain correspond with foci of altered glucose metabolism, due to excessive neuronal and synaptic activity (10).

In our patient, PET-CT scan showed left temporal hypometabolism, demonstrating the connectivity with left heterotopia. The explanation why the activation of cortex was unilateral remains unresolved in our case, due to the lack of neuronal connectivity studies.

From the epilepsy onset, the seizures have not responded to treatment, the frequency being 1-2 per week under treatment with Carbamazepine 800 mg/day and Levetiracetam 2000 mg/day, demonstrating pharmacoresistance. Subsequently, seizures became daily (1-2 seizures/day), which is why the patient was directed to an epilepsy surgery center to determine the opportunity for surgery.

Due to the polymorphism of epileptic seizures, it was concluded that the neural network involved in the initiation and propagation of the epileptic seizures is complex, involving probably both the ectopic cortex and the cortex adjacent to heterotopia, bilaterally. Interesting is the fact that although brain injuries are large and bilateral, the EEG showed preponderant unilateral left interictal and ictal discharge, corresponding with semiological aspects, suggested left temporal lobe epilepsy.

Temporal resections in patients with periventricular nodular heterotopia and intractable focal seizures often yield poor results. In order to define the role of the heterotopic grey matter tissue in epileptogenesis for selective surgery, invasive recordings were necessary up to now. In our case, those investigations were not being performed.

Because of the presence of bilateral and large lesion size, increased operator risk and high rate of recurrence of seizures after surgery, conservative treatment was chosen. The patient is currently undergoing treatment with Levetiracetam 2,000 mg/day, Carbamazepine 1,200 mg/day and Topiramate 200 mg/day (13, 14). It would be of great interest for the patient to benefit from vagus nerve stimulation, but she refused this intervention (12).

Throughout this period, the patient managed to have good school performance, to integrate into society and to have a normal life, but in the last year she accused a decrease in concentration and memory capacity.

CONCLUSIONS

We have presented a rare case of bilateral heterotopia, with pharmacoresistant epilepsy and psychiatric manifestation, in a young girl with normal intellect, with left temporal lobe seizures.

In those cases, the necessity of intracerebral recording and functional imaging is mandatory, to evaluate the connectivity between the heterotopia and adjacent cortex and to predict the benefit of surgery.

Because PNH is bilateral, symmetrical, in a female patient intellectually normal, the genetic test would be of great interest, suggesting the implication of FLNA mutation on X chromosome, but this test was not performed for financial reasons.

The peculiarity of this case is the onset of disease, with psychiatric manifestations and the tendency of recruitment of the left temporal cortex, despite the bilateral heterotopia.

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NEWS

COULD BEHAVIOUR CHANGE AND WEIGHT LOSS BE RESTRICTED ?

It is known that Biochemical remission of type 2 diabetes in the absence of pharmacological or surgical intervention has been shown to be achievable. According to new studies, weight loss of ≥10% in the first few years after diagnosis was strongly associated with remission of Type 2 diabetes at 5 years. The most interesting is that this was achieved without intensive lifestyle interventions or extreme calorie restrictions. These results were obtained from a prospective cohort study on 867 people with newly diagnosed diabetes aged 40–69 years, which had a 5-year follow-up. These findings should inform discussions with people who have newly diagnosed type 2 diabetes as motivation towards remission of the disease without restrictive and sometimes unachievable calorie restrictions (Papakitsou I, Vougiouklakis G, Elisaf MS, Filippatos TD. Differential pharmacology and clinical utility of dapagliflozin in type 2 diabetes. *Clin Pharmacol* 2019; 11: 133-143).

QUALITY OF COLLECTED BIOLOGICAL SPECIMENS – THE INVISIBLE PART OF THE ERROR SOURCES IN HEMOSTASIS FOR THE CLINICIANS

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QUALITY OF COLLECTED BIOLOGICAL SPECIMENS – THE INVISIBLE PART OF THE ERROR SOURCES IN HEMOSTASIS FOR THE CLINICIANS (Abstract)**:** The introduction of modern analyzers in the laboratories that carry out hemostasis tests has led to the devolution of the sources of error from the preanalytical phase, ensuring results with a high level of reliability. The identification of the non-compliant coagulation specimens due to events related to the biological product collection from the clinic are reported by the laboratories that make these determinations. **Aim:** To inform clinicians about these problems, so that through preventive actions we can ensure optimal clinical management of patients. **Material and methods:** Selective research were conducted in the national and international specialized literature. Inclusion criteria were studies presenting the prevalence and impact of preanalytical errors with the determination of hemostasis tests, as well as recommendations regarding the identification and rejection of the non-compliant samples. **Results:** In the study were introduced works that reported the sources of error which may influence the quality of the laboratory results. The errors associated with blood sampling were related to the samples with hemolyzed plasma, insufficient volume taken and the accidental presence of the clot in the erythrocyte sediment. Available literature data suggests that, out of the total of 75% of the errors identified in the laboratory, 26% can have harmful effects on patient's care, which contribute to unnecessary investigations or to the inadequate treatment and implicitly, extension of hospitalization. **Conclusions:** Applying corrective measures can improve the quality indicators of the coagulation specimens and implicitly the quality of the patient's health care by informing clinicians and using educational activities. **Keywords:** HEMOSTASIS, HEMOLYSIS, VOLUME, CLOT, EDUCATION.

Available literature data suggest that errors once appeared with the sampling of the biological product can have very serious consequences on the patients care, since the medical decisions are made in a proportion of 60 to 80% based on the laboratory tests results (1, 2, 3). Because the technique of sampling the biological product is an invasive and complex procedure, it is performed in the clinic and is considered a pre-analytical phase. This aspect represents a major error source in the testing process (4, 5, 6, 7).

In order to minimize possible errors on

the determinations, improvement of the analytical phase in the laboratory was desired by modernizing and automating the methods of coagulation tests determination, being able to provide very precise results (3, 8). In 2005, van Geest-Daalderop *et al.* in their study mentioned that oral administration of the anticoagulants with coumarin derivatives depends on PT/INR (prothrombin time/ international normalized ratio) results, evaluating the effect of preanalytical variables on test determination, including those related to the sampling and transport of samples (9).

In the national literature, in agreement with the international specialized literature, among the causes of rejection of the samples taken for the determination of PT and APTT (activated partial thromboplastin time) tests, are mentioned: insufficient volume taken, less than 90% (0.5 mL blood less) of the recommended volume (4.5 mL blood), intensely hemolyzed plasma and the whole coagulated sample, as described in (fig. 1) (10).

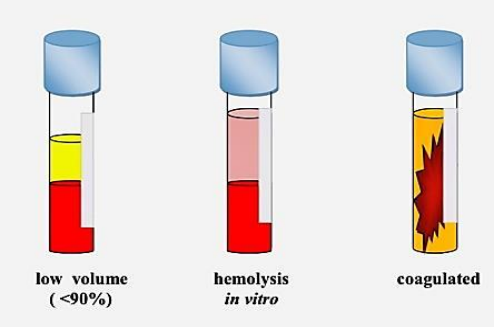

Fig. 1. Representation of the causes of rejection of the coagulation specimens (personal collection)

OBJECTIVES

The purpose of this study is to inform clinicians about the negative interference of error sources from the pre-analytical phase, with the determination of coagulation tests, to better understand the possible discrepancies between the results released by the laboratory during dosing and the monitoring of anticoagulant therapies. Emphasizing the need for educational activities in order to reduce the incidence of preanalytical errors that generate non-compliant blood samples.

MATERIAL AND METHODS

In order to investigate the topics of interest, it was chosen as a means of using existing data to review the literature. It was desired to use primary sources based on evidence, which used different methodologies and theoretical comments, on the effect of preanalytical factors on the samples taken for the determination of hemostasis tests.

To make a comprehensive approach regarding the negative impact of the sources of error from the pre-analytical phase on the determination of coagulation tests PT and APTT we performed a search on PubMed and Google Scholar.

INCLUSION CRITERIA

To select this information, we looked for original studies, reviewed by colleagues that describe the pre pre-analytical and post post-analytical phases in the testing process. We considered the study protocols, conclusions, as well as the reported cases. This study has some limitations represented by the impossibility of approaching all articles dealing with preanalytical factors and the consequences on hemostasis tests. The level of the works number approached is quite small, but despite this fact, based on the selected studies, as well as the presentation of the conduct in our laboratory, we want to draw

attention to the sources of error regarding the quality of the sample taken.

RESULTS

In our research, we were able to introduce studies that analyzed the changes occurred during the test process to determine PT test and APTT in the hemolysate samples, with insufficient volume taken and the accidental presence of the clot in erythrocyte sediment.

The selection of the most recent national and international studies (2014-2019) was abstracted using a table, starting from the design mode, objectives, methods of evaluation, sample size and conclusions; implicitly based on the keywords: pre-analytical, post-analytical, coagulation, errors, hemolysis, volume, clot, procedure, false, rejected, education, patient, clinician, nurse, laboratory medicine, trust; as described in first table.

TABLE I
Relevant studies included

No.	AUTHORS	YEAR	TITLE
1.	Wan Azman WA, Omar J, Koon TS, *et al*. (25)	2019	Hemolyzed Specimens: Major Challenge for Identifying and Rejecting Specimens in Clinical Laboratories.
2.	Delianu C, Foia L, Hurjui LL, *et al*. (31)	2019	Blood Clot Identification Procedures in Clinical Laboratory Sampling.
3.	Delianu C, Tuchilus C, Filip F, Foia L. (30)	2018	Post-analytic clot detection in vitro by transvasation.
4.	Delianu C, Tuchiluș C, Hurjui L, Foia L (45)	2018	In vitro false hypercoagulability-possible source of error from the pre-analytical phase.
5.	Watson ID, Wilkie P, Hannan A, Beastall GH (46)	2018	Role of laboratory medicine in collaborative healthcare.
6.	Wu AHB, Christenson RH, Greene DN, *et al*. (48)	2018	Clinical Laboratory Practice Recommendations for the Use of Cardiac Troponin in Acute Coronary Syndrome: Expert Opinion from the Academy of the American Association for Clinical Chemistry and the Task Force on Clinical Applications of Cardiac Bio-Markers of the International Federation of Clinical Chemistry and Laboratory Medicine.
7.	ZemLin AE. (49)	2018	Errors in the Extra-Analytical Phases of Clinical Chemistry Laboratory Testing.
8.	Delianu C, Foia L, Dobreanu M (43)	2017	The importance of medical personnel training in reduction of pre-analytical phase laboratory.
9.	Al-Ghaithi H, Pathare A, Al-Mamari S, *et al*. (44)	2017	Impact of Educational Activities in Reducing Pre-Analytical Laboratory Errors.
10.	Hernaningsih Y, Akualing JS (19)	2017	The effects of hemolysis on plasma prothrombin time and activated partial thromboplastin time tests using photo-optical method.
11.	Geffen Y, Zaidise I (47)	2017	At the crossroads: the role of laboratory medicine in the patient care process.
12.	Magnette A, Chatelain M, Chatelain B, *et al*. (12)	2016	Pre-analytical issues in the hemostasis laboratory: guidance for the clinical laboratories.

No.	AUTHORS	YEAR	TITLE
13.	Woolley A, Golmard JL, Kitchen S (24)	2016	Effects of hemolysis, icterus and lipemia on coagulation tests as performed on Stago STA-Compact-Max analyzer.
14.	Crous L, Armstrong SJ (42)	2016	The bloody truth: Investigating nurse phlebotomy competencies at a private laboratory in Johannesburg, South Africa.
15.	Freitas F (18)	2015	What's new about sample quality in routine coagulation testing?
16.	David RE, Delianu C, Dobreanu M (35)	2015	Preanalytical errors in Clinic Laboratory Management.
17.	Guder WG (36)	2014	History of the preanalytical phase: a personal view.
18.	Plebani M, Astion ML, Barth JH, *et al*. (51)	2014	Harmonization of quality indicators in laboratory medicine. A preliminary consensus.
19.	S Arora, S Kolte, JS Dhupia (20)	2014	Hemolyzed Samples Should be Processed for Coagulation Studies: The Study of Hemolysis Effects on Coagulation Parameters.
20.	Lippi G, Plebani M, Favaloro EJ (29)	2014	Technological advances in the hemostasis laboratory.

DISCUSSION

Favaloro *et al*. in their study mentioned that between 9 and 15% of errors are the result of a misdiagnosis, with a significant impact on the patients care, and the probability of inadequate care represents between 2-7% of such cases, agreeing with Plebani's study (8, 11). Errors resulting from inadequate sampling, associated with improper handLing and processing of samples, will reflect the status of the sample taken and will not accurately reflect the actual clinical status of the investigated patient, being considered pre-analytical variables (8).

In 2016, Magnette *et al*., point out that the ratio of blood taken and Na citrate of (1:9) for coagulation tests, (12) must be respected, corresponding to 0.5 mL anticoagulant and 4.5 mL blood, quantities recommended in the national literature since 1959 (13). A volume of blood taken less than 89% of the recommended amount (0.5 mL blood missing) interferes with the determination of the APTT test, which is clinically significant, and a volume of 78% (3.5 mL blood taken) affects fibrinogen determination, and less than 67% (lacking 1.5 mL of blood) from the recommended volume affects factor VIII (Antihemophilic A) determination, being in agreement with the subsequent studies (7, 12, 14, 15). In our laboratory, in cases where the sample has a lack of 0.5 mL blood, in order to inform the clinician, the non-compliance is explained textually, once the result is released, with the following comment: "Sample with insufficient volume taken, lack 0.5 mL of blood; possible interference with the determination". Leblanc, Ellouze and Guermazi recommend rejecting the sample, if the vacutainer has a volume of less than 80% (1 mL of blood is missing) (16, 17). In accordance with these recommendations, we reject the determinations made in these samples, explaining to clinician the non-compliance: "Repeat the sampling. Sample with insufficient volume taken, 1 mL of blood is missing".

In medical laboratories, the receipt of samples with plasma hemolysis is a common phenomenon, representing a reason for refusing the determinations (8, 18, 19). Some authors believe that this rejection creates discomfort for the patient, delayed outcomes and additional laboratory costs (19, 20). Most of the time, in the laboratories carrying out hemostasis tests the interpretation of the *in vitro* hemolysis, is performed post-centrifugation, by visual examination (21). From a technical point of view, plasma hemolysis can be classified according to the hemoglobin concentration in the supernatant:

a) non-hemolysis (0.05 g / L, yellow)

b) slightly hemolyzed (from 0.05 to 0.3 g / L, toned yellow to light pink)

c) medium hemolyzed (0.3 to 0.6 g / L, pink to slightly red)

d) hemolysis (0.6 to 2.0 g / L, slightly red)

e) intensely hemolysis (2.0 g / L, red to brown), as exemplified in (fig. 2) (21, 22).

As the assessment is done by the laboratory assistant who prepares the samples for the analysis itself, it would be ideal to use color diagrams for the correct interpretation of the degree of hemolysis (22).

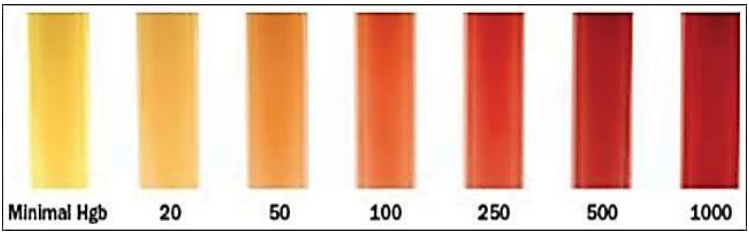

Fig. 2. Color diagram for in vitro hemolysis detection.

The numbers indicate the degree of hemolysis expressed in mg/dL (22).

Favaloro *et al*. draws attention to cell lysis products that include tissue factors which activate coagulation, and, as hemolysis increases, fibrinogen levels may decrease, D-dimers increase, while APTT may increase or decrease. Through the activation effect, PT test values may decrease depending on the fibrinogen decrease (8). Laga *et al*. and Arora *et al*. concludes on the basis of the research carried out on the samples of the healthy volunteers, that the differences of the results were insignificant, and the statistical difference found does not have clinical significance. This hypothesis is safe if the result falls within the normal reference range, with no certainty for the results at the lower or upper limit (20, 23).

Using different reagents for test determination, Woolley *et al*. observed no correlation between variations in results and level of hemolysis (24). Wan Azman *et al.* recommends standardizing the interpretation of hemolyzed plasma samples and drawing up a laboratory guide, thus providing essential information to clinicians (25). However, the results being controversial, further studies on larger groups are needed to determine the effect of hemostasis (19). Rejecting the samples with hemolyzed plasma being considered inadequate, it is recommended to add a brief comment that suggests doubt about the interpretation of the results and their benefits for the patients care (26, 27,

28). In accordance with these recommendations, in the case of hemolyzed plasma samples, we inform the clinician, releasing the result with the following comment: "Hemolyzed plasma sample, possible interference with the determination".

Current analyzers for coagulation tests determination can detect in the pre-analytical phase fibrin, foam, hemolysis plasma, as well as insufficient blood volume is taken (29), without being able to detect the accidental presence of the clot in the erythrocyte sediment, as described in (fig. 3).

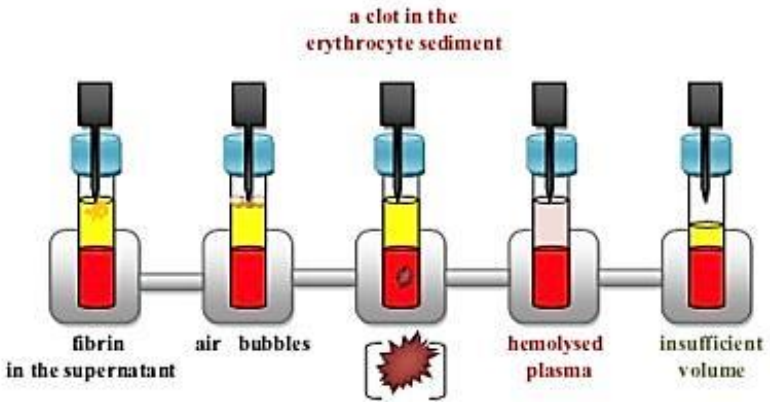

Fig. 3. Detection of fibrin, foam, hemolysis in the supernatant, the insufficient volume is taken and clot in the erythrocyte sediment (personal collection)

Total blood coagulation *in vitro* (fig. 1) is caused by the loss of cellular elements, as well as the factors such as: fibrinogen, prothrombin, proaccelerin, anti-hemophilic A, and differentiated loss of von Willebrand factor, leading to the serum formation (8). Determination of hemostasis tests from partially coagulated samples, as a consequence of the clot presence *in vitro* (fig. 3) may result in false shortening or prolongation of coagulation tests, because the differentiation between plasma and serum is difficult to perform post-centrifugation by visual inspection (8, 28).

In our laboratory, starting with 2014, in order to exclude or confirm the presence of the clot in the erythrocyte sediment during the test, we also re-verify post-analytically by transvasation (fig. 4) (30, 31) the samples with hemolyzed plasma, in agreement with Favaloro *et al*. as well as the samples from which false or possibly false tests are obtained (8). In the case of clotting, the results are canceled and recommended sampling repeat, textually explaining the non-conformity: "Sample with clot. Repeat the sampling".

Fig. 4. Detection of the clot by transvasation (personal collection) (30, 31*)*

Clinical departments ensure the selection and tests recommending, sampling, handLing, transport and preparation of samples for analysis, a process that begins and ends with the patient, that's why we can consider that patients are also part of this team (32). It is important to maintain total control of the testing process in a systematic and transparent manner, encouraging and promoting errors identification, because, if the team fails, patients will suffer the most (32, 33). For patient's safety, nurses must be aware of the influence and possible consequences of preanalytical errors on laboratory results (32).

The recognition of these aspects is a challenge for the nurses who have an important role in the handling and sampling, an activity that should be considered as a necessary competence, and not just a technical trick (35). Being in agreement with previous studies (8, 17, 20, 34), from 2015, we pay special attention in the identification and monitoring of non-compliant samples (35), which represents error source for determining coagulation tests: insufficient volume has taken (lack of 0.5 mL blood), plasma hemolysis, as well as the presence of the clot identified in erythrocyte sediment post-analytical. In this way we can minimize the effects of biological factors that might have a significant impact on patient care.

It is necessary to train the nurses during the service, as well as to introduce in the syllabus the basic theoretical aspects regarding correct sampling technique of the biological product (32). Makitalo and Liikanen state that, through multi-professional cooperation, involving laboratory staff, the number of errors in the pre-analytical phase could be reduced (32). Guder in his article (36) mentions that the term preanalytical phase was included in textbooks in 1983 (37), and in the teaching books of Laboratory Medicine in 1987 and 2005 (38, 39). The lack of a standardized training program and periodic skills assessment has an impact on the quality of the results (40, 41, 42). Crous and Armstrong believe that errors during sampling can produce negative outcomes for the patient, which can vary from financial implications to death, according to the study of Al-Ghaithi *et al.* (41, 42).

In 2017 we presented the importance of the laboratory specialists involvement in errors reducing from pre-analytical phase through a training based management and acquired knowledge verification of the medical personnel (43), being in accordance with Al-Ghaithi's study (44), continuing in 2018 with a study, by which we drew attention to the false hypercoagulability *in vitro*, as a result of highlighting the error sources regarding the quality of the sample taken (45).

The focus of patient care, accepted on internationally level, changes the responsibility and delivery of healthcare (45). Medicine laboratory through a tripartite collaboration with clinicians and patients who are the beneficiaries of these services should include this change (46). The involvement key is enhanced by better communication, knowledge management, information disseminations, and educational support (46, 47). In the paper presented by Wu *et al.*, one of the 10 topics addressed refers to communication with clinicians and to laboratory role in educating clinicians about preanalytical and analytical influences on outcomes (48). Highlighting the importance of these errors is necessary for the clinician, who decides which tests to recommend in the pre-preanalytical phase, following by post post-analytical to decide the

appropriate treatment based on the laboratory results received (49). These aspects are supported by available literature data regarding incorrect diagnosis associated with laboratory test errors (50, 51).

CONCLUSIONS

We wanted to emphasize through this work, that evaluation of medical services quality in laboratories has become more and more important, both for the pressures exerted to reduce the additional costs due to the repetition of the determinations, but also for the evidence of the wrong diagnosis associated with the error sources tests.

The impact in the case of false results, generated by the interference of the error sources with the determination of the coagulation tests, can be especially serious because, in the case of specialized hemostasis tests, these are often considered "diagnostic". That's why we consider being very important identifying error sources before the results are released to the clinician and before to became a real harm for the patient's life.

Establishing a permanent link between clinicians and laboratory specialists would be useful by combining the unique talent of knowing the physiological reason behind the tests and conducting quality laboratory analyzes.

In order to ensure the quality of hemostasis exploration, we strongly support the creation of an initiative for developing the continuous educational programs for health professionals, representing also a priority for medical institutions.

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PATENT FORAMEN OVALE – A RARE CAUSE OF RECURRENT ACUTE OPTIC NEUROPATHY AND CEREBROVASCULAR EVENTS

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PATENT FORAMEN OVALE–A RARE CAUSE OF RECURRENT ACUTE OPTIC NEUROPATHY AND CEREBROVASCULAR EVENTS (Abstract): We report the case of a young female patient who developed recurrent acute bilateral and asymmetric vision loss, associated with pain and paresthesia on the right half of the body. Brain and spinal cord MRI showed inactive gliotic lesions, not suggestive of multiple sclerosis-related diseases. A wide range of autoantibodies were tested to exclude neuromyelitis optica, an autoimmune systemic disease, a paraneoplastic neurological syndrome or an autoimmune channelopathy; all came back negative. Cardiac examination and transthoracic echocardiography revealed persistent foramen ovale (PFO). Paradoxical ocular and brain embolism was the presumed cause of patient's uncommon clinical features. She underwent percutaneous closure with 25mm septal occlude, followed by permanent antiplatelet therapy, with favorable clinical outcome. **Conclusions:** This case is a unique association of recurrent PFO-related ocular and neurologic symptoms and emphasizes the necessity of considering cardiovascular causes among young patients who present with transient vision loss. Medical imaging was extremely important both in diagnosis and minimally invasive treatment, with favorable outcome and social reintegration of the patient. **Keywords:** OPTIC NEUROPATHY, PARADOXICAL EMBOLISM, PERSISTENT FORAMEN OVALE, CEREBROVASCULAR EVENT.

Acute optic neuropathy is the most common cause of vision loss, and it is a serious condition with a profound impact upon patient's quality of life. The etiology of acute optic neuropathy may be inflammatory, infectious, ischemic and traumatic. A careful history of patient's associated conditions and symptoms can help refine and narrow the differential diagnosis and select the appropriate therapeutic approach. In a young patient, a history of eye pain associated with eye movement, prior history of neurological symptoms such as paresthesia, limb weakness, and ataxia is suggestive of demyelinating optic neuritis, or, less frequently, of systemic vasculitis. In children and young adults, if visual loss is preceded by flu-like symptoms, sinusitis, or there is a recent vaccination history, the diagnosis is most probably parainfectious or post-vaccination optic neuritis, respectively. Personal history of atherosclerosis or blood diseases may be associated with ischemic optic neuropathy. Jaw claudication, temporal pain, fatigue, associated biological inflammatory symptoms are signs of giant cell arteritis, which can affect optic nerve perfusion by small vessel

vasculitis. Yet, in some cases, there are diagnostic challenges and an interdisciplinary approach is required.

CASE REPORT

We report the case of a 29-year-old woman with no significant personal history, admitted for sudden loss of vision in her right eye (days), intense photophobia and ocular pain exacerbated by eye movements and bright colors, accompanied by lower back pain and paresthesia on the right half of the body.

The eye exam revealed severely decreased visual acuity, of 0.05 in the right eye (RE), with constricted visual field, both for white light and colors. Ophthalmoscopy of the RE revealed hyperemic optic disk, with mild edema in the superior sector. Left eye (LE) was normal.

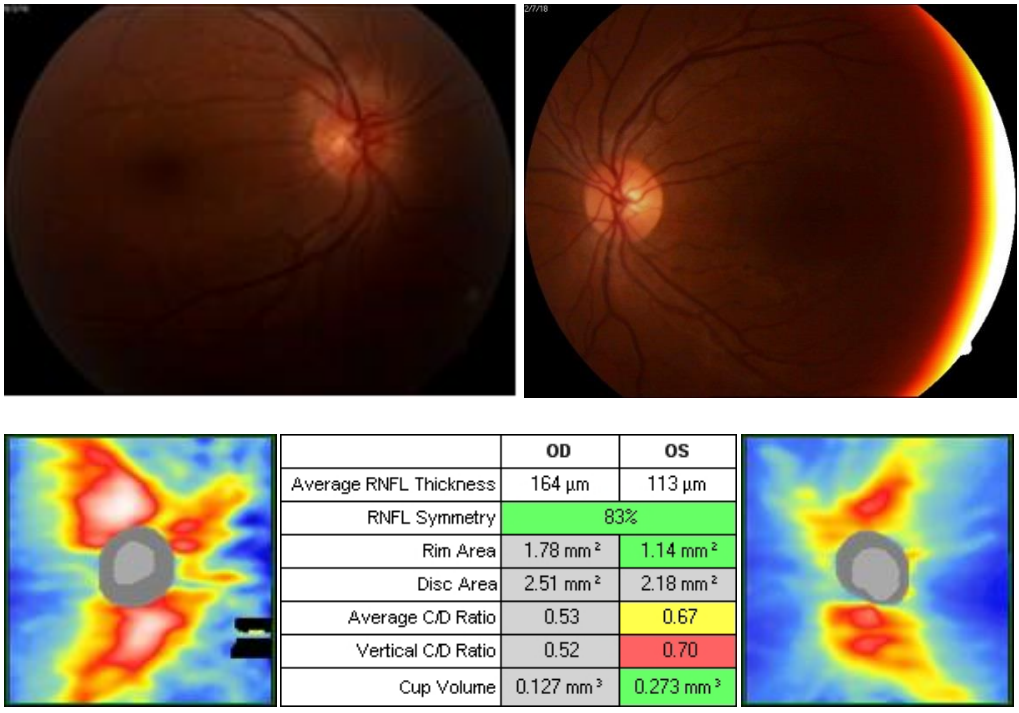

Fig. 1. a) Fundus appearance: RE - mild edema of the superior sector of optic disk; b) LE - normal appearance c) OCT exam of the optic disk: hyperemic optic disk, mild peripapillary retinal nerve fiber layer edema in the RE, superior sector

Based on the clinical ocular and neurological symptoms, the probable diagnosis was: RE acute optic neuropathy, in observation for multiple sclerosis. Other possible causes considered were a systemic autoimmune disease, a neurological paraneoplastic syndrome, reaction to a systemic or regional acute infectious disease, or an ischemic mechanism.

MRI exam showed some small, well-delineated, inactive gliotic lesions, situated deep subcortically, in the parietal and occipital area, not meeting the criteria for an acute demyelinating disease.

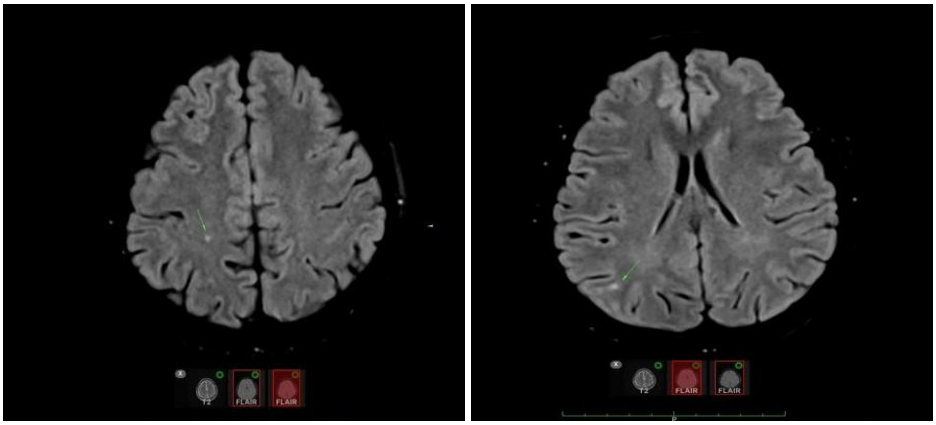

Fig. 2. MRI: small, inactive gliotic lesions, probably of ischemic origin, located occipitally and parietally

All laboratory test results were normal, showing no signs of biological inflammatory syndrome (C3, C4, rheumatoid factor, immunogram, lactate) or systemic infection. The extensive array of immunological tests were all within normal ranges: serum anti-aquaporin- 4 and anti-Mog autoantibodies, anticardiolipin autoantibodies, anti-beta2-glycoprotein autoantibodies, ANA, c-ANCA, p-ANCA, anti-double-stranded DNA autoantibodies, anti-ro (SS-A), anti-La (SS-B), onconeural autoantibodies, IgG4s profile, anti-VgKCA autoantibody.

While still investigated and in process of recovery, the patient presented a bilateral asymmetric acute visual loss, more severe in her RE, urinary functional complains (retention), paresthesia and painful hyperesthesia on the right half of the body, with difficulties holding an object in her right hand, and a walking perimeter limited at about 200 m.

Ophthalmological exam revealed: visual acuity (VA) hand movement in the RE and 0.1 in the LE, and bilateral visual field contraction of 10º in her RE and 30º around fixation point in her LE.

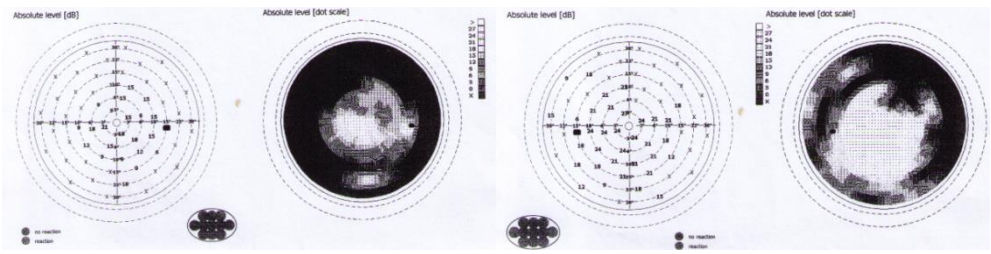

Fig. 3. a) Visual field of the RE; b) Visual field of the LE

The fundus exam in the RE revealed the same optic disk appearance, with mild peripapillary edema, more intense in the upper quadrant, and 2 new small whitish retinal lesions in the super temporal sector, which could be either a sign of retinal vasculitis or micro embolism. LE was normal. Visual evoked potentials revealed increased P100 latency in both eyes (RE 146 ms, LE 123 ms).

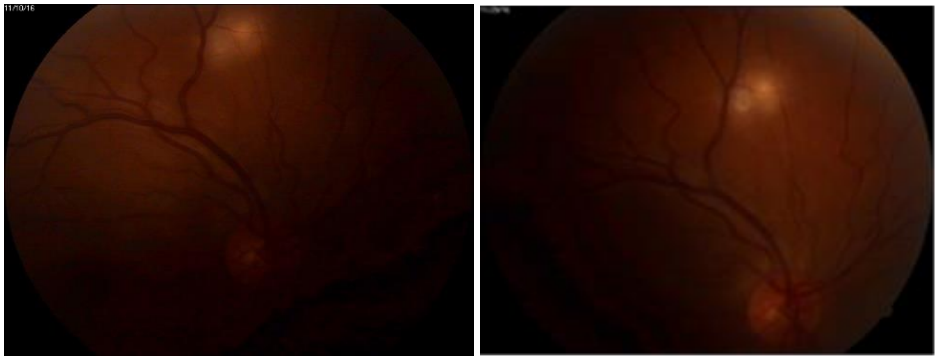

Fig. 4. Fundus Photography of RE: 2 new whitish, ill-defined retinal lesions
(a) with slow progression to focal atrophy
(b) located in the superior sector, centered by a small vessel in the superotemporal sector

As all laboratory results and rheumatological examination excluded a systemic vasculitis, the patient was referred to a cardiology consultation in search of a possible cause of an ischemic event. Physical examination revealed a 1/6 grade systolic murmur and color Doppler echocardiography evidenced a patent foramen ovale 5 mm in diameter.

The final diagnosis was recurrent bilateral optic neuropathy and transient ischemic attack. Persistent foramen ovale. A recurrent paradoxical micro embolism was considered to be the cause of both the ophthalmic and neurologic findings, with immediate indication for percutaneous surgical closure. The 25 mm ASD occlude was introduced loaded and with a delivery guide via a 6F sheath placed in the right femoral vein, progressed via a Cournand catheter and positioned at the level of PFO under 3D echocardiography guidance. She was prescribed permanent antiplatelet therapy (aspirin 75 mg/day) and vitamin B complex supplements for 3 months.

Fig. 5. a) control coronarography of the septal occluder at the end of surgical intervention;
b) follow-up echocardiography 6 month later

The course was favorable, with progressive neurological and ophthalmological recovery. One month after the intervention, she came back in for an ophthalmologic

exam which revealed normal visual acuity and slight peripheral visual field constriction in both eyes. The 2 retinal lesions showed a slow progression to focal atrophy. During the 12-month follow-up period, no relapse was recorded, and the patient could resume her normal daily routine and even work.

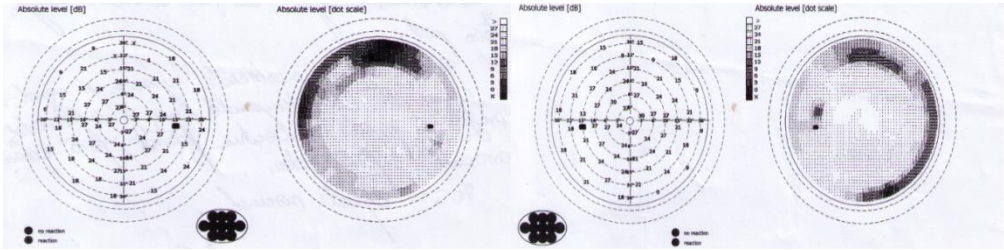

Fig. 6. a) Visual field of the RE – final appearance;
b) visual field of the LE – final appearance.

DISCUSSION

Patent foramen ovale is one of the most frequent congenital cardiac malformations, encountered in about 20-30% of subjects (1, 2). In most cases, it is a benign condition, and surgical closure is not routinely recommended. However, the presence of this abnormal right-left atrium communication can be responsible for paradoxical systemic embolism. Neurologic events are encountered in about 1-2% of persons with PFO requiring surgical closure to alleviate the symptoms, but ophthalmological features are even less frequent (2, 3). The neurologic signs of PFO-related ischemic events are: sudden weakness of face, arm or leg, swallowing difficulties, dizziness, loss of coordination, sudden paralysis or paresis, difficulty speaking and understanding words or simple sentences. On the other hand, among subjects with cryptogenic stroke, the incidence of PFO is of 40%, suggesting a strong cause-effect relationship between the two pathological conditions (4). There are only a few reports of vision loss associated with PFO, caused by central or branch retinal artery occlusion, yet in none of the cases concomitant ocular and cerebrovascular ischemic events were described (5-11).

The efficacy of PFO closure in preventing cryptogenic stroke was a subject of controversies in the last years. Recent studies confirm a significant decrease of the relative risk or neurologic events up to 58% in cases with percutaneous PFO closure vs medical therapy, especially in young patients aged <60 years (12-15). On the other hand, the absolute risk of cryptogenic stroke decreases only with 2.6-4.8% after PFO occlusion (13, 15), suggesting that other factors may be involved, especially in older people, like dyslipidemia or atherosclerosis. Periprocedural risk is estimated at 3% and the possible adverse events described in literature are: hemorrhages, embolic events, arterio-venous fistulas, retroperitoneal bleed, peripheral ischemia or a need for a vascular surgical repair (15). Episodes of atrial fibrillation may be related to local inflammation or device induced arrythmia. Presently, PFO closure indication is restricted to young patients, who experienced cryptogenic stroke (12). In these cases, the long-term benefits are proved to overcome the relative procedural associated risk.

In a young patient with sudden visual loss and neurologic symptoms, the most frequent cause is multiple sclerosis followed by systemic vasculitis; ischemic optic neu-

ropathy is extremely rare. The lack of laboratory data to support the clinical symptoms was a challenge for establishing the diagnostic and therapeutic approach. The ophthalmologic exam documented the acute ischemic disease and oriented the clinical investigations toward finding the cause. The favorable outcome was due to the small areas affected by emboli, which allowed patient's almost full recovery, and also to the early diagnosis and treatment of the underlying cardiac condition.

CONCLUSIONS

This case report presents a unique association of PFO-related recurrent ocular and neurologic ischemic events and emphasizes the necessity of considering cardiovascular causes even among young patients who present with transient vision loss.

Medical imaging was extremely important for diagnosis and minimally invasive treatment, with favorable outcome and social reintegration of the patient.

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ANEMIA DIAMOND-BLACKFAN. CAZURI CLINICE

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DIAMOND-BLACKFAN ANEMIA. CASE REPORT (Abstract): Diamond – Blakfan Anemia (DBA) is a rare condition in pediatric pathology, with more than 500 cases described in literature. It is a congenital eritroblastopenia, with AD/AR/ X-linkage inheritance, 90% were diagnosed by one year of age. Physical appearances were abnormal only in 29%. The current recommendations are corticosteroids, with suportive treatment and bone marrow transplantation. In general, DBA remains a single cytopenia. Long term prognosis remains uncertain, DBA is a preleukemia condition.We present you two cases that we took care of in our clinics.
Key words: BLACKFAN-DIAMOND ANEMIA, CONGENITAL APLASTIC ANEMIA

LAPAROSCOPY IN ACUTE ABDOMINAL DISORDERS

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LAPAROSCOPY IN ACUTE ABDOMINAL DISORDERS (Abstract): In emergency preoperative diagnosis is most accurate in acute cholecystitis and least accurate in pelvic conditions and mesenteric ischemia. Laparoscopy has a double goal: establish or confirm diagnosis and treating the disorders. Laparoscopy identifies those patients who do not need surgery. Our experience includes 2,778 patients with surgical acute abdomen: 1,857 cases with acute cholecystitis, 719 cases with acute appendicitis, 57 cases with perforated peptic ulcer, 10 cases with intestinal obstruction on postoperative adhesions, 19 cases with abdominal trauma, 87 cases with acute adnexal pathologies, 4 cases with acute pancreatitis, 12 cases with mesenteric ischemia, 12 cases with abdominal abscesses and one case with false acute abdomen. Our experience clearly shows the feasibility and safety of the laparoscopic appendectomy. The minimal access approach brings back suture in the treatment of perforated peptic ulcer in the conditions of an efficient postoperative medical treatment. Keywords: LAPAROSCOPY, ACUTE ABDOMEN, UNNECESSARY LAPAROTOMIES.

The recent expansion of both diagnostic and therapeutic laparoscopy has added a new tool to the surgical armamentarium. Laparoscopy is an efficient method for diagnostic in surgical acute abdomen because usually allows a good view of the uterus and adnexa, the appendix, the gallbladder, the stomach, the pylorus, and the first portion of the duodenum.

The laparoscope was introduced to determine whether the laparotomy would be required. With the development of new techniques, it has become possible to perform definitive therapeutic procedures under videoendoscopic control in the setting of the acute abdomen.

Laparoscopic appendectomy and closure of perforated duodenal ulcer have become common procedures, and laparoscopic cholecystectomy is now the standard of care in acute cholecystitis.

Laparoscopy can be used to assess intestinal viability if bowel ischemia is suspected.

MATERIAL AND METHODS

Between January 2013 and March 2020, in the First Surgical Clinic, from "Sf. Spir-

idon" County Clinical Emergency Hospital, Iasi were performed 8,367 laparoscopic interventions; 2,778 interventions of these (33,2%) were performed in emergency.

Laparoscopies establish the following diagnostic: Perforated peptic ulcer – 57 cases; Abdominal trauma – 19 cases; Intestinal infarction – 12 cases; Intestinal obstruction – 10 cases; Acute appendicitis – 719 cases; Acute adnexal pathologies – 87 cases; Acute cholecystitis – 1,857 cases; Acute pancreatitis – 4 cases; abdominal abscesses – 12 cases; False acute abdomen – 1 case.

RESULTS

8031 laparoscopic cholecystectomy was performed in our clinic for various anatomoclinical form of cholecystitis. Laparoscopic cholecystectomy for acute cholecystitis was performed in a series of 1857 patients (71.9% women and 28.1% men) with mean age 50.4 years (between 18 and 82 years). Obesity was encountered in 78% of the cases. Clinical aspect was typical for acute cholecystitis in 93.7% of the cases. In all cases diagnosis was confirmed or suggested at ultrasound examination. In most cases acute cholecystitis was related to gallstones presence; only 5.7% of the cases were not determined by lithiasis.

At pathology, the lesions were: edematous acute cholecystitis (72.3%); phlegmonous acute cholecystitis (8.1%); gangrenous acute cholecystitis (9.5%); plastron (11,1%).

Intraoperative in 80% of the cases we found pericholecystic adhesions. In 31.2% Hartmann pouch was placed behind porta hepatis. This was not surprising because with an inflamed gallbladder the anatomy in the area may be severe distorted. If the dissection was difficult or the ductal anatomy was not clear we converted to open cholecystectomy, because in this conditions the risk of ductal lesions is rather high. Conversion rate in this series was 9.1% (169 cases) as compared to a 2.8% rate in the entire series of laparoscopic cholecystectomies. The rate of biliary lesions was 0.1%.

Decompression of the enlarged gallbladder was needed in 80% of the cases.

We encountered the following intraoperative complications: gallbladder perforation (31.8%); hemorrhage (16.8%); gallstone loss (11.2%).

Mean operating time was 78 minutes (25-130 minutes), much longer than the mean value for laparoscopic cholecystectomy in general. Bottom line is that the intervention was easy in only 7.8% of the cases. In most cases we gave antibioprophylaxis. Postoperative morbidity was low, 5.6%. Mortality was 0.18% (2 cases – the cause was pulmonary thromboembolism). Mean postoperative hospitalization was 3.8 days. Laparoscopic cholecystectomy can be performed routinely for acute cholecystitis. Laparoscopic appendicectomy was performed on a series of 719 patients (81% women and 19% men). Their age was between 12 and 68 (mean 23) years.

Laparoscopic appendicectomy was indicated in the following situations: women at the reproductive age, because of the difficulties of establishing an accurate preoperative diagnosis in their cases; in our series the diagnosis error rate in this group was 20%; obesity, present in 32% of the cases; young patients; sportsmen.

At pathology, the lesions were: edematous appendicitis (61.5%); phlegmonous appendicitis (25.3%); gangrenous appendi-

citis (7.6%); plastron (5.6%).

The technique used was: "in" in 120 cases (16.7%); "out" in 252 cases (35%) and mixed in 298 cases (41.49%). Conversion rate was 6.81% (49 cases).

Morbidity was: 4 cases with parietal hematomas (0.55%); 5 cases with wound infections (0.69%).

Complications encountered were: 2 cases with peritonitis (6th day postoperative) 0.27%. Mean hospital stay was 3.2 days.

In our experience laparoscopic appendicectomy has certain advantages allows diagnosis of other conditions; decreases wound infection rate; deceases hospital stay; decreases recovery time for the patient; best cosmetic results.

Laparoscopic closure of duodenal peptic ulcer was performed in a series of 57 patients (all men) with mean age 28 years (between 18 and 58 years). The presentation was at 1-10 hours after symptoms onset. Preoperative diagnosis was perforated ulcer in most cases, pneumoperitoneum being present in 55 cases. At presentation, the patients had acute abdomen symptoms; perforations were small (< 5 mm) in all cases. Suture closure with an omental patch was performed in 57 cases. It is very important to wash and drainage very well the peritoneal cavity. Laparoscopic intervention offers this possibility. In only one case an inadequate closure leaded to a leak and after two days we performed a classic intervention. There were no other complications, and the morbidity was zero. Mean postoperative hospitalization was 4.6 days. Postoperative all patients received medical treatment for ulcer and sterilization of *Helicobacter pylori*. At follow-up after 3 months and a year there was no recurrent peptic ulcer in all cases.

Open laparoscopy was performed in 10 cases of intestinal subacute obstruction by postoperative adhesions. In only 5 cases was possible to performed adhesiolysis. In all other cases we had to convert to open procedure.

Laparoscopic surgery for abdominal trauma was performed in 19 cases, 5 cases for wound trauma and 14 cases for abdominal blunt trauma (hemoperitoneum). In abdominal blunt trauma intraoperative diagnosis was 3 hepatic lesions (II-III Moore degree) for who was performed exploration and hemostasis with Argon beam and TachoComb® (superficial injury of the liver) and 11 splenic lesions (II-III Moore degree) for who we performed laparoscopic splenectomies in 3 cases (rupture hematoma spleen), laparoscopic diagnosis with conservative treatment and spleen preservation in 2 cases and 6 conversions with spleen preservation in one case (packing with resorbable prosthesis).

Laparoscopic procedures were performed for acute adnexal pathologies in 87 patients. In cases with torsion of the ovary was required oophorectomy. In 6 cases with tubal pregnancies, we performed 3 salpingostomy and 3 salpingectomy, respectively. Otherwise, there were cases of ruptured ovarian cysts hemorrhagic. We did not include cases of pelvic inflammatory disease.

Other causes of acute abdomen in which we intervened by means of laparoscopy were: 4 cases of acute pancreatitis and 10 cases with bowel ischemia. In all this 4 cases an open laparotomy was avoided.

DISCUSSION

Many of the causes of the acute abdomen can be managed by laparoscopic ap-

proach (1). The therapeutic approach in these patients is based on clinical suspicion and on the results of diagnostic laparoscopy. Each procedure started by performing diagnostic laparoscopy to determine the specific abnormality present. Laparoscopy has become a routine procedure in the management of acute abdominal disease and can be considered both an excellent therapeutic and additional diagnostic tool in selected cases (2).

The diagnostic of acute cholecystitis is usually made preoperatively by clinical examination and ultrasound exploration. Sometimes acute cholecystitis may present as an acute abdomen if the gallbladder is gangrenous or perforated. Grasping of the gallbladder can be difficult when an acute state is present. We do not hesitate to decompress the gallbladder to improve the ability to grasp an acute inflamed gallbladder.

Laparoscopic cholecystectomy can be routinely performed in acute cholecystitis, but the surgeon must be prepared to convert to open cholecystectomy. If there is necrosis or the gallbladder cannot otherwise be retracted, then the operation should be converted to open approach.

It is also important to not spend to long with the laparoscopic technique if the dissection is difficult or the ductal anatomy is not clear.

The complications are more frequent in laparoscopic cholecystectomy than in open method. Bleeding from the liver can be a significant problem. Bile leaks can occur the cystic duct where a clip has fallen off.

Endoscopic retrograde cholangiopancreatography is used to identify the site of the ductal leak and to rule out injury to the common bile duct.

Common bile ducts injuries typically occur when the common duct or the right hepatic duct have been misidentified as the cystic duct. This injury is preventable by performing meticulous dissection beginning at the junction of the neck of the gallbladder and the cystic duct.

Indications for laparoscopic appendectomy include patients with an uncertain diagnosis (elderly, reproductive age females), obese patients, and those with suspected perforated appendicitis.

Laparoscopic management of perforated appendicitis is superior to open technique.

Dissection of adhesions and the irrigation of the entire abdominal cavity can be realized without extension of the incision. Simultaneously, another cause of acute abdomen can be identified and operatively managed. Obese patients have an increased wound infection rate and require larger incisions for open procedure. Further advantages of the laparoscopic technique include decreased exposure of subcutaneous tissue to the infected appendix and therefore a lower infection rate (3). It is better to performing appendectomy even if the organ appears normal (sometimes the appendiceal inflammation can be detectable only at the microscopic examination).

The preoperative diagnosis of perforated peptic ulcer is usually simple established by clinical examination and presence of air in peritoneal cavity on the abdominal radiographic films. The laparoscopic approach brings back the suture closure with an omental patch in the treatment of perforated duodenal ulcer. The modern medical treatment of peptic ulcer avoids the recurrent ulcer.

If the perforation is small and surrounding tissue is healthy it is possible to per-

formed direct closure using 3-0 polyglactin suture closure on atraumatic needles. It is better to close the defect in a transverse direction to prevent narrowing of the lumen.

Large perforations or those with large amount surrounding tissue friability and edema can be closed with an omental plug. The peritoneal cavity must be thoroughly irrigated with warm saline solution. Tilting the table in different positions helps with irrigating the entire peritoneal surface (4).

"Kissing ulcers" can occurs in which there is an anterior perforation and posterior penetration with bleeding. In these conditions it may be necessary to convert to laparotomy.

Acute abdominal pain from gynecologic sources is a common problem in surgery. The etiology of this pain in reproductive age females is often obscure (5). Many of these gynecologic sources of acute abdominal pain can be managed laparoscopically. Laparoscopy is indicated when the diagnosis is uncertain, the patient is obese, and the definitive operative management can be handled with the laparoscopic technique. The only contraindication is the patient with hemorrhagic shock.

Another cause of acute abdomen can be the acute pancreatitis. The laparoscopic diagnosis is made by opening the lesser sac with scissors dissection.

Ischemic bowel can be found at laparoscopy for an acute abdomen. It depends on the judgment of the surgeon to determine if it is hopeless situation (6). If the bowel is ischemic and not necrotic, the patient should have emergency mesenteric angiography.

The use of laparoscopy in blunt abdominal trauma has been expanded from merely a diagnostic tool to a therapeutic modality in selected cases (7). This has resulted in a quicker and more specific diagnosis and avoidance of unnecessary laparotomies.

Regarding the outcome measures for cases of the acute abdomen as a whole, Agresta *et al.* (8) included 1,272 patients admitted with acute abdomen who were approached laparoscopically. In comparison with open surgery, there was a significant reduction of total complication in laparoscopic treatment than open surgery (1.9 *vs.*13%).

Hemodynamic instability, huge abdominal distension, fecal peritonitis, and perforated cancer are relative contraindications for the laparoscopic approach. In recent years, abdominal emergencies have increasingly been managed successfully by laparoscopy. In acute appendicitis, acute cholecystitis and perforated peptic ulcer, randomized controlled trials have proven that the laparoscopic approach is as safe and as effective as open surgery, with fewer complications and a quicker postoperative recovery. Other indications such as blunt and penetrating trauma to the abdomen, small bowel occlusion and perforated diverticular disease are under debate, indicating that more randomized controlled trials comparing laparoscopic and open surgery are still necessary (6).

CONCLUSIONS

Laparoscopy is very important in the diagnosis and treatment of acute abdomen conditions. This is due to certain advantages as the ability to examine the entire peritoneal cavity and perform a definitive procedure, and of course when needed there is always the possibility to convert to an open procedure. Laparoscopy also reduces unnecessary laparotomies.

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NEWS

LONG TERM CONTROL IN ACROMEGALY - IS IT ACHIEVABLE?

Acromegaly is a rare endocrine disorder characterized by an excessive chronic secretion of GH and IGF-I, that, in time, results in numerous comorbidities. Biochemical control is not easily achievable but is of paramount importance for a reduction in morbidity and mortality. The presented results come from an extension to a prospective, multicenter, randomized, parallel-group, 6-month core study (Phase III PAOLA study). In the core study, patients were randomized to double-blind long-acting pasireotide 40 mg/28 days or 60 mg/28 days, or continued treatment with open-label long-acting octreotide 30 mg or lanreotide 120 mg every 28 days. As much as half of the enrolled patients had at least three comorbidities (mostly cardiovascular and glucose- or lipid-related disorders) at study entry. The conclusion of this study is that long-acting pasireotide was an effective and consistent therapeutic option for up to 5.8 years of treatment in patients with previously uncontrolled acromegaly and a high prevalence of comorbidities (cardiovascular, metabolic, including glucose-related disorders). (Colao A, Bronstein MD, Brue T, *et al*. Pasireotide for acromegaly: long-term outcomes from an extension to the Phase III PAOLA study. *Europ J Endocrinol* 2020; 182(6): 583-594).

DILEMMAS AROUND NECROTIZING ENTEROCOLITIS IN LATE PRETERM NEONATES

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DILEMMAS AROUND NECROTIZING ENTEROCOLITIS IN LATE PRETERM NEONATES (Abstract): Necrotizing enterocolitis is an acquired, multifactorial, and potentially severe gastrointestinal disease characterized by ischemia, necrosis, and inflammation of the bowel wall. It affects mostly very low birth weight preterm neonates, but approximately 10% of the cases are diagnosed in late preterm and term infants with risk factors for mesenteric ischemia like intrauterine growth restriction, polycythemia, birth asphyxia, chorioamnionitis, sepsis, or congenital gastro-intestinal or cardiac defects. In growth restricted newborns the incidence of necrotizing enterocolitis is approximately twice compared to those with adequate birth weight. In these fetuses blood flow to the brain is preserved at the expense of blood flow to other organs, including the gastrointestinal tract, which leads to mesenteric ischemia. An exclusive human milk diet might be protective due to growth factors, antibodies, cellular immune factors, probiotic bacteria, oligosaccharides, and stem cells, which are all present in fresh breastmilk. **Keywords**: NECROTIZING ENTEROCOLITIS, IUGR, CONGENITAL CARDIAC DEFECT, LATE PRETERM.

Necrotizing enterocolitis (NEC) is an acquired, multifactorial, and potentially severe gastrointestinal disease characterized by ischemia, necrosis and inflammation of the bowel wall. Being one of the most common neonatal gastrointestinal emergencies requiring surgery, this disease presents high mortality rate (10-30%), particularly among preterm infants (1,2). While some studies claim that the incidence of NEC remained stable in recent years (~10%) (3, 4), different studies revealed that due to the enhanced survival of extremely low birth weight (ELBW) newborns, the incidence has increased (11-15%) (5-7).

The risk of developing NEC depends on the gestational age (GA), the lower the GA, the higher the risk (8). For the survivors affected by NEC, there is high associated morbidity with neurodevelopmental sequelae and retinopathy of prematurity (2). Being one of the main causes of mortality and morbidity in neonatal intensive care unit (NICU), a better understanding of the pathogenesis of NEC is critical in order to

have an early diagnosis. Very low birth weight (VLBW) preterm infants represent the vast majority of patients affected by NEC, however about 10% of the cases are diagnosed in late preterm and term infants with risk factors for mesenteric ischemia like intrauterine growth restriction (IUGR), polycythemia, birth asphyxia, chorioamnionitis, sepsis, or congenital gastrointestinal or cardiac defects (9-12). The risk factors for different etiopathogenetic mechanisms of NEC, as well as most common laboratory and radiological findings are summarized in (tab. I).

TABLE I

Risk factors for different etiopathogenetic mechanisms of NEC and common laboratory and radiological findings.

	ISCHEMIC NEC	**INFECTIOUS NEC**
Medical history	Full-term/late preterm infant with IUGR Caesarean section 5-minute Apgar score < 7 Severe metabolic acidosis Hypoglycemia Severe anemia/ polycythemia 36ºC at 1 hr. of life Mother with preeclampsia or diabetes Placental abruption Gastroschisis/omphalocele Congenital heart disease (patent ductus arteriosus, hypoplastic left heart syndrome etc.) (13) Umbilical arterial catheterization (14)	Preterm infants (< 32 weeks GA) VLBW infants (< 1,500 g) Chorioamnionitis Premature rupture of membranes
Drugs	Indomethacin ± dexamethasone Ibuprofen Red blood cell transfusions (15)	H2 blocker (ranitidine/famotidine/nizatidine) (16,17) Empirical antimicrobials
Time of onset	Short term after birth (5.3 days) (11)	Later onset (15.3 days) (11)
Laboratory studies	• Blood culture negative • Negative gastric aspirate • Normal WBC • Low serum bicarbonate (due to poor tissue perfusion - isolated, without metabolic acidosis) • Inflammatory syndrome is absent - normal serial CRP values would favor aborted antibiotic therapy and early resumption of feedings (18).	• Blood culture may be positive in sepsis • Gastric aspirate may be positive (a negative gastric aspirate does not exclude a bacterial infection) • Inflammatory syndrome present -persistently elevated CRP after initiation of appropriate medical management suggests associated complications (18). Moderate to profound neutropenia - sepsis • Low hematocrit or hemoglobin may suggest infection or are low due to blood loses caused by consumptive coagulopathy • Low serum bicarbonate (associated with severe metabolic acidosis in sepsis) • Thrombocytopenia - reaction to gram-negative organisms and endotoxins or as part of consumptive coagulopathy (associated with high levels of PDF) • Fecal volatile organic compounds (VOCs) (19)

	ISCHEMIC NEC	INFECTIOUS NEC
Radiography	• Abnormal gas pattern • Dilated bowl loops • Thickened bowel walls	Radiological signs from ischemic NEC associated with: • Pneumatosis intestinalis • Portal venous gas • Abnormal free air • Intraperitoneal free fluid

CASE REPORT

Prenatal and birth history

Preterm female infant, GA 34 weeks, birth weight 1,800 g, first twin, of a dichorionic diamniotic pregnancy, born vaginally in a level II maternity to a 20 year old mother with inadequate prenatal care. In the last month of pregnancy, the mother had a urinary tract infection with *Escherichia coli* treated with antibiotic (Cefiximum). Apgar scores were of 8/9/9 at 1, 5 and 10 minutes, requiring early CPAP in the delivery room for 15 minutes.

The second twin was also female, with a BW = 2150 g, and Apgar scores of 9 at 1 minute and 10 at 5 minutes. Twin 2 had an uncomplicated course in the neonatal intensive care unit.

Hospitalization course prior to transport

The infant was admitted in the level II NICU at 20 minutes of life and the initial physical exam revealed intercostal and subcostal retractions, oxygen saturation (SpO_2) of 88%, a heart rate (HR) of 135 beats/minute (bpm), respiratory rate of 38 beats/minute and blood pressure (BP) 63/24/35 mmHg. The neonate required free-flow oxygen in order to maintain SpO_2 within normal range, parenteral nutrition, and prophylactic antibiotic (Ampicillin). Enteral feeding was initiated with formula on day of life (DOL) 2.

Initial laboratory studies revealed hemoglobin (Hb), hematocrit (Ht) white blood cell count (WBC) within normal range, negative C-reactive protein (CRP) and initial peripheral cultures (skin, external auditory canal, gastric liquid, and nasopharyngeal swabs).

On the fifth day of life the patient became unstable and clinical examination revealed recurrent desaturations (lowest value of SpO_2 = 50%) and coffee ground followed up by bilious emesis. Blood gas analysis showed mild respiratory acidosis (pH 7.26 and PCO_2 52.7 mmHg). Chest and abdomen X-Ray was normal. Blood and stool were sampled for bacterial cultures (and were negative after 72 hours). Feeds were stopped and ampicillin was replaced by intravenous gentamicin and colistin. At this point then transfer to level III center was requested. During transfer the neonate was stable with free flow oxygen.

Neonatal course at level III facility

The clinical examination at admission in the level III ICU revealed pale-greenish skin, hypotonia, shallow breathing, systolic murmur grade IV/VI, tachycardia (175-181 bpm) and bilious drainage with streaks of old blood. Immediately after admission, she presented severe recurring apneas requiring positive pressure ventilation (PPV) followed by intubation and mechanical ventilation (SIMV). Blood gas analysis showed respiratory acidosis with normal oxygenation (pH = 7.26, PCO_2 = 52.8

mmHg, PO_2 = 58 mmHg). Laboratory workup on admission revealed anemia, leucopenia (I/T = 0.47), elevated CRP and PCT (tab. II).

The X-Ray on admission revealed distended bowels with wall edema. Peripheral and blood cultures, as well as cerebrospinal fluid were collected.

TABLE II

Laboratory workup on admission and during hospitalization in the level III NICU up to DOL 17

Day of Life (DOL)	Hb (g/dl)	Ht (%)	WBC x10^3/mm^3	PLT x10^3/mm^3	CRP	PCT	Direct bilirubin	Urea
6	10.6	33.2	2.8	187	44.1	20.7	0	69.8
8	13	41.1	6.7	104	50.6	6.86	0.2	110.7
9	10.1*	31*						
10	12	37.9	9.8	120	125.2	1.06	0.4	66.3
14	10.6	33.1	17.9	249	87.5	1.11	1.4	79.5
17	14.3	43.7	16.3	174	37.6	0.79	0.9	71
*values from the arterial blood gas bulletin								

A nasogastric tube was placed for decompression of the dilated bowels and total parenteral nutrition (TPN) and intravenous antibiotics with broad-spectrum coverage (colistin, meropenem and gentamicin) were started. The cranial ultrasound revealed supernumerary ventricular cavity. Echocardiography showed Tetralogy of Fallot (TOF) with mild pulmonary stenosis, *patent foramen ovalae* and *ductus arteriosus*. Blood cultures and lumbar puncture were negative, and the peripheral cultures were positive for *Escherichia coli*. The newborn required mechanical ventilation for 21 days, three packed RBC's transfusions (15 ml/kg) for anemia and completed a 15-day course of antibiotic therapy for presumptive sepsis despite negative blood culture. Following treatment, clinical status improved slowly, and the laboratory tests normalized by DOL 17. Enteral feeds were restarted on DOL 15 with full feeding established on DOL 29.

Onward the clinical course of the infant was uneventful, however on DOL 55 the neonate presented marked abdominal distension and the X-ray revealed dilated intestinal loops. Following surgical consult, the newborn was transferred to the pediatric surgery department for further evaluation.

DISCUSSION

The etiology of NEC is not entirely understood as multiple factors appear to be involved, including hypoxia, acidosis, and hypotension, leading to ischemic damage of the mucosal barrier of the small intestine. Gram negative bacterial invasion of the mucosa may be another factor involved in the pathogenesis of NEC. Repeated radiographs are recommended, pneumatosis intestinalis and portal venous gas being pathognomonic signs, caused by anaerobic

bacteria, particularly by Clostridia.

Newborns with very low birth weight (< 1,500 g) and very preterm infants (< 32 weeks) are the most affected by this pathology (3, 20), as they have decreased ability to suppress the exaggerated inflammatory response or are unable to develop an inflammatory response to pathogenic bacteria (21). This case report presents a late preterm infant with a low birth weight and congenital heart disease. Hence, intestine mucosal injury and ischemia might have played a part in the development of NEC.

The signs and symptoms of necrotizing enterocolitis are highly variable, nonspecific and subtle (22). Physical examination findings may include abdominal distention, abdominal tenderness, visible intestinal loops, decreased bowel sounds, palpable abdominal mass, and erythema of the abdominal wall. Systemic findings may include respiratory failure, circulatory collapse, and decreased peripheral perfusion. The clinical course of this patient was marked by multiple days of parenteral nutrition and mechanical ventilation.

The role of inflammation in the pathogenesis of NEC is increasingly recognized. Antenatal inflammatory processes like chorioamnionitis may increase the susceptibility of preterm infants to develop NEC. Decreased intestinal mucosal thickness and increased mucosal permeability, as a result of inflammatory mediators, may explain this association (21). This twin derived from a mother with urinary tract infection in the third trimester, which seems to have complicated the course of the disease.

A recent meta-analysis investigating the outcomes of preterm infants affected by IUGR compared with those without growth restriction found that the incidence of NEC was at least 2.5 times higher in preterm infants with growth restriction (22). It is suggested that in such fetuses, blood flow to the brain is preserved at the expense of blood flow to other organs, including the gastrointestinal tract (23). Although the evidence suggests that newborns with IUGR and abnormal antenatal Doppler are at increased risk of developing NEC, early initiation of breast milk feeds does not appear to increase the incidence of NEC in this population (23).

Generally, this condition occurs after the first week post-partum when the intestine has been colonized (24). In this case, rapid initiation of enteral feeding with formula was a risk factor for NEC, due to changes in enteric blood flow and oxygen requirements during feeding. An exclusive human milk diet might be protective due to growth factors, antibodies, cellular immune factors, probiotic bacteria, oligosaccharides, and stem cells. Fresh maternal breast milk is superior to frozen or donor breast milk (25, 26). A prospective multicenter study concluded that the incidence of NEC was 6-10 times higher in exclusively formula-fed infants when compared with exclusively breast milk-fed newborns, and three times higher when compared with infants who received breast milk and formula (27). Early initiation of enteral feeds in preterm infants with very small non-nutritive volumes (known as trophic feeding or minimal enteral nutrition) is thought to have several advantages over complete fasting as a result of “priming” the gastrointestinal tract and aiding in its maturation processes (28-30). Initially, one of the differential diagnosis considered was feeding intolerance caused by lactose intoler-

ance. Neonatal lactose intolerance syndrome is based on a series of digestive system symptoms caused by the lack of lactase, leading to the inability of digesting the lactose in breast milk or cow milk. Symptoms of lactose intolerance include loose stools, abdominal bloating and pain, flatulence and vomiting (31). In this particular case, the neonate presented gastric residuals, abdominal tenderness, and recurrent apnea/bradycardia, which together with the systemic signs and abnormal blood work raised the suspicion of sepsis.

The reported frequency of NEC in infants with critical congenital heart disease (CCHD) ranges from 3% to 9% (32-35) with no relationship to feeding practices. However, in many centers infants with CCHD are managed with TPN and enteral feedings are deferred in the preoperative period (36, 37) as hypoxia, poor mesenteric perfusion, acidosis and hemodynamic instability are high risk factors for NEC. In our case the diagnosis of congenital heart defect was made on DOL 7, after the onset of symptoms and the deterioration of the infants' clinical status. TOF with mild pulmonary stenosis is unlikely to be a risk factor for NEC, but in this particular case it might have played a part in the intrauterine growth restriction, considering the fact that the other twin without cardiac pathology had an adequate birth weight and an uneventful clinical course. The growth restricted twin was at risk for both NEC and sepsis, making the differential diagnosis extremely challenging, as clinical signs are frequently non-specific and overlapped and laboratory test results may show similar abnormalities. However, the medical treatment in both cases follows the same general principles. The suggested antibiotic regimen includes ampicillin, gentamicin, and either clindamycin or metronidazole. While the patient is NPO, total parenteral nutrition must be provided. Antimicrobial therapy should always be adapted to the pathogen causing the disease. It is recommended to be justified by clinical, paraclinical and imagistic arguments in order to prevent bacterial antibiotic resistance.

If this conservative therapy is effective, infants may resume enteral feedings once signs of infection or abdominal pathology have disappeared. In some cases, this may take several days to a week. The presence of normal bowel movements suggests the return of bowel function. In infants with confirmed NEC who have worsening condition or bowel perforation or who do not respond to medical therapy, surgical intervention is indicated. Laparotomy is the standard approach, surgery being as conservative as possible, with removal only of portions of unquestionably necrotic or perforated intestine in an attempt to preserve as much intestine as possible (38).

For the neonatologist, the decision to refer the case to the pediatric surgeon and the timing of the referral are real challenges when faced with a neonatal patient with clinical deterioration despite maximal medical therapy, in the absence of free air in the abdominal cavity, as in the present case.

CONCLUSIONS

The peculiarity of this case consists in the association of multiple risk factors, as well as several etiological contexts - ischemic and infectious - which make inclusion into classical etiopathogenetic mechanistic patterns extremely difficult, if not impossible.

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SMALL BOWEL ADENOCARCINOMA ARISING IN LONGSTANDING FIBROSTENOTIC CROHN'S DISEASE

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SMALL BOWEL ADENOCARCINOMA ARISING IN LONGSTANDING FIBROSTENOTIC CROHN'S DISEASE (Abstract): Empirical evidence suggesting a link between neoplasia and chronic inflammation was first noted by Virchow more than a century ago, but the intricacies of the involved pathological processes have not yet been entirely elucidated. With a growing number of Crohn's disease (CD) cases worldwide, it is only logical to consider that the complications of this entity would become proportionally more prevalent. We report a case of small bowel adenocarcinoma arising in a patient with longstanding CD and discuss the current differences in paradigms related to surgical decisions in patients with inflammatory small bowel disease. A 56-years-old male patient known with CD was admitted in emergency to our clinic for intense diffuse abdominal pain spontaneously and on palpation, Blumberg +, with signs of peritoneal irritation, episode apparently much more severe compared to the previous ones. Laboratory investigations confirmed the diagnosis of acute abdomen and immediate surgery was performed. Intraoperative findings were peritonitis due to ileal perforation and ileal adenocarcinoma with "signet ring" cells, moderately differentiated (G2), on the background of active inflammatory bowel disease (CD). Segmental enterectomy and ileostomy were performed. The postoperative outcome was relatively favorable, with resumption of digestive tolerance and of intestinal transit at ileostomy level. As increased evidence suggest causality between chronic inflammation and malignant transformation one should contemplate whether the old treatment paradigms applied for other conditions such as fistulae in anal and leg ulcers should not be translated to inflammatory bowel disease (IBD) patients. Are we doing too much or not enough surgery for IBD? Could we diagnose small bowel adenocarcinomas in CD in early stages or there is too much risk for the patients and very few to benefit from a more aggressive surveillance? **Keywords:** CHRON'S DISEASE, SMALL BOWELL ADENOCARCINOMA, EMERGENCY SURGERY, ILEOSTOMY.

Empirical evidence suggesting a link between neoplasia and chronic inflammation was first noted by Virchow more than a century ago but the intricacies of the involved pathological processes have not yet been entirely elucidated (1). Many types of cancers arise at sites with chronic inflammation, such as Marjolijn ulcers in

longstanding venous insufficiency, anal carcinomas in chronic fistulae or gastroesophageal tumors promoted by gastroesophageal reflux disease (GORD) and Barret's esophagus. Dr. Burrill Crohn described the eponymic disease in 1932 but the first case of small bowel adenocarcinoma in an IBD patient was reported in 1957 by Ginzburg.

The growing number of CD cases worldwide justifies us to believe that the complications of this entity would become proportionally more prevalent. Thus, emphasizing their existence by reporting unusual or rare cases would increase the awareness on epiphenomena and peculiar manifestations associated with IBD.

CD, a chronic inflammatory disease located in the wall of the digestive tract, can theoretically affect any segment from the esophagus to the anus, but especially the distal portion of the small intestine (terminal ileitis) and the colon. It is characterized by transmural ulcerative lesions that can cause complications such as strictures or fistulas. It is a disease that affects young people (15-30 years) but can also occur in children (2) or older ages, with equal incidence in women and men. CD is also associated with many extraintestinal manifestations, most triggered by an autoimmune mechanism, little elucidated.

The main manifestations of CD are accentuation of intestinal transit - stools several times per day – persistent over weeks or months (noninfectious chronic diarrhea), recurrent abdominal pain, rectal bleeding, persistent fever, impaired general condition, weight loss. The spread of inflammatory bowel disease together with severe diarrhea may require emergency hospitalization. Sometimes the symptoms resemble acute appendicitis or intestinal obstruction, usually the patients being hospitalized to determine the cause of prolonged diarrhea or persistent febrile syndrome or because they are frightened by rectal bleeding. Most often, CD is a chronic disease with remissions lasting for weeks or years, interspersed with severe exacerbations.

The positive diagnosis is suggested by the above-mentioned symptoms, helped by the presence of the biological inflammatory syndrome (increase in ESR, fibrinogen, C-reactive protein), possibly anemia and it is confirmed by digestive endoscopy (diffuse, discontinuous mucosal involvement, with linear and deep ulcerations; histopathological evidence of deep chronic lymphoplasmacytic infiltrate, based on glandular crypts, architectural distortions of the gastric mucosa, presence of sarcoid-like granulomas, and transmural damage to the intestinal wall).

Complications of CD can be parietal and perianal abscesses, fistulas: enter enteric, enter vesical, enter vaginal, enterocutaneous, with infectious complications caused by the septic content of the intestine, adhesions of the neighboring intestinal loops with the development of tumor masses with obstructive phenomena, toxic dilation of the colon (toxic megacolon) - rare - with imminent danger of perforation, anemia, malnutrition, malignancy (3).

CD is treated with medicines, surgery being usually considered in case of complications (fistula, abscess, occlusion, and tight stenosis, especially in the small intestine). The drugs used address the inflammatory substrate (anti-inflammatory agents: sulfasalazine, corticotherapy) and also immunosuppressants: azathioprine, mercaptopurine, methotrexate, cyclosporine).

The prognosis of CD is variable, depending on the extent of the inflammatory process, presence of complications, treatment followed, and last but not least, patient's compliance; being a chronic disease

it requires regular medical investigations, careful and competent follow-up (4). Fortunately, malignant degeneration is a rather rare complication, its incidence increasing after 10 years of active disease, but with a more unfavorable prognosis.

CASE REPORT

A 64-year-old male patient, known with Crohn disease, diagnosed 3 years earlier in the gastroenterology service, on treatment with sulfasalazine, with repeated sub occlusive episodes, regular hospitalizations in the surgery service, in imminent surgery, successfully timed each time. He presents to the emergency room complaining of significant abdominal pain, accompanied by nausea and vomiting, intestinal transit disorders, initially diarrheal stools, then no stool in the last 48 hours, symptoms progressively accentuated in the last 12 hours. Clinical examination revealed altered general condition, psychomotor agitation, cachexia, pale skin and mucous membranes, BP 110/55 mmHg, AV 88/min. Local abdominal assessment revealed: intense diffuse abdominal pain spontaneously and on palpation, Blumberg +, with signs of peritoneal irritation, episode apparently much more severe compared to the previous one. Biological samples showed leukocytosis (WBS 27,000/uL), moderate anemia (Hg 10 g/dL).

Fig. 1. Pneumoperitoneum, CT image.

Fig. 2. CT images: at the level of the distal small bowel, ileocecal valve, descending colon, and sigmoid, distended intestinal loops, with edematous, thickened circumferential wall; free fluid in medium-large quantity, perihepatic, peri splenic, inter-ileal, parieto-colic and at the pelvic level.

Emergency CT scan showed bilateral subdiaphragmatic pneumoperitoneum (fig. 1), medium-large amount of free fluid at perihepatic, peri splenic, interileal, paracolic and pelvic level, multiple lymphadenopathies with the highest diameter 18/16 mm projected in the hepatic hilum, interaortocaval, lumboaortic regions, bilateral mesenteric and external iliac roots. The intestinal loops are distended and with edematous thickened wall, suggestive of inflammation/ intraperitoneal infection (fig. 2).

Clinical-laboratory investigations confirmed the suspicion of acute abdomen, surgery being the next step. The signed informed consent was obtained after discussing with the patient the probability of a temporary ileostomy, and the possibility of malignancy. The disclosure included the possible medical errors and therapeutic alternatives in a comprehensive information and transparency of the medical attitude (5). Thus, under general anesthesia, immediate surgery was performed. Intraoperatively, generalized peritonitis with multiple abscesses and entero-enteral fistulas of the terminal ileum, as well as a hard tumor mass at the level of ileum, located at about 20 cm from the ileocecal valve were found. Segmental enterectomy (approximately 50 cm) was performed with terminal ileostomy, abundant peritoneal lavage, and multiple peritoneal drainage. The segmental enterectomy specimen had a diameter ranging between 2 and 4.5 cm, which presented at 17 cm from the distal resection edge a 2.5 cm perforation area and multiple micro abscesses in the serosa. At sectioning, the intestinal wall was diffusely thickened (up to 0.8 mm), the mucosa showing atrophic folds, with areas of ulceration alternating with areas with whitish-green deposits; towards the distal edge of the resection the intestinal lumen was stenotic.

The resection specimens were sent for histopathological examination at the pathology department of the same hospital. These were fixed in 10% buffered formalin for 24 hours and processed according to the classical histopathological technique (hematoxylin eosin staining).

Although the macroscopic appearance suggested rather a stenotic area in the intestinal wall, a common lesion found in patients with CD, microscopic examination revealed the presence of an invasive malignant tumor with glandular differentiation that penetrated circumferentially and diffusely the whole intestinal wall, reaching the level of the serosa, and extending to the sub serosal space (fig. 3).

Tumor cells were pleomorphic, with marked cytonuclear atypia and relatively common mitotic figures. Tumor mass present also a part of weakly cohesive tumor cells with the classic appearance of “signet ring” cells (figs. 4 and 5).

In addition to the presence of tumor proliferation, the remaining intestinal wall showed histopathological changes suggestive of active CD: deep erosions of the intestinal mucosa, villoglandular destruction, extensive foci of pseudo pyloric metaplasia, areas of fibrosis, sarcoid-like granulomas located deep in the intestinal wall and even in the vicinity of tumor contingent, marked interstitial edema of the lamina propria and marked basal plasmacytosis (figs. 6, 7, 8). Twenty-five locoregional lymph nodes were identified and evaluated (hematoxylin eosin staining), of which 9 shows tumor invasion (pN1)–lymph node metastasis with cells in the “signet cells” (fig. 6). Adjacent to the glandular tumor

proliferation, desmoplastic reagents were identified in the context in which there were predominantly chronic lympho-plasmacytic inflammatory infiltrates and discoid "signet cells" (10% of tumor mass). Also, the intestinal wall presented a deep ulceration that affected the entire intestinal wall.

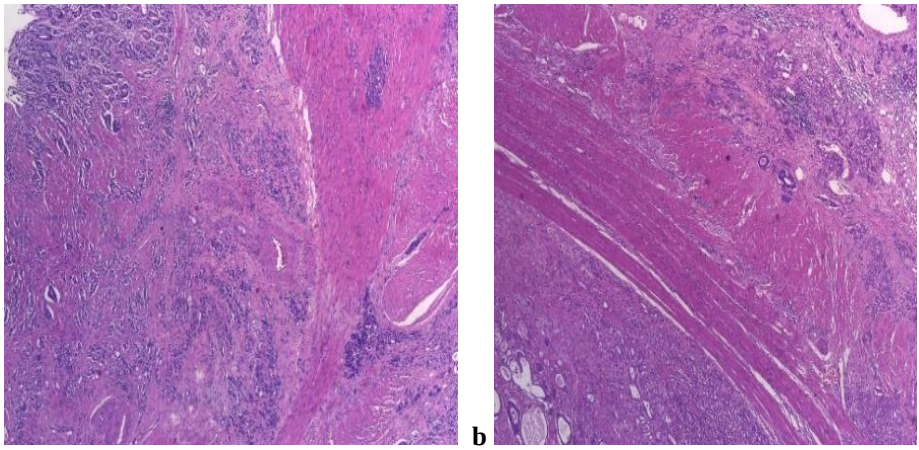

Fig. 3. (a, b). Small bowel adenocarcinoma infiltrating the full thickness of the muscularis propria with massive extension to the subserosa space. (Hematoxylin and eosin staining, ob. 4X)

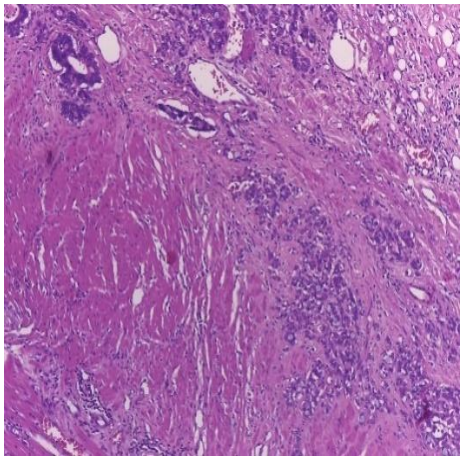

Fig. 4. Small bowel adenocarcinoma showing a mixture of poorly cohesive signet ring cell and glandular structures, with lymphovascular invasion. (Hematoxylin and eosin staining, ob. 10X)

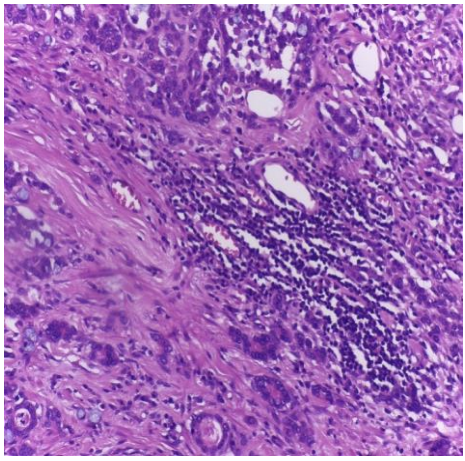

Fig. 5. Tumor cells with predominant signet ring cell features discohesively infiltrating the muscularis propria. They have hyperchromatic nuclei with moderate cytologic atypia. Chronic inflammatory cells (lymphocytes and plasma cells) are also present (Hematoxylin and eosin staining, ob. 20X).

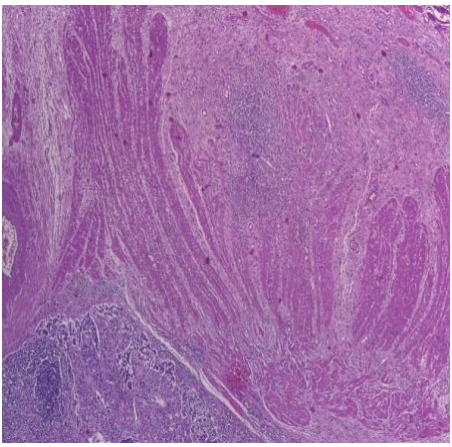

Fig. 6. Small bowel adenocarcinoma in CD. Note the marked distortion of the muscularis propria by fibrosis and transmural inflammation with lymphoid aggregates, sarcoid-like, noncaseating and poorly formed granulomas. (Hematoxylin and eosin staining, ob. 4X)

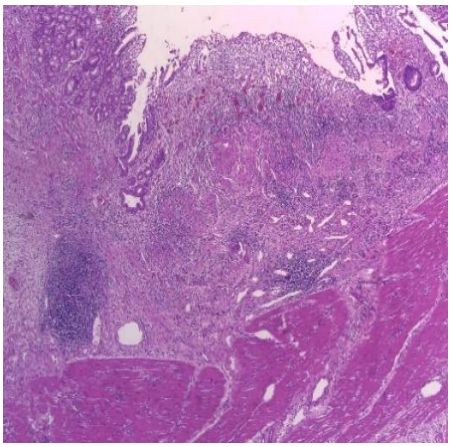

Fig. 7. The remaining intestinal wall had the typical appearance for CD: superficial and deep ulceration with adjacent granulation tissue extending into deep submucosa, lymphoid aggregates, and poorly formed granulomas, fibrosis, and edema of the lamina propria and muscularis propria, reduplication of the muscularis mucosae, and pseudo pyloric metaplasia. (Hematoxylin and eosin staining, ob. 4X).

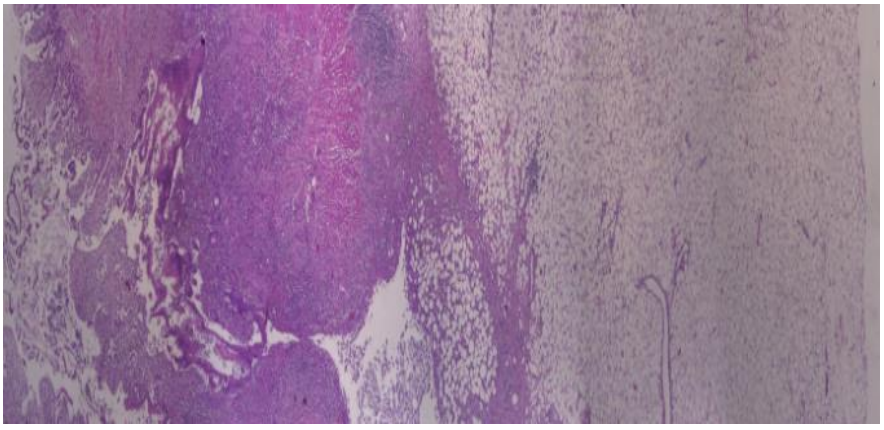

Fig. 8. Deep transmural ulceration of the small bowel wall. Image edited and stitched using Leica imaging software (Hematoxylin and eosin staining, ob. 4X).

The final histopathological diagnosis was intestinal (ileal) adenocarcinoma with the component of moderately differentiated (G2) signet-ring cell carcinoma arising in the setting of active inflammatory bowel disease (CD) - pTNM: pT4pN1Mx (stage IIIA). The advanced neoplasia was an intraoperative finding, during emergency

surgery for acute abdomen and peritonitis caused by ileal perforation.

The patient had a relatively favorable postoperative outcome, with resumption of digestive tolerance and intestinal transit at the level of ileostomy. He was referred to the Oncology Department for postoperative adjuvant chemotherapy.

DISCUSSION

IBDs are lifelong conditions with an individual pattern of flares and remission periods and with permanent potential of complications. Although both ulcerative colitis (UC) and CD (CD) share an immune mediated chronic inflammation, the propensity for malignant transformation and perforations differ considerably (6). UC is not a transmural disease; hence it does not produce strictures or perforations, but it does cause bowel cancer in 20% percent of the patients after 30 years of active disease. CD is notorious for fibro stenotic changes of the bowel, but it rarely elicits malignant transformation. (7) Some studies have shown that the risk of small bowel adenocarcinoma is increased by more than 60-fold in patients with IBD as compared to general population (8). In a meta-analysis by von Roon *et al.*, the relative risk of developing small bowel carcinoma in 9642 patients was estimated at 28.37, and the incidence rate of small bowel carcinoma in 12,740 patients was 15 per 100,000 patient years (9). However, the exact magnitude of the increased risk is hard to establish because data derived from population-based studies, case-controlled studies, physician surveys, and case reports have been combined in most studies regarding the incidence of malignancy in patients with IBD. In the same review by von Roon *et al.*, the mean duration of Crohn's disease before the onset of malignancy was about 9 years. The relative risk of developing small bowel carcinoma compared to the background population was higher in North America (RR = 41.23), the United Kingdom (RR = 40) and Scandinavia (RR = 21.3). The vast majority of patients with IBD-induced small bowel cancers have endured a long course of the disease raising the question whether a more thorough surveillance for malignancies might increase early detection and survival in these patients (10). With regard to perforating complications of CD there is strong evidence that deferring surgery is a helpful strategy in avoiding short bowel syndrome and multiple surgeries related sequels. There is growing evidence that a large array of cytokines, such as interleukins (IL-6, Il-10, Il-22, Il-26), LBP, soluble CD14 receptor plays a crucial part in the uncontrolled intestinal inflammatory process, which is a main characteristic of IBD. Additional testing of inflammatory biomarkers such as Il-6 and LBP could be a useful tool for monitoring the severity of inflammation, but also the onset of a septic intraperitoneal complication (11,12). Acute abdomen due to ileal perforation is a serious, life threating condition. Ileocolic perforation might be favored by stenotic lesions, with upstream dilatation, fistulizing disease, or carcinoma. Emergency surgery is particularly vulnerable to patient safety issues, due to short time of planning and difficulties related to preoperatory investigations and risk assessment (13). The coexistence of pathological associations in emergency supports the need for a thorough intraoperative examination to address to all lesions (14). The use of the laparoscopic approach also in case of diffuse peritonitis is now becoming accepted provided hemodynamic stability and may be

used successfully even in perforation associated with Crohn disease, ensuring a faster recovery and shorter hospital stay (15, 16). In patients with associated malignancy, some authors recommend that the opportunity of primary anastomosis and bypass protection should be judged individually, upon the patient's clinical condition and past history of steroid use. However, when malignancy and perforation are associated, anastomosis is not recommended, being associated with high morbidity and mortality (17-20). Careful examination of the vascularization of anastomosis tranches is important to prevent anastomosis leaks, one of the most frequent causes of postoperative morbidity (21).

Patients with CD are at increased risk of colorectal cancer and a higher mortality from colorectal cancer in comparison with general population (22). Strong *et al.* (23) recommend total proctocolectomy in the surgical management of CD-associated malignancy in cases with high grade dysplasia or multifocal low-grade dysplasia. Several clinical studies documented the high rate (35-40%) of metachronous cancers following segmental or subtotal colectomies (23-25). In the present paper, a less invasive approach was chosen due to the general condition of the patient and the presence of intense peritoneal cavity contamination. According to Ola *et al.* (22), a careful colorectal cancer surveillance was indicated especially in patients diagnosed with CD before the age of 40 years, patients with colon inflammation, and in those who have primary sclerosing cholangitis.

CONCLUSIONS

A high level of suspicion and awareness of malignant transformation in CD is key to early diagnosis. Better outcomes in patients with small bowel adenocarcinoma arising in IBD strictures would be expected with advancements in radiology and refinement of surveillance guidelines. CD remains a condition with a high rate of both benign and malignant transformation.

Further studies on the sequence of metaplasia-dysplasia-cancer in IBDs would bring more evidence to explain the differences between UC and CD in terms of malignant potential and would provide a solid background for improved surveillance guidelines.

CONFLICT OF INTERESTS AND FUNDING

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VARIABLES INFLUENCING MEDICAL DECISIONS IN A CLINICAL REHABILITATION HOSPITAL

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VARIABLES INFLUENCING MEDICAL DECISIONS IN A CLINICAL REHABILITATION HOSPITAL (Abstract): The discharge from the hospital represents a complex process that should assess the patient's ability to adapt outside of the medical setting. **Material and methods:** This paper analyses the medical status of the patients admitted to a rehabilitation clinic, at the moment of discharge, considering a number of influencing factors, such as age, sex, place of origin, length of stay (LOS) in the hospital. In order to achieve this purpose, we conducted a study on 136 patients discharged from the Clinical Rehabilitation Hospital Iasi in 2016 using an econometric modelling. **Results:** Our findings reveal that the medical status of the patients at the time of discharge was not influenced by the sex or place of origin. Also, with an increase in patient's age by one year, the estimated chances of a patient not to be cured at discharge decreases by 3.5%. In addition, with an increase in the patient's length of stay in the hospital by one day, the estimated chances of a patient not being cured at discharge time increases by 8.1%. **Conclusions:** It was found that the age and the length of admission in the hospital has a significant influence on the disease stabilization, even if the patients are not fully cured at discharge time. Because the research only considered data from a single regional hospital, it limits the conclusions that can be drawn from the sample. **Keywords:** MEDICAL STATUS, DISCHARGE, ECONOMETRIC MODELING, LENGTH OF STAY.

Managers put forth significant effort to make acceptable organizational decisions, hence decisions and decision-making processes are at the heart of management (1, 2). Because the efficacy of corporate activities is primarily determined by how decisions are created and implemented, the study of decision-making processes is becoming increasingly essential (3, 4), because decisions affect not only the organization in which they are made, but also the people who work there (5, 6).

Clinical decision-making is at the heart of clinical practice on a daily basis. The application of clinical and biomedical information, problem-solving, calculating probability and possible outcomes, and risk-benefit balance are all part of this

process. One of the most difficult tasks is to strike a balance between personal experience and general knowledge (7).

MATERIAL AND METHODS

The goal of our research was to determine the elements that influence decision-making and the parts actively involved in the process. Taking a careful look at the literature, we can observe that the socio-economic status of our country is crucial, independent of the decisional model adopted.

This paper investigates whether the medical state of patients at discharge is influenced by their age, place of origin, gender, and the LOS in the hospital. Patients hospitalized to the Clinical Rehabilitation Hospital from Iasi, in 2016 constitute up the study population. A total of 136 patients were randomly chosen from the population. The analysis did not contain any clinical data from the patients. The *SPSS* statistical analysis tool was used to process the sample data.

The variables considered in this study were: LOS, place of origin, age, gender, and medical condition at discharge. The study-dependent variable, namely the medical condition of the patient at discharge, is a nominal qualitative variable with two categories: cured and not cured. The independent variables considered in the analysis were divided into continuous quantitative variables (age and LOS) and nominal or qualitative variables (gender and place of origin). Both gender and place of origin variables have two categories: female and male, respectively rural and urban.

Statistical analysis

For the qualitative independent variables, the patient distribution was analyzed by calculating the absolute and relative frequencies. Also, the average percentages for each of these variables were calculated. *Pearson Chi-square* test was used to determine the association between the dependent variable and each of the qualitative independent variables.

The econometric modelling was conducted with logistic regression to determine the link between the medical status of the patient and the independent variables (age, gender, place of origin, and LOS), using as statistical analysis *Pearson Chi-square test*, relative frequencies, assumed risk, probabilities, relative risk, odds and odds ratio.

RESULTS

Patients distribution using qualitative variables

The absolute and relative frequencies of the qualitative characteristics were determined for a full investigation of the patients' distribution. Out of a total of 136 patients, 68 (50%) were cured at the time of discharge and 68 (50%) were not. In terms of gender, 64 (47.1%) of all hospitalized patients were female, while 72 (52.9%) were male. There were 36 (52.9 percent) females and 32 (47.1%) males among the uncured patients. The cured patients were divided into two gender groups: 28 (41.2%) females and 40 (58.8%) males (tab. I).

The distribution of patients by place of origin and medical status at discharge revealed that of the total of 58 (42.6%) rural patients, 27 (46.6%) were uncured patients and 31 (53.4%) were cured. Furthermore, at the time of discharge, 41 (52.6%) cases of uncured patients and 37 (47.4%) cases of cured patients were reported from the urban area (tab. II). Our analysis proved that there was no link between the patient's medical status at the time of discharge and their place of origin.

Our statistical analysis showed that the average percentage of patients who were

cured at the moment of the discharge was equal to the average percentage of uncured patients at the same moment. The average percentage of male patients in the sample studied was 52.9%, whereas the average percentage of female patients was 47.1%. Also, the average percentage of patients in urban areas was 57.4%, while the average percentage of patients in rural areas was 46.2%.

TABLE I

Patient's distribution by gender and medical status

			Gender	
			Female	*Male*
Status	*Not cured*	Count % within medical status % within gender	36 52.9% 56.3%	32 47.1% 44.4%
	Cured	Count % within medical status % within gender	28 41.2% 43.8%	40 58.8% 55.6%

TABLE II

Patient's distribution by place of origin and medical status

			Place of origin	
			Rural	*Urban*
Status	*Not cured*	Count % within medical status % within place of origin	27 39.7% 46.6%	41 60.3% 52.6%
	Cured	Count % within medical status % within place of origin	31 45.6% 53.4%	37 54.4% 47.4%

The relationship and measurements between the dependent variable and each of the qualitative independent factors.

We further calculated using the confidence interval the proportion of uncured patients at the time of discharge from the patients included in the study.

The results showed that the interval [0.415; 0.584] covers the proportion of uncured patients at discharge time with a 95% confidence interval.

Regarding the specific measurements, our results proved the following data.

Measuring the association between the patient's medical status at discharge and the patient's gender:

- Probabilities: The probability of a female patient to be cured at discharge was 0.437, while for a male patient was 0.555.
- Relative risk: The risk of not being cured at discharge was 1.268 times higher for female patients compared to males.
- Odds: The odds for a female patient not to be cured at the time of discharge were 1.285, while for a male patient the odds were 0.8.

- Odds ratio for a female patient not to be cured at discharge when compared to a male patient was 1.265. In other words, female patients have 1.265 more chances of not being cured at discharge comparing to male patients.

Measuring the association between the patient's medical status at discharge and the patient's place of origin:

- Probabilities: The probability that a patient from rural area to be cured at the time of discharge was 0.534, while from an urban area was 0.474.
- Relative risk: The risk of not being cured at the time of discharge was 1.128 times higher for urban patients than for rural patients.
- Odds: The odds for an urban patient not being cured at the time of discharge were 1.109, while the odds for a rural patient were 0.873.
- Odds ratio: The odds for an urban patient not being cured at the time of discharge were 1.109, while for a rural patient were 0.873, identical as for odds.

DISCUSSION

The moment of discharge from the hospital is an essential part of the physician's role in providing comprehensive care. A rehabilitation hospital's physicians must determine whether and when a patient can be discharged in a safe and timely way while maintaining physical mobility and independence.

Matmari *et al.* (8) pointed out that the process of discharge requires an understanding of the involved individuals (patients, families, health care team), the environment (supports and resources required for successful transition to the discharge destination), the processes influencing the plan (facility and provincial policies), and how all these factors interact. In order to discharge patients in the best medical condition possible, Pashley *et al.* (9) emphasized the importance of clinical experience in shaping physician`s processes, practices, and priorities in the discharge decision making.

Studies conducted by Kirk-Sanchez (10) and Shahpar (11) examined the relationship between mobility at the time of discharge in a group of patients with orthopedic diagnoses and other associated influencing factors. The results of these studies indicated that the hours of physical therapy and occupational therapy of a patient with orthopedic diagnoses, as well as the cognitive status at admission, length of stay (LOS), and number of secondary diagnoses, can predict the mobility at the time of the discharge. The age of the patients was not found to be a predictor for the discharge medical condition. Morri *et al.* (12), on the other hand, demonstrated that age and sex are major predictive factors for patients' levels of autonomy at discharge.

In 2008, Berges *et al.* examined the effects of the patient's gender on rehabilitation outcomes following hip replacement surgery. The study highlighted the importance of gender in the medical condition of the patients at discharge, finding statistically significant differences in functional achievements among women compared to men. In this study, women began inpatient rehabilitation with lower ratings of Functional Independence Measure (FIM) than men but had greater improvements in mean FIM rating from admission to discharge (13).

Mathijssen *et al.* employed a multiple regression model to estimate the LOS as a function of a variety of independent variables to determine which factors influence

the LOS in hospitals, which directly affects the discharge outcome. Other characteristics such as age and ethnic group, in addition to the type of condition, were found to influence patient LOS (14).

According to Pashley *et al.* (9), patients may not achieve their full rehabilitative potential as a result of an early or poorly planned discharge, or they may have serious injuries that generate secondary impairments. As indicated by Henke *et al.* (15), premature discharge may result in hospital readmission. Furthermore, early hospital discharge does not always imply cost savings if patients require more intensive health care in the future. A solid quality evaluation should serve as the foundation for providing healthcare services in medical practice as well as in healthcare administration, and it could be expressed in dimensions such as: effectiveness, accessibility, professional competence, continuity, security, physical infrastructure, efficiency, interpersonal relations, comfort, choice of supplier and type of insurance or treatment (16, 17).

By evaluating the correlation between the dependent variables and each of the qualitative independent variables, the *Pearson Chi-square* analysis showed that the medical status of the patient at the time of discharge was not influenced by gender and place of origin. Our results revealed the fact that half of the sample patients were cured, and half were not; in addition, among the cured patients, there were more men than women. More specifically, the risk of not being cured at discharge was 1.268 times higher for female patients compared to males. Moreover, the estimated risk of not being cured at discharge time is 1.154 times higher for female patients from rural areas compared to males from the same place of origin.

Inadequate individual patient care could result from a physician's lack of grasp of non-clinical elements when making management decisions. Even if physicians should strive to act in a reasonable, evidence-based, and professional manner at all times, they should also consider the particular challenges of individual patients in their clinical practice and respect their values and principles. In order to provide the finest quality healthcare to all patients, treatment policies should support management of all patients equally, without discriminating between them based on gender, age, sexual orientation, religion, or race (18).

CONCLUSIONS

In our study, using econometric modeling with logistic regression, we found that the age and the length of admission in the hospital has a significant influence on the disease stabilization, even if the patients are not fully cured at discharge time. This means that with an increase in patient's age by one year, the estimated chances of a patient not being cured at discharge decrease by 3.5%. Also, with an increase in the patient's LOS in the hospital by one day, the estimated chances of a patient not being cured at discharge time increase by 8.1%. Because the research only considered data from a single regional hospital, it limits the conclusions that can be drawn from the sample. Future work should aim to extend the analysis to more hospitals, also implying a larger number of patients.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SOMATOSTATIN RECEPTORS SCINTIGRAPHY PRIORITIZATION CRITERIA BASED ON NET PATIENT EPIDEMIOLOGICAL PROFILE: A BI-CENTRIC STUDY

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SOMATOSTATIN RECEPTORS SCINTIGRAPHY PRIORITIZATION CRITERIA BASED ON NET PATIENT EPIDEMIOLOGICAL PROFILE: A BI-CENTRIC STUDY (Abstract): This study aimed to create a generic profile of patients with NETs and to establish the prioritization criteria for somatostatin receptors scintigraphy (SRS), within the NETs diagnostic algorithm, being the first study of this kind conducted in Romania. **Material and methods:** A bi-centric, retro-prospective study comprising 103 patients with NETs, evaluated through ^{99m}Tc-TOC SRS, in two nuclear medicine departments from "Sf. Spiridon" County Clinical Emergency Hospital Iasi, Romania and Center of Nuclear Medicine, University Clinical Center of Kragujevac, Serbia. We analyzed the clinical-paraclinical features of included patients, as a starting point for streamlining the selection of patients for SRS. **Results and discussion:** The total group of patients included 47.57 % men and 52.43 % women, the age varying between 21-83 years. The most frequent primary NETs were represented by pNETs, and GI-NETs and the symptoms were nonspecific for majority of analyzed patients. Both prospective and retrospective analysis showed that SRS was followed by positive results. For prospectively analyzed patients, due to the used customized sheets, it was extremely easy to identify four SRS prioritization criteria. **Conclusions:** This study could outline the profile of patients with NETs, as the starting point for streamlining patient selection for SRS. The four prioritization criteria proposed by us could guide the diagnostic algorithm. **Keywords:** SOMATOSTATIN RECEPTORS SCINTIGRAPHY, NEURO-ENDOCRINE TUMORS.

As in the rest of the world, both in Romania and Serbia, the NETs incidence is relatively low and the nonspecific symptoms of onset are added to the diagnostic and to the therapeutic difficulties (1). Late presentation to the physician, followed by a diagnostic protocol not very well defined could contribute to the delaying a NET personalized diagnosis (2).

A minimally invasive functional imaging technique for diagnosing patients with NETs, using the prioritization criteria, could facilitate the personalized diagnostic process, with specific influences on NETs management. Nowadays, possible diagnostic errors could occur, followed by medical

ethics issues, because the imaging assessment of patients with NETs doesn't follow a very well-structured diagnostic algorithm. These observations were the basis of this study, which aimed to establish the prioritization criteria for scintigraphy of somatostatin receptors (SRS), within the diagnostic algorithm, for patients with NETs.

Our primary objective was to create a generic profile of patients with NETs, by analyzing the clinical-paraclinical features, as a starting point for streamlining the selection of patients for SRS. Secondary, the post-PRRT nephrotoxicity was analyzed. It is the first study of this kind conducted in Romania, in connection with the functional imaging of NETs.

The final results could lead to the adoption of the prioritization criteria in the current medical practice or even their implementation in the future NETs screening.

MATERIAL AND METHODS

We conducted a bi-centric, retro-prospective, study, between January 2020-May 2021, on NET patients, who performed ^{99m}Tc-SRS for diagnosis in two departments of nuclear medicine: "Sf. Spiridon" County Clinical Emergency Hospital Iasi, Romania and Center of Nuclear Medicine, University Clinical Center of Kragujevac, Serbia.

The study was included in a Ph.D. study approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi.

For all patients, the same inclusion/exclusion criteria were applied (tab. I).

TABLE I
Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
-diagnosed NETs	-the patient's refusal
-morphologically imaging evaluated within three months	-hypersensitivity to HYNIC- [D-Phe1, Tyr3-Octreotide] trifluoroacetate/another excipient
-positive biochemical NETs markers – patients > 18 years old	-hypersensitivity to sodium pertechnetate solution
-no surgery before SRS	-inflammatory digestive diseases
-no SA before/during the SRS	-pregnancy, breast-feeding

The patients were divided in two groups: group 1, prospectively analyzed (27 patients) and 2, retrospectively analyzed (80 patients). From 107 initially included patients, were finally selected 103 cases of NETs (fig. 1), four patients not being eligible due to the exclusion criteria met.

In the prospective analysis, which finally included 25 patients, we developed "*^{99m}Tc-TOC SRS personalized file*", a personalized electronic monitoring sheet, where we included personalized pts. Using these, we identified and proposed four ^{99m}Tc-TOC SRS prioritization criteria (tab. II).

Patients' preparation: discontinuation of treatment before ^{99m}Tc-TOC scan: 2-3 days for short-acting and 3-6 weeks for long-acting somatostatin analogues; in significant renal impairment, ^{99m}Tc-TOC is not recommended because the reduced /absent renal function will lead to greater radiation exposure; in hepatic impairment, no dose reduction is required; when the abdomen was the area of interest, the use of laxatives was considered in the evening before and in the evening following tracer's administration and a liquid-based diet two days before SRS (3).

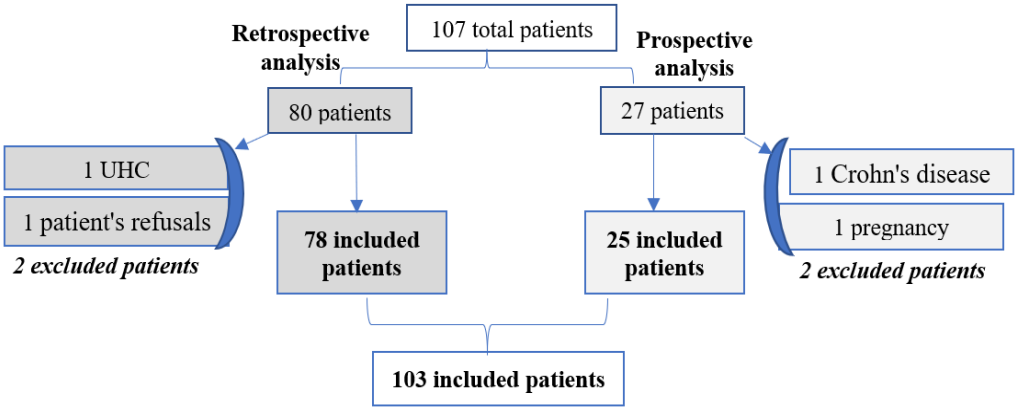


Fig. 1. The process of selecting patients with NETs
Legend: UHC (ulcerative hemorrhagic colitis); somatostatin receptor scintigraphy (SRS)

TABLE II
SRS prioritization criteria

Criteria 1	Criteria 2	Criteria 3	Criteria 4
-surgery -positive histology and minimum two positive IHC markers -negative morphologi-cal imaging (3 months post-surgery)	-without surgery -metastasis (structural imaging) -positive biochemical markers at two successive assessments -negative morphological imaging for primary NETs	-without surgery -suggestive symptomatology -metastasis (structural imaging) -negative morphological imaging for primary NETs	-suggestive symptomatology -positive biochemical markers at two succes-sive assessments; -negative morphological imaging for primary NETs

Two gamma cameras, low-energy, all-purpose, parallel hole collimator, energy window of 20%, set to 140 keV +/- 15% were used: SIEMENS E.CAM signature series, Dual-Head, Variable Angle, Cardio, 2007, Siemens, Medical Systems Inc. USA (Romania) and SIEMENS E.CAM, Dual-Head, Variable Angle, Syngo, 2006, Goldseal, Siemens, Medical Systems Inc. USA (Serbia).

Imaging protocol: In prospective analysis, the images were obtained early, at 2, 4 and 24 h and in retrospective analysis at 4 h after tracer administration: whole body (256 x 1024 matrix, 6 cm/min bed movement), planar localized (256 x 256 matrix), SPECT (128 x 128 matrix, 180° rotation detector head, 132 images, 1 image/second) images.

PRRT nephrotoxicity assessment: For patient who performed at least one single PRRT cycle, the renal function was evaluated through ^{99m}Tc-DTPA dynamic scans.

RESULTS

Prospective study

The age of the patients varying between 30-69 years, with a mean of 51. The distribution of NETs by age showed NETs' increased after 40 years old, with a maximum in the 60-69 years age group (36%). Analyzing by gender, the same increase in incidence was observed after the age of 50 in both categories, the number of female

patients exceeding the male ones in the age groups 50-59 and 60-69 years (fig. 2).

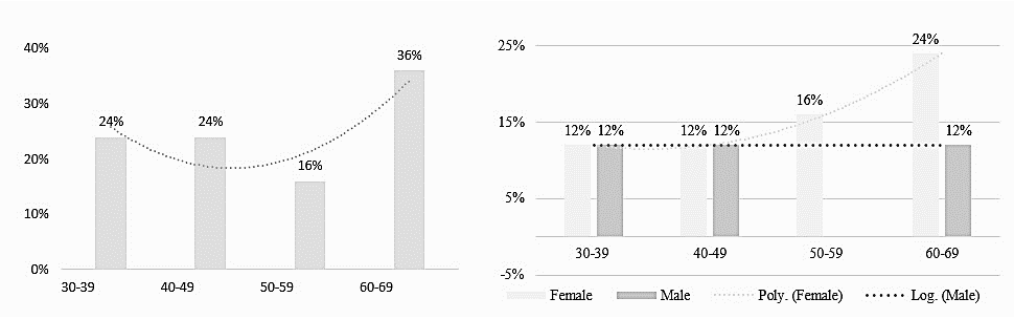


Fig. 2. Incidence of NETs by age groups. Incidence of NETs by age groups and gender

The most frequent diagnosis was GI NETs and more rare diagnosis Pheo (fig. 3).

Regarding the primary NETs sites, the pancreas was the most frequent primary site (40%), unlike adrenal gland which was less represented (4%), the thyroid and ileum primaries represented 32 % and duodenum and lung 24 %.

In all patients, the symptoms were non-specific, the most common symptoms being abdominal pain (44%), abdominal bloating (52%), transit disorders (28%), flushing (16%), weight loss (9.52%).

Although all patients were evaluated by ultrasound, at 88% of them the results were irrelevant in supporting the diagnosis of NETs. The entire group of patients was evaluated through CT imaging, but in 44% of cases were negative CT results. Five patients were evaluated by MRI, and only one patient had a pathological MRI image (right intrasellar pituitary adenoma).

48% of the patients had known or newly diagnosed associated pathology. The hypertension was observed in 58.3% of all patients and type II diabetes mellites in four cases.

Diagnosis	*No of patients*	*% of patients*
pNETs	n = 6	24%
GI NETs	n = 5	20%
Gastrinoma	n = 4	16%
MTC	n = 4	16%
Lung	n = 3	12%
Insulinoma	n = 2	8%
Pheo	n = 1	4%

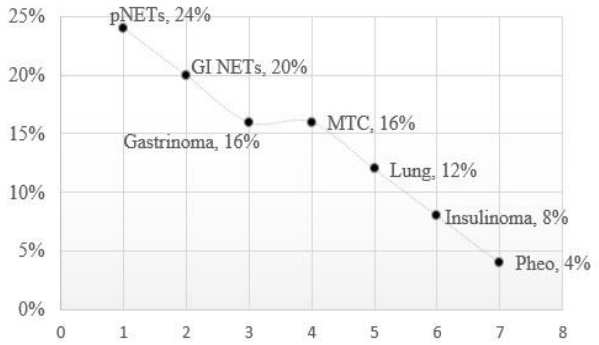


Fig. 3. Frequency of different types of NETs

SR expression

SRS had a sensitivity of 95.65% and a specificity of 50%, 22 out of 25 patients included in the study having ^{99m}Tc -TOC positive lesions.

^{99m}Tc-TOC SRS revealed a pharmaco-

kinetic profile, SR presence being evaluated due to the activity tracer concentration. Below, we present some serial images: early, at 2, 4 and 24 hrs. after injection, on which the uptake pattern can be visualized (fig. 4). The tumor uptake was seen early, at 2, and 4 hrs., at which time muscle and blood activity was markedly reduced. At 24 hrs., the pathological lesions were still visible, although the image quality is poor due to the low count rate produced by the rapid decay of ^{99m}Tc.

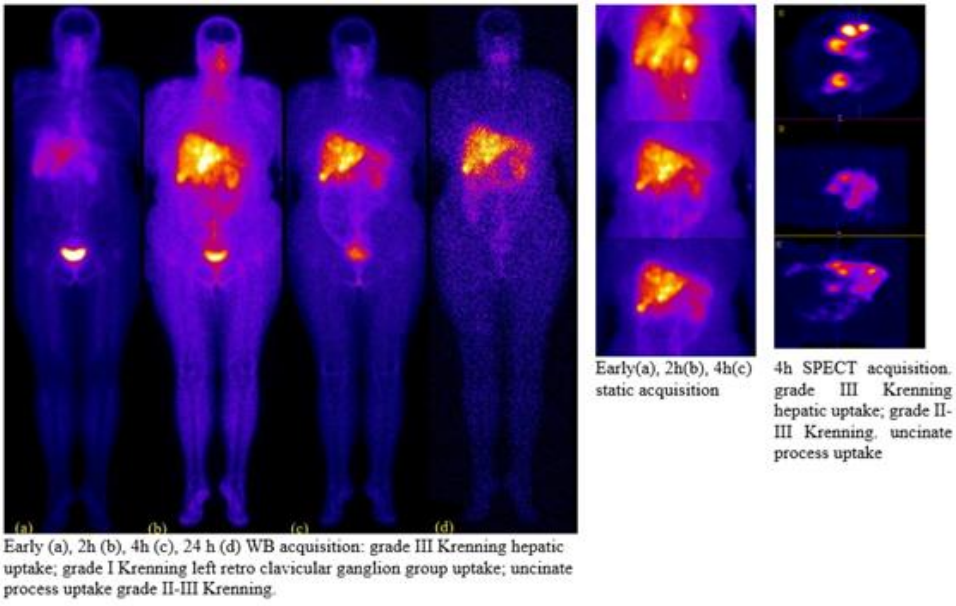


Fig. 4. Serial images: early, at 2, 4 and 24 hours after injection.

The distribution regarding the four prioritization criteria, was as follows:

- ten patients were included in Criteria 1: personalized patient files included surgery performed as the first therapeutic intention, in order to eradicate the primary NETs (five enterectomies, two pancreatectomies and three thyroidectomies), increased values of NE markers (Cg A, 5-HIIA, serotonin, calcitonin), for which morphological imaging examinations, performed at least 3 months post-surgery, could not objectify the presence of primary NETs;

- one patient with lung NET was included in Criteria 2: high values of Cg A, structural imaging showed only hepatic disseminations, without highlighting the primary pulmonary site of NET;

- nine patients met Criteria 3: personalized records of these nine patients showed the symptoms suggestive of NETs, negative structural imaging methods performed to identify the primary NET site and no history of surgery;

- the last five patients fulfilled Criteria 4: symptoms suggestive for NET but with negative neuroendocrine biochemical markers and positive structural imaging for the presence of secondary but negative

results for primary NETs.

Retrospective analysis

From 80 consecutive patients with NETs, two patients were excluded from the analysis (one patient refused the SRS and another one had ulcerative hemorrhagic colitis) and 78 patients constituted the final population. The group of patients was formed by 51.28% male, respectively 48.71% female, and the average age was 59.18 +/- 11.53 years. The most frequent diagnosis was represented by GI NETs and more rare diagnosis by Pheo (fig. 5).

Diagnosis	*No of patients*	*% patients*
GI NETs	n = 24	30.77 %
PNETs	n = 17	21.8 %
Lung	n = 14	17.9 %
MTC	n = 13	16.66 %
Rare NETs	n = 6	7.69 %
Pheo	n = 4	5.13 %

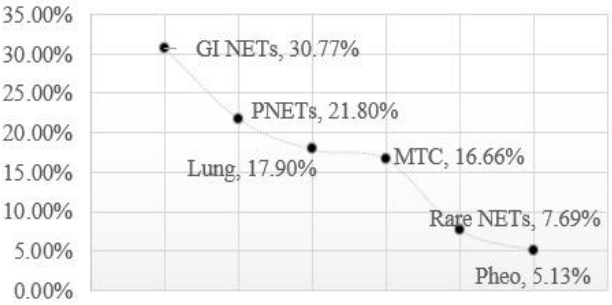
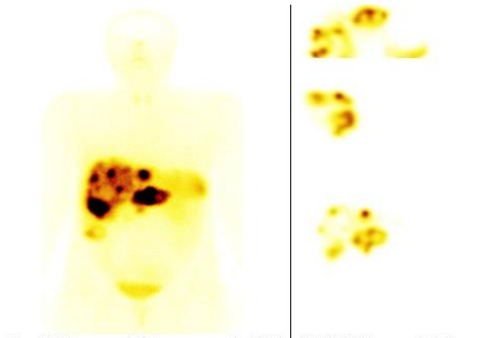


Fig. 5. Frequency of different types of NETs

Regarding associated pathology, 12.82% of the patients had known or newly diagnosed associated pathology. Eight of ten patients had synchronous neoplasms, one patient had Hashimoto's thyroiditis and the last one had iron deficiency anemia.

SR expression



4h WB acquisition: grade IV Krenning pancreatic uptake; liver metastasis | SPECT acquisition

Fig. 6. pNETs

SRS had a sensitivity of 97.44% and a specificity of 50%. Below, we presented some images from NETs patients on which the uptake pattern is visualized (fig. 6).

PRRT ^{99m}Tc-DTPA nephogram analysis

The three patients treated with PRRT, renal function was monitored by ^{99m}Tc-DTPA nephograms.

Case 1: nephrogram showed minor changes, with discrete elongated elimination for both kidneys; a moderate tracer retention in both kidneys, on late post-micturition renal static imaging.

Case 2: nephrogram showed a slight decrease in mGFR.

Case 3: nephrogram showed a decrease in mGFR and moderate elongation of the nephrographic curves of both kidneys; moderate tracer retention in the middle calyx of the right kidney.

DISCUSSION

NETs are generally quite rare tumors,

known to have low incidence of 1-5 / 100,000 (4) and prevalence of 35 / 100,000/year (5) in the general population. However, nowadays increased studies are beginning to show an increase in the incidence of NETs.

Our NET epidemiological study realized in Romania shows that NET is still a much-underdiagnosed pathology, with an obvious incidence increase in these two centers, in accordance with the data literature. The results confirm the generic profile of the patient with NETs, presented in the literature: age over 50 years, high BMI, a general condition with comorbidities, a history of surgery and primary NETs located especially in the digestive tract, pancreas, thyroid and lung (6).

In both countries, the annual number of new cases has increased quite a lot in recent years, this increase being related also with the possibility of performing SRS. Analyzing the results, we noticed that in both centers, the highest number of NETs appears in the 57-67 age group and that the incidence of NETs in the female population in Romania was higher than in Serbia.

One of the findings of our study is that the age of the patients correlates with the incidence of NETs. The literature describes a maximum incidence after the age of 65 years old (7), fact also observed in our study.

Observing the smaller percentage of patients with NETs aged under 40 years (24%), it can be stated that the incidence of NETs in young adult patients is relatively low. However, we believe that attention should be paid to people aged under 40 years, even if, in absolute values, their representation in the total number of patients is not very high. Currently, no screening is performed for NETs and physicians tend not to consider people under 40 years in the risk category for the occurrence of NETs (taking also into account the current difficulty of guiding the diagnostic process). Furthermore, the low incidence may be caused by the low addressability of patients with NETs to the physician, due to the extremely nonspecific incipient NETs symptoms.

Due to this fact, patients with NETs could be diagnosed more than seven years after the onset of symptoms.

Nonspecific NET symptomatology is an impactful factor in the management of the patient with NETs, making the diagnostic process very difficult, for the doctor being quite difficult to orient the diagnostic process in the right direction.

The clinical status of the patient, along with the presence of possible associated comorbidities, are important elements to consider in the diagnostic process. There are studies in the literature that show that high BMI and an existing metabolic syndrome contribute to increased incidence of NETs (8). In our research, 19 patients out of 25 had BMI above the upper limit, one of the patients even suffering of morbid obesity.

In both analyzes, the first two places were ranked with the same two types of NETs, GI-NETs and pNETs, and the last place by Pheo. As an associated pathology, prospective analysis showed that some of the included patients had hypertension or diabetes mellitus; retrospective analysis showed that a fairly high percentage of patients had synchronous neoplasms.

Prospective analysis showed clearly that SRS was followed by positive results, even when the structurally imaging investigations were irrelevant from NET diagnostic point of view.

The use of customized sheets for all 25 prospective analyzed patients made extremely easy the inclusion of patients with

NETs in one of the four prioritization criteria, and implicitly to achieve, more easily, their correct management.

In the studied group of PRRT nephrotoxicity, the nephrograms showed a slight decrease in GFR, with the elongation of the elimination phases of the curves, confirming the data literature (2).

CONCLUSIONS

Starting from the analysis of the database created within the Romania-Serbia collaboration, the conclusions of this study can outline the profile of patients with NETs, as the starting point for streamlining patient selection for SRS. The four prioritization criteria proposed could guide the diagnostic algorithm, being able to contribute to the orientation of the investigating physician towards imaging investigations with decisive request, ^{99m}Tc-TOC SRS.

A standardized and well-oriented diagnostic process could positively impact the NETs diagnosis costs, reducing them only as absolutely necessary expenses, and eliminating diagnostic interventions which are consuming any kind of resources: financial, time, medical staff.

This conclusion is of particular importance for the possibility of implementing SRS prioritization criteria that could completely change the diagnostic and therapeutic personalized management for NET patients.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SOFTWARE APPLICATION FOR ESTIMATING BIOMECHANICAL PARAMETERS OF THE HUMAN BODY

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SOFTWARE APPLICATION FOR ESTIMATING BIOMECHANICAL PARAMETERS OF THE HUMAN BODY (Abstract): Knowledge of the biomechanical parameters of human body segments is necessary for solving various problems in biomechanics and applications in this field. This paper presents a software tool designed for estimating biomechanical parameters of the human body, such as length of segments, center of mass, and radius of gyration, using anthropometric data of body mass and height. **Material and methods:** The software tool was designed and implemented in LabVIEW™ and was developed to determine the values of biomechanical parameters in the study of the human body, to facilitate the design of elements of orthoses, prostheses, exoskeletons, and robots. The application performs automatic calculations of biomechanical parameters of interest, based on anthropometric tables made by different investigators. **Results:** The result is a graphical interface that allows the introduction of general data of the human subject to be analyzed or an anatomical model, an interface that includes specific digital areas to display different values of anthropometric data and automatically calculated biomechanical parameters. The software was tested using different input values, and the results were compared with the data developed by classical calculation methods. **Conclusions:** Developing an experimental method that provides precise positioning of such important data as the center of gravity of the body segments and that can be widely available to scientists may allow further development in their research, without needing to use sophisticated or time-consuming equipment. The application developed by us performs automatic calculations of biomechanical parameters of interest, based on anthropometric tables made by different investigators, and can be a very useful tool for researchers. **Keywords:** MASS MOMENT OF INERTIA, RADIUS OF GYRATION, BIOMECHANICS, ORTHOSIS, DESIGN.

From a biomechanics viewpoint, human morphology can be described in terms of the following problems: biomaterials, structure, construction, geometry, and inertia (1). According to Erdmann, mass, density, the radius of the center of mass, the moment of mass (static moment), the moment of inertia, the radius of gyration, and deviation moment are inertial quantities and are known as anthropometric data (1, 2). Among the above quantities, the Mass Moment of Inertia (MOI) is of particular importance in the study of the biomechanics of the human body. Mass, Centre of Gravity (COG) and radius of gyration of each body segment are the inertial quanti-

ties required for the estimation of MOI.

An anthropometric table, useful in determining these quantities, has been generated over time, following the results of research on corpses and living subjects by several investigators: Borelli - 17th century, Weber brothers - 1836, Harless – 1860 (2), Braune and Fisher - 1889, Dempster - 1955, Clauser *et al.* – 1969, but also others (3, 4). The data compiled by Braune and Fisher were the most accurate and used by scientists.

According to Clauser (3), in 1936, Steinhausen reported a series of attempts by contemporary researchers to develop data on the weight of anatomical segments and the center of mass on living subjects. He quoted a work by Hebestreit (unpublished) that worked with a modified Borelli balance.

This device was first attributed to Borelli (1679) and later modified by du Bois-Reymond and Basler (1900 and 1931, respectively). The composition of the device consists of a rigid plate supported by an edge of the knife at one end and a sensitive dial scale at the other end (fig. 1) and was used in their studies on the total center of mass of the human body.

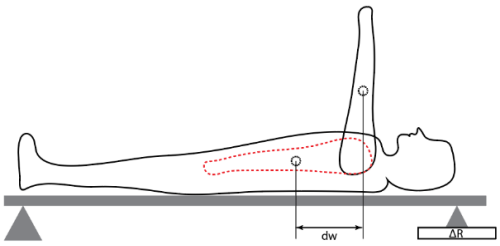

Fig. 1. Estimation of a segment's weight by the method of reaction change (Adapted from C. Clauser - 3)

The subject to be measured is positioned on the support panel of the device and knowing the weight of the subject and the distance between the supports, the center of mass of the subject can be determined by monitoring the reaction of the scales to body weight.

For example, equation 1 is used to determine the forearm weight:

$$W = \frac{D \cdot (\Delta R)}{dw} \quad (1)$$

W – Weight of forearm;

ΔR – Difference between scale readings;

D – Distance between supports;

d_w – Displacement of center of mass of forearm.

For this method to be useful for accurately determining the weight or center of mass of body segments, Bernstein concluded that the center of mass of a segment could be considered coincident with its center of the volume, since the volume and center of volume of the of a segment can be determined experimentally live. This idea was put into practice by studying frozen corpses, in order to eliminate the errors resulting from the leakage of fluids from the body (3).

In 1955, Dempster generated data on weight, volume, COG, and MOI for each segment of the human body (fig. 2). The limb segments were separated at each of the primary joints and the torso was divided into shoulder, neck, thorax and an abdominopelvic unit. The segmentation plans were similar to those established by Braune and Fischer, except that before dismemberment, the joints were flexed, which Dempster believed would provide an equitable distribution of tissue mass in each segment. After flexion, the joints were frozen before being bisected. After sectioning, each segment went through a series of five steps:

1. the segment was weighed;
2. the center of mass of the straight-

ened part was established on a balanced plate;

3. the oscillation period was determined (for the subsequent determination of the moment of inertia);

4. the volume was measured by the Archimedes method;

5. the parts were then re-frozen and prepared for further segmentation.

The segmental centers of mass were located using a balanced plate specially designed for Dempster's study (3).

In 1957, Bartner used data obtained by Braune & Fischer, Fischer and Dempster to estimate the weights of body segments from a subject's body weight (4).

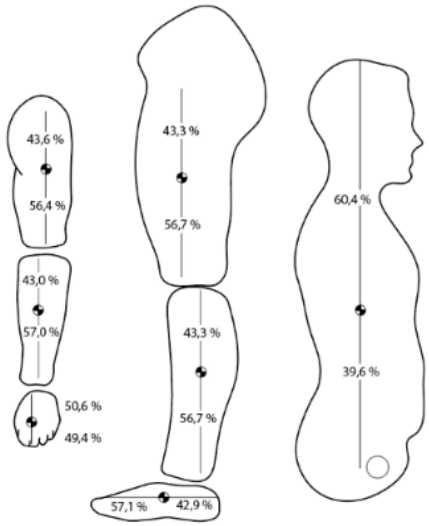

Fig. 2. Location of mass centers of body segments (Adapted from W. Dempster - 7)

The studies of Santchi *et al.* demonstrated that in a three-dimensional body such as the human body, several axes can pass through the COG, so the results lead to several MOIs (approximately three orthogonal axes defined as the intersection of three anatomical planes of the body, ie sagittal, frontal and transversal) (2). In a uniform field of gravity, the center of mass (COM) is the same as the center of gravity (COG) (5).

Knowing the biomechanical parameters of a subject's body segments (e.g., mass, volume, center of mass, mass moment of inertia) facilitates an analysis of motor activity and thus emphasizes how it can perform high-performance motor functions safely. It is important to find an experimental method that provides a precise positioning of such important data as the center of gravity of the body segments, widely available to scientists and that allows further development in their research, without the need of sophisticated or time-consuming equipment.

A good knowledge of the parameters of the body segment helps to facilitate optimal design of elements of orthoses, prostheses, exoskeletons, and robots.

This paper presents a software tool that we designed for estimating biomechanical parameters of the human body, such as length of segments, center of mass, and radius of gyration, using anthropometric data of body mass and height.

MATERIAL AND METHODS

The software application for the automatic calculation of biomechanical parameters of interest was made based on anthropometric tables made by different investigators. For example, Table I contains segment weights (head and neck, torso, upper arm, lower arm, hand, thigh, calf, foot) as a percentage of body weight reported by different investigators (Harless, Braune and Fisher, Fisher, Dempsters, Clauser *et al.*, Hall) (2). Table II contains segment lengths as a percentage of body height as compiled by Hall, and segment COG expressed as a percentage of segment length is provided in Table III (2, 6).

TABLE I

Segment weights as percentage of body weight (2, 6)

	Anatomic segment							
Investigators	**Head & neck**	**Torso**	**Upper arm**	**Lower arm**	**Hand**	**Thigh**	**Calf**	**Foot**
Harless	7.6	44.2	3.2	1.7	0.9	11.9	4.6	2.0
Braune &Fischer	7.0	46.1	3.3	2.1	0.8	10.7	4.8	1.7
Fischer	8.8	45.2	2.8	-	-	11.0	4.5	2.1
Dempster	8.1	49.7	2.8	1.6	0.6	9.9	4.6	1.4
Clauser *et al.*	7.3	50.7	2.6	1.6	0.7	10.3	4.3	1.5
Hall	8.2	46.84	3.25	1.8	0.65	10.5	4.75	1.43

TABLE II

Segment length as percentage of body weight (2)

Segment	Length in percentage
Head & neck	10.75
Torso	30.00
Upper arm	17.20
Lower arm	15.70
Hand	5.75
Thight	23.20
Calf	24.70
Foot	14.84

TABLE III

Distance of segment centre of gravity from proximal end as percentage of segment length (2)

Segment	COG distance in percentage
Head & neck	56.7
Torso	56.2
Upper arm	43.6
Lower arm	43.0
Hand	46.8
Thight	43.3
Calf	43.4
Foot	50.0

These data were implemented in LabVIEW™ (Laboratory Virtual Instrumentation Engineering Workbench), a platform and a development environment for the visual programming language from National Instruments (8).

Thus, the program generates a series of estimated results regarding the values of the moment of inertia, of the radius of gyration and of the positioning of the center of gravity of each segment. These results are calculated based on the general information of the human body (mass and height) which is entered by the user of the software.

A Graphical User Interface (GUI) is an interface between the user and electronic devices such as computers, portable devices, electronic devices, and some medical or non-medical equipment. To present all available information and actions, a graphical interface provides icons and visual indicators, as opposed to text-based user interfaces, which provide command names to be entered or scanned in the text (9, 10). It is intended to facilitate the user's work by eliminating the process of writing software code or entering repetitive data, and thus reducing the risk of errors by the user of the tool, which can sometimes lead to erroneous results. Due to the advantages of graphical interfaces, this concept was also used in this paper.

RESULTS AND DISCUSSION

The resulting graphical interface consists of a control panel and input of general data about a subject or anatomical model, a panel for displaying different values of anthropometric data and a panel displaying the calculated biomechanical parameters, as follows:

a) **Subject parameter control panel** is the area where the user of the application enters the general data that refers to the mass (kg) and height (m) of the subject (fig. 3);

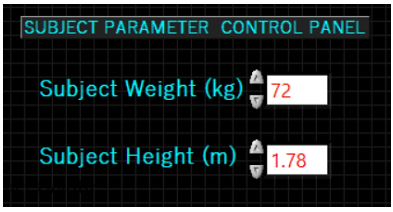

Fig. 3. Subject parameter control panel

b) **Anthropometric data display panel** displays the values of mass (kg) and length (m) of the segments of the human body with reference to the points a, b, c, d, e, f, g, h (fig. 4). This area of the application is for viewing only, not for control.

c) **Biomechanical parameters display panel** allows the visualization of the values of the biomechanical parameters calculated with the anthropometric data obtained in panel b). Similar to the previous one, panel c) displays the results for Radius of Gyration – *k* (m), Mass Moment of Inertia – *I* (kg·m^2), Centre of Gravity Location – *COG* (m). The first two sets of values are calculated for three planes of the human body, namely sagittal, frontal and transverse (fig. 5).

d) The complete interface of the application also contains a graphical representation of the human body with references for each body segment for easy orientation, as follows: a – head and neck; b – torso; c – upper arm; d – lower arm; e – hand; f – thigh; g – calf; h – foot (fig. 6).

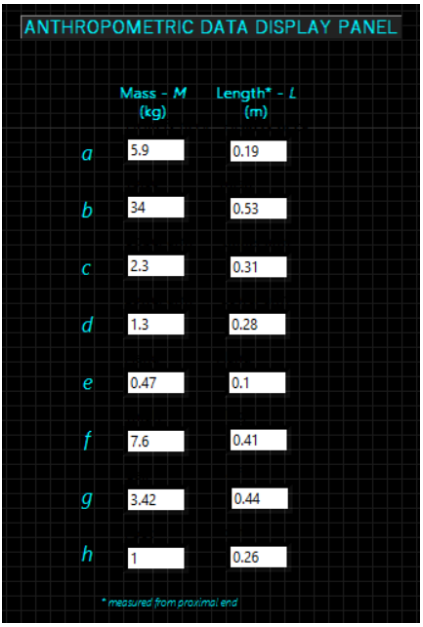

Fig. 5. Anthropometric data display panel

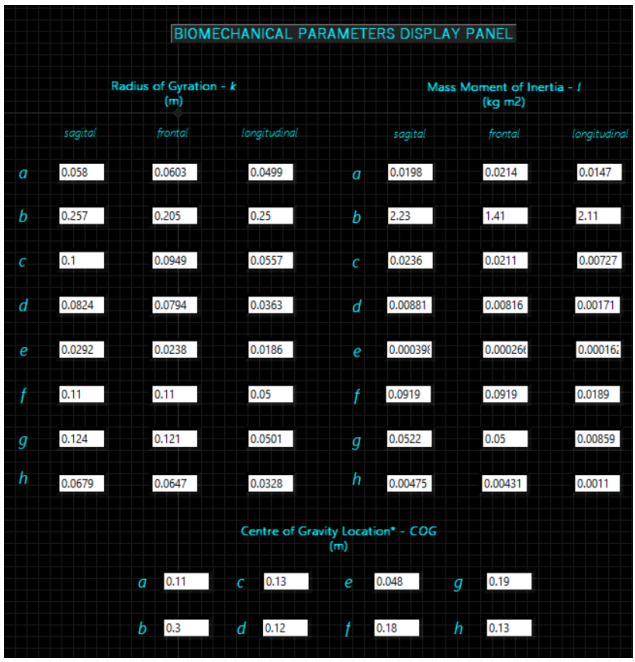

Fig. 6. Biomechanical parameters display panel (includes values for Radius of Gyration – *k* (m), Mass Moment of Inertia – *I* (kg·m^2), Centre of Gravity Location – *COG* (m))

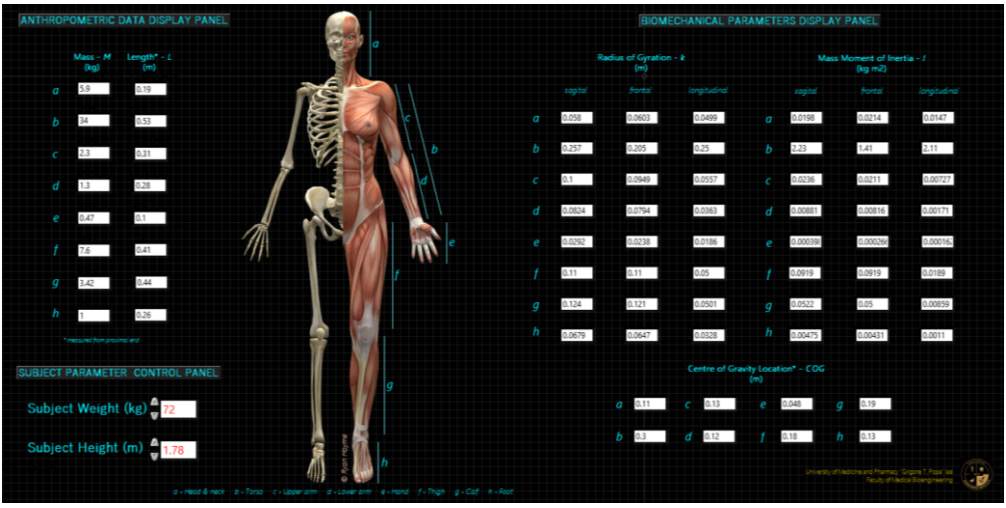

Fig. 7. Graphical user interface of the application

For parameters relating to the length of the human body segment and the location of the center of gravity, the measurement shall be made from the proximal end of the corresponding segment.

To validate the values calculated through the developed application, a group of 18 subjects was analyzed. The subjects were

weighed, and their height was measured. In this verification we were interested in the values of the length of three segments (thigh length - f, calf length - g and leg length - h).

These values are necessary for the design and development of knee braces. Table IV shows the 18 subjects examined, the values measured directly on the patient and the values calculated using the software program developed.

TABLE IV

Comparison between the values of the measured segment lengths and the determined ones (MV-measured values, DV-determined values)

Subject ID	Weight (kg)	Segment length (m)			
	Height (m)		f	g	h
S1 - CF	69.00	MV	0.43	0.445	0.265
	1.70	DV	0.42	0.44	0.27
S2 - IF	59.00	MV	0.40	0.42	0.245
	1.69	DV	0.39	0.42	0.25
S3 - AF	59.00	MV	0.39	0.41	0.24
	1.65	DV	0.38	0.41	0.24
S4 - MA	58.00	MV	0.40	0.42	0.23
	1.68	DV	0.39	0.41	0.25
S5 - FM	81.00	MV	0.40	0.42	0.24
	1.69	DV	0.39	0.42	0.25
S6 - AM	62.00	MV	0.35	0.38	0.23
	1.56	DV	0.36	0.39	0.23
S7 - MV	90.00	MV	0.41	0.44	0.26
	1.77	DV	0.41	0.44	0.26
S8 - MP	110.00	MV	0.45	0.47	0.29
	1.90	DV	0.44	0.47	0.28
S9 - LC	69.00	MV	0.40	0.40	0.25
	1.66	DV	0.39	0.41	0.25
S10 - LP	86.00	MV	0.40	0.42	0.25
	1.75	DV	0.41	0.43	0.26
S11 - CF	94.00	MV	0.43	0.44	0.26
	1.83	DV	0.42	0.45	0.27
S12 - EF	70.00	MV	0.36	0.38	0.23
	1.58	DV	0.37	0.39	0.23
S13 - CI	75.00	MV	0.38	0.42	0.25
	1.69	DV	0.39	0.42	0.25
S14 - CM	80.00	MV	0.43	0.45	0.25
	1.85	DV	0.43	0.46	0.27
S15 - VP	97.00	MV	0.41	0.39	0.23
	1.63	DV	0.38	0.40	0.24
S16 - LG	99.00	MV	0.41	0.43	0.27
	1.71	DV	0.40	0.42	0.25
S17 - TT	38.00	MV	0.31	0.34	0.19
	1.40	DV	0.32	0.35	0.21
S18 - MP	97.00	MV	0.40	0.40	0.24
	1.66	DV	0.39	0.41	0.25

CONCLUSIONS

Experience in recent years has shown that the proper development of orthoses, prostheses, rehabilitation devices, and medical instruments requires a thorough understanding of the biomechanical principles of human body performance.

The design of rehabilitation devices such as active orthoses can be optimized for better customization and human-machine compatibility.

The application developed by us performs automatic calculations of biomechanical parameters of interest, based on anthropometric tables made by different investigators, and can be a very useful tool for researchers.

Knowing the biomechanical parameters of a subject's body segments (e.g., mass, volume, center of mass, mass moment of inertia) facilitates an analysis of motor activity and thus emphasizes how it can perform high-performance motor functions safely. It is important to find an experimental method that provides precise positioning of such important data as the center of gravity of the body segments, widely available to scientists which may allow further development in their research, without needing to use sophisticated or time-consuming equipment.

Given the growing interest in "controller/agent" medical systems, it is necessary to have a good knowledge of the parameters of the body segment to design the necessary medical equipment. The data can allow the design of more comfortable equipment and chairs, for example for the high comfort of the surgical specialist who is in charge of the "source" module of a medical device. Also, control systems, such as the pedals of a piece of equipment, can be customized according to the situation and the anthropometric data of the user. In many situations, it is necessary to simulate the human body, so it is necessary to manufacture a test dummy with real anatomical dimensions for self-crash studies on seat belts, for first aid maneuvers, robots for exploring dangerous spaces, or others.

In this application, there must be complete data of body parameters. The uses of body segment parameter data are indeed quite numerous. The complete evaluation of these quantities by using a software application can be an important contribution in the field of biomechanics.

CONFLICT OF INTEREST AND FUNDING

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NEWS

EARLY IDENTIFICATION OF CAROTID VULNERABLE PLAQUE IN ASYMPTOMATIC PATIENTS

Atherosclerosis (AS) is the root cause of cardiovascular and cerebrovascular diseases, including coronary artery disease and stroke. Atherosclerosis is an independent risk factor for cardiovascular and cerebrovascular diseases. Plaque rupture and subsequent thrombosis are the main causes of myocardial infarction and stroke. With the continuous improvement of people's living standards, the occurrence of cardiovascular and cerebrovascular events is showing a younger trend, and some senile diseases such as hypertension, cerebral infarction, and myocardial infarction appear earlier, increasing the mortality and disability rate of people. Therefore, early detection and effective intervention of patients with atherosclerotic cardiovascular and cerebrovascular disease (ASCCVD) is an important measure to reduce cardiovascular and cerebrovascular disease and its complications. AS is a systemic, long-term, slowly progressing inflammatory disease. The basic lesions are lipid deposition in the intima of the artery, focal fibrosis of the intima, and atheromatous plaque formation. Atherosclerotic plaques are divided into stable plaques and unstable plaques, unstable plaques are also called vulnerable plaques. The methods of identifying vulnerable plaques early include: multi-slice spiral CT, nuclear magnetic resonance, radionuclide imaging, optical coherence tomography, etc. There are few studies on early identification and intervention of carotid vulnerable plaques in patients with asymptomatic health checkups and there is no standardized diagnosis and treatment recommendations at present. The purpose of this study is to explore the influencing factors of atherosclerotic plaque formation and stability in patients with asymptomatic carotid atherosclerotic plaques. Also, the authors proposed to identify the vulnerable plaques and find the patients of asymptomatic ASCCVD earlier through combining the non-invasive carotid artery ultrasound and the detection of serum plaque stability markers so as to conduct the clinical intervention, delay the progress of plaque, avoid thrombosis and prevent the occurrence and development of ischemic cardiovascular and cerebrovascular diseases (Jiao Y, Qin Y, Zhang Z, *et al.* Early identification of carotid vulnerable plaque in asymptomatic patients. *BMC Cardiovascular Disorders* 2020; 20: 429).

DESIGN AND OPTIMIZATION METHOD FOR OBTAINING PIOGLITAZONE AND CURCUMIN-LOADED CHITOSAN NANOPARTICLES

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DESIGN AND OPTIMIZATION METHOD FOR OBTAINING PIOGLITAZONE AND CURCUMIN-LOADED CHITOSAN NANOPARTICLES (Abstract): **Aim**: The aim of the present study was to design and optimize the method used for preparation of CS NPs loaded with pioglitazone (P) and curcumin (C), as a new multi-target polymeric nanosystems, for type 2 diabetes mellitus therapy. **Material and methods**: CS NPs loaded with pioglitazone, curcumin and pioglitazone-curcumin were prepared using ionic gelation method. After setting the optimal parameters, the developed nanosystems (CS_P NPs, CS_C NP and CS_P_C NPs) were characterized in terms of morphological aspects, using Scanning Electronic Microscopy (SEM) and of average particle size and poly dispersity index, using Dynamic Light Scattering (DLS) measurements. In addition, the zeta potential values, an important parameter for physical stability of the NPs, was also studied. **Results and discussion**: The following parameters were set as optimal, which assure the obtaining of NPs with proper characteristics: CS 0.1% (wt/v):TPP 0.1% ratio of 3:1, tween 80 0.05% (wt/v), DMSO as solvent, sonication during 30 min, rotational speed of 1,400 rpm. **Conclusions**: The developed CS_P_C NPs are stable nanosystems with proper physical characteristics (PS = 215 nm, PI = 0.300, ZP = 11.13 mV), which support to continue our studies, using *in vitro* and *in vivo* assays, to prove their efficiency as multi-target polymeric nanosystems. **Keywords**: CHITOSAN, NANOPARTICLES, PIOGLITAZONE, CURCUMIN.

Diabetes mellitus (DM) is one of the most serious chronic diseases, which is considered an important public health problem and a social-economic one due to the increased prevalence from last decades (1). Worldwide, diabetes causes nearly 4 million deaths annually, while another 1.5 million new cases are diagnosed and it is estimated that in 2040 one in ten people will suffer from this disease (2).

The most common form of diabetes is type 2 diabetes mellitus (T2DM), representing 85% of diagnosed cases. Its prevalence having increased in recent decades because of the careless lifestyle of the people. Also, it is worrying that the incidence among children and youngers is increasing (3).

The chronic hyperglycemia is the major cause for serious complications in various organs or tissues. The complications can be acute (diabetic ketoacidosis, hypoglycemia) or chronic, microvascular (diabetic retinopathy, neuropathy and nephropathy, diabetic foot) and macrovascular (cardiovascular diseases) (4).

The treatment of T2DM, which is depends on the stage of disease, include different classes of drugs: sulfonylureas, biguanides, inhibitors of alpha-glucosidase, glinides, thiazolidinediones and modulators of incretins (agonists of GLP-1 receptors and inhibitors of DPP IV enzyme). Thiazolidinediones (pioglitazone, rosiglitazone and lobeglitazone) is a relatively new class of oral antidiabetic agents which acts as selective agonists on peroxisome proliferator-activated receptors-gamma (PPAR-gamma), thus improving the endogenous insulin sensivity in the liver, muscles and adipose tissue (5). Pioglitazone significantly decreases the glycosylated hemoglobin (HbA1c), reduces fasting and postprandial blood glucose levels, as well as improves pancreatic beta-cell function. Unfortunately, this drug has a low oral absorption, a low water solubility and a short half-life (3-6 h), which limit its efficiency.

It is known that the hyperglycemia generates reactive oxygen species (ROS) which can produce the destruction of the cellular structure. Moreover, it was proved that the oxidative stress acts as a mediator of the insulin resistance and glucose intolerance, being involved in the development of diabetes mellitus and its micro and macrovascular complications (6). In this context curcumin, a natural polyphenolic compound, has been shown to have significant antioxidant activity and a potential hypoglycemic effect in experimental diabetic animal models (7). Unfortunately, it presents some disadvantages including hydrophobicity, poor solubility in water, low absorption and faster metabolism in the gastrointestinal tract which contribute to its poor bioavailability (8).

In the last time the researchers focused their studies on nanotechnology field, as a new strategy for enhancing the solubility, improving the biological performance and decreasing the toxicity of the drugs. Some intensely studied nanosystems are nanoparticles (NPs) which are used as drug delivery systems due to small particle size (between 1-100 nm) and large surface area. These nanosystems present numerous advantages such as high stability, feasibility of incorporation of different drugs, even hydrophobic ones, and high carrier capacity. There nanosystems are able to reduce the doses frequency, to provide sustained and controlled release of encapsulated substances, to increase the stability, bioavailability and absorption of the drugs (9). In addition, they can be used for targeting drugs to cells and tissue through the capacity to penetrate into cells by endocytosis or receptor-mediated transcytosis (10, 11).

The chitosan (CS), frequently used to

develop NPs, is a natural polysaccharide which is considered the largest biomaterial, after cellulose, used in various medical and pharmaceutical applications (12). It attracted the interest of the researchers firstly due to its unique properties: biocompatibility, biodegradability and low toxicity and secondly due to its important biological effects in diabetes and its complications therapy including antioxidant, antimicrobial, hypocholesterolemiant and antitumoral effects (13, 14).

This paper aims to describe the optimization of the method used for preparation of CS NPs loaded with pioglitazone and curcumin, as a new multi-target polymeric nanosystem, able to increase the bioavailability of the loaded drugs and so improve the pharmacological effects, targeting T2DM.

MATERIAL AND METHODS

Materials: chitosan low molecular weight (CS LMW, molecular weight 50-190 kDa, 75-85% deacetylation degree, viscosity 20-300 cP), pentasodium tripolyphosphate (TPP), acetic acid (min. 99.8%, p.a. ACS reagent), tween 80, curcumin, pioglitazone, were purchased from Sigma Aldrich. All other reagents were analytical grade and were used as received without further purification.

Preparation of pioglitazone/curcumin/ pioglitazone-curcumin-loaded CS NPs

The drugs (pioglitazone - P, curcumin - C, pioglitazone-curcumin) were loaded into CS NPs using ionic gelation method (15). Briefly, CS LMW 0.1% (wt/v), was dispersed in acetic acid solution 0.15% (v/v) and the mixture was stirring for 2 h at room temperature to obtain a clear polymeric solution. To 3 mL of CS solution, pioglitazone (2 mg/3.5 mg), curcumin (5 mg/6 mg) or pioglitazone-curcumin (2 mg/5 mg, 3,5 mg/6 mg) dissolved in 0.5 mL of methanol or DMSO was added. Then 1 mL of TPP aqueous solution 0.1% (wt/v) was added drop wise with a micropipette under stirring. The formation of colloidal solution indicates the reticulation of NPs.

În order to optimize the reticulation method for obtaining stable pioglitazone-loaded CS NPs (CS_P NPs) and curcumin-loaded CS NP (CS_C NPs), different parameters were changed, CS 0.1% (wt/v):TPP 0.1% (wt/v) ratio (3:1, 3:1.5); presence or absence of surfactant, tween 80 0.5% (wt/v); presence or absence of sonication; type of solvent (methanol, DMSO) and the rotational speed (1,000 rpm, 1,400 rpm). The optimized parameters were applied further to obtain the pioglitazone-curcumin-loaded CS NPs (CS_P_C NPs).

Characterization of pioglitazone/curcumin/pioglitazone-curcumin-loaded CS NPs

The scanning electron microscopy method (SEM), using a Desktop SEM (Phenom, The Nederland), was applied to study the morphological aspects of the NPs. The average particles size (PS) of the colloidal NPs was determined using Dynamic Light Scattering (DLS) and the poly dispersity index (PI) was evaluated via light scattering measurements. The zeta potential (ZP) of the NPs was determined using a Easier Nano ZS90 instrument (Malvern Instruments, Malvern, UK) by placing NPs, after dispersing in distilled

water at 25°C, in disposable zeta cells. The electrophoretic mobility between the electrodes was converted to a zeta potential, depending on the Smoluchowski diffusion equation. All measurements were performed in triplicate.

RESULTS AND DISCUSSION

The influence of the concentration of the drug (pioglitazone, curcumin), CS 0.1% (wt/v): TPP 0.1% (wt/v) ratio, of type of solvent, of using or not of sonication, of presence or absence of tween 80 0.05% (wt/v), as surfactant and of the rotational speed, on formation of CS_P NPs and CS-C NPs respectively, in terms of particle size (PS) and poly dispersity index (PI) is presented in tables I and II.

TABLE I.

The parameters used to optimize the preparation of pioglitazone loaded CS NPs

CS 0.1%: TPP 0.1%	P (mg)	Tween 80 (%)	Sonication (min)	Solvent	Rotational speed(rpm)	PS (nm)	PI
3: 1	3.5/2	-	30	MeOH	1000	1382/1352	0.971
					1400	1362/1342	0.746
				DMSO	1000	1278/1261	0.902
					1400	1256/1239	0.839
3:1.5	3.5/2	-	-	MeOH	1000	3500/3800	0.831
					1400	3300/4550	0.927
				DMSO	1000	2980/2867	0.852
					1400	1982/2866	0.658
3: 1	**3.5/2**	**0.05**	**30**	MeOH	1000	1952/1848	0.792
					1400	1852/1749	0.845
				DMSO	1000	496/504	0.371
					1400	**254/217**	**0.349**
3:1	3.5/2	0.05	-	MeOH	1000	3251/3167	0.924
					1400	2951/2678	0.893
				DMSO	1000	2162/2063	0.932
					1400	1735/1620	0.841
3:1.5	3.5/2	0.05	30	MeOH	1000	382/327	0.426
					1400	362/349	0.303
				DMSO	1000	334/328	0.313
					1400	318/315	0.245
3:1.5	3.5/2	0.05	-	MeOH	1000	4429/2964	0.961
					1400	3400/3271	0.782
				DMSO	1000	2184/2093	0.951
					1400	2125/2084	0.872

TABLE II.

The parameters used to optimize the preparation of curcumin loaded CS NPs

CS 0.1%: TPP 0.1%	CUR (mg)	Tween 80 (%)	Sonication (min)	Solvent	Rotational speed (rpm)	PS (nm)	PI
3: 1	6/5	-	30	MeOH	1000	2711/2698	0.853
					1400	2192/2631	0.961
				DMSO	1000	1472/1392	0.482
					1400	1583/1429	0.531
3:1.5	6/5	-	-	MeOH	1000	5671/4713	1.067
					1400	6718/6451	1.326
				DMSO	1000	3692/3289	0.735
					1400	3029/3071	0.856
3: 1	**6/5**	**0.05**	**30**	MeOH	1000	503/471	0.462
					1400	391/375	0.412
				DMSO	1000	282/216	0.232
					1400	**277/234**	**0.286**
3:1	6/5	0.05	-	MeOH	1000	4723/4645	0.813
					1400	4524/4425	0.745
				DMSO	1000	3712/3152	0.956
					1400	3417/3267	0.942
3:1.5	6/5	0.05	30	MeOH	1000	794/756	0.451
					1400	731/714	0.531
				DMSO	1000	374/361	0.509
					1400	360/348	0.315
3:1.5	6/5	0.05	-	MeOH	1000	4521/4241	0.724
					1400	4276/4123	0.641
				DMSO	1000	3519/3281	0.524
					1400	3197/3278	0.471

In both cases, CS_P NPs and CS_C NPs, the optimal parameters were set as follow: CS 0.1% (wt/v):TPP 0.1% ratio of 3:1, tween 80 0.05% (wt/v), DMSO as solvent, sonication during 30 min, rotational speed of 1400 rpm. Using these parameters, the smaller NPs were obtained: 217 nm (CS_$P_{2\ mg}$ NPs), 257 nm (CS_$P_{3.5mg}$ NPs) and 234 nm (CS_C_{5mg} NPs) and 277 nm (CS_C_{6mg} NP), respectively (fig. 1). For CS_P_C NPs the recorded PS were 215 nm (CS_P_{2mg}_C_{5mg} NPs) and 382 nm (CS_$P_{3.5mg}$_C_{6mg} NPs), respectively. For all these NPs the smallest PI was recorded (fig. 1).

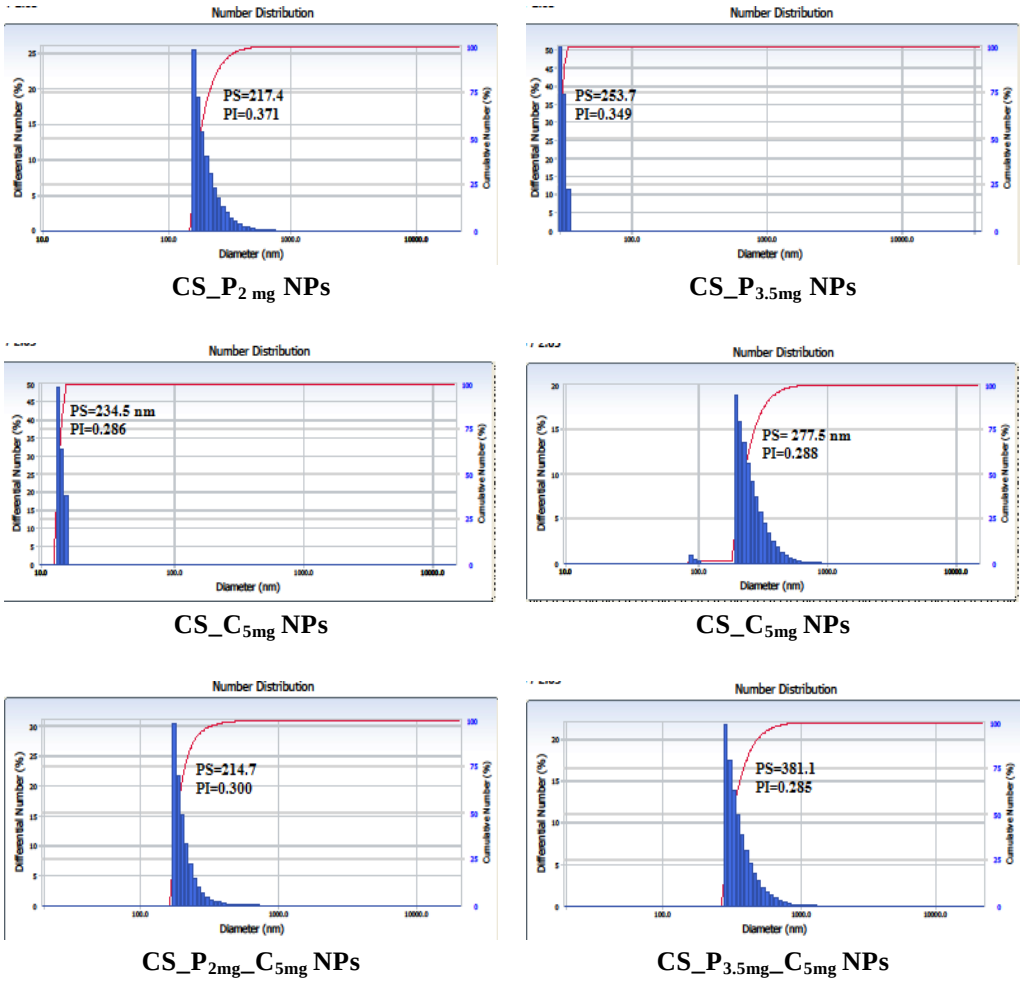
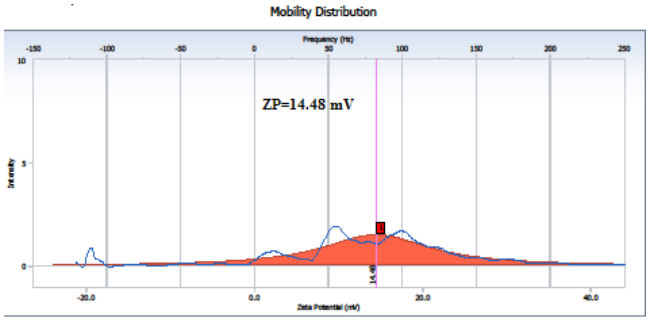
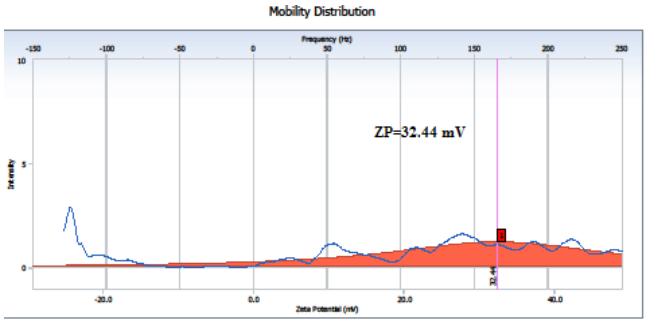


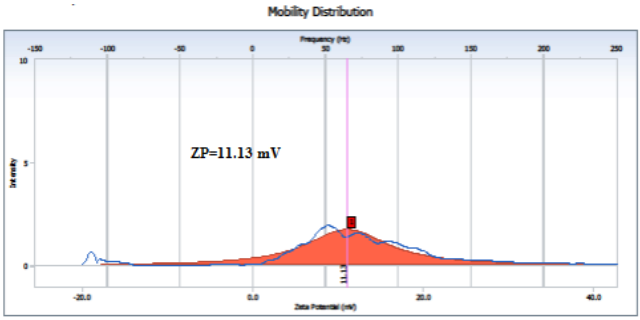
Fig. 1. Particle size (PS) distribution and poly dispersity index (PI) of optimized CS_P NPs, CS_C NPs, CS_P_C NPs at different concentrations.



CS_P$_{2mg}$ NPs



CS_C$_{5mg}$ NPs



CS_P$_{2mg}$_C$_{5mg}$ NPs

Fig. 2. Zeta potential (ZP) values for CS_P$_{2mg}$ NPs, CS_C$_{5mg}$ NPs and CS_P$_{2mg}$_C$_{5mg}$ NPs.

The physical stability of the developed NPs (CS_P NPs, CS_C NPs and CS_P_C NPs) was evaluated in term of ZP, a parameter closely related to the charge of the particles. It is well documented that the aggregation of the NPs is depending on following factors: the small particle size, the large surface contact and the free energy (15). The ZP value shows the repulsion degree between the electrically charged and dispersed NPs. A high ZP value means a certain charge value of the NPs which gives them a sufficient repulsive force to prevent the particle aggregation. If the ZP has a low value, the attrac-

tion force of the particles exceeds of that of repulsion and as result the particles adhere to each other to form agglomerates (8). The optimal value of ZP, which assure the stability of the NPs, what means a low risk for aggregation and increasing of the PS is between 20-40mV (11). As is observed from fig. 2 the ZP of the developed NPs was lower than 40 mV, that means they have increased stability. The ZP values recorded for these NPs was as follow: 14.48 mV (CS_P_{2mg} NPs), 32.44 mV (CS_C_{5mg} NPs) and 11.13 mV (CS_P_{2mg}_C_{5mg} NPs).

In addition, the SEM images (fig. 3) proved the formation of the NPs using the optimized parameters.

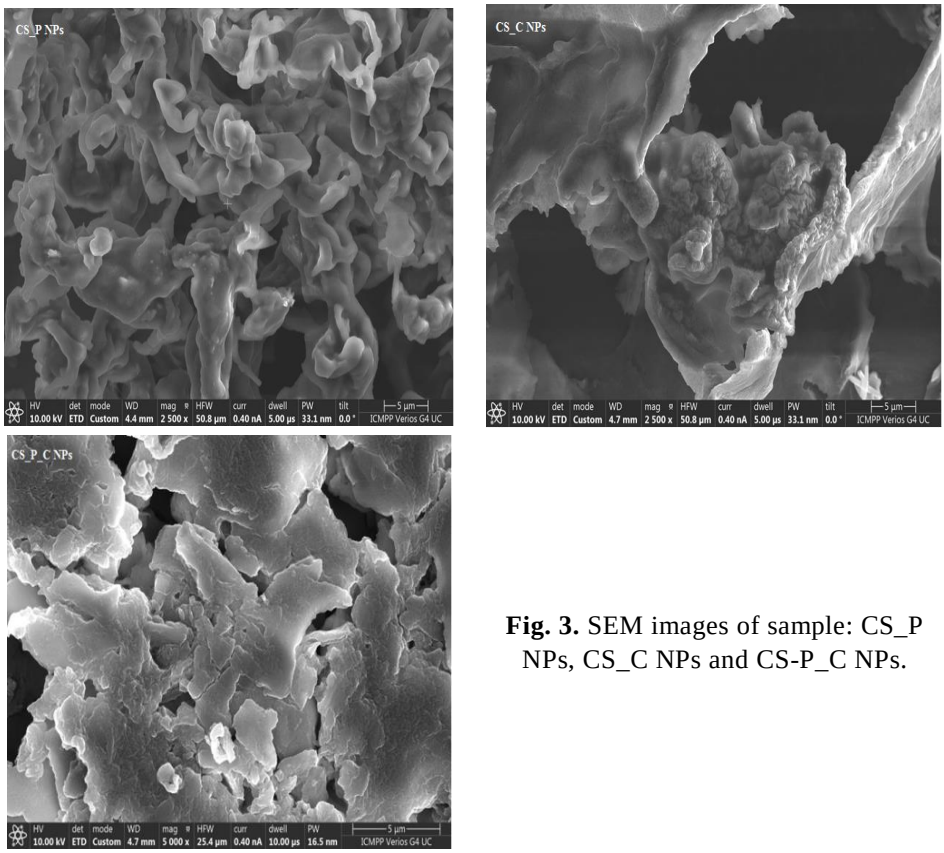


Fig. 3. SEM images of sample: CS_P NPs, CS_C NPs and CS-P_C NPs.

CONCLUSIONS

New CS NPs loaded with pioglitazone (CS_P NPs) and curcumin (CS_C NPs) were designed and their obtaining process was optimized, considering the size of the NPs and the poly dispersity index as key elements to successful preparation. The ionic gelation method, used for preparation, was optimized by changing different parameters such as: concentration of the drugs, polymer: reticulation agent ratio (CS:TPP), presence/absence of surfactant, presence/absence of sonication, type of solvent and rotational speed. Based on optimization process of CS_P NPs and CS_C NPs respectively, new CS NPs loaded with pioglitazone

and curcumin (CS_P_C NPs) were prepared and characterized. The results support the successful preparation of the CS_P_C NPs based on small particle size, proper poly dispersity index and low zeta potential, which assure the physical stability of the developed nanosystems. These nanosystems will be further tested, using *in vitro* and *in vivo* assays as new potential candidates for management of T2DM.

CONFLICT OF INTEREST AND FUNDING

Authors declare that there is no conflict of interest.

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CONTEMPORARY ASPECTS OF PROVISIONAL FIXED PROSTHESIS

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CONTEMPORARY ASPECTS OF PROVISIONAL FIXED PROSTHESIS (Abstract): Provisional prosthetics in the rehabilitation algorithm with fixed restorations has a number of particularly important roles in transmitting the data absolutely necessary to the dental laboratory for the achievement of the definitive work, which influences the healing of periodontal tissues and, definitively, the occlusal scheme and the resizing of the vertical occlusal dimension. The **aim** of this study is to analyze the factors involved in the choice of provisional prosthesis and the implications this has for the definitive prosthesis according to the particularities of each individual case. **Material and methods:** A total of 86 patients diagnosed with partially reduced edentulousness, i.e., coronal recessed dental lesions, were included in the study group and clinically and paraclinically evaluated for prosthetic rehabilitation by different types of definitive restorations from the current prosthodontic range, prefaced within the therapeutic algorithm of provisional restorations. **Results:** The current clinical-technological algorithm of rehabilitation with fixed restorations, provisional prosthetics represents an extremely important step in order to conform the periodontal tissue on the one hand, to protect the prepared substructures on the other hand, and last but not least to prefigure the shape of the future prosthetic rehabilitation in the context of dento-somatic-facial harmony. **Conclusions:** Prosthetic restorations made by the digital technique are extremely precise, their design is laborious considering all biomechanical aesthetic criteria so that we have individualized restorations that lead to successful final prosthetics. **Keywords:** TEMPORARY FIXED PROSTHESES, PMMA, DIGITAL APPROACH, AESTHETICS, GENERAL STATUS.

Over time, fixed prosthetics has gone through important evolutionary stages in terms of the biomaterials used and the technological line approached, with particular attention being paid to the therapeutic algorithm used in contemporary society. Thus, we cannot talk about fixed prosthesis without being preceded by provisional protection, this stage being particularly important both from a morphological and functional point of view (1).

Provisional prosthetics in the rehabilitation algorithm with fixed restorations has a number of particularly important roles in transmitting the data absolutely necessary to the dental laboratory for the achievement of the definitive work, which influences the healing of periodontal tissues and, defini-

tively, the occlusal scheme and the resizing of the vertical occlusal dimension. It is important to mention that the provisional prosthesis configures the correct morphology for the performance of the phonatory function at optimal parameters as well as for the design of the aesthetic aspects related to the shape and its integration into the dento-somato-facial harmony (2, 3).

Successful clinical outcome is dependent on a whole range of factors in which the effectiveness of the provisional protection is essential and is also a relevant starting point for the patient expectations related to prosthetic work in provisional prosthetic restorations may be of shorter duration or long-lasting, being recommended in different clinical situations where laborious therapeutic interventions are performed in the periodontal territory (4, 5, 6).

For a long time considered as an accessory, the provisional prosthesis has become, during the evolution of theoretical and practical knowledge and therapeutic resources, one of the "key" stages of fixed prosthetic restorations. A quality transitional prosthesis is a prosthesis that fulfils its roles: aesthetically, functionally, therapeutically.

The aesthetic role of the transitional restoration is the most spectacular result of the transitional prosthesis, the restoration has the advantage that it can be retouched in such a way that the laboratory receives a model that accurately reproduces the shape, volume, placement of convexities, accompanied by explanatory notes on color, shade, make-up (7, 8, 9).

The functional role of the provisional prosthesis includes three aspects: dental, periodontal, functional. Dental appearance refers to the morphological resolution of the edentulousness and the recovery of the primary function affected by the edentulousness: aesthetic for the frontal area or masticatory for the lateral area. With preparation, the biomechanical value of the teeth decreases, whether vital or not, mechanical protection against chemical or microbial aggression must be ensured. The provisional prosthesis acts as a barrier between the prepared tooth and the oral environment. A tight denture-prosthetic seal, even if it is provisional, is a conditioning factor for isolation from the oral environment. Although imperfect, thermal insulation allows the vitality of the teeth to be maintained, thereby also contributing to patient comfort. Occlusal aspect, using provisional prostheses restores static and dynamic occlusal contacts, the point of contact with antagonists and neighbors. Dental migrations after preparation, impression and occlusal or proximal retouching of the definitive prosthesis are limited. The provisional prosthesis allows quality control of the preparation and volume of the definitive restoration, avoiding occlusal interferences. The periodontal aspect aims to maintain the marginal gingiva in a physiological position after the preparation of dental substructures (10, 11, 12).

AIM

The aim of this study is to analyze the factors involved in the choice of provisional prosthesis and the implications this has for the definitive prosthesis according to the particularities of each individual clinical case.

MATERIAL AND METHODS

A total of 86 patients diagnosed with partially reduced edentulousness, i.e., coronal recessed dental lesions, were included in the study group and clinically and para-

clinically evaluated for prosthetic rehabilitation by different types of definitive restorations from the current prosthodontic range, prefaced within the therapeutic algorithm of provisional restorations.

RESULTS

In prosthetic therapy by conjunctive means, the provisional prosthesis is an indispensable element in both single and large restorations. Without excluding the protective role of the prepared substructures, the subtle occlusal, periodontal, and functional requirements of temporary restoration remain an indisputable fact. The "dialogue" provisional prosthesis - practitioner - patient has as its motto but, and as a conclusion at the same time, a definitive piece correctly integrated into the functionality of the dental system.

Regarding the distribution of the group of patients according to sex, a percentage of 56% for women and 44% for men was recorded, the sex of the patient correlated with age, general status and aesthetic demands directs us towards a certain type of prosthetics to be grafted according to the individuality of each clinical case with the demands of contemporary aesthetics (fig. 1).

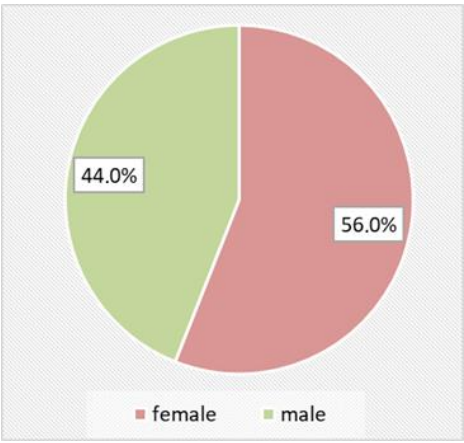

Fig. 1. Distribution of patients according to gender

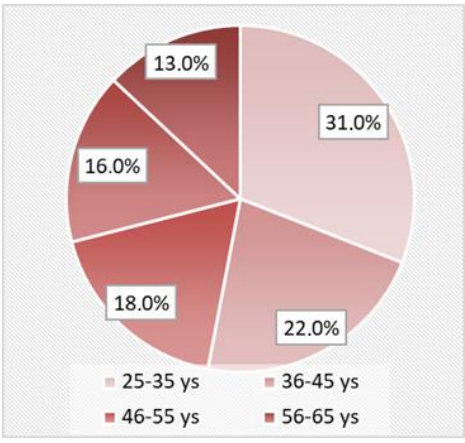

Fig. 2. Distribution of patients according to age

Regarding the age of the patients we have a wide range from 25 to 75 years as prevalence of coronary dental lesions and partially reduced edentulousness, this pathology is found in a percentage of 31% in the age range 25 - 35 years, a percentage of 22% is found in the age range 35 - 45 years, while for the age range 45 and 55 years a percentage of 18% is found, for the age range 55 - 65 years a percentage of 16% is found and a percentage of 13% is found in the age range 65 - 75 years (fig. 2).

The general condition of the patient has always been an extremely important starting point for dental therapy, as many general diseases influence the structure of the oral cavity and the medication for each pathology, thus decisively influencing the therapeutic algorithm and the choice of the type of preparation and the type of prosthesis. Thus, cardiovascular diseases were recorded in the highest percentage in the group analyzed in

a percentage of 32%, it is important to note that of this percentage a quarter were taking anticoagulant medication, as for metabolic diseases, they are found in a fairly high percentage of 28% within the general pathology followed by respiratory diseases in a percentage of 15% and digestive diseases in a percentage of 13%. A percentage of 12% was recorded in patients with general oncological diseases which are often associated with long-term temporary prostheses such as PMMA (fig. 3).

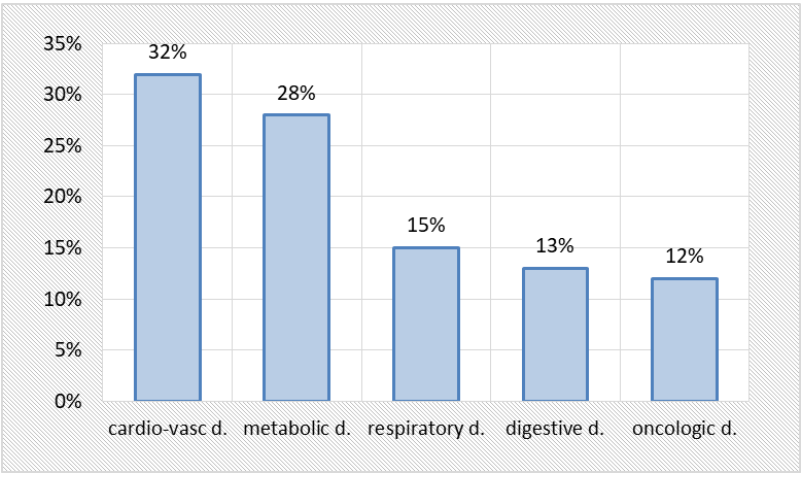

Fig. 3. Distribution of patients according to the assessment of the general condition

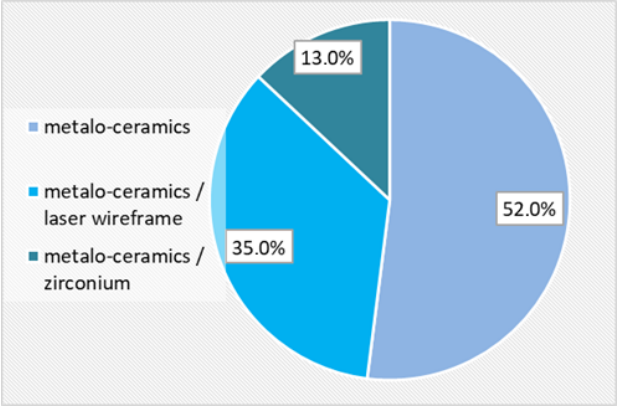

Fig. 4. Distribution of the types of performed fixed prostheses

As far as definitive restorations are concerned, classical metal-ceramic restorations prevailed as a percentage, followed by metal-ceramic restorations with laser frameworks, a current technology which is extremely precise and which provides the best periodontal reports, with a percentage of 13% of zirconium-ceramic restorations made with digital techniques. As for the type of provisional prosthesis, it was anchored both in the digital and in the classical territory (fig. 4).

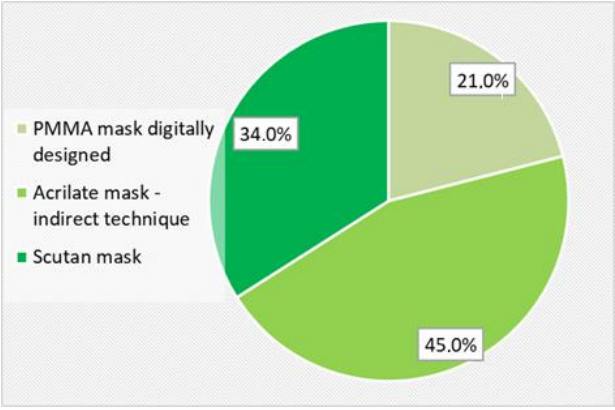

Fig. 5. Distribution of temporary prostheses performed

The design of provisional prostheses by means of digital technologies was carried out in a 21% percentage of patients, followed by classical acrylic prostheses which were found in a percentage of 45% and Scutan mask carried out in the dental surgery was found in a percentage of 34% (fig. 5). As regards the location of temporary restorations, 64% of the restorations were placed at the frontal level, of which 30% were temporary restorations that were preceded by gingivectomy-type prosthetic preparations; 36% were placed at the lateral level .The accuracy of adaptation of the transitional denture margins to the preparation limits is the key factor in periodontal protection. It allows, by its anatomy, dento-periodontal hygiene, so it must respect the "shapes" of a definitive restoration. At the cervical level, its morphology must fit in with the morphology of the neighboring teeth, respecting the natural convexities, which on the one hand divert the food bolus away from the marginal gingiva and at the same time allow, through the play of muscles, self-cleaning. For Parkinson, "the gingival super contour protects the dental plaque, not the gingiva". Over contours appear to be much more harmful than under contours (for definitive restorations), especially if the restorative boundary is placed subgingival. The dimensions of the periodontal biological space are maintained by correctly shaped provisional denture margins, the volume of the margins compatible with a correct emergence profile and the surface condition of the material perfectly polished, thus avoiding inflammatory reactions. Provisional prosthetics by the digital method was extremely important in cases that had prosthetic work with which the patient was not very satisfied, by means of this prosthesis, exploring all the possibilities of functional morphological reconfiguration and occlusion ratios, so that the patient's expectations were assured. A concrete case in this regard was the realization of a PMMA prosthesis that was applied after a gingivectomy, the patient having been previously restored with a zirconia-supported ceramic work, the future restoration being also zirconia-supported ceramic. The frontal curvature will be completely restored, and the shape of the teeth modified according to the patient's facies so that

the final results will be as expected (fig. 6).

Another clinical situation was represented by a digital restoration, also applied in the frontal area, an area of maximum exigency. This time the method was represented by a long-term provisional prosthesis until the patient's general status could be balanced so that the absolutely necessary prosthetic preparation steps could be carried out in this clinical case (fig. 7).

Fig. 6. Digital and clinical aspects of PMMA provisional rehabilitation

Fig. 7. Aspects of long-term provisional prosthesis

DISCUSSION

It is also necessary for the patient-practitioner-technician trio: for the patient it compensates for aesthetic and/or functional disability, for the practitioner because it has therapeutic value, authorizing him to continue treatment, and for the technician because it is a source of clinical data that allows him to comply with the highest demands of the patient and the practitioner. The lifetime of this prosthesis is limited in time and the degree of development depends on the goals and duration of treatment. The provisional prosthesis is of so much interest in joint therapy that its absence may be surprising. The biological integration of the fixed prosthesis, i.e., the congruence of the prosthetic part (non-biological in terms of the method, design, and materials from which it is made) with the supporting biological elements, is conditional on achieving bio functional stability (occlusal, periodontal, aesthetic).

CONCLUSIONS

In the current clinical-technological algorithm of rehabilitation with fixed restorations, provisional prosthetics represent an

extremely important step in order to conform the periodontal tissue on the one hand, to protect the prepared substructures on the other hand, and last but not least to prefigure the shape of the future prosthetic rehabilitation in the context of dento-somatic-facial harmony. Prosthetic restorations made by the digital technique are extremely precise, their design is laborious considering all biomechanical aesthetic criteria so that we have individualized restorations that lead to successful prosthetics.

The clinical reality gives the transitional prosthesis essential functions of control and management of occlusal, periodontal, and aesthetic-functional parameters.

CONFLICTS OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest to disclose, and they received no funding regarding this scientific research.

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DEVELOPMENT AND EVALUATION OF INDOMETHACIN LIPOSOMAL SYSTEMS

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DEVELOPMENT AND EVALUATION OF INDOMETHACIN LIPOSOMAL SYSTEMS (Abstract): **Aim:** Treating the inflammation with oral indomethacin might be in some situations inadequate for patients that cannot dispense the medication on their own, a fact that conduct to the necessity to develop new pharmaceutical formulations administered through different pathways, such as the transdermal application. To trespass the skin layers, this study aimed to develop and evaluate an indomethacin liposome formulation that will be included in a Carbopol gel, subsequently. **Material and methods:** To develop indomethacin liposomes egg lecithin and cholesterol were used in order to confer membrane stability and elasticity. Two technologies of liposome-sizing were used: extrusion and sonication, the obtained vesicles being incorporated in a Carbopol gel. The *in vitro* release profiles were conducted on both liposomes and gels using the Franz cell method and two types of membranes (M1 and M2). **Results:** Liposome sizing through extrusion proved to be a better method compared to the sonication one. The microscopic evaluation of the liposomal vesicles showed that through extrusion smaller liposomes are obtained, a fact that is reflected also in their capacity for API releasing. **Conclusions:** Via the *in vitro* releasing study differences of 10% were observed regarding the amounts of API liberated, through both the extruded liposomal dispersion and the gel containing this dispersion in comparison with the sonicated liposomes. The type of releasing membrane used influenced the amount of API released, observing a higher concentration of API released after 8 hours through M2 (the skin-like membrane). **Keywords:** INDOMETHACIN, LIPOSOMES, TRANSDERMAL, FRANZ CELL.

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most prescribed classes of drugs in the world for treating rheumatoid polyarthritis and osteoarthritis. Indomethacin (Ind) is a well-known NSAID used as a long-term treatment in these kinds of pathologies, thus, its main disadvantage during oral administration is represented by the gastric side effects such as ulcer, digestive hemorrhage, allergy, and respiratory and hematology disorders. Ind is an indole derived compound, with the IUPAC name 2-{1-[(4-Chlorophenyl)carbonyl]-5-methoxy-2-methyl-1H-indol-3-

yl}acetic acid having the following physico-chemical properties: white-yellowish powder, photosensitive, with the molecular mass of 357.8 g/mol, insoluble in water, partly insoluble in alcohol. 90% of the NSAIDs users are patients over 65 years old, the fact that implies a risk regarding the side-effects increase (1-3).

To decrease the risk and to improve the bioavailability of the medicines, the pharmaceutical industry started developing new pharmaceutical formulations such as liposomes. Besides the minimization of the well-known side-effects belonging to an NSAID, the transdermal administration of formulations in which the API is processed as liposome can facilitate skin penetration, improving bioavailability. A major advantage of the liposomes is given by their capacity to encapsulate different types of API and biocompatibility (4-8). The API liposomal systems have the advantage of incorporating this API, therefore, protecting it from degradation and transformation into inactive metabolites, also, the API is vectorized to the unhealthy cells, providing a gradual release. In this manner, the toxicity caused by a conventional administration decrease, and the distribution to other organs is hindered or reduced. The liposomes do not represent a pharmaceutical formulation lacking disadvantages, the loss or fusion of the APIs entrapped, or the oxidation of the phospholipids in the composition being some of them. Liposomes, named also, microparticle lipidic vesicles, bi-layered lipidic membranes, are constituted of lipids, their lipophilic parts being oriented through the internal and external part of the membrane, forming a mixture of lipidic substances, more often being used the phospholipids (phosphatidylcholine, phosphatidylserine), octadecyl amine and cholesterol (9-14). Taking into consideration the exposed introduction, this study aimed to develop and evaluate Ind liposomes that will be furthermore processed in a Carbopol gel for transdermal administration.

MATERIAL AND METHODS

***Materials for liposome preparation*:** indomethacin (Sigma Aldrich Milan, Italy), egg lecithin (AppliChem GmbH, Germany), cholesterol (Fagron, Greece), ethanol (Chimreactiv, Romania), phosphate saline buffer pH 7.4.

In first table is presented the composition of the Ind liposomal dispersion.

TABLE I.
Liposomal composition

Ingredient (g)		Function
Indomethacin	0.2	active pharmaceutical ingredient
Egg lecithin	0.2	stability and elasticity of the liposomal membrane
Cholesterol	0.018	stability and elasticity of the liposomal membrane
Ethanol	25	solvent
Phosphate saline buffer	25	adjusting pH

Liposomes preparation method: In the first step the lecithin, cholesterol, and Ind are dissolved in ethanol under continuous stirring for 20 minutes at 300 rpm (Heidolph RYR1 stirrer, Germany, fig. 1). During the second step, the dispersion is added gradually, using an Eppendorf pipette, drop by drop, in 25 g of phosphate saline buffer pH 7.4. A liposomal dispersion of 0.4% Ind is obtained.

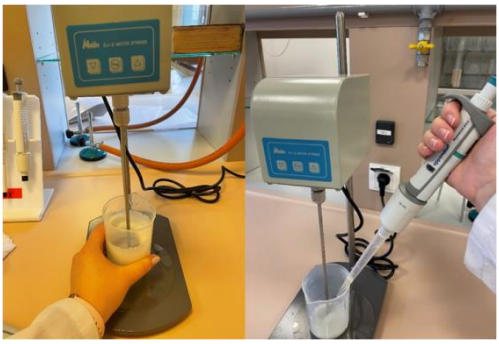

Fig. 1. Liposomes preparation method

Decreasing the liposomes vesicle size:

Extrusion: The liposomal dispersion was sized by the 8-time passage through two syringes of 250 µL belonging to the Mini Extruder (fig. 2, left), provided with a supported filter at one of the ends. The passage from one syringe to another was realized through a support membrane located between the two syringes. The extruded liposomal dispersion will be furthermore coded as Lex.

Sonication: The liposomal dispersion was subjected to the ultrasound action, in an iced water bath (fig. 2, right) (t=2.5-4°C) for 20 minutes, in innings of 5 minutes with pauses between them of 5 minutes. The extruded liposomal dispersion will be further coded as Lsn.

Fig. 2. Liposomes sizing equipment

Liposomes aspect: The liposome aspect was evaluated microscopically, using a digital binocular microscope with a camera (Optika Microscopes, Italy), the objective of the camera of 100 X.

***Materials for Carbopol gel preparation*:** carbopol 940 (BF Goodrich), sodium hydroxide solution 10% (Merck, Germany), distilled water.

In Table II is presented the composition of the Carbopol gel.

TABLE II.

Carbopol gel composition

Ingredient (g)	
Carbopol 940	1
Sol. NaOH 10%	3
Distilled water	96

Carbopol gel preparation method: The carbopol gel was prepared using the classic method of polymer hydration and neutralization with NaOH 10% to increase the viscosity and the pH.

Carbopol gels with indomethacin liposomes preparation method: 2 formulations of Ind gels were prepared, the first one consisting of liposomes obtained through extrusion (Gex), whilst the second one was prepared with sonicated liposomes (Gsn).

The quantity of liposomal dispersion incorporated ensures an amount of API of 40 µg/g gel.

Rheological evaluation of Carbopol gel

To determine the rheological behavior

of the gels a rheometer Rheotest RV was used (Rheotest RV, Germany), supplied with coaxial cylinders S/S1 and 12 shear rates.

In vitro release study:

Samples (1 g): Lex; Lsn; Gex; Gsn.

Apparatus: series of Franz cells (PermeGear, Germany), 11.28 mm clear jacketed with flat ground joint, 8 ml receptor volume, pinch clamp, and stir bar.

Acceptor phase: phosphate buffer pH 7.4, maintained at 37°C±1°C.

From the acceptor compartment of the Franz cell, samples of 0.8 mL were withdrawn at different time intervals for a period of 8 hours. The acceptor phase was immediately replaced with fresh phosphate buffer maintained at 37°C±1°C.

Tested membranes: two types of membranes were used to evaluate the release profiles of the drug from the analyzed samples:

-M1: Teknokroma membrane filters, nylon, 0.45 µm thickness, 25 mm diameter; requires hydration before use

-M2: Strat-M Membrane, 0.45 µm thickness, 25 mm diameter; has two polysulphonated layers (corresponding to epidermis and dermis) similar to the human skin layers, and polyolefin layers that simulate the porous aspect of the skin.

The cumulative amounts from the tested formulations were quantified by a validated HPLC method at 237 nm: injected volume: 10 µL, flow rate: 1 mL/min., mobile phase: formic acid solution 0.1% (25 %) + methanol (75 %), retention time: 2.9 min, stationary phase: Nucleodur C18 Gravity 3 µm column.

RESULTS AND DISCUSSION

Liposomes aspects

The microscopic evaluation of the liposomes obtained through extrusion (fig. 3) showed that through this method smaller vesicles are obtained in comparison with the ones sized through the sonication method (fig. 4). This aspect can suggest a better dermal penetrability of the extruded liposomes.

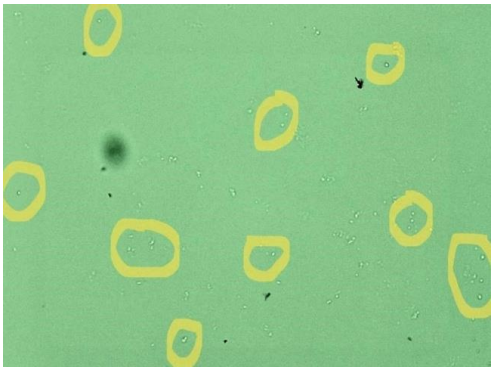

Fig. 3. Extruded liposomes aspect

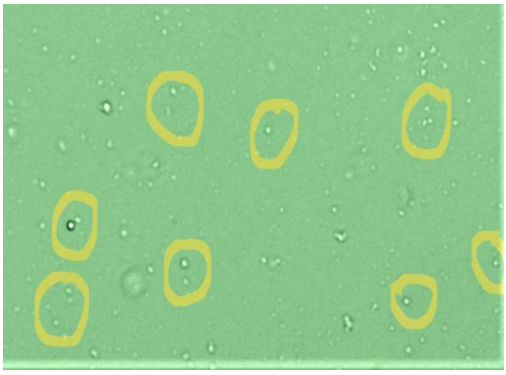

Fig. 4. Sonicated liposomes aspect

Rheological evaluation of Carbopol gel

The rheological analysis of the Carbopol gel shows a plastic behavior (fig. 5) characterized by a modification of the structure only after the tangential tension reaches a certain limit (flow threshold). Over this threshold and during the shear rate is increasing the viscosity starts to decrease.

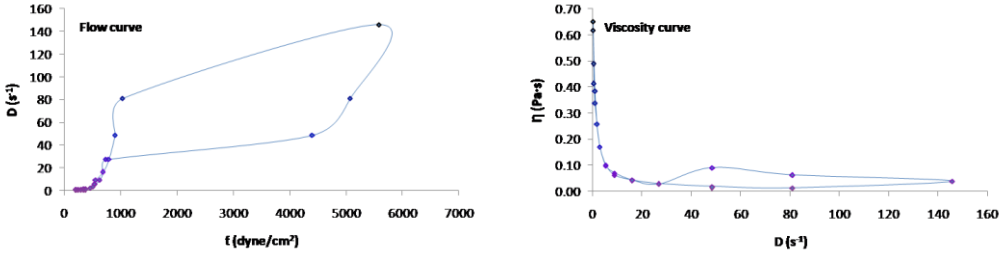

Fig. 5. Rheological behavior of the Carbopol gel

In vitro release study

The etalon scale and typical chromatograms are included in figure 6.

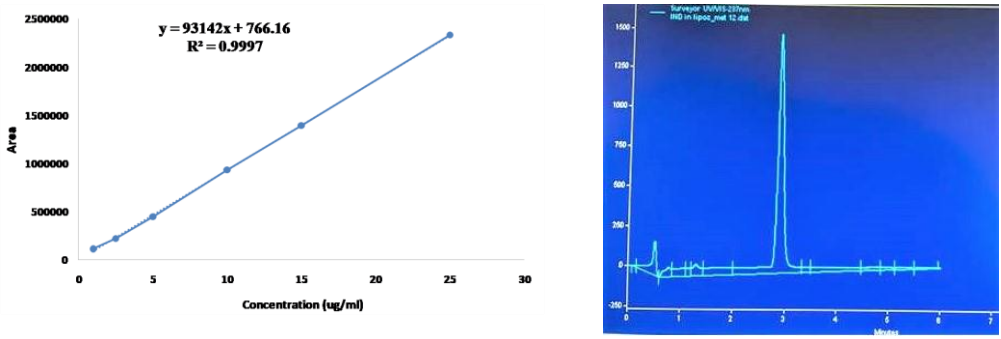

Fig. 6. Standard curve of indomethacin and a typical chromatogram

Through the API *in vitro* releasing study from the liposomal dispersions, it has been noticed after 8 hours that the extruded vesicles are easily trespassing the tested membranes in comparison with the ones obtained through sonication. Also, the type of membrane tested in the study differentiates the amount of Ind released. It can be observed that in the case of the extruded liposomes with the M2 membrane (skin-like membrane), 13% more of the API is released in comparison to the M1 membrane. Similar behavior is retrieved in the case of the sonicated liposomes, the difference in the amount of API released being 10 % (fig. 7).

Ind passaging through the membrane from the carbopol gels that included liposomes maintained the same behavior manifested also at the releasing study from the liposomal dispersion, with the distinction that after 8 hours the concentration of API released was in general, lower. This behavior might be explained by the initial necessity to release API from the gels followed by the passage of the testing membrane. Also, in the case of the liposomal gels, it was observed the importance of the membrane tested during the *in vitro* releasing study, noticing that the gels with extruded liposomes and the ones with extruded liposomes, that via the M2 membrane a higher amount of Ind is released (10% higher) compared to M1 (fig. 8).

These results suggest that in physiological conditions Ind might passage the skin layers to ensure a systemic anti-inflammatory effect.

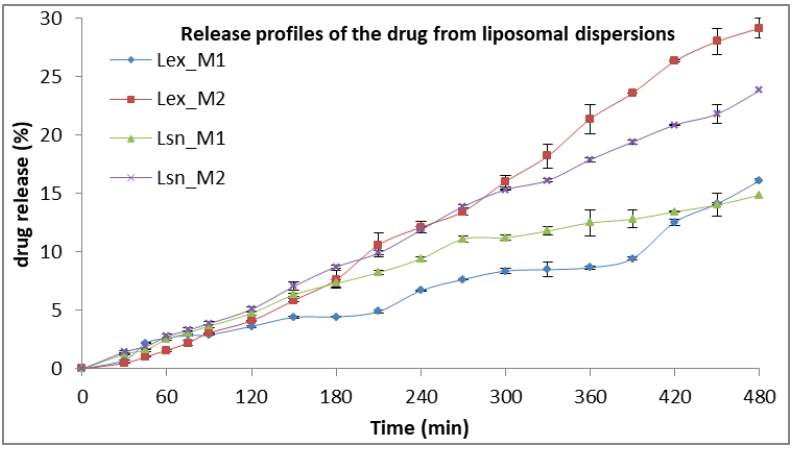

Fig. 7. Release profiles of indomethacin from liposomal dispersions across M1 and M2

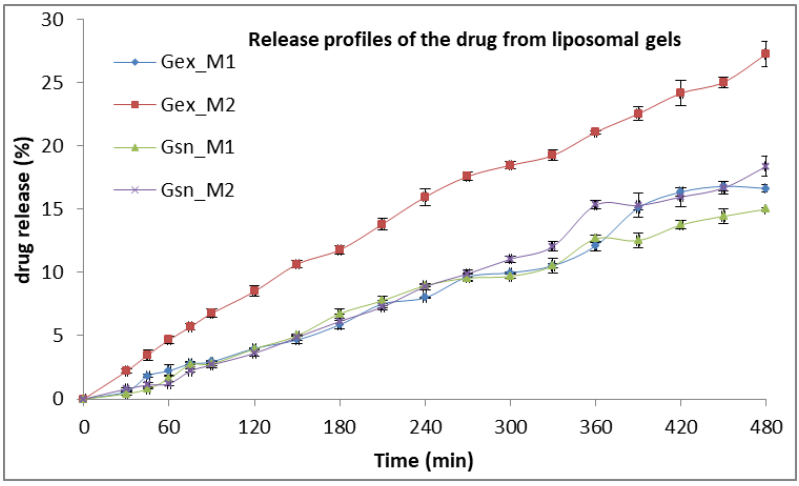

Fig. 8. Release profiles of indomethacin from liposomal gels across M1 and M2

CONCLUSIONS

The oral anti-inflammatory treatment can be in some situations inadequate for some patients that can administer by themselves the medication. In this manner and to minimize the Ind side-effects, the formulation of transdermal gels with medicinal liposomes can be an adequate solution for the pathologies treatment that includes an inflammatory process.

The liposome-sizing through extrusion proved to be a better method compared to the sonication one. The microscopic evaluation of the liposomal vesicles showed that through extrusion smaller liposomes are obtained compared to the ones obtained through sonication, a fact that is reflected also in their capacity for API release.

Through the *in vitro* releasing the study, differences of 10% of API released were observed for both extruded liposomes and gel with extruded liposomes compared to the sonicated liposomes. The releasing membrane type also influenced the amount of API released, observing a higher concentration of API released after 8 hours through M2 (the skin-like membrane).

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and this work was supported by the "George Emil Palade" University of Medicine, Pharmacy, Science and Technology of Targu Mures, Research Grant No. 10127/15/17.12.2020.

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ANTIOXIDANT AND ENZYME INHIBITORY EFFECTS OF *MORUS SP.* EXTRACTS

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ANTIOXIDANT AND ENZYME INHIBITORY EFFECTS OF *MORUS SP*. EXTRACTS (Abstract): *Morus* leaves water extracts are used for a long time for its anti-diabetic, anti-inflammatory, and cardiovascular disease-treating properties. Our research's **objective** was to evaluate the hypoglycemic and antioxidant effects of *Morus* leaves extracts obtained by using different solvents. **Materials and methods**: The extracts from *Morus nigra* and *Morus alba* leaves were obtained by ethanol-water extraction, respectively hot water extraction. We assess the enzyme inhibition (α-amylase and α-glucosidase) ability of extracts from *Morus sp*. leaves, and we correlate these effects with polyphenols and flavonoids content. **Results**: Total polyphenols content ranged between 89.4 mg gallic acid equivalents/g ethanol-water extract of *Morus nigra* leaves and 46.81 mg gallic acid equivalents/g aqueous extract of *Morus alba* leaves. Ethanol-water extracts contain more polyphenols and flavonoids than aqueous extracts. The most important effect on alpha-amylase was presented by the ethanol-water extract from *Morus alba* leaves (EC50 88.26±0.52 µg/mL) and on alpha-glucosidase by the ethanol-water extract from *Morus nigra* leaves (EC50 69.86±0.03 µg/mL mL). From the point of view of antioxidant effects, the extracts inhibit lipoxygenase, with the maximum effect in the case of the ethanol-water extract from *Morus alba* leaves (EC50 23.75±1.77 µg/mL). The ethanol-water extracts have a greater capacity to chelate the ferrous ion compared with aqueous extracts, the most active being the ethanol-water extract from the *Morus nigra* (EC50 213.32±0.26 µg/mL). **Conclusion**: The conducted study highlights the ability of the analyzed extracts to control the enzymes involved in the digestion of carbohydrates and to reduce oxidative stress. **Keywords:** *MORUS ALBA*, *MORUS NIGRA*, ANTIOXIDANT ACTIVITY, ANTIDIABETICS.

Diabetes mellitus is the most common endocrine disease affecting over 537 million adults worldwide in 2021 with a predicted rise to 643 million by the end of the decade (1). Type II (T2DM) is the major form of diabetes, accounting for about 90% of the cases. Diabetes is caused by impaired insulin secretion, insulin resistance or a combination between the two which leads to a dysregulated metabolism of the

carbohydrates and lipids (2). Chronic hyperglycemia induces oxidative stress, affects many biological structures, and represents the most important cause for diabetes consequences such as peripheral neuropathy, diabetic retinopathy, diabetic nephropathy, or diabetic ketoacidosis.

The management of diabetes involves, among lifestyle changes, administration of oral hypoglycemic substances which are associated with side effects such as hypoglycemia, weight gain (insulin, sulphonyl urea), lactic acidosis (biguanide), and gastrointestinal disturbances. Therefore, herbal medicines present an attractive alternative in the management of diabetes as they are generally accepted to present less adverse effects compared to modern conventional pharmaceutics (2). Moreover, considering that more than three quarters of adults living with diabetes are in low- and mid-income countries both the patients and the national healthcare institutions could benefit for herbal alternative medicines as they are generally less expensive than synthetic pharmaceutics (1, 2). In the recent years, the number of studies on using plants as a source of diverse biologically active substances with anti-inflammatory, diabetic, cardiovascular diseases, anti-cancer, and antioxidant effects have increased (3, 4). The bioactive substances from plants, such as: polyphenols, phenolic acids, flavonoids, flavonols, diterpenes, tannins, phytosterols, fatty acid esters, phenylpropanoids, alkaloids, glycosides, etc., have attracted significant interest in medicinal chemistry and natural product research (5, 6).

Polyphenols are divided into four main classes: flavonoids, phenolic alcohols, stiblins, lignans and represent the biologically active substances found in plan-based diets. The structure and proprieties of these compounds depend on several factors, such as plant cultivar/genotype, growing environment conditions, soil characteristics, harvest time, and storage conditions. These substances come from plants, such as fruits, vegetables, cereals, and coffee, and are an integral part of the human diet. Polyphenols are also recognized as a preventative for degenerative illnesses (7).

Polyphenols and flavonoids, by their hydroxyl groups can scavenge free radicals, release hydrogen that will neutralize different oxidants and also, they have the ability to interact with different enzymes and to control activity of these (7). Depending on the source and combination, they may exhibit diverse properties such as specific enzyme inhibition activity.

α-Amylase and α-glucosidase are two important enzymes responsible for catabolizing starch, glycogen and disaccharides to glucose that will be absorbed into the blood. Inhibition of these enzymes is often employed for controlling blood glucose level by decreasing the rate of intestinal carbohydrate metabolism.

Oxidative stress participates in the onset and worsening of diabetes. The identification of compounds that have both antioxidant effects and the ability to control the enzymes involved in the digestion of carbohydrates represents an advantage for the patient with diabetes because the number of drugs administered to him is reduced.

Morus alba, *Morus nigra* and *Morus rubra* are woody plant from the Moraceae family that has been cultivated worldwide in sericulture from ancient times. They have been used in Chinese medicine for several purposes such as antifever, diuretics, protection of the liver, improvement of eyesight, and to prevent cardiovascular diseases. *Morus sp*. main active compo-

nents and the compositions of phenol, alkaloid, and flavonoid, have been thoroughly investigated for their noteworthy characteristics (8). Mulberry fruits are used as a natural treatment to reduce the blood pressure, to treat fever or to prevent liver and kidney damage (9, 10, 11). The antithrombotic, anti-obesity, antiinflammatory, anti-carcinogenic and neuroprotective effects of mulberry fruit flavonoids are registered in the Pharmacopoeia of the People's Republic of China (12).

Morus sp. leaves polyphenols are functional compounds possessing various beneficial effects against cardio metabolic diseases, including anti-hyperglycemic, antihyperlipidemic, anti-obesity, antihypertensive, anti-oxidative, anti-inflammatory, anti-atherosclerotic, and cardioprotective effects (4). Leaves of *M. nigra* are commonly used by women during menopause as a replacement for the conventional hormonal substitute therapy, with a similar effect to the estrogens (13). Roots, bark, stem twigs, and fruits of *Morus sp.* possess valuable bioactive ingredients that can be explored in pharmaceutical, health care, cosmetic and food industries (8).

The aim of our study was the assessment of antioxidant and enzyme inhibition (α-amylase and α-glucosidase) ability of extracts from *Morus sp*. leaves and to correlate these effects with polyphenols and flavonoids content.

MATERIAL AND METHODS

Chemicals and reagents

Reagents: Folin-Ciocalteu's Reagent, gallic acid, sodium nitrite, aluminum chloride, acarbose, alpha-glucosidase, alpha-amylase, 4-nitrophenyl α-D-glucopyranoside (pNPG), sodium hydroxide, acetate buffer (pH 5.25), ferrous sulphate, ferrozine, potassium ferricyanide, trichloroacetic acid, ferric chloride, 3,5-dinitrosalicylic acid were purchased from Sigma Aldrich (Germany). Dimethyl sulphoxide (DMSO), methanol, ethanol, hydrochloric acid were purchased from Merck (Germany). The water used in this study was ultrapure water generated with Milli-Q PLUS (Millipore Corporation).

Preparation and analysis of the extracts

Morus alba and *Morus nigra* leaves were collected from their natural habitat in Iasi (Coordinates 47°9′24″N 27°29′10″E), Romania, in May 2022. Vegetal samples were air-dried and were ground with an IKAA1 analytical mill. Voucher specimens are deposited at the Department of Pharmacognosy, Faculty of Pharmacy, "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Romania.

Preparation of aqueous extract: 10 g of dried and powdered leaves were extracted twice with 150 mL boiling distilled water and was maintained 20 minutes to 80° C. After cooling the extract was filtered on Whatman filter paper no.1 and the combined extracts were lyophilized in order to obtained lyophilizate that was used for analysis (14).

Preparation of the ethanol-water extract: 2 g of dried and powdered leaves were extracted with 2 x 50 mL ethanol-water 70:30 on water bath, at reflux, for 45 minutes. The extracts were filtered and completed to 100 mL using the same solvent. The extracts were dried using a rotary evaporator.

Dried extracts were solved in dimethyl sulfoxide (for ethanol-water extract) or water (for lyophilizate extract) in order to obtain solutions with concentrations between 0.0097 and 20 mg/ml that have been

used for the tests (14). We obtained and analyzed four extracts: MAE (*Morus alba* ethanol-water extract), MAA (*Morus alba* aqueous extract), MNE (*Morus nigra* ethanol-water extract), MNA (*Morus nigra* aqueous extract).

Determination of total phenols content: 1 ml diluted solutions of extracts were mixed with 1 mL of Folin-Ciocâlteu's Reagent, allowed to stand for 5 minutes at 25ºC before adding solution of sodium carbonate 20%. For 120 minutes, the samples were kept in the dark, before measuring the absorbance at 750 nm. Gallic acid was used as standard. The results were expressed as gallic acid equivalents (GAE) (mg/1 g dry extract) (15).

Determination of flavonoids content: 0.25 mL diluted extract were mixed with 1.525 mL distilled water, 0.075 mL solution sodium nitrite 5%- and 0.15-mL solution aluminum chloride 10%. After 5 minutes 0.5 mL solution sodium hydroxide 1M was added and the absorbance of solution was determined at 510 nm against sample blank. The results were expressed as rutoside equivalents (mg RE/1 g dried extract) (16).

Antioxidants and enzyme inhibition tests

Antioxidant activity of the extracts was determined by iron chelating test, ferric ions reducing test, lipoxygenase inhibition.

Iron chelating test: 0.2 mL diluted extracts, 0.74 mL 0.1 M acetate buffer (pH 5.25), 0.02 mL 2 mM ferrous sulphate solution and 0.04 mL of 5 mM ferrozine solution were mixed. After 10 in the dark medium the absorbance of the solutions was determined at 532 nm. The iron chelating activity was determined using the following formula: Activity % = 100 × (A_C – A_S)/(A_C), where A_C is the absorbance of the control solution (without chelating compounds) and A_S is the absorbance of the sample solution. Rutoside was used as positive control. The assay was conducted in triplicate. The EC_{50} was calculated for each extract that present a chelating activity over 50% and is expressed as mg sample/mL final solution (17).

Ferric ions reducing test: 0.5 mL diluted extracts, 0.5 mL phosphate buffer (0.2 M, pH 6.6) and 0.5 mL potassium ferricyanide solution 1% were mixed and maintained at 50ºC for 20 minutes. After cooling the solutions were mixed with trichloroacetic acid solution 10%, centrifuged at 3,000 rpm, and an aliquot of the upper layer was mixed with distilled water and ferric chloride solution 1%. The absorbance of solutions was determined at 700 nm. Rutoside was used as positive control (18).

Lipoxygenase inhibition test: 0.05 mL diluted extracts were mixed with 0.05 mL lipoxygenase buffer solution (pH 9) and kept for 10 minutes at room temperature. Afterwards, 2 mL linoleic acid buffer solution 0.16 mM (pH 9) were added. The absorbance was determined at 234 nm for 120 seconds. The results were calculated using the following formula: % activity = (A_E – A_{ES}) x 100/A_E, where A_E is the difference between the absorbance of the enzyme solution alone after 90 seconds and the absorbance of the same solution after 30 seconds and A_{ES} is the difference between the absorbance of the enzyme solution mixed with the sample after 90 seconds and the absorbance of the same solution after 30 seconds (19). Rutoside was used as positive control.

The capacity of extracts to control carbohydrates digestion was evaluated by alpha-amylase and alpha-glucosidase inhibition tests.

Alpha-amylase inhibition test: 0.04 mL diluted extracts were mixed with 20 mM phosphate buffer pH 6.7, 0.08 mL alpha-amylase solution (2 UI/mL), 0.2 mL 0.5% starch solution in 20 mM phosphate buffer pH 6.7 and were maintained at 37°C for 10 minutes. The reaction was stopped with 0.32 mL 96 mM 3,5-dinitrosalicylic acid and the mixture was maintained at 100°C for 15 minutes. After cooling was added distilled water and the absorbance of each solution was measured at 540 nm. The percentage of alpha-amylase inhibition was calculated as follows: $(1–A_C/A_S) \times 100$, where A_C is the absorbance of control and A_S is the absorbance of the sample. Acarbose was used as positive control (20).

Alpha-glucosidase inhibition test: 0.1 mL diluted extracts were mixed with alpha-glucosidase (1 U/mL), phosphate buffer, pH 7.0, and were maintained at 37°C for 5 minutes. After that 0.25 mL p-nitrophenyl-alpha-D-glucopyranoside solution was added and the mixture was maintained again at 37°C for 10 minutes. The absorbance of the final solution was determined at 405 nm. The percent of alpha-glucosidase inhibition was calculated as follows: $(1–A_C/A_S) \times 100$, where A_C is the absorbance of control and A_S is the absorbance of samples containing extracts. The inhibitory concentration of the extract required to inhibit the activity of the enzyme by 50% (EC_{50}) was calculated by regression analysis. Acarbose was used as positive control (21).

For each test, the concentration of the extract required to obtain a positive effect over 50% (EC_{50}) was calculated by regression analysis and expressed as µg sample/mL final solution.

Statistical analysis: all determination were conducted in triplicate and the results are expressed as mean ± standard deviation. Statistical analysis of the data was performed using ANOVA test.

RESULTS

Radojkovi and co-workers investigated the effects of various extraction parameters on the extraction yield of phenolic compounds and the antioxidant activity of the extracts from *Morus nigra* leaves using Response Surface Methodology (RSM). It was shown that three factors, namely ethanol concentration, temperature and liquid/solid ratio have the most significant influence (22). Starting with the data collected from this specialty field, we have chosen to do extractions using 70% ethanol and boiling distilled water.

Morus sp. extracts can be obtained through different extraction processes such as: pressurized liquid technique, supercritical-fluid, microware-assisted, ultrasound-assisted, in the solid phase, but the solid-liquid extraction is the most advantageous compared to other methods due to the reduced processing and the convenience of operation (23, 24, 25). These methods often use toxic solvents and may require an evaporation or concentration step. Large amounts of solvents are needed, which could be hazardous to the human health and the environment, difficult and time-consuming to completely remove. Furthermore, the possibility of thermal degradation of the bioactive ingredients cannot be neglected due to the high temperatures of the solvents during the prolonged extraction time. Considering these conditions and also the fact that in traditional medicine are used the aqueous extracts from the *Morus sp*. leaves, we decided to realize and analyze the aqueous extracts resulted after concentration through lyophilization.

Chemical composition of extracts

The total phenolic contents of extracts from *Morus alba* and *Morus nigra* leaves ranged from 46.80 to 95.20 mg GAE/g (tab. I). Ethanol-water extracts obtained from *Morus* leaves contain more polyphenols and flavonoids than aqueous extracts. This is explained by the ability of ethanol-water solution to extracts some lipophilic compounds from the vegetal samples. In traditional medicine *Morus* leaves aqueous extracts are used for diabetic patients and the ratio between vegetal material and water is lower than that was used by us to prepare the aqueous extracts.

These results were in accordance with those reported by other researchers that also found higher total polyphenolic content for *M. nigra* than for *M. alba* (26).

The variation of phenolic compounds in the leaves extracts depends on many factors, such as degree of maturity at harvest, genetic differences and environmental conditions but also the solvent type, extraction time, temperature in the different extraction procedures and analytical methods used in each work (7).

Suriyaprom and coworkers observed that the aqueous extracts of *Morus* leaves exhibited the high antioxidant and antibacterial activity, which was associated with a higher phenolic and anthocyanin content (27).

TABLE I.

Total phenolic and flavonoids content of *Morus sp*. extracts

No	Sample	Polyphenol (mg GAE/g)	Flavonoids (mg RE/g)
1.	MAA	46.81 ± 0.01	3.25 ± 0.01[a]
2.	MNA	61.31 ± 0.02	7.28 ± 0.01
3.	MAE	62.53 ± 0.02	7.16 ± 0.005[a]
4.	MNE	89.46 ± 0.07	11.22 ± 0.01

MAE - *Morus alba* ethanol-water extract; MAA - *Morus alba* aqueous extract.
MNE - *Morus nigra* ethanol-water extract; MNA - *Morus nigra* aqueous extract;
[a]p <0.0001 (ethanol-water extract *vs.* aqueous extract)

Yazdankhah and co-workers investigated total phenol content, antioxidant activity and anthocyanin components of ethanol-water *Morus nigra* extract and they noticed that *Morus nigra* extract had a good antioxidant potential and the main detected anthocyanin by HPLC method was cyanidin-3-glucoside (28).

The assessment of antioxidant activity

Iron 2+ is essential for human body but at the same time, depending on concentration could be involved in toxic processes. This ion participates in toxic conversion of hydrogen peroxide and superoxide anion to hydroxyl radical that affects proteins, nucleic acids and lipids by oxidation (29). Uncontrolled oxidative processes induce different pathological conditions and increase the risk for cardiovascular disease, diabetes, inflammatory state, and cancer. Natural compounds that have the ability to block iron 2+ will control oxidative processes and will reduce the intensity of oxidative stress.

Morus sp. extracts have the ability to block iron ions depending on extract concentration. Ethanol-water extracts from *Morus nigra* are seven times more potent than lyophilized extracts from the same species. For Morus alba extracts, the ethanol-water are four times more potent (fig. 1).

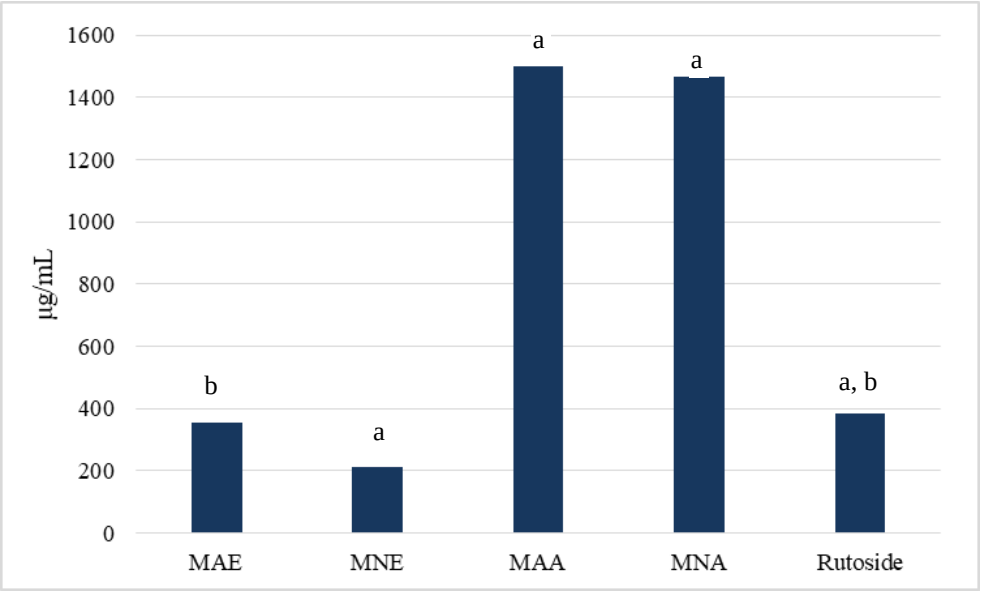

Fig. 1. The variation of the EC_{50} values of the *Morus sp*. extracts in the iron chelating test MAE - *Morus alba* ethanol-water extract; MAA - *Morus alba* aqueous extract; MNE - *Morus nigra* ethanol-water extract; MNA - *Morus nigra* aqueous extract; sample *vs.* positive control - a: p<0.0001 extremely statistically significant; b: p < 0.05 statistically significant

Compared to the Rutoside used as a positive control, the ethanol-water extracts have a greater capacity to chelate the ferrous ion (fig. 2), which suggests the involvement in this process of other compounds present in the plant extracts (30).

Iron 3+ is another form of iron that participates in redox processes in the human body. The high ratio Fe^{3+}/Fe^{2+} induced oxidative stress (31). Compounds with hydroxyl groups have the ability to transform Fe^{3+} to Fe^{2+} and finally to chelate Fe^{2+}.

15-lipoxygenase is a non-heme iron enzyme that catalyze the oxidation of unsaturated fatty acids to peroxides involved in physiological and pathological processes. High rate of its activity is correlated with inflammation and uncontrolled degradation of lipids and membrane structures.

The activity of enzyme can be modified until it is blocked by compounds that reduce Fe^{3+} to Fe^{2+}, chelate Fe^{2+} or modify the three-dimensional structure of the enzyme. Organic compounds such as polyphenols and flavonoids have the ability to release protons and electrons that modify the enzyme activity (32).

All extracts influence the enzyme activity in a concentration-dependent manner (fig. 3). The less active is *Morus alba* aqueous extract with maximum inhibition of 47.91±0.88%.

Morus alba ethanol-water extract has the most important effect on lipoxygenase inhibition test, but this extract doesn't contain the most important quantities of polyphenols or flavonoids. This effect sustains the necessity to establish the chemical composition of extracts and to evaluate the interactions between the most important groups of active compounds and enzyme.

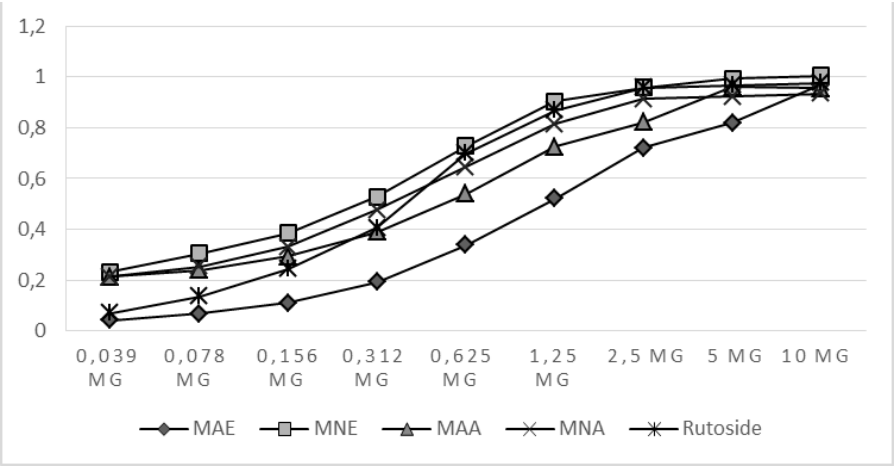

Fig. 2. The variation of absorbance for the ferric ions reducing test
MAE - *Morus alba* ethanol-water extract; MAA - *Morus alba* aqueous extract; MNE - *Morus nigra* ethanol-water extract; MNA - *Morus nigra* aqueous extract

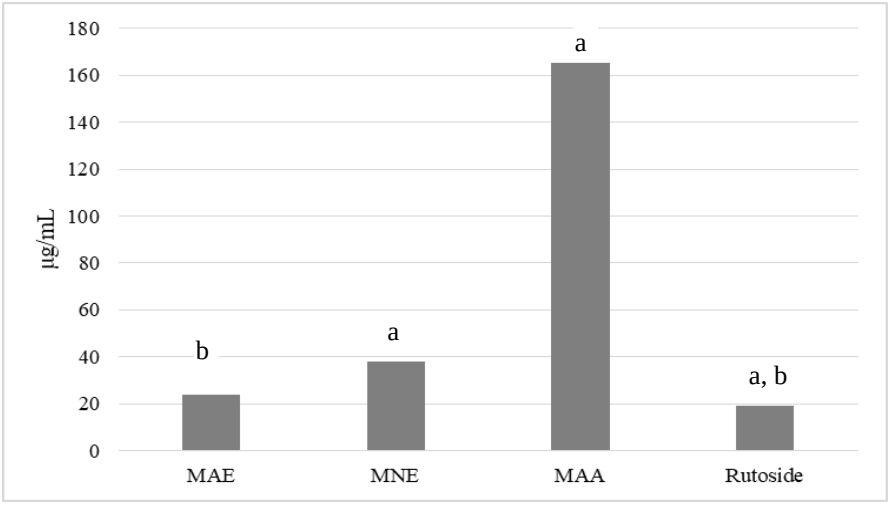

Fig. 3. The variation of the EC_{50} values of the *Morus sp.* extracts in the lipoxygenase inhibition test. MAE - *Morus alba* ethanol-water extract; MAA - *Morus alba* aqueous extract; MNE - *Morus nigra* ethanol-water extract; MNA - *Morus nigra* aqueous extract; a - $p<0.0001$ extremely statistically significant; b - $p < 0.05$ statistically significant

The ability to control carbohydrates digestion

Alpha-amylase and alpha-glucosidase are the most important digestive enzymes for carbohydrates transformations in the small intestine. All compounds that have the ability to control these enzymes will control the value of postprandial hyperglycemia by lowering carbohydrates hydrolysis and glucose absorption.

Long-term hyperglycemia is difficult to control, and this phenomenon is correlated with high risk for diabetes and obesity. Some studies suggest the necessity to con-

trol these enzymes in order to control diabetes onset (33).

The values of EC_{50} determined for alpha-amylase and alpha-glucosidase inhibition tests are presented in figures 4 and 5.

All extracts reduce alpha amylase activity with more important effects for ethanol-water extracts compared to aqueous ones. The EC50 values determined for the ethanol-water extracts are approximately three times lower compared to those for acarbose.

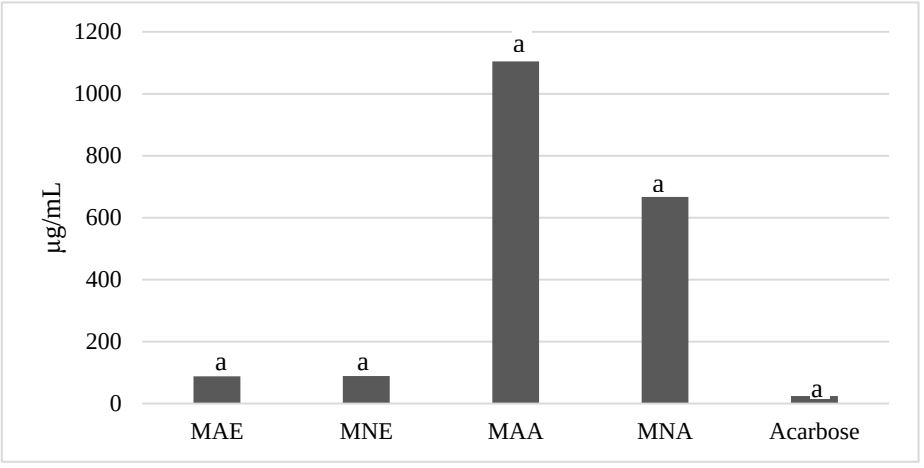

Fig. 4. The variation of the EC_{50} values of the *Morus sp.* extracts in alpha-amylase inhibition test. MAE - *Morus alba* ethanol-water extract; MAA - *Morus alba* aqueous extract; MNE - *Morus nigra* ethanol-water extract; MNA - *Morus nigra* aqueous extract; a - p<0.0001 extremely statistically significant

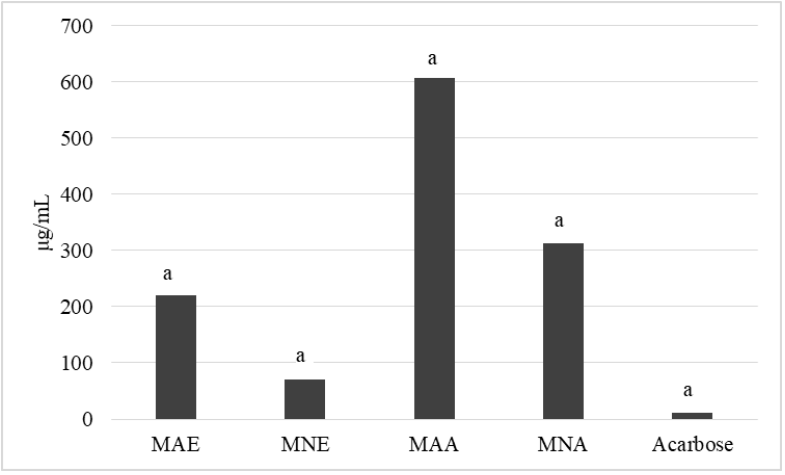

Fig. 5. The variation of the EC_{50} values of the *Morus sp.* extracts in alpha-glucosidase inhibition test. MAE - *Morus alba* ethanol-water extract; MAA - *Morus alba* aqueous extract; MNE - *Morus nigra* ethanol-water extract; MNA - *Morus nigra* aqueous extract; a - p<0.0001 extremely statistically significant

DISCUSSION

The extracts control alpha-glucosidase activity in special manner, the effect is not so important until concentration below 0.625 mg/mL and reach almost total inhibition at concentration above 10 mg/mL for ethanol-water extracts.

In the alpha-amylase inhibition test, the ethanol-water extract from both species have similar effects. It also observed that the effect of the same extracts is different in the alpha-glucosidase inhibition test with a stronger effect for extracts from *Morus nigra*. The enzyme inhibition effects are correlated with polyphenols content. Extracts from the two species can inhibit enzymes differently, so Eruygur and colleagues observed a more intense action for *Morus alba* in the alpha-glucosidase inhibition test and for *Morus nigra* in the alpha-amylase inhibition test (34).

Morus sp. extracts control digestive enzymes like acarbose that is one of the most important drugs used for this effect. The use of acarbose is limited by therapeutic compliance due to side effects such as flatulence, diarrhea, bloating and liver toxicity. This side effects are induced by the aggressive action of acarbose on enzymes, for this reason moderate inhibitors are more acceptable by the patients (35).

Another interaction between active compounds from extracts is with starch that is the substrate for alpha-amylase. Plant extracts rich on polyphenolic compounds modify the physicochemical properties of starch molecule and reduce it digestibility on the small intestine (36). Polyphenols interact with starch structure and realize hydrogen bonds with hydroxyl groups of this (37).

Depends on concentration isolated polyphenol carboxylic acids such as gallic acid can increase starch transformation by reducing the value of pH. The use of these acids in pharmaceutical forms is limited by this effect (38).

Chlorogenic acid, rutin, isoquercitrin, and quercitrin are present in *Morus alba* leaves and have been shown to have hypoglycemic properties and an ameliorating effect on diabetic nephropathy, insulin resistance, and dyslipidemia in rats. In extensive work, Hunyadi and his collaborators have demonstrated that chlorogenic acid and rutin play a major role in the in vivo anti-diabetic activity of *Morus alba* leaf extract (39). The preliminary analysis by HPLC highlights the presence of polyphenols in our samples, thus in future research the quantification of these compounds and the highlighting of their biological implications will be conducted

The obtained results allow the establishment of correlations between the extraction conditions, the composition of the extracts and their biological actions. The results also support the use of extracts for in vivo studies, with the aim of highlighting their ability to control blood glucose levels.

CONCLUSIONS

Morus leaves contain a variety of polyphenols that have antioxidant effects and free radical scavenging *in vitro*.

The analyzed extracts have an antioxidant activity and capacity of controlling the enzymes involved in the digestion of the carbohydrates, but in order to raise the therapeutically efficiency and also to reach optimal effects by administrating lower quantities of vegetal extracts, it’s necessary to fraction these extracts and an advanced separation of the compounds with important biological effects.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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DIFFICULTIES IN IMPLEMENTING THE DIETARY REGIMEN IN OBESE LITHIASIC PATIENTS

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DIFFICULTIES IN IMPLEMENTING THE DIETARY REGIMEN IN OBESE LITHIASIC PATIENTS (Abstract): Obesity and kidney stones are two related diseases. We initiated a program that involved monitoring patients who had an episode of urinary lithiasis in the past three months and providing a personalized diet regimen adapted to the type of kidney stone. **Material and methods:** Of the 687 patients diagnosed with urinary lithiasis and obesity between January 2021 and February 2023, only 243 accepted a first evaluation. We enrolled 115 females and 128 males, aged between 32 and 74 years, whit a mean age of 54.51 (SD+/- 11.13). **Results:** Of the 243 patients who accepted the first evaluation, 49.79% (n=121) followed the recommended plan, which included a diet, drinking at least 2.5 liters of water daily, and frequent measurement of urinary pH. Of these, 34.97% (n=85) patients measured their urinary pH twice daily, while 10.69% (n=26) measured it only once in the morning. It is important to note that every patient who adhered to the dietary and urological recommendations was able to sustain an average weight reduction rate of roughly 1 kg/week. Additionally, body weight and urine pH were negatively related, with larger body weights corresponding to lower pH readings. A bodyweight surplus of less than 30 kg had no impact on lipid constants, but over this amount, the average went over the upper limit. Working with obese urolithiasis patients might be difficult, but it's essential to get results. **Conclusions:** However, patients who adhered to the guidelines and the weight reduction program had positive outcomes, which encouraged us to stick with this strategy. Additionally, we discovered that no patient gained weight again throughout a time spanning from 4 months to 2 years while under intensive surveillance and counseling. This proves that our weight loss plan is successful and long-lasting. **Keywords**: KIDNEY STONES, OBESITY, WEIGHT LOSS, DIET.

Obesity is a complex multifactorial disease, defined as an abnormal or excessive accumulation of adipose tissue that causes a major health risk (1). It represents a serious global problem because it significantly increases morbidity and mortality due to the physio-pathological and metabolic implications for cardiovascular, pulmonary,

rheumatological, and oncological conditions but also due to mental health impairment (2, 3).

Approximately 39% of adults worldwide are overweight, of which 13% are obese. (4). In the European Union, 52.7% of adults are overweight, of which 16.5% are obese. Comparatively, in Romania, the data show that approximately 58.7% are overweight, of which 10.9% are obese (4).

On the other hand, urinary lithiasis is a common multifactorial disease in the general population, with a global incidence of approximately 10-11% (5). Different studies indicate that obesity increases the risk of developing and recurrence of urinary stones, with a significant association between BMI, abdominal circumference, and the risk of kidney stones, both for adult and young populations (6, 7). The relationship between obesity and lithiasis is complex and can be explained by multiple mechanisms. People with obesity are prone to excrete higher calcium and uric acid concentrations.

Trying a multidisciplinary approach to the complex problem of binom obesity - lithiasis, we encountered numerous difficulties that we tried to overcome. We aimed to evaluate the compliance of obese lithiasic patients to the lifestyle changes program

MATERIAL AND METHODS

We initiated at the Urology Clinic of "Dr. C.I. Parhon" Clinical Hospital in Iasi a Program for Evaluating and Monitoring Lithiasic Patients with Obesity (PEMLPO) The inclusion criteria include patients under 75 years of age with BMI>24.99 and diagnosed with at least one episode of urinary lithiasis. The program involved monitoring patients who had an episode of urinary lithiasis in the past three months and providing a personalized diet regimen adapted to the type of kidney stone.

Patients were excluded from the program if they were uncooperative, noncompliant, refused to be included, had various forms of dementia/oligophrenia, were pregnant, had associated tumor pathology, or opted for specialized nutritional and medical counseling in another medical center.

Patients were clinically and anthropometrically evaluated at the initial examination, laboratory tests were performed, renal and vesical ultrasound was performed, and a KUB was performed if necessary. After analyzing the dietary pattern and macro- and micronutrient requirements, a personalized diet was developed based on the body's needs, offering foods with low concentrations of oxalates/urates or that reduce the inflammatory syndrome.

Based on the medical history and interpretation of paraclinical results, other diseases associated with urinary lithiasis were identified (endometrial neoplasm, ovarian neoplasm, hypertension, diabetes, dyslipidemia, irritable bowel syndrome), and patients were referred to specialized medical consultations.

Patients received written recommendations regarding dietary restrictions and conduct specific to the type of stone, paraclinical data results, and a personalized nutritional plan for 7-14 days. The nutritional plans were calculated and verified based on the ideal weight according to the modified Lorentz formula for age.

The nutritional plans were developed in a specialized program that respected the proportions of macronutrients (protein 15-20%, fat 25-30%, carbohydrates 50-55%) and the requirement of micronutrients (vit-

amins and minerals), as well as the requirement for water and physical exercise (ideally outdoors). Patients were asked to send pictures of their three main meals and two daily snacks and additional information about blood pressure, blood glucose, urinary pH, and regular body weight measurements to a dietitian. After the first two weeks of the nutritional plan, each patient received a personalized physical exercise program based on age and sex. To ensure adequate monitoring, patients were regularly re-evaluated, and nutritional plans were adjusted based on the new measurements.

RESULTS

Between January 2021 and February 2023, we identified 687 patients diagnosed with urinary lithiasis and obesity who were invited to be included in PELO. Out of these patients, 223 accepted a first evaluation. At the same time, 444 declined the invitation due to reasons such as a lack of interest in losing weight, not considering obesity as a disease, not wanting to follow a dietary program, not having the time and desire to be monitored for a long period, living far away from Iasi, or residing in rural areas.

We enrolled 105 females and 118 males, aged between 32 and 74 years, whit a mean age of 54.51 (SD+/- 11.13). Of the 223 patients who accepted the first evaluation, 49.77% (n=111) followed the recommended plan, which included a diet, drinking at least 2.5 liters of water daily, and frequent measurement of urinary pH. Of these, 76.57% (n=85) patients measured their urinary pH twice daily, while 23.42% (n=26) measured it only once in the morning. Additionally, 39 patients followed only the dietary plan and frequently communicated with the nutritionist through phone or messages. At the same time, 17.11% (n= 19) monitored their urinary pH carefully but did not adhere to the recommended dietary plan and consumed between 2-2.5 liters of water daily.

On the other hand, 54 patients only came for the first evaluation. They withdrew from the program later, citing lack of time, demanding work schedules, or difficulty following a balanced diet. The majority of patients had a surplus body weight of over 20 kg and were between 31-70 years old, with the highest number of patients aged between 51-60 years old.

Regarding comorbidities, out of the 223 enrolled patients, 41 (18.39%) were diagnosed with type 2 diabetes. At the same time, nine were found to have altered basal glycemia during the initial paraclinical evaluation, and five were put on Metformin treatment. Out of the 41 patients with diabetes, 29 collaborated with the program. Furthermore, 49 patients were diagnosed with hypertension for which they received medical treatment, 46 had dyslipidemia, and 68 had hyperuricemia.

During the initial evaluation, several comorbidities were discovered, such as one case of endometrial cancer, one case of ovarian cancer, four cases of irritable bowel syndrome, and seven cases of hypertension. Five patients were diagnosed with type 2 diabetes and referred to a specialist for further consultation.

Regarding the evolution of known comorbidities, 11 patients under oral antidiabetic treatment and following dietary recommendations normalized their glycemic values, while five patients reduced their medication doses under direct supervision from their diabetologist. Of the 49 hypertensive patients, 23 were able to re-

duce their medication doses under cardiologist supervision as their blood pressure values improved with weight loss. Among the 46 patients with dyslipidemia, 28 stopped taking their lipid-lowering medication under medical supervision as their values improved, while 9 reduced their medication doses. Moreover, out of the 68 patients with hyperuricemia, 29 normalized their serum uric acid values. Finally, one patient with hepatic fibrosis resulting from chronic infection with hepatitis C improved from F2 to F1 according to the results of their periodic FibroScan.

It is worth saying that all patients who followed the recommended nutritional and urological guidelines were able to maintain an average weight loss rate of about 1 kg/week. Furthermore, urinary pH was inversely proportional to body weight, meaning that the higher the body weight, the lower the urinary pH values. However, lipid constants were unaffected by a bodyweight surplus of less than 30 kg, while above this value, the average exceeded the upper limit.

Of all the patients who collaborated with us, 45 have reached their ideal weight, +/- 2 kilos. However, the most satisfying aspect was that they have managed to maintain it until now.

DISCUSSION

Although there are several interventions available to manage obesity and prevent urinary stones, their effectiveness is highly dependent on patient engagement and adherence. Our study investigated the difficulties in implementing a dietary regimen in obese lithiasic patients. The findings reveal several important challenges that healthcare providers and patients face in achieving the two main goals: normal weight and nonrecurrence of kidney stones.

One major obstacle is the difficulty in collaborating with obese lithiasic patients. Patients who struggle with obesity often have difficulty adhering to dietary recommendations and may lack the motivation to make lifestyle changes. As a result, healthcare providers must employ creative strategies to engage patients and encourage compliance with dietary guidelines.

Additionally, healthcare providers must address the psychological barriers patients may face in adhering to a dietary regimen. Patients may struggle with feelings of deprivation or boredom when following a restricted diet, which can lead to noncompliance or binge eating. Nutritionists can address these challenges by providing patient education, support, and motivation to maintain adherence to the dietary regimen.

In a large South Korean cohort, Lim *et al.* found that approximately 27% of patients who had undergone the initial evaluation were determined to take serious measures to lose weight (8). This is an optimistic result because in our study, only 17% fully complied with the recommendations and the diet and can be considered to have taken serious measures to lose weight. Moreover, the authors reported that 84% of patients considered obesity to be an important factor in their general health, but some patients who refused enrollment indicated that they did not consider obesity to be a problem. The authors propose that patients can be persuaded to acknowledge the true consequences of obesity through informative and easy-to-understand materials (8). The study also found that 78% of patients had made at least one serious attempt to lose weight in the past, but they did so on their own, without the support of

a monitoring program. In our study, only 16.8% of obese patients agreed to be enrolled. This lack of support may have contributed to their failure and reduced their confidence in future attempts. Therefore, the article suggests that providing patients with the necessary support may help improve their chances of success (8).

We think patients can achieve weight loss through adherence to different diets, but the type of diet should be chosen based on the individual's needs and preferences to which the type of twist should be added.

In conducting studies and implementing weight loss programs, enrolling participants is difficult. As demonstrated by Rounds *et al*., participation in weight loss programs is often low, with only a small percentage of eligible individuals opting to enroll. This highlights the need for strategies to increase enrollment and engagement, such as targeted outreach efforts and tailored interventions based on patient preferences (9).

Another study, Caterson *et al.* showed that people with obesity are aware of their health problems and that many are motivated to lose weight. However, there is a difference between perception and reality regarding obesity and weight loss. For example, while 88% of patients consider obesity a disease and 46% are concerned about the impact of excess weight on their health, only 48% are motivated to lose weight (10).

The yo-yo effects represent another issue regarding weight loss and maintaining weight loss. These refer to repeated cycles of weight loss and weight gain, which can have a negative impact on physical and psychological health. Although weight loss is important for overall health, it is important to provide patients with information and support to maintain long-term weight loss and avoid the yo-yo effect (11).

Another challenge is the need to address the complex nutritional needs of obese lithiasic patients while promoting weight loss. Weight loss is a critical component of a successful dietary regimen for obese lithiasic patients, as excess body weight can contribute to the formation of kidney stones. However, losing weight can be difficult, particularly for patients with failed weight loss attempts.

One strategy to address this challenge is reducing calorie intake while ensuring that patients receive adequate nutrients. This can be accomplished through a diet high in fruits, vegetables, and whole grains while limiting high-fat and high-sugar foods.

A study by Shai *et al.* aimed to determine the efficacy and safety of different types of diets for weight loss. The study included 322 obese individuals, and it was conducted over two years. The study found that the adherence rate to the diet was high, and the effect on glycemic control was better with the Mediterranean diet, while the effect on lipid levels was better with the low-carbohydrate diet (12). Tuomilehto *et al*. aimed to prevent type 2 diabetes by making changes to diet and lifestyle. The study included 522 individuals, and the incidence of diabetes was reduced by 58% through changes to diet and lifestyle (13).

In our program, 11 out of 26 diabetic patients were able to normalize their blood glucose levels, and five others were able to achieve significant reductions in their blood glucose levels. This shows that the implementation of lifestyle changes can help prevent and manage diabetes.

In a relatively large cohort, Borghi *et al.* identified an increased urinary volume as a factor that can reduce the risk of develop-

ing kidney stones. The study included 300 patients, and it was found that an increased water intake can help prevent the recurrence of kidney stones (14). According to Siener *et al*., a vegetarian diet that promotes urine alkalization and thus increases pH reduces the risk of uric acid stone formation (15). It is important to avoid foods with high oxalate concentrations, regardless of their type: spinach, nuts, chocolate, or sweet potatoes. Additionally, we need to differentiate between vegetarian and vegan diets, the latter being associated with uric acid stones due to hyperuricemia (16). Another dietary aspect to consider is calcium intake, which reduces the risk of stone formation. Therefore, milk and dairy products are recommended (17).

In patients with calcium phosphate stones which often develops in alkaline urine, the goals of nutritional therapy were to acidify the urine and reduce the intake of calcium phosphates. In such cases, we implemented a hypocaloric diet based on white bread, bran bread, muffins, dried fruits, pumpkin puree, vegetable oils, fruit jelly, and meat (poultry, fish). To reduce the intake of phosphates, we excluded foods such as smoked meat and products (sausages), dairy and cheese, pickles, marinades, coffee, tea, and canned food. We also contraindicated alcohol and food consumption. One aspect that requires further study is the use of phosphates as food additives (calcium phosphate - E341). Although approved by the European Union, they could contribute to the development of stones. Further studies are needed to evaluate this risk. The macronutrient ratio was subsequently adjusted individually (18).

The dietician developed a balanced and varied meal plan to address the patient's preference for meat and prevent uric acid stone attacks. This plan included a variety of protein sources, including small amounts of lean meat (up to 100 g per day) and protein-rich plant alternatives such as legumes, nuts, and seeds. Larger portions of vegetables, salads, and whole grains must accompany meat. By avoiding foods high in purines, such as animal organs and certain types of fish and seafood (Sardines in oil - about 480 mg of purines, Anchovies - about 410 mg of purines, Herring - about 370 mg of purines, Mussels - about 350 mg of purines, Fresh sardines - about 320 mg of purines, Squid - about 260 mg of purines, Oysters - about 230 mg of purines, Octopus - about 220 mg of purines), the patient could reduce the risk of uric acid crystal formation. The patient was encouraged to choose complex carbohydrates such as whole grains, legumes, and vegetables over refined and high glycemic index carbohydrates. The diet was accompanied by the introduction of an adapted physical activity program (such as walking briskly for 30 minutes after each meal) (19).

The diet for a patient with uric acid stones should aim to reduce purine intake and increase fluid intake. According to studies, a low-purine diet can significantly decrease urinary uric acid levels and the risk of uric acid stone formation. The diet should include plenty of fruits and vegetables, whole grains, and low-fat dairy products. Protein sources should be limited to low-purine options such as chicken, fish, and tofu, while high-purine sources like red meat, organ meats, and seafood should be avoided (20). Although according to our previous data, overweight and obese patients treated in our center had the most frequent calcium oxalate kidney stones (21).

We found that some patients did not

understand the importance of adequate hydration. Our data suggest that providing information about hydration's importance can help prevent kidney stone recurrence. This is more important, taking into account that in obese patients, the efficiency of shock wave lithotripsy is relatively ineffective, and more invasive procedures should be taken into account (22). Paradoxically, a higher BMI does not seem to influence infectious complications after percutaneous lithotripsy (23).

It is essential for patients with kidney stones to consume enough water to prevent the formation of new stones or to eliminate existing ones. The recommended amount of water depends on the level of physical activity, sweating, and loss of fluids through urine. To provide patients with a deeper understanding of the role of water in eliminating and preventing the formation of kidney stones, a multidisciplinary team may be involved, including urologists, dietitians, and psychologists who can offer personalized solutions for individual patient issues.

For patients who have difficulty consuming enough water, there are several solutions. If the patient does not like the taste of water, various products can be added to change the taste, such as lemon, cucumber, mint, basil, or fruits. Patients who experience nausea or vomiting may be prescribed medication by their doctor to help alleviate symptoms that interfere with water intake. In our study, one difficulty was regarding patients with overactive bladder. We encountered 17 such patients, and after the prescription of anticholinergics, their voiding frequency was reduced to normal, allowing them to have adequate water intake.

Complying with recommendations to consume enough fluids may be challenging for patients with a busy schedules or physically demanding jobs. In this situation, patients can carry a water bottle with them and consume it during activity and take bathroom breaks during their breaks. Patients with digestive problems or a hectic lifestyle can be helped by always carrying a water bottle and setting reminders on their phones to drink water regularly.

If the patient cannot afford enough water or other hydrating drinks, alternatives such as tea, unsweetened fruit compote, and fruits and vegetables with a high-water content can be used. If the patient does not feel the need for hydration, the urologist and dietitian can investigate the reason and offer advice to encourage the patient to consume increasingly larger quantities of fluids and water-rich foods. A multidisciplinary team can provide personalized solutions for patients with kidney stones who have difficulty consuming enough fluids. It is important to recognize and address dysfunctional habits and patterns and the patient's vicious behavior to motivate them to change. In our study, of all 243 patients, 179 have maintained a daily water intake of a minimum of 2.5 liters, which is a strong suggestion that patients can understand the necessity of drinking enough water. Once a daily habit of drinking water is established, the problem of adequate hydration is less prevalent.

Our study highlights the complex challenges healthcare providers and obese lithiasic patients face in implementing a hygienic-dietary regimen.

Another issue highlighted in the literature is the relationship between obesity and urinary stone disease. Obesity is a well-established risk factor for urinary stones, likely due to changes in urine composition

and increased urinary excretion of calcium and other stone-forming substances. BMI and urine pH have an inverse relationship. Because individuals with higher BMI have a lower increase in urine pH under treatment with potassium citrate, it is suggested that most benefits may derive from weight loss (24). Alkalinization of the urine proved to be effective not only for kidney stone prophylaxis but also with similar results as alfa-blocker for some ureteral stones as medical expulsive therapy (25).

Urinary pH plays an important role in the formation of urinary stones. Therefore, monitoring and maintaining urinary pH within a certain range can help prevent the formation of urinary stones. This can be achieved through dietary changes, increasing water intake, and medications if necessary.

There are several reasons why some obese lithiasic patients do not monitor their urinary pH in our monitoring program, such as lack of awareness - some patients are not aware of the importance of urinary pH and how it affects the formation of kidney stones; cost or difficulty in obtaining urinary pH testing kits - some testing kits can be expensive or hard to obtain, which can discourage patients from using them; mismatch with their lifestyle - monitoring urinary pH can be a difficult task for some patients, especially those with a busy lifestyle; lack of motivation - some patients may be demotivated in monitoring their urinary pH because they do not see immediate benefits or do not understand the connection between pH level and stone formation.

The solutions found include explaining the importance of continuous monitoring of urinary pH and how it can help prevent the formation of urinary stones through dietary and hydration interventions; emphasizing the importance of preventing recurrence and how monitoring urinary pH can help in this regard; teaching patients how to collect urine samples and perform tests correctly; providing free kits through the PEMLPO program to eliminate reasons related to cost or availability of kits; encouraging communication with the PEMLPO team in case of monitoring problems or concerns related to results and providing personalized advice; encouraging patients to keep a journal of pH values to see progress and gain confidence in their interventions. The importance of pH monitoring is significant as pH directly reflects whether patients are indeed following dietary and fluid recommendations.

When patients have a limited budget, it is recommended that they consume healthy and nutritious foods that are cheaper. They are suggested to buy and prepare foods in larger quantities and divide them into smaller portions to save time and money. If they cannot access fresh foods due to financial or geographic limitations, patients should buy frozen vegetables, beans, fruits, and dehydrated vegetables. Information should be provided on food assistance programs and free or reduced-price food resources to assist patients with limited food needs. Additionally, it may be beneficial to collaborate with community organizations and local health centers to find personalized solutions for each patient.

A study in Australia approximated the costs of a diet for a family of four, and the presented results show that a healthy diet is 21% cheaper than an unhealthy one. However, both diets exceed the budget for families with low to very low financial situations. Additionally, discounted prices through various offers reduce costs by

approximately 3% (26).

Contributing to the failure of weight loss diets, some authors consider the endocrine response that occurs with weight loss, consisting of increased appetite and decreased satiety (27). Another aspect is sedentary behavior, both at work and in daily life, and the modernization of transportation with the use of personal vehicles also contributes to this (27)

A meta-analysis of 29 studies shows that over 50% of patients return to their initial weight within two years and over 80% within five years. The reasons are unclear, but the lack of a regular activity and exercise program is suggested (28). Another study proposes that the reason for returning to the initial weight is a too-rapid initial weight loss followed by a period of no further weight loss (29). Grodstein *et al.* concluded after a 3-year weight loss program that only 12% of patients managed to maintain at least 75% of the weight they lost, while 40% gained more than they had initially (30).

Another study led by Wing *et al.* concluded that long-term success has better outcomes for patients who maintain their lost weight for 2-5 years. The best predictor for long-term success is weight maintenance for two years. Additionally, another predictor highlighted by Wing *et al.* is represented by low levels of dietary disinhibition, or the ability of individuals to not overeat in the presence of factors such as emotional stress and appetizing foods (31).

Finally, the study found that strict supervision and counseling can ensure compliance with dietary guidelines and prevent weight gain. Patients who received regular supervision and support were able to maintain weight loss over an extended period, suggesting that ongoing support and monitoring may be necessary for long-term success.

Regarding weight loss strategies, an important aspect is to provide patients with adequate support for long-term changes in dietary and exercise habits. In this regard, multidisciplinary approaches involving physicians and specialists in nutrition and psychology can be effective in helping patients achieve and maintain weight loss goals.

CONCLUSIONS

Following this study, we have reached some important conclusions. Firstly, collaborating with obese urolithiasis patients can be challenging but crucial to achieving positive results. However, patients who followed the instructions and the weight loss program achieved good results, encouraging us to continue with this approach. We also found that under strict supervision and counseling, no patient gained weight back within a period ranging from 4 months to 2 years. This demonstrates that our weight loss program is effective and sustainable in the long term.

Finally, it is important to note that no patient who became “stone-free” after ESWL or URS experienced urolithiasis recurrence. This suggests that weight loss and an appropriate diet can be powerful tools in preventing kidney stone formation and maintaining renal health in the long term.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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CHEMICAL ASSESSMENT AND BIOLOGIC POTENTIAL OF A SPECIAL *LESPEDEZA CAPITATA* EXTRACT

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CHEMICAL ASSESSMENT AND BIOLOGIC POTENTIAL OF A SPECIAL LESPEDEZA CAPITATA EXTRACT (Abstract): Secondary metabolites are natural compounds synthesized by plants as an adaptation to environmental factors. Humans use such components for the prevention and treatment of minor or mild conditions. *Lespedeza capitata* is a medicinal and melliferous plant known for its various beneficial properties (antioxidant, antiaging, antiedematous, etc.). **The objectives** of this study were to identify and quantify the secondary metabolites from a selective *L. capitata* extract. Moreover, the evaluation of the antioxidant activity was part of the present research. **Material and methods:** Identification and semi-quantification were assessed by liquid chromatography (HPLC/MS), and the total flavonoids and polyphenols were determined by spectrophotometry according to European Pharmacopeia indications. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and iron chelation test were used to establish the antioxidant putative effects of the investigated extract. **Results**: Among the identified compounds different quercetin derivatives, chlorogenic acid, and rosmarinic acid were found. The total polyphenols amounted to 174.24 mg/g GAE and flavonoids measured 95.65 mg/g rutoside equivalents. The antioxidant activity showed a good capacity to scavenge free radicals and a higher potential to chelate ferrous ions. The intensity of the effect was correlated to concentration. All in all, the investigated *L. capitata* extract demonstrated a polyphenol and flavonoid-rich content with good antioxidant potential. **Keywords**: LESPEDEZA, HPLC/MS, TOTAL POLYPHENOLS, ANTIOXIDANT ACTIVITY.

Actual living conditions are commonly related to a variable exposure to polluted environments which determines the alteration of homeostasis, the overloading of body defense mechanisms, and immunosuppression with a negative impact on health and lifestyle. In this context, plant secondary metabolites represent frequent options as antioxidants that can counteract the harmful effects of pollution and overexpression of oxygen-reactive species (1, 2, 3). The chemical conformation of flavonoids and polyphenols presents chemical patterns that allow them to donate protons to quench free radicals. Various studies confirm the antioxidant and antiradical properties of natural

and synthetic flavonoids (1, 4, 5). Moreover, certain compounds were found to have possible applications in the prevention of hypertension, vascular problems, degenerative disorders, and gastrointestinal and urinary tract diseases (6, 7, 8).

In the last decade, forage legumes come into the spotlight due to their beneficial effects on domesticated animals and the environment (phytoremediation). Hence, many plant species belonging to the Fabaceae family were screened for their rich content of polyphenols, a chemical group of metabolites, generally recognized as antioxidants (4, 5). *Lespedeza* species represent a group of wild plants used as legumes, native to the Americas and oriental countries as part of their traditional remedies. Some of the reports showed that five species (*L. tomentosa* (Thunb.) Maxim, *L. bicolor* Turcz., *L. hedysaroides* (Pall.) Kitag., *L. cyrtobotria* Miq., and *L. davurica* (Laxm.) Schindl.) are also native to Russia (5, 9, 10, 11). These species were found to contain flavonoid glycosides, lignans, sterols, alkaloids, organic acids, coumestans, and terpenoids.

Lespedeza capitata Michx. (Roundhead *Lespedeza)*, native to eastern North America and common in meadows, is an understudied legume with a tradition in folk medicine as a diuretic, anti-inflammatory, and antirheumatic both in the USA, Korea, and China. Native Americans used the roots or extracts from roots as an antidote for poisoning (5). Among the *Lespedeza* species, *L. capitata* is less studied, although extracts from leaves and stems are used for urinary tract and kidney disorders.

This study aimed to evaluate the chemical spectra of an industrial extract obtained from the aerial parts of *Lespedeza capitata* and to establish the existence of an antioxidant profile.

MATERIALS AND METHODS

Plant extract: The plant extract was obtained by direct collaboration with Vanelli company, which are single importer of *Lespedeza capitata* dried extract (4:1) in our country.

Chemical profile: The identity of the compounds found in the investigated extract was evaluated by liquid chromatography using a Transcend TLX-1 Vanquish Flex system coupled with the Orbitrap Exploris 480 high-resolution mass spectrometer and Compound Discoverer 3.3.1 software for the identification of small compounds, part of the CENEMED platform. The method used formic acid 0.1% (A) and methanol (B) in a ramp from 95:5 to 10:90 in 15 minutes, partial loop injection of 10 µL, a debit flow of 300 µL, Hypersil Gold C18 column (50 x 2.1 mm, 1.9 µm) at 40°C, and the instrument settings were electrospray ionization (ESI), negative ion mode (-p), mass range m/z from 100 to 1500 at 5 Hz frequency, negative voltage of 3.0 kV and an ionization temperature of 350°C. Integration and detection were subjected to Compound Discoverer 3.2 software for evaluation in targeted and untargeted modules. The peaks with a rating of at least 7.4 were taken into account and verified against Thermo m/z Vault, NIST, and Chem spider databases.

Total phenolic content evaluation: A colorimetric method (proposed by Singleton and Rossi) using the Folin-Ciocalteu reagent was conducted to determine the total amount of polyphenols that are capable of forming blue complexes in alkaline conditions, following a method previously described (13, 14). Gallic acid (amounts of 3-8 µg/mL) was used to obtain a calibration curve and the final results were expressed as gallic acid equivalents (GAE), quantified as milligrams of gallic acid per g dry

extract (3, 14).

Total flavonoid content evaluation: Similarly**, t**he quantification of total flavonoids used a spectrophotometric method using aluminum chloride in alkaline conditions, when flavones and their derivatives form yellow fluorescent complexes that can be measured at λ = 430 nm (12). Rutoside was used for calibration and the results were denoted as rutoside equivalents (mg rutoside/ g dry extract).

Antioxidant potential: The antioxidant effects of the extracts were evaluated by two well-known methods DPPH radical assay and iron chelation assay. For this, 2,2-diphenyl-1-picryl-hydrazyl (DPPH) was used to estimate the radical scavenging effect of *Lespedeza capitata* extract. previously described (3, 14). Ion ions (Fe^{2+}) may be complexed by ferrozine to form a magenta complex measured at 562 nm. The compounds that have an iron chelation effect block the complex formation thus resulting in a lower absorbance. All steps were followed as previously described by Nani *et al.* and Iancu *et al.* (15, 16), Ascorbic acid was used as a positive standard in both tests.

All determinations were in triplicate, and the presented results were expressed as mean ± standard deviation error.

RESULTS AND DISCUSSION

Chemical profile

The general compound profile showed the major presence of flavonoid derivatives (especially quercetin derivatives) and some polyphenol carboxylic acids [chlorogenic acid (m/z = 353.08) and rosmarinic acid (m/z = 359.16)]. The general aspect of the chromatogram is given in first figure.

Different studies indicated the major presence of similar compounds, in particular flavonoid glycosides and condensed tannins (2, 5, 6, 17), however a more elaborate chemical composition of this species is not yet available. Some of the aforementioned studies are dated before 2005. For our results, it should be taken into account that the data is strictly related to the investigated extract (which is a commercial, industrially obtained, and selective extract) and cannot comprise the full spectra of components that belong to the plant.

Total phenols amounted to almost 175 mg GAE for each gram of extract, whereas flavonoids were less (95.65) (tab. II).

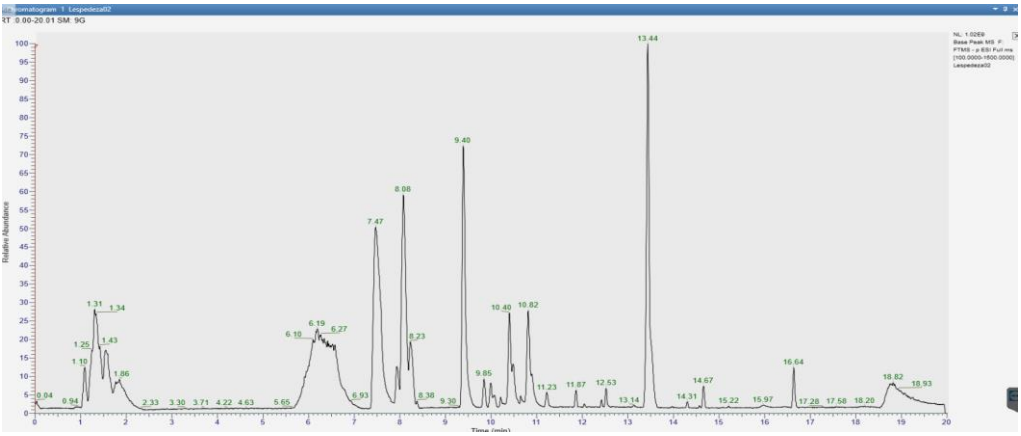

Fig. 1. General LC-ESI-MS chromatogram for *Lespedeza capitata* extract

TABLE I.

Tentative identification of the components from *Lespedeza capitata* extract

No	$[M-H]^-$ (m/z)	MS/MS (m/z)	Compounds
1	353.08	353.08, 191	Chlorogenic acid
2	179.02	341, 179, 135	Caffeic acid-3-glucoside
3	301.03	301.03, 179.0, 150.9	Quercetin-3-D-galactoside
4	515.10	515.10, 353, 191, 179	1,3-Dicaffeoylquinic acid
5	287.05	333, 287.05, 151	Dihydroluteolin
6	269.04	269.04	3,3',4'-Trihydroxyflavone syn. 5,7-Dideoxyquercetin
7	317.06	317, 299, 175	6-Methyldihydroquercetin
8	359.16	359, 161.02, 197	Rosmarinic acid

TABLE II.

Major classes of compounds for the *Lespedeza capitata* extract

Sample	Total phenols (mg gallic acid/g)	Total flavonoids (mg rutoside/g)
Lespedeza capitata	174.24± 0.63	95.65± 0.31

Results expressed as mean ± standard error of the mean (n =3) of three determinations

Despite the long tradition and use in folk and modern medicine, *Lespedeza capitata* has no scientific data available regarding the total polyphenols and flavonoids. By searching Scopus/Elsevier databases for *lespedeza AND capitata AND total AND polyphenols OR flavonoids* no matching documents were found. Nevertheless, other Lespedeza species such as Korean *L. cuneata* were proven to contain 102.7 mg gallic acid/g and 62.8 mg quercetin/g (18). Also, data on the aerial plant product (*L. bicolor*) from Pakistan indicated the presence of 3.321 mg GAE/g of dried weight and 0.148 mg/g of dried weight (19).

Antioxidant potential

The obtained data for both antioxidant tests are given in fig. 2 and are expressed as the inhibitory concentration of 50 % of DPPH radicals and iron complexes respectively.

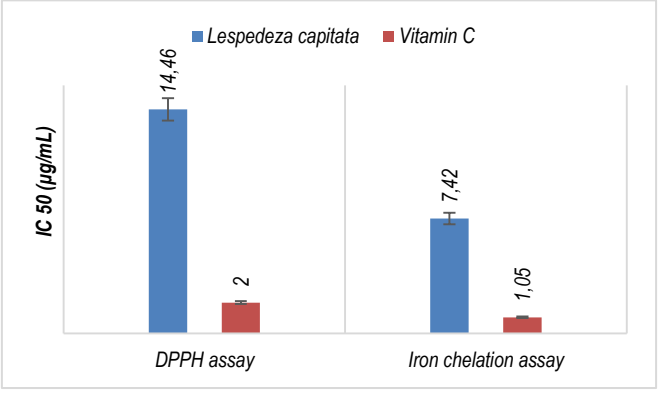

Fig. 2. Results for the antioxidants assays (represented as IC50) for *L. capitata* extract

The results indicated that the investigated extract has a lower activity than ascorbic acid used as a positive standard, however, the IC50 values were expressed in μg/mL of extract. Such amounts are extremely low and prove that the selective *L. capitata* extract has great potential as an antioxidant, but the intensity of the effect depends on the mechanism of action. The phytocomplex found in the extract exhibited twice as much activity against ferrous ions than against DPPH radicals. This is an interesting aspect since ferrous ions are involved in the Fenton reaction that generates hydroxyl radicals in living organisms, such radicals are known to be aggressive and damaging (15, 16).

Other *Lespedeza* species (*L. bicolor*) showed good antioxidant activity, with IC50 values ranging from 50 to 200 μg/mL, but the root samples were better than the aerial parts (19).

More recent research investigated the capacity of an *L. capitata* extract to inhibit collagenase activity which is related to skin cell ageing. The results showed that the extract possesses potential benefits for dermatologic use and wound healing similar to some other Brazilian plants (12).

CONCLUSIONS

Our study represents a starting point for further research in assessing the putative activity of *L. capitata*. The investigated extract is already used in some formulations (along with silymarin) as food supplements recommended for maintaining good liver and kidney functionality. The obtained results indicated a good chemical profile and an interesting antioxidant potential, especially active against ferrous ions. In conclusion, more data is still needed to confirm the actual benefits of *Lespedeza capitata*.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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PREDICTABILITY OF DIGITAL SET-UP IN ORTHODONTICS

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PREDICTABILITY OF DIGITAL SET-UP IN ORTHODONTICS (Abstract): The orthodontic setup is a three-dimensional simulation that allows the teeth to be aligned in the appropriate position according to a previously established treatment plan previewing the final result. The main purpose of this study was to evaluate the accuracy of the digital orthodontic set-up by comparing it with the result of the orthodontic treatment. The secondary purpose is to compare the correlation between the parameters obtained after performing the digital setup with the analysis of the study models at the end of the orthodontic treatment. **Materials and methods:** The study models of 40 patients were digitized. In a subsequent phase, the digital set-up was performed using the BlueSky Plan software (Libertyville, IL United States) to obtain appropriate intermaxillary relations according to the treatment plan. **Results:** The intercanine and intermolar distances, and the length of the dental arches were measured both in the initial, final models, and after the set-up in order to be able to compare them. Statistically significant differences were observed between the intercanine width (p=0.007) and arch length (p=0.02) of the final models compared with the set-up models. Regarding the other evaluated parameters of the final models, although the values were decreased compared to the set-up models, the differences were not significant. **Conclusions:** the comparison made between the digital set-up and the patient's final model gives confidence in choosing the set-up as a reliable tool for treatment planning. **Keywords:** DIAGNOSTIC, DENTAL MODEL, SET-UP, INTERCANINE WIDTH.

Diagnosis, in orthodontics, as in other fields of medicine, requires the collection of a database with concrete information about the patient's main dento-alveolar problems. Important information can be obtained from direct observations, the study of the patient's photographs, radiographs, CT scans, study models, intraoral scans, but also by creating a set-up that allows simulating the alignment of the teeth following the orthodontic treatment (1, 2).

The dental field has always been in step with the developments in the world of technology. During the last century, several

revolutionary leaps that gave rise to new theories and hypotheses were introduced. The last important discovery can be considered the use of CAD-CAM systems (3, 4).

The use of the intraoral scanner in the daily routine offers the possibility of obtaining 3D models of the dental arches with impeccable precision. The use of these 3D models has made it possible to modify the diagnostic approach, improving and simplifying classic orthodontic analyses, thus allowing various virtual simulations (5, 6).

The biggest revolution introduced by the digital set-up consists in the fact that it allows the treatment to begin by previewing its "end", orienting the practitioner strictly in the direction of the desired result (7, 8).

Set-up in orthodontics

Until the recent past, the focus in orthodontic treatment planning was mainly based on the analysis of photographs, models and profile teleradiograph. The contemporary approach involves a much more detailed clinical examination, where many aspects of the treatment plan are discovered through a functional and aesthetic assessment of the patient. (9)

The orthodontic set-up represents a three-dimensional simulation of the orthodontic teeth movement based on specific treatment objectives. It is thus a valuable diagnostic tool that allows the comparison of several proposed treatment plans, highlighting the risks and benefits of each one, indicating the possible need for surgical interventions or dental extractions, thus facilitating communication with the patient (9,10).

The main advantage of this procedure is that it allows the clinician to assess whether the planned treatment plan is really viable for that case (11, 12).

Digital Set-up

Over the years, with the advancement of the digital age, the orthodontic set-up has gone through several phases of evolution (13). Now we can talk about the existence of two types of set-up: classic and digital. So, the "laboratory work" in which the teeth were cut and aligned with the help of wax, is now reduced to some virtual segmentation techniques (14, 15).

Although it presents multiple advantages, the digital set-up has a long learning curve, requiring an additional effort of the practitioner. In addition, most of the software allows the strict evaluation of the dental crown movement, without considering the position and condition of the roots (16, 17).

Classic or Digital, it is universally accepted that the setup makes the treatment plan less speculative, providing the clinician with reliable information that can reduce biological damage to the patient.

OBJECTIVES

The main purpose of this study was to evaluate the precision of the digital orthodontic set-up by comparing it with the result of the orthodontic treatment.

The secondary aim was to compare and correlate the parameters obtained after performing the digital set-up with the analysis of the models after the completion of the orthodontic treatment. Thus, comparatively analyzed the intercanine, intermolar distance and the length of the dental arches for both the upper and lower arches.

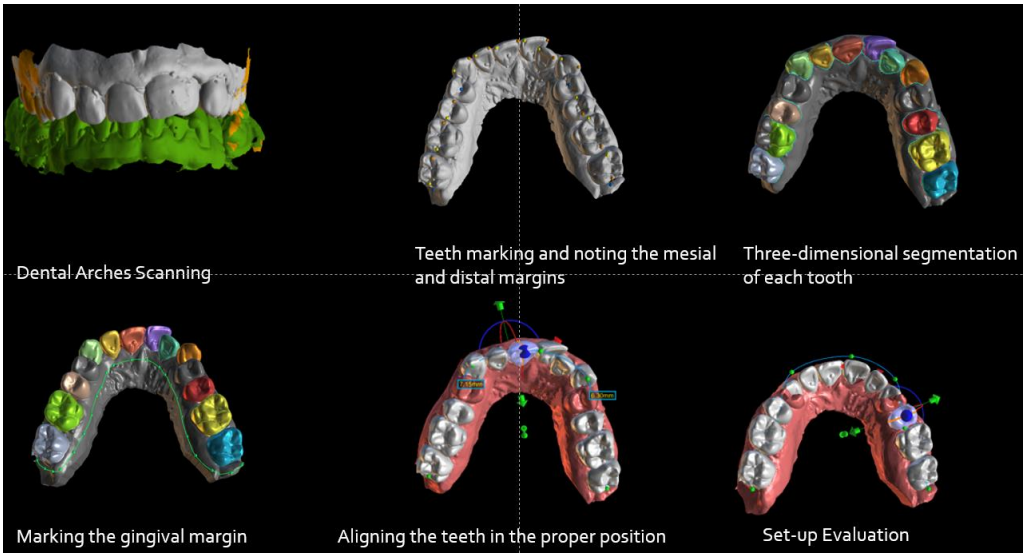

Fig. 1 Digital Set-Up Step by Step

MATERIALS AND METHODS

To achieve the proposed objectives, we studied a sample consisting of 80 conventional study models (40 initial models and 40 final models after the orthodontic treatment) and 40 digital models. Patients were treated during the years 2016-2021 at the Dental Clinic "Natural Smile by Dr. Pop", coming from both urban and rural areas. The inclusion criteria for our study were as follow:

1. The patient has completed the orthodontic treatment;

2. The treatment required the extraction of one permanent tooth from each hemi arcade;

3. Permanent or mixed dentition at the beginning of treatment;

4. The treatment does not require major prosthetic and surgical interventions;

5. The patient does not need orthognathic surgery;

6. Patient models are in proper condition and have been manufactured correctly.

Thus, following a rigorous selection, 40 patients who m*et all* these requirements were included in the study. The main goal after the orthodontic treatment, as well as the digital set-ups, was to obtain:

- correct occlusal relationships, respecting the fundamental principles of occlusion,
- arch of appropriate shape,
- cusp-pit occlusal contacts,
- overbite and overjet within acceptable limits,
- appropriate relationships of the inclined occlusal plane at the level of molars and premolars,
- appropriate sagittal Spee and transverse Wilson curves.

The initial and final models were scanned using the Medit i500 (Seoul, South Korea) high-precision scanner which uses a laser beam and intraoral cameras, which allowed them to be saved in STL (Standard Triangle Language) formal. Next, the digital set-up was carried out using the BlueSky Plan software, using the "Orthodontics" section.

The steps followed are approximately the same for all the software, with small differences depending on the complexity of the analyzed data (fig.1):

1. Scanning dental arches or study models
2. Entering data and scans in the setup software
3. Calling the teeth and marking their mesial and distal ends
4. Three-dimensional segmentation of each tooth
5. Aligning the teeth in the proper position
6. Evaluation of the setup

The teeth were moved to their ideal position according to the treatment plan, considering the limits imposed and the teeth considered extracted achieving stable and functionally correct interocclusal relationships. At the end, the set-up was saved as digital models, to allow the measurements necessary for the study to be carried out and then the comparison with the data obtained from the analysis of the final models (fig. 2).

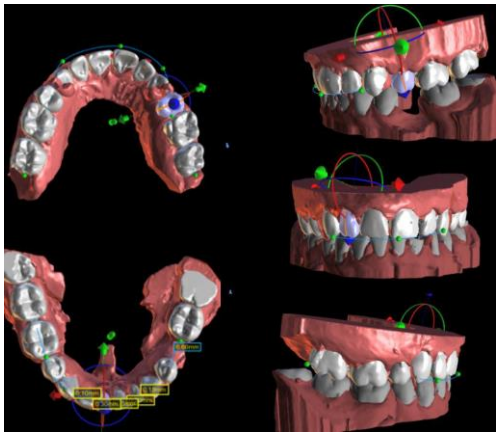

Fig. 2. Dental Arches After the Set-Up

In order to make a proper analysis of the results obtained by comparing the digitally created set-up and the final model of the patient, the following measurements were made: the length of the dental arches; upper and lower intercanine distance; upper and lower intermolar distance. All the virtual measurement was made online on webceph.com (Seongamsi, South of Korea) and the conventional casts were measured using a digital caliper (fig. 3).

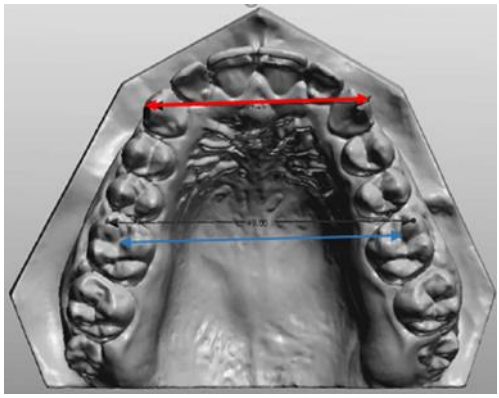

Fig. 3 Measurements on the Models

Student T-test and Wilcoxon, level of significance $p<0.05$ was used to statistically analyze the results. Also, ANOVA relevance test allowed the simultaneous evaluation of the three cases.

RESULTS

Forty patients were included in this study: 18 male and 22 female aged 11-43 years. The patients were distributed into groups according to the dental classes. By calculating the average values recorded as a result of the measurements performed and the standard deviation, obtained after the elimination of extreme outlier type values, the data were entered in first table to be able to be further compared descriptively and analytically from a statistical point of view.

TABLE I.
Mean and Standard Deviation

	Initial Model		Final Model		Set-up	
	Mean (mm)	SD (mm)	Mean (mm)	SD (mm)	Mean (mm)	SD (mm)
Upper Inter-Molar Distance	46,84	3.07	44.51	2.06	47.65	2.10
Lower Inter-Molar Distance	48.44	2.92	47.36	2.23	48.84	2.86
Upper Inter-Canine Distance	34.84	3.12	35.12	1.93	37.42	1.84
Lower Inter-Canine Distance	28.04	2.29	26.8	1.34	28.42	1.83
Upper Arch Length	118.07	9.24	111.14	1.00	113.97	6.25
Lower Arch Length	110.05	12.04	107.01	5.38	108.18	4.27

The statistical tests showed a significant difference for the comparison of the Lower Inter-Canine Distance (p=0.007) and Upper Arch Length Distance (p=0.02) values. The difference for the Lower Inter-Canine Distance was confirmed further in comparing all the 3 situations simultaneously (p=0.03).

DISCUSSION

The significant difference between the setup and the final model at the level of the length of the upper arch, through might be explained by an effect of "shortening of the dental arch". The oscillation between the mean values (2.83 mm) led to the emergence of significant differences by applying statistical tests, which could be explained by the fact that the degree of retrusion of the frontal group, as well as the molar mesialization after extraction was much higher than was previewed via the digital set-up. Another, more pronounced difference can be observed at the level of the lower intercanine distance. This variation poses problems from the point of view of maintaining the stability of the result obtained after orthodontic treatment for a long term.

A similar study was performed by Monica S. Barretoa *et al.*, (18,19) who made the same measurements in order to evaluate the differences obtained. Analyzing their results, a statistically significant difference is observed in the intercanine distance of the upper arch ($p<0.001$). Comparing the data described in their study with those obtained in ours, a similarity of the values was observed, the greatest variation being obtained at the level of the intercanine distance. From a clinical point of view, their research shows that especially in cases that require the extraction of premolars to obtain functional intermaxillary relations, the risk of relapse following orthodontic treatment is directly proportional to the changes in the intercanine distance. Therefore, it is extremely important to keep this value as close as possible to the initial one, thus minimizing the risk of relapse. (20,21)

Another more complex research made by Takuya Kihara *et al.* (22) analyzed the clinical data of 5 patients to demonstrate the importance of root position preview in the digital set-up. A significant difference was detected in the root distance between anterior and posterior teeth ($p < 0.05$). Takuya Kihara emphasizes through this study the importance of previewing the final result of orthodontic treatment from the perspective of the position and condi-

tion of the dental roots. He believes that this is one of the most common complications of orthodontic treatment, so special attention should be paid to this aspect (23).

Another study was carried out by Fabio Federici Canova *et al.* (24), which evaluates the predictability of the set-up of the same patient performed with four different programs, including the one used by us - Blue Sky Plan. It remains one of the most current at the moment, because it possesses a wide range of "tools" that make the work of the practitioner easier, facilitating the clinical applicability of the implemented changes (24, 25).

New technologies make orthodontic therapy more efficient and predictable. And the results of this study demonstrate that the use of digital set-ups is a reliable method of evaluating the treatment plan, as it provides a three-dimensional preview of the patient's occlusion at the end of the orthodontic treatment. This allows for greater trust and strengthens the doctor-patient relationship.

CONCLUSIONS

The comparison made in this study between the digital set-up and the patient's final model gives confidence in choosing the setup as a reliable tool for treatment planning.

Preserving the intercanine distance following orthodontic treatment is an important factor for relapse prevention.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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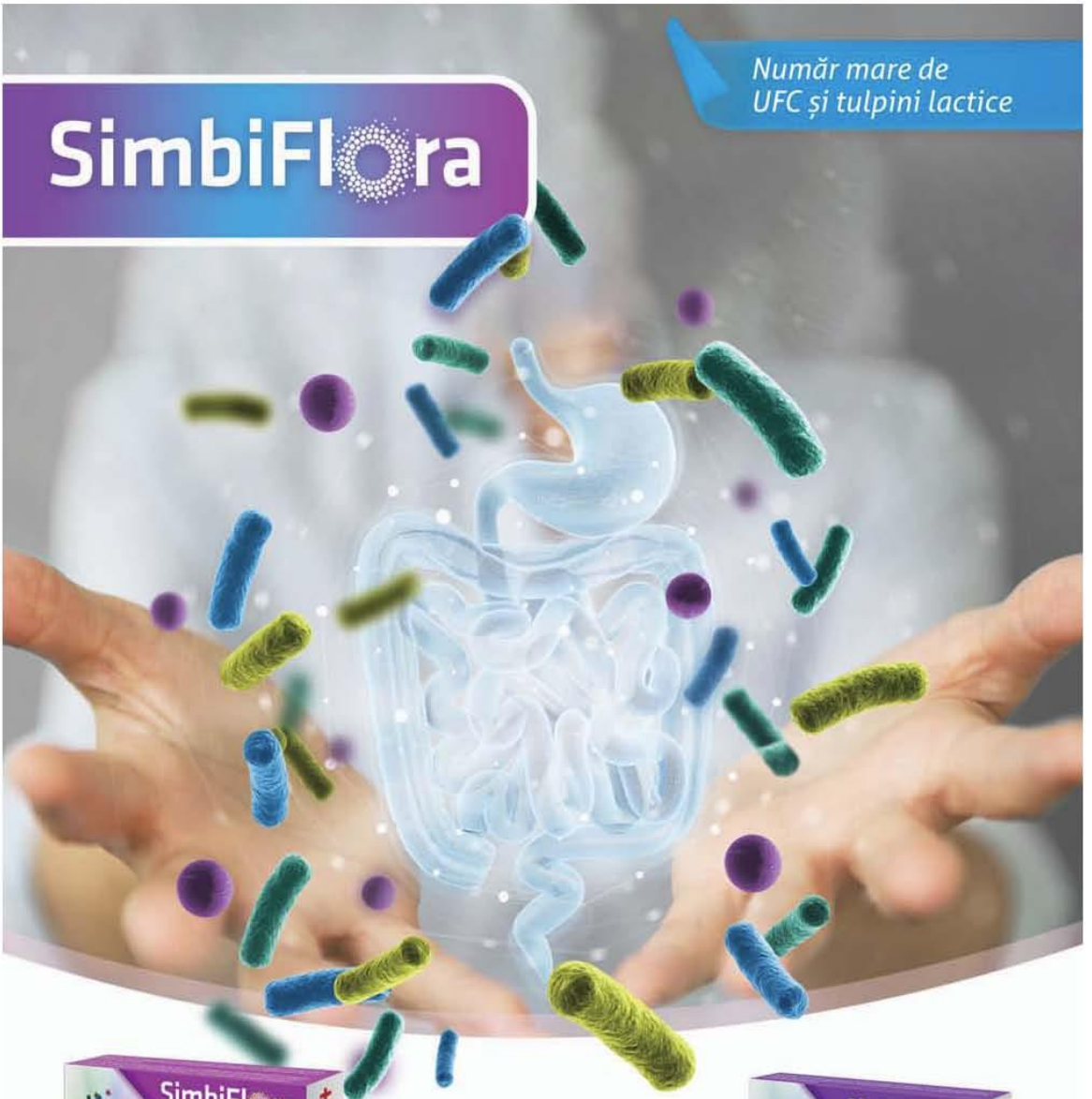
SimbiFlora

Număr mare de UFC și tulpini lactice



SIMBIOZA care reglează *DISBIOZA*

Antibiotice a+

THE BENEFIT OF MOLECULAR MICROBIOLOGICAL ASSAYS IN OPTIMAL MONITORING OF PERIODONTAL DISEASE. A RETROSPECTIVE STUDY

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THE BENEFIT OF MOLECULAR MICROBIOLOGICAL ASSAYS IN THE OPTIMAL MONITORING OF PERIODONTAL DISEASE. A RETROSPECTIVE STUDY (Abstract): Periodontal disease is among the most frequent pathologies worldwide, with multiple consequences to the quality of daily patient life. **Aim:** To assess the prevalence and the DNA load of etiologic agents in selected patients with periodontal disease. **Materials and methods:** 55 saliva samples from periodontal disease patients were analyzed by multiplex Real Time PCR, using specific primers for: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola*. The positive sampled were further analysed for the evaluation the antibiotic susceptibility to Clindamycin, Metronidazole, Amoxicillin, Doxycycline, Ciprofloxacin, and Clarithromycin. **Results:** The most frequent bacteria detected were *Treponema denticola*, in samples from 47 patients (85.5%), followed by *Porphyromonas gingivalis*, in 43 patients (78.2%) and *Aggregatibacter actinomycetemcomitans*, in 5 patients (9.1%). Besides these 3 periodontal pathogens, one patient was positive for *Tannerella forsythia, Prevotella intermedia, Eubacterium nodatum and Capnocytophaga gingivalis.* **Conclusions:** The microbiologic diagnosis using sensitive molecular assay is compulsory for modern and optimal periodontal diseases assessment. **Keywords:** PERIODONTAL PATHOGEN, CLINICAL GUIDELINE, REAL-TIME PCR, DIAGNOSIS, TOOTH SURVIVAL.

Periodontal disease is the most frequent chronic noncommunicable human disease worldwide, with important implications in the daily life of diagnosed patients, as tooth loss and disability lead to lower quality-of-life, has been associated with diabetes, cardiovascular diseases and with increased medical expenditure. The global burden of severe periodontitis has been substantial and increasing over the past three decades. In 2019, there were registered 1.1 billion prevalent cases of severe periodonti-

tis globally (1).

Many groups of researchers elaborate guidelines, for optimization of disease prevention, detection, and efficient therapy. Periodontal specialists elaborated a UK version of the European Federation for Periodontology S3-level clinical practice guideline which include consensus-based clinical recommendations of direct relevance to the dental community in the UK and encompasses 62 clinical recommendations for the treatment of stage I-III periodontitis, based on a stepwise process mapped to the 2017 classification system (2).

The UK healthcare experience recognize the benefit of Metronidazole + Amoxicillin for periodontal infections, but at the same time, the authors mention the risk of side effects regarding antibiotics overuse. These can lead to an increase in antimicrobial resistance genes, dysbiosis, and impacts on the microbiome. Given the potential harm vs. benefit considerations on the use of antibiotics (stewardship issues), the evidence-based recommendation supports the use of locally administered sustained-release antibiotics as an adjunct to subgingival instrumentation in patients with periodontal disease (2).

A more recent guideline of The European Society of Endodontology was elaborated by Duncan HF *et al*., which have used a robust, rigorous, and transparent process including the analysis of relevant comparative research in 14 systematic reviews. The guideline describes a series of clinical recommendations the effectiveness of diagnosing apical periodontitis, to preserve teeth over a patient's lifetime, according to the best comparative evidence currently available (3).

The understanding of etiopathogenesis of periodontal diseases, motivated the European researchers to evaluate the benefit of systemic periodontal antibiotic therapy versus the use of antimicrobials to target bacteria in subgingival biofilms in the periodontal pocket. Even though systemic therapy seems to be efficient, the current recommendations are limited because of the very well-known emergence of antibiotic resistance. The authors named their review very suggestive: “Europe’s contribution to the evaluation of the use of systemic antimicrobials in the treatment of periodontitis” (4).

These guidelines motivated us to evaluate patients in our area and publish our findings in this study.

AIM: to assess the etiology of periodontal pathogens in a study group of Romanian periodontal disease patients, and to follow up the efficiency of antibiotic therapy.

MATERIALS AND METHODS

For the accurate determination of periodontal pathogens, in the period 12.03.2018 - 17.11.2022, we have tested 55 patients clinically diagnosed with periodontitis or peri-implantitis, using PET (periodontitis / peri-implantitis pathogens test). From each patient we collected samples of plaque from the periodontal pockets or affected implants of patients with the use of small paper tips. These samples of plaque are not sensitive to the effects of transportation, as we analyzed DNA, which is stable in such conditions. The first step of the PET assay used involved the DNA purification. A highly sensitive molecular biology technique, real-time PCR was used in the case of every individual sample, using specific primers for our tested etiologic agents. With this method, the genetic material (the DNA) of the bacteria which it contains was processed following the classical known steps of qRTPCR: DNA denaturation, primer annealing and extension. The purified DNA was tested by multiplex Real Time PCR for the next known etiologic agents of

periodontal disease: *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Prevotella intermedia, Peptostreptococcus micros, Fusobacterium nucleatum, Eubacterium nodatum* and *Capnocytophaga gingivalis.* For positive samples, the antibiogram was performed for Clindamycin, Metronidazol, Amoxicillin, Doxycycline, Ciprofloxacin, and Clarithromycin, using a CFU (colonies forming units' assay) based assay. We analyzed our data with SPSS 20.0 software package, for descriptive statistics.

The institutional approval for the study was not applicable considering the retrospective design of the study.

RESULTS

The mean age of the study group was 35.15 years old (limits 13 - 79), 38 (67.9%) being female. Repartition of patients per age group and gender are reported in first table.

TABLE I.

Demographic data: age group - gender crosstabulation

		Gender		
		F	M	Total
Age_group		7	4	11
	11 - 20	2	0	2
	21 - 30	10	6	16
	31 - 40	10	4	14
	41 - 50	4	2	6
	51 - 60	3	1	4
	61 - 70	1	0	1
	71 - 80	1	0	1
Total		38	17	55

The Real Time PCR assay included a qualitative and quantitative assessment of the detected pathogens. The detected bacterial load varied between 10^1 - 10^9. The highest bacterial load was detected for *Porphyromonas gingivalis.* The ratio of the different pathogens was also determined. This approach is driven by the fact that some pathogens pose a risk only when present in large numbers or when they coexist with other pathogens, while others can lead to disease progression even when present only in small amounts.

Treponema denticola was detected in 47 patients (85.5%). The highest frequency was detected for the bacterial load of 10^4 in saliva: 20 (36.4%). *Porphyromonas gingivalis* was detected in 43 patients (78.2%). The highest frequency was detected for the bacterial load of 10^5 in saliva: 18 (32.7%). *Aggregatibacter actinomycetemcomitans* was detected in 5 patients (9.1%), each of them with different concentration. *Prevotella intermedia* was detected just in one patient (1.8%), with a load of 10^4. *Peptostreptococcus micros* and *Capnocytophaga gingivalis* were detected just in one patient, (1.8%), each with a concentration of 10^3. *Fusobacterium nucleatum* was not detected in any patients. *Tannerella forsythia, Prevotella intermedia, Eubacterium nodatum* and *Capnocytophaga gingivalis* were present just in one female patient, with a 10^4 bacterial load for the first two species, and 10^3 for the last two.

The highest prevalence for *Porphyromonas gingivalis* was detected for group age 31 - 40 (7/18, 38.9%), and the highest bacterial load (10^6) was detected in the 21 - 30 age group, 4/20, 20% (Figure 1). The highest prevalence for *Treponema denticola* was detected for group age 21 - 30 (19/20, 95%), and the highest bacterial load (10^6) was detected in the 21 - 30 age group, 1/20, 5% (Figure 2).

The prevalence for *Aggregatibacter actinomycetemcomitans* was equally detected for each age group (1, 29.1%), and the highest bacterial load was also in 31 - 40 age group: 10^6, 1/14 (7.1%). We found no significant statistical association (tabs. II, III).

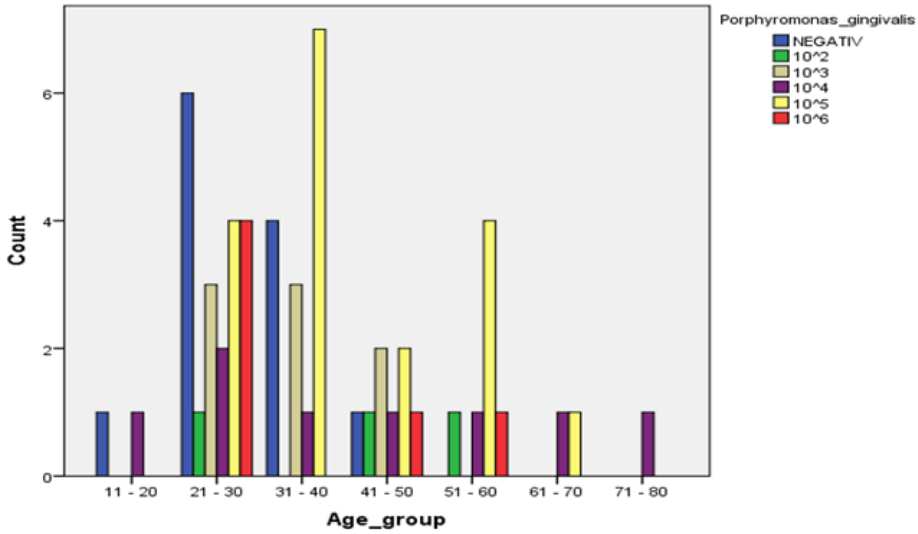


Fig. 1. Crosstabulation between age group and *Porphyromonas gingivalis* bacterial load

TABLE II.

Statistical analysis between age group and *P. gingivalis* load

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	27,166[a]	30	,615
Likelihood Ratio	29,957	30	,468
N of Valid Cases	55		

a. 41 cells (97,6%) have expected count less than 5. The minimum expected count is ,05.

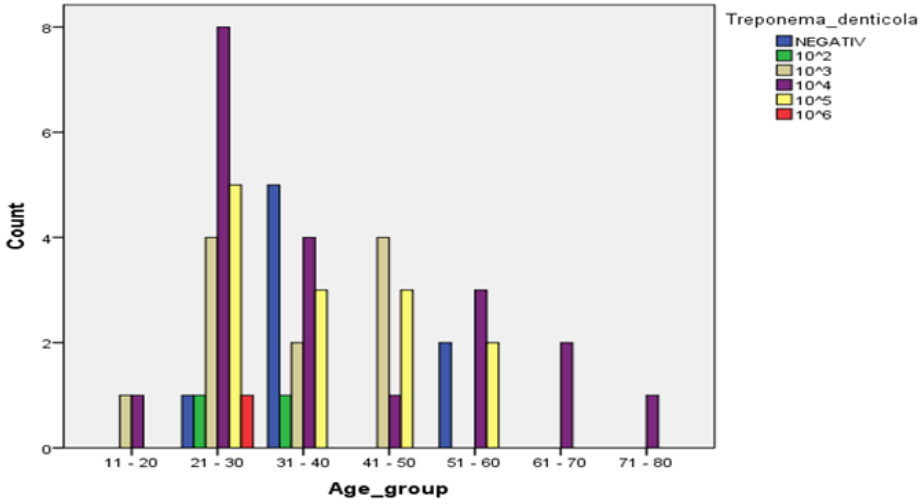


Fig. 2. Crosstabulation between age group and *Treponema denticola* bacterial load

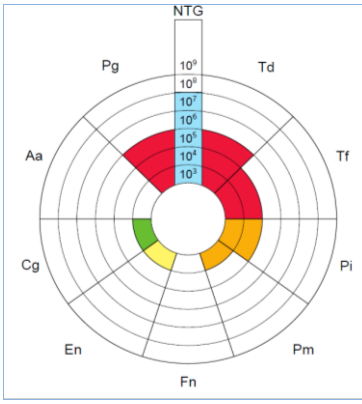
TABLE II.

Statistical analysis between age group and *T.denticola* load

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	24,764[a]	30	,736
Likelihood Ratio	27,958	30	,573
N of Valid Cases	55		

a. 40 cells (95,2%) have expected count less than 5. The minimum expected count is ,02.



Quantity of of pathogens in the sample	Percentage of pathogens %	#Pathogen complex	Pathogen name
$5,9 \times 10^7$	100		Total number of germs (NTG)
n.n.	n.n.	Aa	Aggregatibacter actinomycetemcomitans (Aa)
$*4,3 \times 10^5$	*0,73	red	Porphyromonas gingivalis (Pg)
$*1,9 \times 10^5$	0,32	red	Treponema denticola (Td)
$*6,7 \times 10^4$	0,11	red	Tannerella forsythia (Tf)
$*3,2 \times 10^4$	0,05		Prevotella intermedia (Pi)
$1,9 \times 10^3$	0,00	orange	Peptostrep. (Micromonas) micros (Pm)
n.n.	n.n.		Fusobacterium nucleatum (Fn)
$1,2 \times 10^3$	0,00	orange associated	Eubacterium nodatum (En)
$4,8 \times 10^3$	0,01	green	Capnocytophaga gingivalis (Cg)

#according to Socransky et al., 1998
n.n.= undetectable, n.t.= not tested
*bacteria for which their number is relevant for the action and treatment

Fig. 3. Example of a significant increase in the number of bacteria belonging to the red and orange complexes.

We found no statistical difference between age group and the most frequent bacteria identified in our samples.

For the patient with the above qPCR results (fig. 3), the suggested therapy according with antibiogram results, was Clindamycin, 2 x 600 mg/day, 7 days in association with Metronidazole 3 x 400 mg/day, 7 days.

DISCUSSION

In this study we have evaluated the prevalence of periodontal pathogens in 55 patients diagnosed with periodontal disease, using Real Time PCR. Our data are in accordance with those of other groups of researchers who used the same molecular assay.

The etiology of periodontal diseases has been evaluated by many researchers worldwide. Ergün E *et al.*, used Real Time PCR assay to evaluate the microbial content of periodontal abscesses, using 16S rRNA Universal primers. They detected *Porphyromonas gingivalis, Prevotella intermedia* and *Fusobacterium nucleatum* in all 30 tested samples. Other bacterial species were detected with lower frequency: *Porphyromonas endodontalis, Tannerella forsythia, Actinomyces naeslundii* (5). Reddahi S *et al.*, from Morocco investigated periodontal pathogens in saliva from 53 patients with qPCR and they identified a significant difference in *P. gingivalis* and *A. actinomycetemcomitans JP2* clones in the saliva of periodontitis patient compared to the control group. The researchers consider testing of

etiologic agents of periodontal disease a good potential as diagnostic markers for periodontal disease (6). A more comprehensive study was realized by Chigasaki O. *et al*, in Japan, which tested almost 1000 periodontal disease patients with Real Time PCR. The authors found that *P. gingivalis* was strongly associated with an increase in the rate of sites of 4 mm or greater probing pocket depth, bleeding on probing, and 1.5 mm or more of bone loss on dental radiographs sites. For this study group, the amount of *P. gingivalis* was strongly associated with the severity of periodontal disease. Other bacteria from red-complex as *T. denticola* and/or T. *forsythia* showed heightened progression of periodontitis (7).

Eick S. *et al.*, tested by RT-PCR Swiss adolescent subjects, and detected 10 % to be positive for *P. gingivalis*, 23% for *A. actinomycetemcomitans* and *T. forsythia*, and 47.5% for *T. denticola* (8).

Wang P *et al.*, evaluated through the same assay, qPCR, periodontal pathogens in 25 patients with chronic periodontitis. Our study shows comparable results to theirs, *Treponema denticola* having the highest prevalence. The authors identified that human beta-defensins may play an important role by limiting an increase of bacterial load at the initial stage of periodontitis (9).

In a recent study from 2023, Castillo Y. *et al*. aimed to standardize a qPCR assay for periodontal pathogens, in 55 subgingival biofilm clinical samples, from different stages of periodontitis. The results obtained using qPCR were compared with next-generation sequencing (NGS) results, and the researchers considered that this qPCR test was standardized with efficiencies between 90% and 100%. *Eubacterium saphenum, Eubacterium brachy, Desulfobulbus oralis,* and *Filifactor alocis* which are periodontal pathogens difficult to be cultivated, could be easily detected by this standardized qPCR assay (10).

Limitations of the study: considering the sample population of the current study, further research is necessary to validate our findings. Nowadays, in the era of new technology, there are other modern molecular assays which are used for the detection of periodontal pathogens, understanding new pathogenic mechanisms, finding therapeutic target genes (11) or testing biomarkers in periodontal disease associated conditions, such as diabetes (12). For example, a research team from Greece combined Real Time PCR with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) and they detected *Aspergillus* and *C. albicans* in 60 cases of apical periodontitis (13). Rams TE *et al*., from the USA, used MALDI TOF for subgingival isolates from 38 adults with chronic periodontitis and all *P. gingivalis* subgingival isolates were confirmed as *P. gingivalis* with MALDI-TOF. The authors considered MALDI-TOF as fast phenotypic identification of cultivable *P. gingivalis* in human subgingival biofilm specimens (14). Jeong J *et al*., from Korea used next-generation sequencing (NGS)-based 16S metagenome profiling data to confirm the presence of *Porphyromonas gingivalis* in periodontal patients (15).

CONCLUSIONS

Molecular methods as Real Time PCR are compulsory in optimal management of periodontal diseases since many of the etiological agents are non-cultivable or require accurate quantitative assessment to understand their clinical significance. With sensitive, specific, and clinically validated detection assays and targeted therapy, evo-

lution to more severe consequences could be stopped.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding

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PERCEIVED STRESS AND COPING MECHANISMS AS DETERMINING FACTORS OF THE QUALITY OF LIFE FOR CAREGIVERS OF PATIENTS WITH CHRONIC DISEASES IN ROMANIA

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PERCEIVED STRESS AND COPING MECHANISMS AS DETERMINING FACTORS OF THE QUALITY OF LIFE FOR CAREGIVERS OF PATIENTS WITH CHRONIC DISEASES IN ROMANIA (Abstract): **The objective of this study** was to identify how the impact of perceived stress and the coping mechanisms used by caregivers of patients with chronic diseases in Romania determine their quality of life (QoL). **Materials and methods**: 69 caregivers of patients with chronic diseases participated in the study. The data resulted from the completion by the caregivers of the Perceived Stress Questionnaire (PSQ), the COPE scale, and the World Health Organization Quality-of-Life Scale (WHOQOL-BREF), along with a series of questions related to demographic data, such as gender, age, the environment of origin, the educational level, the civil status, the degree of kinship with the patient, the time since finding out the diagnosis, and the patient's diagnosis. **Results**: The findings indicate that there was no significant correlation between perceived stress and QoL. High scores on planning were associated with high scores on QoL, and high scores on restraint from action were associated with low scores on QoL. As for carers, spouses had higher QoL scores compared to the children of the patient with a chronic disease, but also in comparison with the caregivers who were the mother or the father. Caregivers with high scores for pessimism regarding the diagnosis obtained higher QoL scores compared to caregivers who were hopeless. Within the predictive model composed of perceived stress and coping styles, the predictors were restraint from action (β = .416), denial (β = .397), mental passivity (β = .155), and recourse to alcohol-drugs (β = -.238), all of which were significant predictors, and the strongest predictor was restraint from action (β = .416). **Conclusions**: Caring for a patient with a chronic diagnosis is often a powerful experience for caregivers. The additional tasks taken on with this role, the changes in the previously known rhythm of personal life, and the intensity of the felt stress left important traces in the mental and physical state of the caregivers. The way they perceive stress and the coping mechanisms they use can positively influence their QoL, adjusting their intensity of the new role. **Keywords:** COPING MECHANISMS, QUALITY OF LIFE, CAREGIVERS, CHRONIC DISEASES.

INTRODUCTION

Caring for a patient with a chronic disease can be an overwhelming experience for caregivers, involving personal reframing in the context of patient care. Chronic diseases are characterized by a long-term evolution with persistent or recurrent symptoms and require complex management in order to maintain health and improve the patients' QoL. In the category of chronic diseases there is a wide range of ailments (e.g., neurological, oncological, cardiac, and mental) for which a personalized and integrated approach is necessary. Chronic disabling diseases are one of the world's leading causes of disability, affecting millions of people (1). These diagnoses cause an important limitation of both movement and general functioning of the patients (2). Studies have identified a significant association between patients with chronic diseases and the decrease in the QoL of their caregivers. Factors such as the nature and severity of the diagnosis, as well as the treatments and recuperative therapies followed by the degree of disability developed, considerably affect the QoL. In the case of diseases that require aggressive treatments (e.g., oncological diseases), the physical and mental functionality of the patients is significantly influenced (2). For patients with neurological conditions such as cerebral palsy (CP), stroke, Parkinson's disease, Alzheimer's, dementia, multiple sclerosis, etc., the limitation of cognitive status and the degradation of motor function lead to important challenges in daily activities, limiting independence, and the activities are often impossible to carry out without the help of another person.

Quality of life (QoL) is a "subjective assessment of individual well-being, which may include aspects such as physical and mental health, family and social satisfaction, personal achievements and the subjective level of happiness and satisfaction" (3). QoL is a multidimensional construct that reflects the level of physical, psychological, and social functioning of the person. QoL is influenced by factors such as health status, and social or economic conditions (4). Each person perceives and evaluates the QoL through their own standards and criteria, and these vary due to various factors, such as age, health status, or culture. The aspects that build the QoL are either objective (e.g., living standards, health status) or subjective (e.g., the individual's perception of his own life) (5). QoL is a broad concept, resulting from the interaction between the subjective and objective perspectives and influencing the individual's perception of his own life (6).

The caregivers the person who provides support and assistance to a family member or a person diagnosed with an illness, being responsible for the patient's care and well-being, regardless of the type of illness or its severity. Being the main caregiver of a sick person can be compared to having a full-time job (7). Caregivers provide physical care, social support, and emotional support, coordinate medical care, and administer the financial and administrative aspects associated with the diagnosis (8, 9, 10).

Caregivers contribute to the sustainability of health systems by delaying or preventing institutionalized care (11). Carers' responsibilities subsequent to the patient's care can often lead to personal sacrifices, significantly limiting their QoL on multiple levels, including their professional life, interpersonal relationships, or emotional state (12, 13). The long-term impact of patient care is felt by primary caregivers both physically and emotionally. Due to the demanding and difficult role they undertake, which requires important personal

sacrifices, they present a high degree of risk for mental health problems, making them more susceptible to developing depression or anxiety (14, 15). Taking on the multifaceted care of a patient with a chronic illness exposes them to elevated levels of stress, anxiety, and physical exhaustion (13, 16). The uncertainty about the diagnosis's prognosis and the constant, sustained effort to meet the patient's complex needs further intensify this state (17).

The degree to which caregivers believe that their own or external resources are insufficient to meet demands, real or imagined, determines the level of stress experienced by them. The specialized literature has shown that the caregivers of patients with chronic diseases face high levels of perceived stress. Increased levels of stress can lead to the emergence of dysfunctional emotional reactions, which can be exacerbated by feelings such as powerlessness or lack of control over the situation in which they find themselves (18). The stress that caregivers experience as a result of their ongoing effort to provide for the patient's requirements is frequently frustrating and overwhelming, to the point of physical and mental exhaustion (19, 20).

Thus, physical as well as emotional exhaustion can be the direct consequence of the high levels of persistent stress associated with caring for patients with chronic diseases, affecting the ability of caregivers to carry out the necessary daily responsibilities.

Regarding coping mechanisms, research has highlighted the strategies used by caregivers of patients with chronic diseases to cope with the stress associated with diagnoses. The normal or pathological reaction to stress depends on the coping capacities of each individual and the way in which they adapt to the stressful context (21).

Personal experiences, the individual's social or cultural context, and the resources available all influence the development of coping mechanisms as a personal response to stressful or challenging situations.

Coping mechanisms represent an important base of tools for the caregivers of patients with chronic diseases, with a significant impact on their QoL (22). Thus, the present study aims to analyze the QoL of the carers of patients with chronic diseases, through the lens of coping mechanisms and the stress perceived by them.

MATERIALS AND METHODS

Participants and procedure

The study participants were people who were patients with chronic diseases (e.g., Alzheimer's, cancer, stroke, dementia, multiple sclerosis, etc.).

The data collection procedure was carried out using the Google Forms research platform. After uploading the questionnaire, the link generated by the platform was shared among the caregivers of chronic disease patients. The participants were informed in advance about the conditions of participation (voluntary participation; withdrawal from the study at any time without any consequences; anonymity and confidentiality of answers) and the general objective of the study.

Instruments

Socio-demographic data

A series of questions were related to demographic data, such as gender, age, background, educational level, civil status, degree of kinship with the patient, time since finding out the diagnosis, and the patient's diagnosis.

Psychological data

- **Coping strategies.** To measure this variable, we used the COPE Questionnaire (Carver *et al*., 1989) which consists of 53 items, rated on a 4-point Likert scale, from 1

(I usually don't do this) to 4 (I often do this). Additionally, the questionnaire consists of 14 dimensions representing all coping styles: active coping ("I engage in additional activities that can contribute to solving the problem."), planning ("I try to formulate a strategy about what I have to do."), elimination of recurring activities ("I put aside other activities to focus on the problem."), withholding from action ("I force myself to wait for the right moment to react."), seeking social-instrumental support ("I ask people who have been through similar situations what they did in that situation."), seeking social-emotional support ("I tell someone how I feel."), positive reinterpretation ("I look for something good in what happens to me."), acceptance "I'm learning to live with my problem."), denial ("I refuse to believe what happened."), emotional discharge ("I'm in a bad mood and I'm externalizing my emotions."), religious orientation ("I'm looking for God's help."), mental passivity ("I engage in work or other activities to take my mind off the problem."), behavioral passivity ("I give up trying to get what I want."), resorting to alcohol-medication ("I drink alcohol or take medication to think less about the problem."). The Alpha Cronach coefficients of each dimension were in the range of .600 - .900, which indicates a satisfactory level of internal consistency of the items. The score was made on each dimension, and a high score indicates a high level of the respective coping style.

- **Perceived stress.** To measure this variable, we used the Perceived Stress Questionnaire (Levenstein *et al.*, 1993). The instrument consists of 30 items ("I feel rested"; "I feel that I cannot manage to achieve my personal goals"; "I do many things because I have to do them and not because I like them.") rated on a 4-point Likert scale from 1 (almost never) to 4 (almost always). Cronbach's Alpha coefficient was .856, which means that there was a good level of internal consistency. The total score was made summative, and a high score indicates a high level of perceived stress.

- **Quality of life.** To measure the variable "quality of life", we used the **WHOQOL** questionnaire developed by the World Health Organization (1996). The instrument consists of 26 items covering several aspects related to life in general, such as: subjective well-being, sleep, workplace, personal home, public health services, financial situation, etc. In the present study, only 8 items were used: ("How would you rate your quality of life?"; "Have you got enough money to meet your needs?"; "Have you got enough money to meet the patient's needs?"; "How satisfied are you with the support you get from your friends?"; "How satisfied are you with the conditions of your living place?"; "How satisfied are you with your access to health services?"; "How satisfied are you with your transport?"; "In the last 2 weeks, how often have you had negative feelings such as dark mood, despair, anxiety, depression?"). Items were rated on a 5-point Likert scale from 1 (not at all) to 5 (very much). Cronbach's Alpha coefficient was .767, which indicates a satisfactory level of item consistency. The total score was made summative, and a high score indicates a high level of QoL.

Statistical analysis

The statistical analyses were performed using IBM Statistical Package for Social Sciences (SPSS) for Windows, version 24 (SPSS Inc., Chicago, IL, USA). Results for descriptive statistics were expressed as means and standard deviations (SD).

To test the hypotheses of the study, de-

scriptive statistics including average, standard deviation, minimum score, maximum score, and indices of normality of distribution Skewness and Kurtosis were performed. Given that the Skewness and Kurtosis indices ranged between -1 and +1 for Skewness and -3 and +3 for Kurtosis, we can conclude that the data distribution is normal.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee of Faculty of Psychology and Educational Sciences, "Alexandru Ioan Cuza" University Iasi, Romania No. 511/2024.

RESULTS

Socio-demographic data

In total, there were 69 participants aged between 25 and over 65, the majority being female participants (10 participants were male and 59 participants were female).

Most participants reported being married (N = 60), from an urban environment (N = 51), and of Orthodox religion (N = 65).

Regarding the degree of relationship to the patient, 35 participants reported being the patient's spouse, 22 participants reported being the patient's mother or father, 11 participants reported being the patient's child, and only one participant reported being the patient's cousin.

Psychological data

The results of the psychological instruments used for the present research (experienced stress, quality of life and, coping mechanisms) among caregivers of chronic disease patients are presented in first table.

TABLE I.

Descriptive statistics (N = 69)

	M	SD	Min	Max	Skewness	Kurtosis
Perceived stress	79.53	11.63	59	100	-.06	-1.26
Active coping	13.79	1.95	8	16	-.68	-.06
Planning	13.98	1.95	8	16	-.85	.17
Elimination of competing activities	12.82	1.96	7	16	-.54	.16
Restraint from action	11.60	1.94	6	16	-.22	.10
Search for social-instrumental support	12.11	2.63	4	16	-.11	1.63
Seeking social-emotional support	8.78	3.06	4	16	.50	-.47
Positive reinterpretation	11.04	3.03	5	16	.05	-1.05
Acceptance	11.31	3.21	4	16	-.27	-.64
Denial	10.01	3.90	4	16	-.08	-1.31
Emotional discharge	10.10	2.66	4	16	-.20	-.06
Orientation towards religion	11.66	3.48	4	16	-.55	-.50
Mental passivity	8.97	2.28	4	16	.41	.50
Behavioral passivity	7.95	2.89	4	15	.40	-.66
Resorting to alcohol-drugs	1.44	.88	1	4	.87	.31
Life quality	23.27	3.91	14	29	-.49	-.53

Hypotheses

H1. There was a significant and negative correlation between perceived stress and QoL.

H2. There was a significant correlation between coping styles and QoL.

H2.1. There was a significant and positive correlation between active coping and QoL.

H2.2. There was a significant and positive correlation between planning and QoL.

H2.3. There was a significant and negative correlation between elimination of recurrent activities and QoL.

H2.4. There was a significant and negative correlation between restraint and QoL.

H2.5. There was a significant and positive correlation between seeking social-instrumental support and QoL.

H2.6. There was a significant and positive correlation between seeking social-emotional support and QoL.

H2.7. There was a significant and positive correlation between positive reinterpretation and QoL.

H2.8. There was a significant and positive correlation between acceptance and QoL.

H2.9. There was a significant and negative correlation between denial and QoL.

H2.10. There was a significant and positive correlation between emotional discharge and QoL.

H2.11. There was a significant and positive correlation between religious orientation and QoL.

H2.12. There was a significant and negative correlation between mental passivity and QoL.

H2.13. There was a significant and negative correlation between behavioral passivity and QoL.

H2.14. There was a significant and negative correlation between alcohol-medication use and QoL.

H3. There were significant differences in QoL between spouse, child, and mother or father who were caregivers for the patient with a chronic illness.

H4. There were significant differences in QoL since the diagnosis was made (less than 6 months, 6 months to 1 year, 1 year to 3 years, and more than 3 years).

H5. There were significant differences in QoL depending on how caregivers relate to the patient's diagnosis (worried, pessimistic, hopeless, confident, and resigned).

H6. Perceived stress and coping styles significantly predicted QoL.

Hypothesis testing

H1. There was a significant and negative correlation between perceived stress and QoL.

The results obtained indicated that there was no significant correlation between perceived stress and QoL ($r = .011$, $p = .929 > .05$).

H2. There was a significant correlation between coping styles and QoL.

To test hypothesis 2, we used the Pearson correlation.

H2.1. There was a significant and positive correlation between active coping and QoL. The obtained results indicated that there was no significant correlation between active coping and QoL ($r = .214$, $p = .078 > .05$).

H2.2. There was a significant and positive correlation between planning and QoL. Results obtained indicated that there was a significant and positive correlation between planning and QoL ($r = .376$, $p = .001 < .01$), in the sense that high scores on planning were associated with high scores on QoL.

H2.3. There was a significant and negative correlation between eliminating competing activities and QoL. The results generated by the data analysis indicated that there was no significant correlation between the elimination of competing activities and QoL ($r = .012$, $p = .922 > .05$).

H2.4. There was a significant and negative correlation between restraint and QoL. Statistical analysis of the data indicated that there was a significant and negative correlation between restraint and QoL ($r = -.304$, $p = .011 < .05$), meaning that high restraint scores were associated with low QoL scores.

H2.5. There was a significant and positive correlation between seeking social-instrumental support and QoL. The obtained results showed that there was no significant correlation between the search for social-instrumental support and the QoL ($r = .145$, $p = .235 > .05$

H2.6. There was a significant and positive correlation between seeking social-emotional support and QoL. The obtained data demonstrated that there was no significant correlation between the search for social-emotional support and the QoL ($r = -.031$, $p = .802 > .05$).

H2.7. There was a significant and positive correlation between positive reinterpretation and QoL. Research results indicated that there was no significant correlation between positive reinterpretation and QoL ($r = -.025$, $p = .841 > .05$).

H2.8. There was a significant and positive correlation between acceptance and QoL. Results obtained showed that there was a significant and positive correlation between acceptance and QoL ($r = .280$, $p = .020 < .05$), in the sense that high scores on acceptance were associated with high scores on QoL.

H2.9. There was a significant and negative correlation between denial and QoL. Data analysis proved that there was no significant correlation between denial and QoL ($r = -.073$, $p = .549 > .05$).

H2.10. There was a significant and positive correlation between emotional discharge and QoL. The obtained results showed that there was a significant and positive correlation between emotional discharge and QoL ($r = .246$, $p = .042 < .05$), in the sense that high scores on emotional discharge were associated with high scores on QoL.

H2.11. There was a significant and positive correlation between religious orientation and QoL. The obtained results demonstrated that there was no significant correlation between religious orientation and QoL ($r = -.073$, $p = .550 > .05$).

H2.12. There was a significant and negative correlation between mental passivity and QoL. The obtained results indicated that there was no significant correlation between mental passivity and QoL ($r = -.025$, $p = .839 > .05$).

H2.13. There was a significant and negative correlation between behavioral passivity and QoL. We identified that there was a significant and negative correlation between behavioral passivity and QoL ($r = -.343$, $p = .000 < .01$), in the sense that high scores on behavioral passivity were associated with low scores on QoL.

H2.14. There was a significant and negative correlation between alcohol-medication use and QoL. The analysis of the obtained data proved that there was a significant and negative correlation between the recourse to alcohol-medicines and the QoL ($r = -.330$, $p = .006 < .01$), in the sense that high scores on the recourse to alcohol-medicines were associated with low QoL scores. The results obtained are presented in second table.

TABLE II.

Correlations between coping styles and QoL

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1.Active coping	-														
2.Planning	.287*	-													
3.Elimination of competing activities	.150	.640**	-												
4.Restraint from action	.299*	.421**	.264*	-											
5. The search for social-instrumental support	.040	.311**	393**	.432**	-										
6.Seeking social-emotional support	.084	.251*	.192	.260*	.109	-									
7.Positive reinterpretation	-.168	-.106	-.082	-.226	-.044	.464**	-								
8. Acceptance	-.525**	.019	.062	-.194	.371**	-.030	.395**	-							
9. Denial	-.401**	.064	.012	-.238*	.243*	-.159	.255	.702**	-						
10.Emotional discharge	.509**	.182	.199	.434**	.103	.360**	-.055	-.537**	-.707**	-					
11.Orientation towards religion	.173	.106	-.070	.057	.033	.267*	.458**	.045	.005	.191	-				
12.Mental passivity	-.226	-.164	-.161	.028	.091	.118	.245*	.391**	.391**	-.352**	.276*	-			
13.Behavioral passivity	.355**	.174	.125	.202	.298*	-.021	.115	-.036	-.033	.267*	.180	-.144	-		
14. Recourse to alcohol-medicines	.390**	-.176	-.253*	.063	.052	.128	.027	-.169	-.109	.277*	.071	-.104	.414**	-	
15. QoL	.214	.376**	.012	-.304*	.145	-.031	-.025	.280*	-.073	.246*	-.073	-.025	-.343**	-.330**	-

Note **p< .01; *p< .05

H3. There were significant differences in QoL between the spouse, child and mother or father who were caregivers for the patient with a chronic illness.

To test hypothesis 3, we used One-Way ANOVA. The results indicated that there were significant differences between caregivers who were the spouse (M = 24.74, SD = 3.12) and caregivers who were the child (M = 21.36, SD = 4.29) of the patient with a chronic illness ($F(2,68) = 5.807$, $p = .005 < .05$, Mdif = 3.37, $p = .029 < .05$), in terms of QoL, meaning that spouses had higher QoL scores compared to caregivers who were the child of the patient with a chronic disease.

Additionally, there were significant differences between the caregivers who were the husband or wife (M = 24.74, SD = 3.12), and the caregivers who were the mother or father (M = 21.95, SD = 4.07) of the patient with a chronic illness (F(2,68) = 5,807, p = .005 < .05, Mdif = 2,78, p = .018< .05), in terms of QoL, in the sense that spouses had higher QoL scores, compared to caregivers who were the mother or father of the patient with a chronic disease.

TABLE III.

Differences between spouses, children and mothers or fathers in terms of QoL

Relationship type		Mdif	SD	p
Husband / wife	Child	3.37	1.26	.029
	Mother/father	2.78	.98	.018
Child	Husband/wife	-3.37	1.26	.029
	Mother/father	-.59	1.34	1.000
Mother / father	Husband/wife	-2.78	.98	.018
	Child	.59	1.34	1.000

The obtained results indicated that there were no significant differences between the caregivers who were the mother or father (M = 21.95, SD = 4.07) and the caregivers who were the child (M = 21.36, SD = 4.29) of the patient with a chronic disease (F(2,68) = 5.807, p = .005 < .05, Mdif = -.592, p = 1.000 > .05).

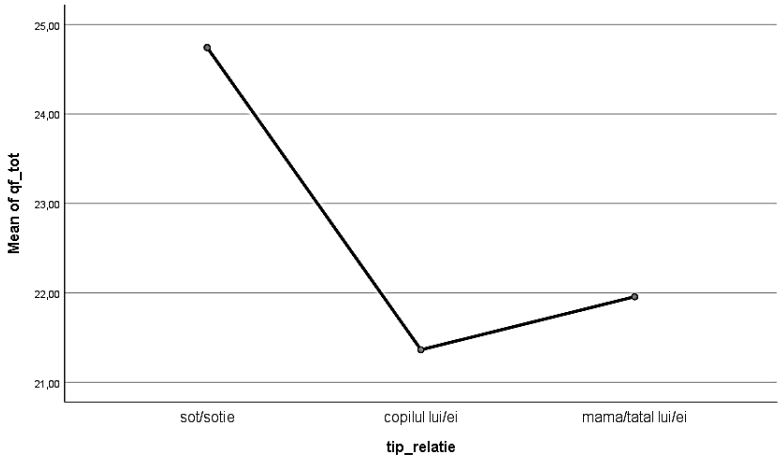

Fig. 1. Relationship type

H4. There had been significant differences in QoL since the time of learning the diagnosis (less than 6 months, 6 months to 1 year, 1 to 3 years, and more than 3 years).

The obtained results indicated that there were no significant differences in the QoL of caregivers of patients with chronic diseases, depending on the time since learning the diagnosis (F(3, 68) = 2.77, p = .049).

TABLE IV.

Differences in the level of QoL according to the time since finding out the diagnosis

Diagnosis time		Mdif	SD	P
less than 6 months	6 months - 1 year	1.71	1.55	1.000
	1-3 years	3.25	1.35	.113
	more than 3 years	2.68	1.08	.094
6 months - 1 year	less than 6 months	-1.71	1.55	1.000
	1-3 years	1.54	1.71	1.000
	more than 3 years	.967	1.51	1.000
1 - 3 years	less than 6 months	-3.25	1.35	.113
	6 months - 1 year	-1.54	1.71	1.000
	more than 3 years	-.574	1.30	1.000
more than 3 years	less than 6 months	-2.68	1.08	.094
	6 months - 1 year	-.96	1.51	1.000
	1-3 years	.57	1.30	1.000

H5. There were significant differences in QoL depending on how caregivers related to the patient's diagnosis (worried, pessimistic, hopeless, confident, and resigned).

The obtained results indicated that there were significant differences between the caregivers who were worried (M = 24.08, SD = 3.06) and the caregivers who were hopeless (M = 18.14, SD = 2.19) in terms of QoL ($F(4, 68) = 4.24$, $p = .004 < .05$, Mdif = 5.94, $p = .003 < .05$), meaning that caregivers who were worried had higher QoL scores compared to caregivers who were hopeless.

TABLE V.

Differences in the level of QoL depending on how caregivers relate to the diagnosis of the patient with a chronic disease

Relating to diagnosis		Mdif	SD	p
Concern	Pessimism	-.66	1.47	1.000
	Hopeless	5.94*	1.54	.003
	Confident	.71	1.11	1.000
	Resignation	.50	1.27	1.000
Pessimism	Concern	.66	1.47	1.000
	Hopeless	6.60*	1.85	.007
	Confident	1.38	1.51	1.000
	Resignation	1.16	1.63	1.000
Hopeless	Concern	-5.94*	1.54	.003
	Pessimism	-6.60*	1.85	.007
	Hopeless	-5.22*	1.58	.016
	Resignation	-5.44*	1.70	.022
Confident	Concern	-.71	1.11	1.000
	Pessimism	-1.38	1.51	1.000
	Hopeless	5.22*	1.58	.016
	Resignation	-.21	1.32	1.000
Resignation	Concern	-.50	1.27	1.000
	Pessimism	-1.16	1.63	1.000
	Hopeless	5.44*	1.70	.022
	Confident	.21	1.32	1.000

Significant differences were found between caregivers who were pessimistic about the patient's diagnosis (M = 24.75, SD = 3.37) and caregivers who were hopeless (M = 18.14, SD = 2.19) regarding QoL (F(4 , 68) = 4.24, p = .004 < .05, Mdif = 6.60, p = .007 < .05), meaning that caregivers who were pessimistic about the patient's diagnosis had higher QoL scores compared to caregivers who were hopeless.

The results obtained indicated that there were significant differences between caregivers who were hopeless (M = 18.14, SD = 2.19) and caregivers who were confident (M = 23.36, SD = 3.80) in terms of QoL (F(4, 68) = 4.24, p = .004 < .05, Mdif = -5.22, p = .016 < .05), meaning that caregivers who were confident had higher QoL scores compared to caregivers who were hopeless.

Statistical analysis of the data indicates significant differences between caregivers who were resigned to the diagnosis of the patient with a chronic disease (M = 23.58, SD = 3.89) and caregivers who were hopeless (M = 18.14, SD = 2.19) in terms of QoL (F(4, 68) = 4.24, p = .004 < .05, Mdif = 5.44, p = .022 < .05), meaning that caregivers who were resigned had higher QoL scores compared to caregivers who were without hope.

H6. Perceived stress and coping styles significantly predict QoL

The obtained results indicated that the predictive model made up of perceived stress and coping styles (coping, planning, elimination of recurring activities, restraint from action, search for social-instrumental support, search for social-emotional support, positive reinterpretation, acceptance, denial, emotional discharge, orientation towards religion, mental passivity, behavioral passivity, and recourse to alcohol-drugs) was a significant predictive model (F(15, 68) = 4.12, p = .000 < .05) that explains 53.8% of the variance of the dependent variable - the QoL of patients with a chronic disease. The predictors restraint from action (β = .416), denial (β = .397), mental passivity (β = .155), and recourse to alcohol-drugs (β = -.238) were significant predictors. The strongest predictor was restraint from action (β = .416).

TABLE VI.

The predictive model of the QoL for caregivers of patients with a chronic disease

	B	*SE(B)*	*B*
Perceived stress	-.04	.04	-.12
Active coping	.19	.31	.09
Planning	.46	.27	.23
Elimination of competing activities	-.50	.27	-.25
Restraint from action	.83	.29	.41*
Seeking instrumental social support	-.05	.22	-.03
Seeking social emotional support	.41	.20	.32
Positive reinterpretation	-.38	.23	-.29
Acceptance	.16	.22	.13
Denial	.39	.18	.39*
Emotional discharge	-.21	.18	-.14
Orientation towards religion	-.17	.14	-.15
Mental passivity	-.44	.21	-.26*
Behavioral passivity	.21	.18	.15
Resorting to alcohol-drugs	-1.29	.54	-.29*
R^2	.538**		

*p < .05; **p<.001

DISCUSSION

The present study analyzed the relationship between perceived stress and coping strategies as determining factors of the QoL of caregivers of people with chronic diseases. The obtained results indicate a significant correlation between the stresses perceived by the patients' caregivers and their QoL in the sense that stress was able to negatively influence the QoL and was felt multidimensional. Perceived stress changed the level of personal functioning, both in terms of mental and physical health, in interpersonal relationships, in the financial or professional sphere, leading to a decrease in the QoL and the establishment of a general dysfunctional state.

Regarding the link between coping styles and QoL, the analysis of the 14 coping styles that follow the COPE scale reveals a significant and positive correlation between active coping and QoL, in the sense that active coping targeted concrete actions, with the aim of removing the stressor and its effects. As a conscious action, this coping style contributed to the QoL of caregivers of patients with chronic diseases, by constantly adjusting to stress factors (23, 24, 25). A significant and positive correlation also resulted from planning as a coping strategy and QoL. Thus, the active orientation towards identifying some modes of action was associated with a high degree of QoL of the carers.

Analyzing the data regarding the elimination of competing activities, the results showed that there was no significant correlation between this coping strategy and QoL. Therefore, the QoL was unaffected by avoiding distraction from stressful situations and focusing on solving the problem. A strong and unfavorable relationship was found between QoL and action restriction. This was a kind of active coping in that it involved focusing on the stressor but also acting passively until conditions permitted it (26).

The results indicate that there was no significant correlation between instrumental social support seeking and QoL or social support seeking.

Thus, the tendency to request information from those around or advice to improve the degree of stress did not change the QoL of the caregivers, although existing studies analyzing this correlation resulted in an increased QoL as a result of social support (27). The results of our study showed that the need for moral support or understanding from the social or family environment to reduce stress did not influence the QoL. Regarding the tendency to look on the positive side even in an undesirable situation as a form of stressor perception, there was no significant correlation between positive reinterpretation and QoL, while there was a significant and positive correlation between acceptance and QoL. Thus, an approach based on accepting reality and being aware of the present period increases the QoL (28, 29). At the same time, the results showed that there was no significant correlation between denial and QoL. As a coping mechanism, denial refers to positioning in the refusal to acknowledge that the new reality means changing the course of life as it was before the installation of the stress factor. The obtained results indicated that there was a significant and positive correlation between emotional discharge and QoL. Thus, expressing emotions reduces the level of distress, which contributes to the QoL (24). In the coping strategy regarding religious orientation as a form of situational reinterpretation, there was no significant correla-

tion between religious orientation and QoL. Although our result is in contrast with the existing studies, a possible explanation may reside in the lack of such research on the Romanian population, the existing studies being carried out on other populations (30, 31, 32). Similarly, analyzing the results regarding withdrawal in other activities, as a technique to avoid focusing on problems, there was no significant correlation between mental passivity and QoL (33). A significant and negative correlation resulted between behavioral passivity and QoL. Reducing effort or giving up action in favor of achieving the goal was associated with low QoL scores. A significant and negative correlation was identified between alcohol-medication use and QoL. Thus, the use of alcohol-medicines by patients' caregivers to alleviate negative states was associated with low QoL scores (34, 35, 36). It was also identified that planning as a coping strategy as well as emotional discharge correlates significantly and positively with a high QoL, while restraint from action correlates significantly negatively with QoL.

Marital or family status was also important. Regarding the QoL between the spouse, child, and mother or father caregivers of the patient with a chronic disease, the results indicate that there were significant differences between the spouse caregivers and caregivers who were the child of the chronic disease patient regarding life quality. Thus, the caregivers who were the husband or the wife had higher scores on the QoL compared to the caregivers who held another degree of kinship. A possible explanation is the emotional connection and the commitment of marital status, which can lead to a greater understanding and empathy between the partners of the couple. Studies on couple relationships showed a high adaptability to life's changes and challenges compared to people with other degrees of kinship (37, 38, 39). Additionally, the family caregivers who were the husband or wife had higher QoL scores compared to the family caregivers who were the mother or father of the patient with a chronic disease, but there were no significant differences between the family caregivers who were the mother or father and the family caregivers who were the child of the patient with a chronic disease (40). Thus, for the children who assumed the role of the main relative of the patient with a chronic disease, the QoL had a low level compared to the people who were the husband or wife of the patient. Young people who found themselves in this role presented a lower level of life compared to older caregivers (41, 42). At the same time, parents who were the main caregivers had a lower standard of living compared to the patient's husband or wife (43).

Regarding the QoL of caregivers of patients with chronic diseases depending on the time since learning the diagnosis, there were no significant differences. A possible explanation could be the adjustment of the caregivers to the new role and subsequent responsibilities and the development of resilience. By adjusting the strategies for managing the situation over time, once the shock associated with the diagnosis subsides, the family caregivers developed the inner resources and new mechanisms that support them in a good management of the situation, which can positively influence the QoL.

The results from the differences in dealing with the situation - how the caregivers deal with the patient's diagnosis (worried, pessimistic, hopeless, confident and re-

signed) showed that there were significant differences between anxious and hopeless caregivers in terms of QoL, in that anxious caregivers score higher on QoL compared to hopeless caregivers. A possible explanation could be that caregivers who were concerned proactively seek coping mechanisms, whereas those who were hopeless regress to a state of withholding action.

Likewise, caregivers who were pessimistic about the patient's diagnosis had higher QoL scores compared to caregivers who were hopeless. Finally, the results showed that caregivers who were resigned to the patient's diagnosis of a chronic illness had higher QoL scores compared to caregivers who were hopeless. The obtained results are in congruence with those identified in the specialized literature. Resignation is reflected in the change of one's own perspective and the assumption of adaptation to the new reality, while the lack of hope was felt as the despair and responsibility resulting from the new responsibilities, which significantly changed the level of the QoL of the caregivers (44). Research results on perceived stress and coping styles as predictors of QoL demonstrated that the predictive model composed of perceived stress and coping styles (coping, planning, elimination of recurring activities, restraint from action, seeking social-instrumental support, seeking social support -emotional, positive reinterpretation, acceptance, denial, emotional discharge, orientation towards religion, mental passivity, behavioral passivity, and recourse to alcohol-drugs) is a significant predictive model and explains 53.8% of the variance of the dependent variable - the QoL of caregivers patients with a chronic disease. Predictors such as withholding from action, denial, mental passivity and resorting to alcohol-drugs were identified as significant predictors. The strongest predictor was restraint from action. Restraint from action as a coping mechanism is correlated with a low QoL as proactive coping strategies can minimize potential stressors in the future (45).

Strengths and limitations of the study. The importance of the present study lies in its uniqueness. No study has investigated the link between perceived stress and coping mechanisms as predictors of the QoL of caregivers of patients with chronic diseases in Romania. As limitations, we can mention a larger number of participants that could provide generalized results for the topic addressed.

CONCLUSIONS

The experience of caring for a patient with a chronic diagnosis is often a powerful one for caregivers. The additional tasks taken on with this role, the changes in the previously known rhythm of life, and the intensity of the stress felt leave important traces in the mental and physical state of the carers. Through active coping, concrete actions with a role in reducing the stressor and constant adjustment, a higher degree of the QoL of the caregivers can be felt. By knowing the coping mechanisms suitable for the life context and by identifying the appropriate ways for the individual to act, the caregivers can go through the stage of patient care more easily and with a more positive positioning.

In addition to planning as proactive action, emotional release plays another important role in QoL, while behavioral passivity such as refraining from action or resorting to alcohol-drugs decreases perceived satisfaction.

In comparison to the carers who are the

mother and father of the patient and their child, the experience of the husband or wife of the patient with a chronic disease may be assimilated with a lesser intensity due to the nature and complexity of the relationship, but as an individual perception, the experience is lived in a way unique.

Regarding the QoL of the caregivers of patients with chronic diseases, depending on the time since learning the diagnosis, no significant changes were reflected in the QoL of the caregivers. As situational positioning and reporting, caregivers who were anxious, resigned, or pessimistic reported a more satisfying QoL, compared to hopeless caregivers.

CONFLICT OF INTEREST AND FUNDING

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DISTRIBUTION OF ASTROCYTIC TUMORS AND BRAIN METASTASES BY ANATOMICAL LOCATION: A STUDY CONDUCTED ON 91 PATIENTS

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DISTRIBUTION OF ASTROCYTIC TUMORS AND BRAIN METASTASES BY ANATOMICAL LOCATION: A STUDY CONDUCTED ON 91 PATIENTS (Abstract): This retrospective study examines the distribution of astrocytic tumors and brain metastases based on anatomical location. **Materials and methods:** the study included a cohort of 91 patients treated at "Prof. Dr. N. Oblu" Emergency Clinical Hospital in Iasi, Romania, between 2011 and 2023. The study focuses on tumors localized in the frontal, parietal, and temporal lobes, with a particular emphasis on the association between these tumors and pre-diagnosis psychiatric symptoms. **Results:** Data were collected from pathology records, including patient demographics, tumor type, location, and histopathological characteristics. The analysis reveals a significant prevalence of astrocytic tumors in the frontal lobes for both male and female patients, with glioblastoma multiforme being the most common tumor type. The study also highlights the impact of tumor location on the presentation and progression of psychiatric symptoms, underscoring the need for tailored treatment approaches. **Conclusions:** These findings contribute to a better understanding of the relationship between tumor location, patient outcomes, and the potential for targeted therapeutic strategies. **Keywords:** ASTROCYTIC TUMORS, BRAIN METASTASES, ANATOMICAL LOCATION, GLIOBLASTOMA, HISTOPATHOLOGICAL ANALYSIS.FRONTAL LOBE TUMORS, TEMPORAL LOBE TUMORS, PARIETAL LOBE TUMORS, TUMOR DISTRIBUTION.

Astrocytomas are a group of brain tumors that most commonly affect individuals between the ages of 30 and 40, with a slightly higher incidence in men. High-grade tumors, such as glioblastomas, are the most common malignant brain tumors, accounting for approximately 54% of all gliomas and 16% of all primary brain tumors (1, 2). Symptoms of low-grade astrocytomas may be subtle, as the brain can temporarily compensate for the slow growth of the tumor. In contrast, high-

grade tumors can cause acute and severe symptoms, such as headaches, seizures, and personality changes (3, 5).

The treatment of astrocytic tumors requires a multidisciplinary approach, involving surgical interventions, chemotherapy, and radiotherapy. For low-grade tumors, complete surgical resection is often curative, with radiotherapy used in cases of tumor recurrence or progression (7). High-grade tumors typically require a combination of maximal surgical resection and radiochemotherapy (8). Despite technological advancements and the identification of molecular markers that have improved prognostic stratification, managing these complex tumors remains challenging (9, 10).

This study aims to investigate the anatomical distribution of astrocytic tumors and brain metastases in the frontal, parietal, and temporal lobes, analyzing the impact of these locations on pre-diagnosed psychiatric symptoms. The study contributes to a better understanding of tumor behavior and the improvement of personalized therapeutic approaches.

SIGNS AND SYMPTOMS

The signs and symptoms of grade I and grade II astrocytomas are difficult to observe, as the brain can temporarily adapt to the presence of a slow-growing tumor. The signs of grade III and IV astrocytomas can appear suddenly and can strongly affect the body (5). The symptoms and signs of brain tumors result in a general increase in intracranial pressure, focal changes in brain function, or, in some cases, a combination of both (6).

Headache is one of the main symptoms often associated with brain tumors (7). The usual pain is felt bilaterally in the frontal area (8), is non-throbbing in quality (9), and is moderate in intensity. Headache is often accompanied by other symptoms that indicate a diverse impairment of intracranial structures, such as personality changes, seizures, and/or specific neurological signs, along with signs of increased intracranial pressure (nausea, vomiting, optic nerve swelling, blurred vision) (7).The exact location of the tumor cannot be predicted just based on the location of the headache, but a predilection for frontal headaches is observed in supratentorial and skull base tumors, while occipital headaches are more commonly found in infratentorial tumors (9). According to a study conducted by Lowry and his colleagues (10), headaches were much more common in patients under the age of 45 suffering from a malignant primary brain tumor, compared to those aged 65 or older.

Cranial tumors can cause severe ocular manifestations and symptoms in addition to the neurological consequences caused by increased pressure inside the cranium, nerve damage or brain compression (11). Common initial symptoms include gradual decrease in visual acuity, possible optic nerve atrophy, visual field impairment, and paralysis of the extraocular nerves (12).

TREATMENT

A team consisting of oncologists, radiologists, and neurosurgeons with individualized experience make treatment decisions. The treatment is determined by the type and location of the tumor, the degree of malignancy, the age, and the health condition of the patient (13). Chemotherapy, radiotherapy, and surgical intervention are the three most commonly used treatment options. In cases where brain tumors are benign, surgical intervention can be effective (14).

Pilocytic astrocytoma has a low rate of malignant progression. The main treatment is surgical resection. Radiotherapy is indicated for the area affected by the tumor in the absence of other surgical options and if there are radiographic evidence of progression (15). The removal of grade II tumors is achieved through microsurgical intervention. Radiation therapy may be recommended for patients who have undergone partial surgical intervention, have observed tumor growth, or cannot undergo surgery (16). Radiochemotherapy may be postponed until disease progression, as surgical intervention is potentially curative (17).

The maximum safe resection of grade III tumors, followed by radio chemotherapy, is considered standard in their treatment (18). Temozolomide administered together with radiotherapy does not have a significant additional effect (19). Current technological and conceptual advances have increased the safety of surgical procedures, while also expanding the possibilities and indications for surgery in the treatment of grade IV tumors (20). Usually radiotherapy follows surgery in most cases. Chemotherapy can sometimes be administered together with or after radiotherapy. However, there is no standard chemotherapy treatment protocol, and each regimen must be customized for each patient (21).

Low grade tumors can be approached through various methods, such as the administration of anticonvulsant/antiepileptic drugs, steroids or surgical interventions. Studies have found that between 30-50% of individuals diagnosed with brain tumors experience at least one seizure, while 10-30% of them face repeated convulsions (22). Nowadays, all those diagnosed with brain tumors should receive treatment with antiepileptic drugs due to the frequency of seizures associated with these tumors (23).

The therapy for neuropsychiatric symptoms involves both medications and non-pharmacological therapies. Non-pharmacological interventions require collaboration efforts from doctors, nurses, and family/caregivers (24). In order to provide the best psychopharmacological and psychotherapeutic intervention, it is essential to assess patients' symptoms through a rigorous neuropsychological and psychiatric evaluation in specialized clinics. Regarding patients' families, the importance of an educational, supportive, and psychosocial-oriented intervention has been consistently highlighted (25, 26). Oncological psychology and social oncology are, compared to biomedical therapies for cancer, new additions in contemporary cancer care (27).

MATERIALS AND METHODS

This retrospective study included 91 patients treated at the "Prof. Dr. N. Oblu" Emergency Clinical Hospital in Iasi, Romania, between February 17th, 2011, and May 8th, 2023. All patients underwent pathological examination of tissue samples removed during surgery. The Pathology Department's biopsy registry recorded the patients' identification details (name, gender, age), the tumor's location and type, and the stains used. Patients were informed about the necessity of histopathological tests and consented to the use of biological material for diagnostic and research purposes.

RESULTS

In this study, we analyzed the distribution of tumors based on their anatomical location, utilizing cerebral MRI and native CT scans for diagnosis. The findings revealed that, for male patients, the left and right frontal lobes were the most common

sites for tumor occurrence, each accounting for 40% of the cases (Fig. 1). This indicates a significant concentration of tumors in these regions compared to other anatomical areas. The next most frequent site was also within the frontal lobe, but with a considerably lower incidence of 7%. This distribution pattern highlights the frontal lobe as a primary site of tumor development in men.

Just like in the case of men, in women most of the tumors are found in the left and right frontal lobes, the percentage being 32% (fig. 2). This finding shows a significant predilection for these areas compared to other anatomical regions. The following areas by frequency are the right fronto-temporal lobe with 4 cases (9%) and the right fronto-parietal lobe with 3 cases (6%). Other observed locations, each with 2 cases (4%), include the left fronto-parietal lobe, the left fronto-temporo-insular lobe, and the left fronto-parieto-temporal lobe.

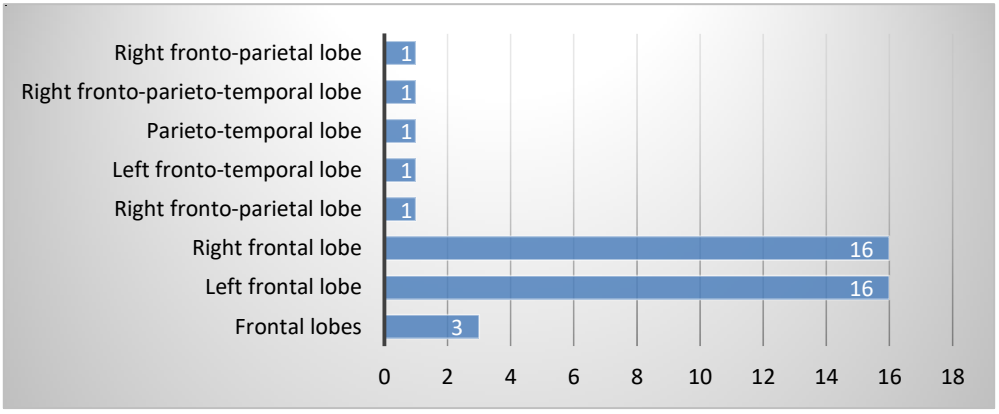

Fig. 1. Distribution of tumors by location in men

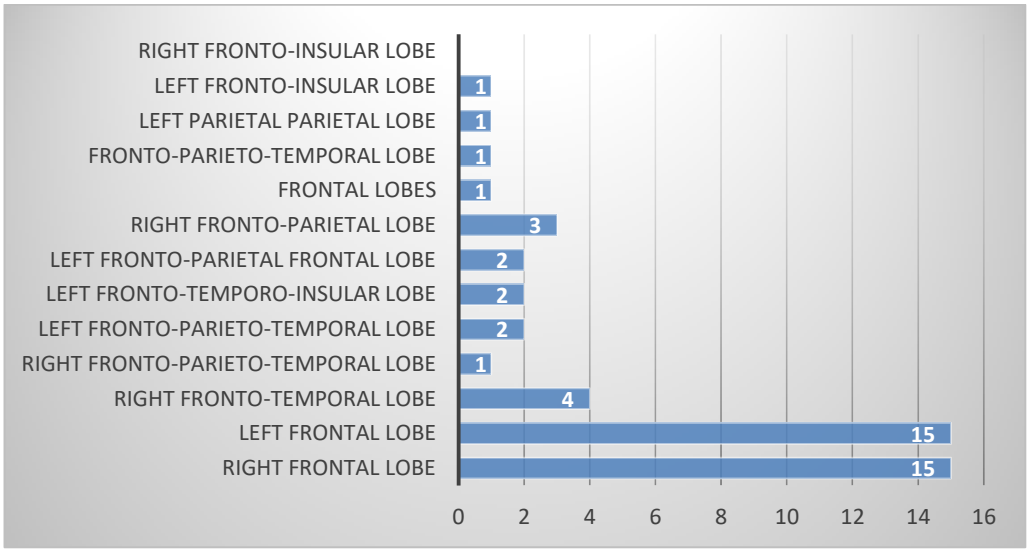

Fig. 2. Distribution of tumors by location in women

Next, we analyzed the distribution of different types of non-metastatic brain tumors, in order to provide a comprehensive perspective on the diversity and frequency of these conditions in our study context (fig. 3).

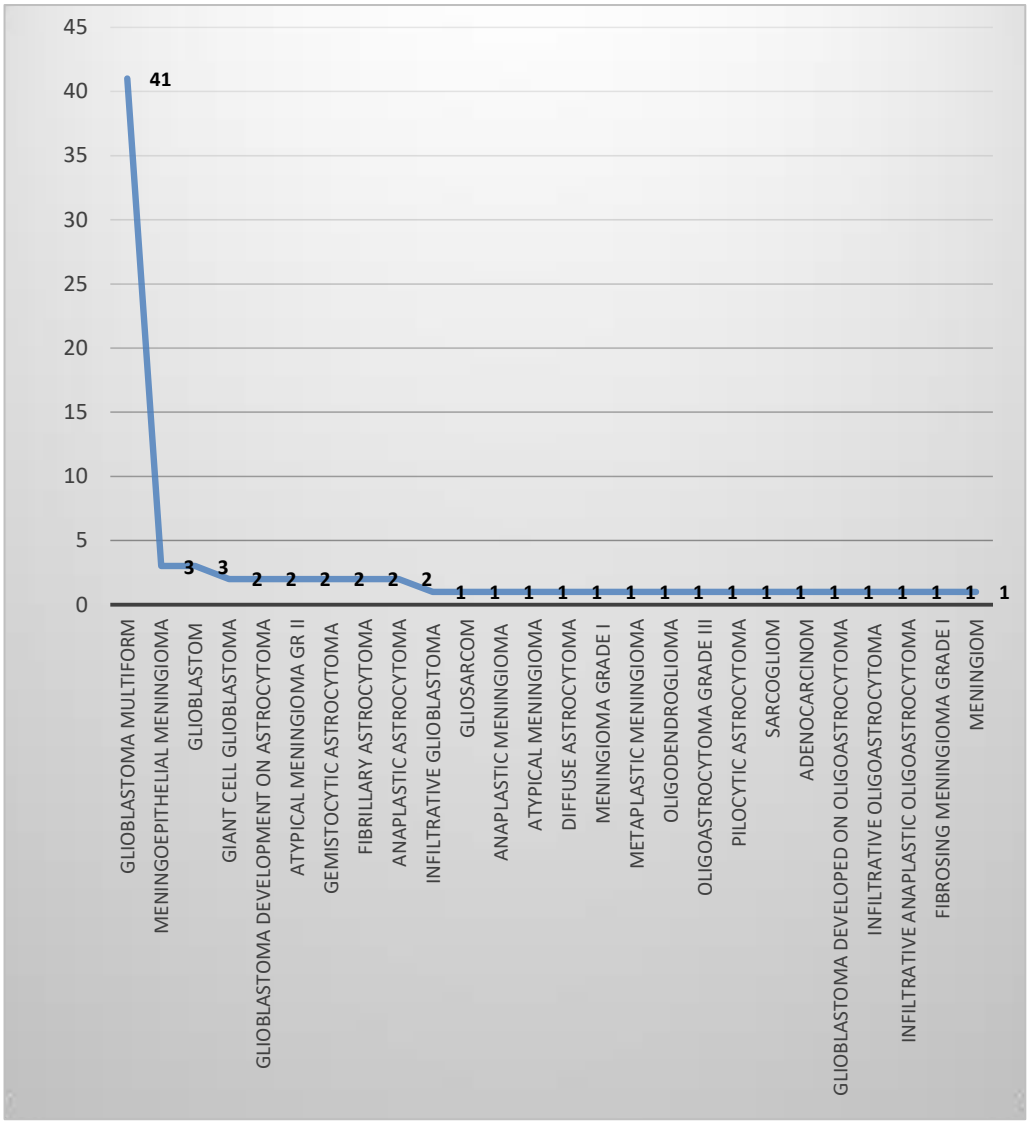

Fig. 3. Distribution of non-metastatic brain tumors

Examining the graph shows that "Glioblastoma Multiforme" is the most common type of tumor, constituting a significant part of the total cases studied, with 41 incidences (55%). Following in second place, at a significant distance, are "Meningoepithelial Meningioma" and "Glioblastoma", each having 3 cases (4%), while "Giant

Cell Glioblastoma" and "Differentiated Glioblastoma on Astrocytoma" have 2 cases each (3%). This distribution highlights the typological variety of non-metastatic brain tumors, with a clear predominance of glioblastoma multiforme.

To complete our analysis, it is essential to examine the distribution of brain metastases according to the affected lobes. This approach allows us to better understand not only the incidence and types of primary tumors, but also how they spread and impact different regions of the brain (fig. 4) illustrates the distribution of brain metastases, providing a clear picture of their prevalence and location within the studied population.

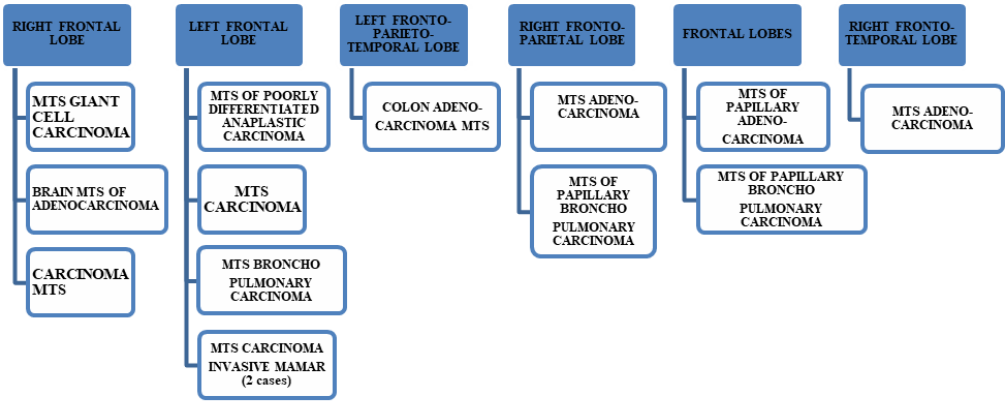

Fig. 4. Distribution of brain metastases (MTS = METASTASIS)

The right and left frontal lobes present the highest diversity of types of metastases, indicating a greater susceptibility of these regions to different types of cancer. In the right frontal lobe, the majority of affected patients are men, which may suggest a higher incidence of brain metastases in this region in men. On the other hand, metastases in the right frontoparietal and left frontoparietotemporal lobes seem to mainly affect women. Metastases from adenocarcinoma and papillary bronchopulmonary carcinoma are common in multiple regions of the brain, demonstrating an increased capacity of these types of cancer to spread to the brain. The frontal lobes (right and left) and frontoparietal lobes are critical regions for cognitive and motor functions. Metastases in these areas can lead to significant neurological deficits, such as movement problems, executive function, and behavior. Metastases in the left frontoparietotemporal lobe can affect language functions and spatial perception, having a major impact on the quality of life of patients. Metastases of adenocarcinoma are common and occur in various regions of the brain, indicating the aggressive and invasive nature of this type of cancer.

To create a more detailed and comprehensive overview of the distribution of non-metastatic brain tumors based on loca-

tion and sex, we have included a table summarizing this information (tab. I). The distribution of these tumors in various regions of the brain is presented for both men and women, offering a comprehensive perspective on the demographic and anatomical differences and similarities of the cases studied.

TABLE I.
Distribution of non-metastatic brain tumors by location and sex

Tumor type	Location	Number of cases	Gender distribution
GLIOBLASTOMA	Frontal lobes	2	M=2
	Right fronto-parietal lobe	1	M=1
	Right frontal lobe	14	W=7; M=7
	Left frontal lobe	18	W=8; M=10
	Right fronto-temporal lobe	2	W=2
	Right fronto-parieto-temporal lobe	2	W=1; M=1
	Left fronto-parieto-temporal lobe	1	W=1
	Left fronto-temporo-insular lobe	2	W=2
	Left fronto-temporal lobe	1	M=1
	Left parietal lobe	1	W=1
	Left fronto-parietal frontal lobe	3	W=1; M=1
	Right fronto-parietal lobe	1	W=1
	Right fronto-insular lobe	1	W=1
	Parieto-temporal lobe	1	W=1
MENINGIOMA	Right fronto-parietal lobe	2	W=2
	Right frontal lobe	6	W=5; M=1
	Fronto-parieto-temporal lobe	1	W=1
	Left frontal lobe	2	W=1, M=1
ASTROCYTOMA	Left frontal lobe	3	W=1; M=2
	Right frontal lobe	3	W=2, M=1
	Right fronto-temporal lobe	1	W=1
	Right fronto-insular lobe	1	W=1
OLIGOASTROCYTOMA	Left frontal lobe	2	W=2
	Right frontal lobe	1	M=1
GLIOSARCOMA	Right frontal lobe	1	W=1
ADENOCARCINOMA	Left frontal lobe	1	M=1
OLIGODENDROGLIOMA	Right frontal lobe	1	M=1
SARCOGLIOMA	Right frontal lobe	1	M=1

W- woman, M- man

Glioblastoma is the most common type of brain tumor in the study, with a total of 50 cases. This suggests a high prevalence of this type of tumor among the patients analyzed. Its distribution is varied in multiple regions of the brain, with the majority of cases being located in the frontal lobe. Glioblastoma is almost equally distributed among men and women. However, a slight predominance of cases in the left frontal lobe in men is observed. These data highlight the importance of carefully analyzing the anatomical distribution of glioblastoma in order to better understand its behavior

and prognosis. There are cases of glioblastoma in various combinations of lobes (e.g. fronto-parieto-temporal), indicating the complexity and extent of these tumors.

Meningioma also appears quite frequently in the dataset, being especially located in the right frontal lobe. This tumor is much more commonly found in women, highlighting their predisposition to developing meningioma compared to men. Specifically, meningioma is predominantly located in the frontal lobe, with a significant concentration on the right side. This trend indicates that women have a higher prevalence for developing this type of tumor in certain regions of the brain.

Oligoastrocytoma, gliosarcoma, adenocarcinoma, oligodendroglioma, and sarcomato-glioma are fewer common tumors, but present, with a variable distribution between sexes and locations, having notable cases, especially in the frontal lobes.

The frontal lobe is generally the most frequent location for tumors in the table, with multiple cases for all types of tumors. Both the right and left frontal lobes are the most common location for tumors. This may indicate a vulnerability or predisposition of this lobe to develop tumors. The distribution of tumors between sexes is relatively balanced for certain types of tumors, such as astrocytoma, but disproportionate for others, such as meningioma.

DISCUSSION

The results of this study highlight a predominant distribution of astrocytic tumors in the frontal lobes for both men and women. This finding aligns with existing literature, which suggests a preference for astrocytoma, particularly glioblastoma, in this brain region (1, 2, 3). The similar distribution between sexes, with a slight predominance in the left frontal lobe in men, emphasizes the need for personalized treatment approaches considering both anatomical location and sex differences.

Our analysis of brain metastases revealed a higher susceptibility of the frontal lobes to various cancer types, particularly metastases originating from adenocarcinoma and papillary bronchopulmonary carcinoma. These findings underscore the complexity of tumor-brain interactions, highlighting the necessity of a multidisciplinary approach to managing both primary tumors and metastases.

The study also found a correlation between tumor location and pre-diagnosed psychiatric symptoms, suggesting that frontal lobe involvement can significantly impact patients' cognitive and behavioral functions. These findings emphasize the importance of rigorous neuropsychiatric evaluations in managing brain tumor patients to optimize their quality of life.

Our study contributes to a better understanding of the anatomical distribution of astrocytic tumors and brain metastases, offering valuable insights for personalized therapeutic approaches. However, further research is needed to explore the underlying biological mechanisms and to validate these findings in larger clinical studies.

CONCLUSIONS

This study presents a detailed exploration of the anatomical distribution of astrocytic tumors and brain metastases, focusing predominantly on the frontal lobes. The results indicate that the frontal lobes are the most common site for these tumors, particularly in men, suggesting a potential anatomical predisposition. The significant presence of glioblastoma multiforme further emphasizes the aggressive nature of

these tumors in this region. These findings underline the critical need for location-specific therapeutic strategies that consider both the type and site of the tumor to enhance patient outcomes.

Additionally, the study draws attention to the importance of using advanced imaging techniques, such as MRI and CT scans, for accurate diagnosis and localization of tumors, which is crucial for effective treatment planning. The variability in tumor types across different brain regions also suggests that personalized treatment protocols are essential to address the unique challenges presented by each tumor's location and characteristics.

Future research should aim to validate these findings in larger cohorts and explore the biological mechanisms that may contribute to the observed distribution patterns. Understanding these mechanisms could lead to the development of more targeted therapies, ultimately improving the prognosis and quality of life for patients with astrocytic tumors and brain metastases.

CONFLICTS OF INTERES AND FUNDING

The authors have declared that there are no conflicts of interest related to this research. Additionally, no external funding was obtained or secured for the study.

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MESENCHYMAL STEM CELL'S OSTEOGENIC DIFFERENTIATION IS DEPENDENT ON ENVIRONMENTAL STIMULI

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MESENCHYMAL STEM CELL'S OSTEOGENIC DIFFERENTIATION IS DEPENDENT ON ENVIRONMENTAL STIMULI (Abstract): Mesenchymal stem cells derived osteoblasts or mesenchymal stem cells osteogenic differentiation could represent an important way to increase the healing after dental implantation. **Aim**: This research investigated the effects of various environmental stimuli (*in vitro*) on the osteogenic differentiation of MSCs and the subsequent development of calcium deposits by these cells. Materials and m**ethods**: Calcium phosphate deposition was quantified using the Alizarin red method via spectrophotometry. **Results**: The calcium deposited solely by mesenchymal stem cells derived osteoblasts was considered to be 100% all the time. When 1% platelet-rich plasma was added in the culture medium, the calcium deposits increased by 20% (on average). Different amounts of calcium deposits were developed by MSCs-derived osteoblasts when platelet-rich plasma was administered concurrently with: rosavin, rapamycin, metformin, "young" exosomes, "adult" exosomes, lower power CO_2 laser, extracellular shock waves, and PI3K/HDAC11 inhibitor. Conversely, platelet-poor plasma did not significantly modify the calcium deposits realized by MSCs-derived osteoblasts. **Conclusions**: All the obtained data suggest that calcium deposits resulting from the functioning of mesenchymal stem cells-derived osteoblasts could be significantly and especially increased by cellular mechanisms related to "young" exosomes, rosavin, acetylation, rapamycin, "adult" exosomes and metformin. **Keywords:** MSC, MSC-iPSC, OSTEOGENIC DIFFERENTIATION, EXOSOMES, PLATELET-RICH PLASMA.

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent cells capable of self-renewal and differentiation into a variety of cell types, and they play an important role in tissue repair and regeneration. Cell treatment and tissue engineering primarily use bone marrow-derived mesenchymal stem cells (BMSCs). It is essential for BMSCs to mobilize from bone marrow and further migrate into injured tissues during the healing process via peripheral circulation. The migration of bone marrow stem cells is influenced by both mechanical and chemical factors during the trafficking process. Numerous studies have emphasized the impact of key regulatory factors on BMSC migration and the associated mechanisms. The focus should follow two essential functions of BMSCs: directed differentiation and paracrine signaling in tissue repair. Additionally, it will explore the connection

between BMSC migration and tissue repair, offering valuable guidance for clinical applications aimed at optimizing BMSC migration for enhanced tissue repair outcomes (1).

The aging process causes a variety of cellular alterations, such as high amounts of reactive oxygen species (ROS) in numerous cell types, including MSCs. The link between reactive oxygen species (ROS) and decreased healing capacity is unclear, as is the effect of ROS suppression on the rejuvenation of old MSCs. The regeneration of old MSCs has lately received attention in regenerative medicine. Extracellular vesicles (EVs) may represent a unique technique for stem cell rejuvenation, as they can allow gene transfer with effects throughout the body and are associated with a high degree of therapeutic safety. The data revealed that aged MSCs had lower levels of superoxide dismutase (SOD)1 and SOD3, which led to an increase in reactive oxygen species (ROS) and a decrease in the MEK/ERK pathways. This limitation hampered the MSCs' ability to diminish necrotic area in the skin flap model. Furthermore, the treatment with the antioxidant Edaravone or co-overexpression of SOD1 and SOD3 reduced ROS levels and cellular senescence in old MSCs, resulting in better functioning. Infant MSC-derived EVs significantly revitalized aged MSCs by reducing ROS production, decreasing cellular senescence, and increasing proliferation and in vivo activities in both type 1 and type 2 diabetic mice (2).

MSCs are multipotent stem cells that have garnered considerable interest in regenerative medicine. The differentiation potential and paracrine properties of MSCs render them a significant choice for tissue repair. Consecutively, the paracrine functions of MSCs involve the secretion of soluble factors and the release of extracellular vesicles, including exosomes and microvesicles. Extracellular vesicles primarily originate from endosomes and carry a cargo of miRNA, mRNA, and proteins, which are transferred from donor cells to recipient cells. Recent findings indicate that extracellular vesicles are solely responsible for the therapeutic effects of mesenchymal stem cells in numerous animal disease models. Therefore, MSC-derived extracellular vesicles could serve as an alternative MSC-based therapy in the field of regenerative medicine (3).

Alveolar bone deficiency presents a significant clinical challenge in maxillofacial reconstructive surgery. The existing surgical techniques for extracortical bone augmentation exhibit a degree of unpredictability and often fail to meet expectations. Several studies sought to quantify extracortical bone augmentation and tissue mineral density (TMD) following the co-transplantation of peripheral blood-derived endothelial progenitor cells (EPCs) and, the same time, of bone marrow-derived MSCs using microcomputed tomography (micro-CT). Micro-CT analysis revealed that the co-transplantation of EPCs and MSCs significantly enhanced bone formation and mineral density (4).

BMSCs possess the capability to differentiate into adipocytes and osteoblasts. External stimuli, such as environmental contaminants, heavy metals, dietary factors, and physical influences, significantly affect the pathway decision of BMSCs regarding adipogenesis or osteogenesis. The equilibrium between osteogenesis and adipogenesis is essential for bone homeostasis, and disruptions in the lineage commitment of BMSCs are linked to various

health problems, including fractures, osteoporosis, osteopenia, and osteonecrosis. Further research is required to comprehend the effects of these external stimuli on bone health and to clarify the mechanisms underlying the differentiation of BMSCs. This knowledge will guide initiatives aimed at preventing bone-related diseases and formulating therapeutic strategies for treating bone disorders linked to diverse pathological conditions and implants (5).

This research investigated the effects of various environmental stimuli (*in vitro*) on the osteogenic differentiation of mesenchymal stem cells and these cells' subsequent development of calcium deposits.

MATERIALS AND METHODS

The laboratory study sought to quantify calcium deposition resulting from the osteogenic differentiation of mesenchymal stem cells in response to various environmental stimuli and different culture mediums. Calcium phosphate deposition was quantified using the Alizarin red method via spectrophotometry (6).

MSCs were isolated as outlined in prior research (7). Additionally, mesenchymal stem cell-derived induced pluripotent stem cells (MSC-iPSCs) were developed using the episomal technique with iPSC reprogramming vectors and the designated Stemline culture media (Invitrogen), adhering to the manufacturer's guidelines. MSC-iPSCs were utilized to isolate exosomes characterized as "young". Conversely, standard MSCs were utilized to derive "adult" exosomes.

The osteogenic differentiation of the MSCs followed a straightforward and effective method (6). Cell viability was assessed using Evans blue exclusion.

The preparation of the platelet-rich plasma (PRP) gel was conducted as outlined in previous studies (8), with a delivery concentration of 1%. The PRP gel was compared to the PPP (platelet-poor plasma).

The culture medium of pluripotent stem cells derived from rat mesenchymal stem cells was subjected to centrifugation at 100,000xg for exosome separation (9).

The compounds, preparations, and methods utilized in the tests and protocols for mesenchymal stem cells' osteogenic differentiation included: 1 µM rosavin (adaptogen), 1 µM rapamycin, 1 µM metformin, "young" and "adult" 0.01% exosomes, lower power CO2 laser, 1% platelet-rich plasma, 1% platelet-poor plasma, extracellular shock waves (1,000 pulses at an energy of 0.3 mJ/mm^2 at 24 and 72 hours), and 1 µM PI3K/HDAC11 specific inhibitor.

Data were verified through additional methods, including epifluorescence, confocal microscopy, and gene expression analysis using the Profiler 725 antibody microarray.

All experimental results from the current study were analyzed using established statistical methodologies, employing various optimized tests recognized in medical research. Notably, the One-Way ANOVA test was utilized, occasionally supplemented by the Student-Newman-Keuls test.

All experiments received approval from the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iaşi.

RESULTS

The optimal qualitative and quantitative development of pluripotent stem cells, experimentally reprogrammed from mesenchymal stem cells, and mesenchymal stem cells, initiated from the second passage in culture, was achieved when cultured in a

preconditioning medium with 1% O2, as previously demonstrated. A 100% increase was consistently assumed (n=5 experiments per set, data not presented, personal observation).

The calcium deposited solely by mesenchymal stem cells derived osteoblasts (aka osteoblasts) was considered to be 100% all the time (control, Ctrl, 7 experiments, as average). When PRP 1% was added in the culture medium, the calcium deposits increased by 20% (on average, Ctrl PRP, fig. 1). By contrast, PPP did not significantly modify the calcium deposits realized by mesenchymal stem cells derived osteoblasts.

In the first series of tests, mesenchymal stem cells derived osteoblasts were treated with 1 µM rosavin and 1% platelet-rich plasma (PRP). The deposited calcium, evaluated through Alizarin red, showed an increase of 36% as compared to the control PRP (on average, fig. 1).

In the second series of tests, a separate batch of mesenchymal stem cells derived osteoblasts was treated with 1 µM rapamycin and 1% platelet-rich plasma. Mesenchymal stem cells derived osteoblasts showed an average 27% increase in calcium deposits as compared to control PRP (on average, fig. 1).

In the third series of tests, a new number of derived osteoblasts were treated with 1 µM metformin and 1% platelet-rich plasma. We observed a 23% increase in calcium deposited by mesenchymal stem cells derived osteoblasts as compared to control PRP (on average, fig. 1).

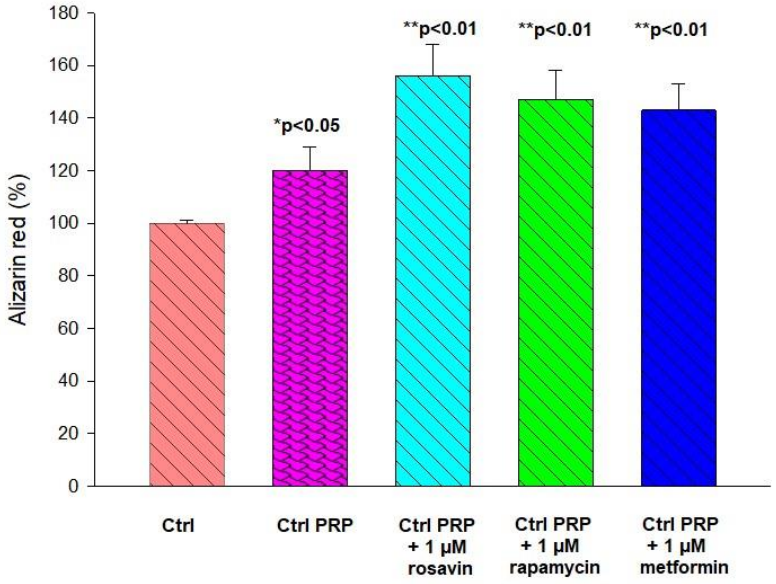

Fig. 1. Determination of calcium deposited by mesenchymal stem cells derived osteoblasts (Ctrl), in the presence of 1% PRP (Ctrl PRP), as well treated with rosavin, rapamycin, and metformin. (*p<0.05 as compared to the solely derived osteoblasts. **p<0.01 as compared to the solely mesenchymal stem cells derived osteoblasts)

In the fourth series of studies, a completely separate batch of derived osteoblasts was treated with 0.01% "young" exosomes obtained from MSC-iPSC supernatant and 1% PRP. In this case, we observed an average of 42% augmentation in calcium deposits as compared to control PRP (on average, fig. 2).

In the fifth series of tests, mesenchymal stem cells derived osteoblasts were treated with 0.01% "adult" exosomes obtained from MSC supernatant and 1% platelet-rich plasma. We observed a 25% growth in deposited calcium as compared to control PRP (on average, fig. 2).

In the sixth series of studies, a separate batch of derived osteoblasts was treated with lower power CO_2 laser for 1 minute, 7 pulses in one day, and 1% platelet-rich plasma. We thus observed a modest 5% augmentation in deposited calcium as compared to control PRP (on average, fig. 2).

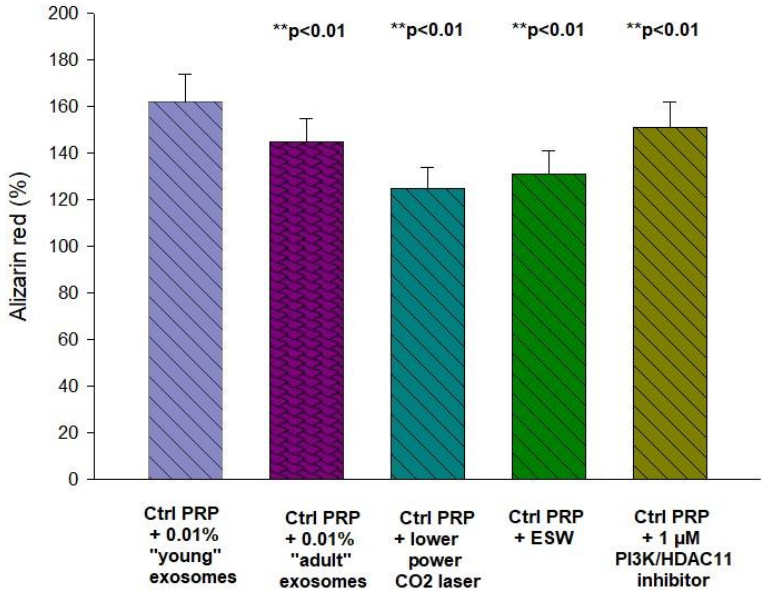

Fig. 2. Measurement of calcium deposited by mesenchymal stem cells derived osteoblasts in the presence of 1% PRP (Ctrl PRP) and as well treated with 0.01% "young" exosomes, 0.01% "adult" exosomes, lower power CO_2 laser, extracellular shock waves, 1 μM PI3K/HDAC11 specific inhibitor (**p<0.01 as compared to the solely mesenchymal stem cells derived osteoblasts)

In the seventh series of tests, a different number of osteoblasts were treated with extracellular shock waves (1000 pulses at an energy of 0.3 mJ/mm^2 at 24 and 72 hours) and 1% PRP. We observed an average increase of 11% of deposited calcium by mesenchymal stem cells derived osteoblasts as compared to control PRP (on average, fig. 2).

In the eighth series of trials, a completely separate batch of osteoblasts was treated with 1 μM PI3K/HDAC11 specific inhibitor and 1% platelet-rich plasma. We observed a 31% augmentation of calcium

deposited by mesenchymal stem cells derived osteoblasts as compared to control PRP (on average, fig. 2).

DISCUSSION

Our research was based on the fact that the differentiation of mesenchymal stem cells into osteoblasts is commonly conducted in research laboratories. Traditional methods utilized to demonstrate this differentiation utilize steps such as cell fixation, cell lysis, or cell scraping. Limited research addresses the gentle dissociation of mesenchymal stem cells during the osteo differentiation process. Consequently, the cells undergoing differentiation established one layer in neurogenic differentiation, two layers in adipogenic differentiation, and a minimum of four layers in osteogenic differentiation. The upper layers, supported by a collagen I extracellular matrix, can be dissociated with collagenase I, whereas the lowest layer, adhering to the dish bottom, is only sensitive to trypsin-versene. Collagenase I exhibit greater efficiency prior to the mineralization of the extracellular matrix. The collagenase-sensitive and trypsin-sensitive layers exhibit differences in their cluster of differentiation expression. The dissociation of the cells on day 15 indicates that they can resume growth (increase in cell number) and rapidly differentiate into osteoblasts within 2 weeks, rather than the previously observed 4 weeks. Cells from the upper layers exhibited increased mineralization. MSCs that undergo osteogenic differentiation develop multiple layers, each exhibiting unique osteogenic characteristics. This approach may enable investigators to utilize the upper layers for the rapid production of differentiated osteoblasts, while the lower layer can sustain growth and differentiation until subsequent dissociation occurs (6).

Our experimental data showed osteoblasts derived from mesenchymal stem cells being able to deposit calcium in higher amounts, approximately 36%, in the presence of rosavin and platelet-rich plasma (PRP). Rosavin, a phenylpropanoid found in *Rhodiola rosea's* rhizome, functions as an adaptogen and is known to improve the body's reaction to environmental stresses. It has a profound impact on cellular metabolism in both health and disease, particularly the metabolism of bone tissue. In vitro studies show that rosavin inhibits osteoclastogenesis, disrupts F-actin ring formation, and decreases the expression of osteoclastogenesis-related genes like cathepsin K, calcitonin receptor (CTR), as well as tumor necrosis factor receptor-associated factor 6 (TRAF6), but also tartrate-resistant acid phosphatase (TRAP), and matrix metallopeptidase 9 (MMP-9). It blocks bone resorption by inhibiting signaling pathways such as NFATc1, c-Fos, NF-κB, and MAPK. Rosavin also enhances osteogenesis and osteoblast differentiation by raising the expression of mouse runt-related transcription factor 2 (Runx2) and osteocalcin (OCN). In vivo studies show that it improves bone mineral density (BMD) in postmenopausal osteoporosis (PMOP) mice by decreasing osteoclast maturation and boosting the proportion of active osteoblasts in bone tissue. It modulates mRNA expressions by enhancing eukaryotic translation elongation factor 2 (EEF2) and reducing histone deacetylase 1 (HDAC1), thus activating osteoprotective epigenetic mechanisms. It alters serum markers, lowering CTX-1, TRACP5b, RANKL, M-CSF, and TRAP while increas-

ing ALP and OCN. Combining zinc and probiotics reduces pro-osteoporotic MMP-3, IL-6, and TNF-α levels while increasing anti-osteoporotic IL-10 and TIMP3 expression (10).

Rhodiola rosea, a perennial herb of the Crassulaceae family, contains the active component salidroside, which is recognized as an adaptogen with significant therapeutic promise for bone metabolism. Salidroside promotes osteoblast proliferation and differentiation by activating critical signaling pathways, and we can highlight the bone morphogenetic protein-2 and adenosine monophosphate-activated protein kinase, which both are essential for bone production and growth. Moreover, it enhances osteogenic activity by increasing alkaline phosphatase activity and mineralization indicators, as well as upregulating important regulatory proteins such as runt-related transcription factor 2 and osterix. Salidroside stimulates angiogenesis via the hypoxia-inducible factor 1-alpha and vascular endothelial growth factor pathways, which are critical for connecting bone formation and vascular supply. The antioxidant qualities prevent bone loss by reducing oxidative stress and promoting osteogenic differentiation through the nuclear factor erythroid 2-related factor 2 pathway. Salidroside can reduce the negative effects of glucocorticoids on bone cells and prevent steroid-induced osteonecrosis. Furthermore, it displays complicated anti-inflammatory actions, mainly by suppressing the expression of tumor necrosis factor-alpha and interleukin-6, while boosting the expression of interleukin-10 (11).

Cells detect mechanical signals from the extracellular matrix to modulate cellular activities and preserve tissue homeostasis. The nucleus serves as a crucial mechanosensor, directly affecting chromatin organization, epigenetic modifications, and gene expression. Nuclear mechanosensing dysregulation has been associated with various diseases, including bone degeneration. Some studies utilized photo-stiffening hydrogels to modulate nuclear mechanosensing in human mesenchymal stem cells (hMSCs) *in vitro*. The findings indicate that human mesenchymal stem cells (hMSCs) react to matrix stiffening by enhancing nuclear tension, which leads to an increase in histone acetylation through the deactivation of histone deacetylases (HDACs). This leads to the commitment to an osteogenic pathway. Disruption of nuclear mechanosensing through the disconnection of the nucleus from the cytoskeleton leads to the upregulation of HDACs and inhibits osteogenesis. Restoring HDAC activity to normal levels improves the epigenetic and osteogenic response in hMSCs exhibiting pathological nuclear mechanosensing. Bone from patients with osteoarthritis exhibits comparable deficiencies in nuclear mechanosensing. The results indicate that nuclear mechanosensing regulates the osteogenic potential of hMSCs through HDAC epigenetic remodeling, suggesting its relevance to bone-related diseases (12).

The secretomes of MSCs represent a unique approach to growth factor administration in tissue regeneration. The purpose of some studies was to compare the efficacy of conditioned media derived from bone-marrow mesenchymal stem cells (MSC-CM) when used with collagen barrier membranes to conditioned media derived from leukocyte- and platelet-rich fibrin (PRF-CM), a modern growth-factor therapy, for guided bone regeneration. Both

MSC-CM and PRF-CM showed a variety of bone-related proteins; however, MSC-CM had a larger increase in differentially expressed proteins, particularly those linked with extracellular matrix components. Micro-CT study of rat calvaria defects revealed that the MSC-CM group had better total bone covering at both 2 and 4 weeks. Histological investigation revealed that both groups had a mix of conventional and 'hybrid' new bone, with the latter generated within the membrane compartment and distinguished by the incorporation of mineralized collagen fibers. Histomorphometry study of central defect sections revealed that the MSC-CM group had more hybrid bone area, although the total new bone area was equivalent across all groups. In vitro and in vivo studies indicate that membrane functionalization with MSC-CM is a viable technique for improving GBR (13).

Moreover, rapamycin, a modulator of autophagy and mTOR pathways, was able to increase the calcium deposition of derived osteoblasts by 27% (on average) in our experiments. Autophagy is crucial for the self-renewal and differentiation of stem cells; however, its impact on BMSCs is not well understood. Several studies have focused on examining the relationship between autophagy and osteogenic differentiation through the use of rapamycin (RAPA), a well-known autophagy agonist with osteo-regulatory properties. The basal autophagy level in BMSCs progressively declined during osteogenic differentiation, characterized by a reduction in BECN1 levels, a decrease in the ratio of lipidated (LC3-II) to unlipidated (LC3-I) microtubule-associated protein 1 light chain 3, and an elevation in the expression of the selective autophagic target p62. Conversely, it rose with higher RAPA concentrations. Additionally, 2 nM RAPA facilitated BMSC osteogenic differentiation on days 7 and 14, whereas 5 nM RAPA suppressed osteogenesis on days 14 and 21. The use of the autophagy inhibitor 3-methyladenine may diminish the osteogenesis-promoting effect of RAPA on BMSCs. The basal autophagy level in BMSCs diminished progressively throughout the process of osteogenic differentiation. An appropriate RAPA concentration facilitated BMSC osteogenic differentiation through the activation of autophagy (14).

Therefore, metformin, which increases and decreases histone acetylation, histone and DNA methylation, and gene expression, augmented the calcium deposition of derived osteoblasts by 23% (on average), as demonstrated by our experiments. Periodontitis is the sixth leading cause of tooth loss in diabetic patients. Metformin stimulates the osteogenic differentiation of stem cells. So, periodontal ligament stem cells (PDLSCs) are the best stem cell type for periodontal tissue regeneration. The purpose of the study was to see how metformin might affect the proliferation, migration, and osteogenic differentiation of periodontal ligament stem cells (PDLSCs) in vitro. PDLSCs were isolated by limiting dilution, and their properties were determined using colony formation assays and flow cytometry. Metformin's effects on cell proliferation and migration were evaluated using cell counting and migration assays. The osteogenic differentiation capacity of PDLSCs was assessed using alkaline phosphatase (ALP) activity and Alizarin Red S staining. Gene expression was measured using a quantitative real-time polymerase

chain reaction (qRT-PCR), and protein levels of osteogenesis-related indicators were assessed using western blotting. Metformin therapy at 10 μM did not impact PDLSC proliferation. However, at 50 and 100 μM, metformin gradually increased PDLSC proliferation, leading to a substantial increase in cell counts after 5 and 7 days of stimulation ($P < 0.05$). Metformin at 50 μM had the strongest effect on migration, ALP activity, and mineral deposition ($P < 0.05$). Metformin at 50 μM significantly increased gene expression for ALP, BSP, OPN, OCN, and Runx2, as well as protein expression for ALP and Runx2 (15).

We observed that there is a clear difference between the effects of „young" exosomes, obtained from the supernatant of mesenchymal stem cells-induced pluripotent stem cells, and "adult" exosomes, isolated from the supernatant of mesenchymal stem cells, on calcium deposition of derived osteoblasts (42% *vs.* 25% on average). The aging global population leads to a higher prevalence of osteoporosis and related fragility fractures, which considerably affects patient quality of life and healthcare expenditures. The acute inflammatory response is crucial for initiating the healing process following injury. Aging is linked to "inflammaging" which denotes the existence of systemic low-grade chronic inflammation. Chronic inflammation hinders the onset of bone regeneration in elderly individuals. Aged macrophages exhibit heightened sensitivity and responsiveness to inflammatory signals. M1 macrophages are activated during the acute inflammatory response; however, the effective resolution of inflammation requires the repolarization of pro-inflammatory M1 macrophages to an anti-inflammatory M2 phenotype, which is linked to tissue regeneration. In aging, chronic inflammation persists due to the failure of M1 to M2 repolarization, resulting in heightened osteoclast activation and reduced osteoblast formation. This imbalance increases bone resorption while decreasing bone formation during the healing process. Inflammaging can hinder the capacity of stem cells to facilitate bone regeneration, contributing to the reduction in bone mass and strength associated with aging. Modulating inflammaging represents a promising strategy for enhancing bone health in the aging population. MSCs exhibit immuno-modulatory properties that could enhance bone regeneration during inflammatory conditions. Preconditioning mesenchymal stem cells with pro-inflammatory cytokines influences their secretory profile and osteogenic capacity. MSCs cultured in hypoxic environments exhibit enhanced proliferation rates and elevated secretion of growth factors. The local delivery of anti-inflammatory cytokines for the resolution of inflammation represents a potential therapeutic approach for bone regeneration in the context of inflammaging. Scaffolds incorporating anti-inflammatory cytokines, unmodified MSCs, and genetically engineered MSCs possess potential therapeutic applications. MSC exosomes enhance the migration of MSCs to fracture sites and promote osteogenic differentiation and angiogenesis. In conclusion, inflammaging may hinder the effective initiation of bone regeneration in the elderly. Modulating inflammaging represents a promising strategy for enhancing impaired bone healing in the elderly population (16).

Conversely, the lower power CO_2 laser, which may exhibit bio stimulatory effects

on cells viability, presented modest effects on calcium deposition by derived osteoblasts (5% on average). Low-level laser treatment (LLLT) has been found to promote fibroblast vitality. Secretomes can be added to culture media using conditioned medium made from BMSCs. Some studies looked at the effect of low-level laser therapy (LLLT) as well as hBMSCs' conditioned medium on the vitality of human dermal fibroblasts (HDFs) cultivated in a high-glucose (HG) medium. For four days, the HDFs were grown in either standard glucose (5.5 mM/l) or high glucose media (15 mM/l). Low-level laser therapy (LLLT) used a continuous-wave helium-neon laser (632.8 nm) with a power density of 0.00185 W/cm² and energy densities of 0.5, 1, and 2 J/cm². Human dermal fibroblasts' high glucose culture media received around 10% of the conditioned medium from human bone marrow-derived mesenchymal stem cells. The vitality of HDFs was assessed using the dimethylthiazol-diphenyltetrazolium bromide (MTT) assay. Laser treatment of HG HDFs at 0.5 or 1 J/cm² resulted in considerably higher cell viability compared to control groups. HG-treated HDFs had considerably reduced cellular viability than LLLT + HG HDFs, hBMSCs condition medium-treated HG HDFs, and LLLT + hBMSCs' condition medium-treated HG HDFs. Finally, both hBM-MSC-conditioned medium and low-level laser therapy improved the survival of hyperglycemic human dermal fibroblasts. The combination of hBMSCs' condition medium with LLLT produced better results than the conditioned medium (17).

Furthermore, extracellular (extracorporeal) shock waves, known to modulate the Ca^{2+} ionic channels, has a slight effect on calcium deposition by obtained osteoblasts (11% on average). Extracorporeal shock wave therapy (ESWT) has been shown to enhance the differentiation of osteoblasts. The impact of ESWT on osteoclast differentiation remains unclear. ESWT has been shown to enhance the process of bone formation. However, the detailed mechanism has not been fully elucidated. Some studies demonstrate that ESWT enhances osteoblast formation *in vitro*. Localized ESW treatment of the femur enhanced *in vivo* bone formation. Moreover, altering the density or frequency of energy did not yield a statistically significant difference in osteogenic differentiation. Local ESW therapy effectively alleviated bone loss and enhanced the quantity of bone trabecular in a rabbit model of osteoporosis, while also promoting endogenous SMAD2 protein expression levels. ESWT may serve as a potential therapy by facilitating osteoblast maturation via the TGF-β/SMAD2 pathway (18).

As a final mention, PI3K/HDAC11 inhibitor, known to act selectively through the inhibition of HDAC11, thus modulating cellular acetylation processes, increased the calcium deposits by 31% on average. Histone deacetylase (HDAC) is an important target in cancer treatment. HDAC inhibitors' key anticancer actions include the stimulation of terminal differentiation and the reduction of cell growth. This study looks into the role of HDAC in sustaining self-renewal and cell proliferation in MSCs either from adipose tissue or umbilical cord blood, using valproic acid (VPA) and sodium butyrate (NaBu). VPA and NaBu altered the shape of MSCs and hindered their proliferation. VPA and NaBu promoted p21 (CIP1/WAF1) transcription by boosting

histone H3 and H4 acetylation, resulting in cell cycle arrest at the G2/M phase. After treatment with an HDAC inhibitor, the expression of p16(INK4A), a cyclin-dependent kinase inhibitor associated to cellular senescence, remained constant. Controlled differentiation into bone, fat, cartilage, and nerve tissue was used to determine the role of HDAC in MSC pluripotency for differentiation into functional tissues. VPA and NaBu reduced the effectiveness of adipogenic, chondrogenic, and neurogenic development, as demonstrated by specific staining and reverse transcription-polymerase chain reaction. Treatment with HDAC inhibitors increased osteogenic differentiation (19).

CONCLUSIONS

The calcium deposited solely by mesenchymal stem cells derived osteoblasts (aka osteoblasts) was considered to be 100% all the time. When 1% platelet-rich plasma was added in the culture medium, the calcium deposits increased by 20% (on average). Different amounts of calcium deposits were developed by mesenchymal stem cells derived osteoblasts when platelet-rich plasma was administered concurrently with: rosavin (a phenylpropanoid in *Rhodiola rosea*'s rhizome, and an adaptogen, +36% on average); rapamycin (a modulator of autophagy and mTOR pathways, +27% on average); metformin (which increase and decrease histone acetylation, histone and DNA methylation, and gene expression, +23% on average); „young" exosomes (obtained from the supernatant of mesenchymal stem cells-induced pluripotent stem cells, +42% on average); „adult" exosomes (isolated from the supernatant of mesenchymal stem cells, +25% on average); lower power CO_2 laser (may exhibit bio stimulatory effects on cells viability, +5% on average); extracellular (extracorporeal) shock waves (modulates the Ca^{2+} ionic channels, +11% on average); and PI3K/HDAC11 inhibitor (by selectively inhibiting HDAC11, it modulates cellular acetylation processes, +31% on average). Conversely, platelet-poor plasma did not significantly modify the calcium deposits realized by mesenchymal stem cells derived osteoblasts.

All of these findings suggest that calcium deposits resulted from the functioning of mesenchymal stem cells derived osteoblasts (aka osteoblasts) could be significantly and especially increased by cellular mechanisms related to "young" exosomes, rosavin, acetylation, rapamycin, "adult" exosomes and metformin.

CONFLICTS OF INTEREST AND FUNDING

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RISK FACTORS OF ANASTOMOTIC FISTULAS AFTER ESOPHAGEAL AND GASTRIC CANCER SURGERY: A RETROSPECTIVE STUDY

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RISK FACTORS OF ANASTOMOTIC FISTULAS AFTER ESOPHAGEAL AND GASTRIC CANCER SURGERY: A RETROSPECTIVE STUDY (Abstract): Anastomotic fistulas remain one of the most severe complications following esophageal and gastric cancer surgery, significantly impacting morbidity and mortality rates. **Materials and methods**: This retrospective study evaluates the risk factors associated with anastomotic fistula formation and assesses the efficacy and complications of esophageal stenting as a treatment modality. Data from 110 patients treated at the Surgery Clinic of "Sf. Maria" Hospital in Bucharest, between 2019 and 2023 were analyzed. The study population was divided into two groups: patients with postoperative anastomotic fistulas who underwent endoscopic esophageal stenting and those who did not develop fistulas. **Results:** Key findings indicate that advanced age, smoking, alcohol consumption, tumor size, TNM stage, hypertension, diabetes, and preoperative radiotherapy were significantly associated with an increased risk of fistula formation. Additionally, hypoalbuminemia, anemia, and impaired renal function emerged as critical predictors. While esophageal stenting proved to be an effective intervention, it was associated with specific complications requiring careful patient selection and monitoring. **Conclusions:** This study identifies multiple risk factors associated with the development of anastomotic fistulas after esophageal and gastric cancer surgery. Understanding these risk factors is crucial for identifying high-risk patients and implementing targeted perioperative strategies to improve surgical outcomes and reduce postoperative complications. **Keywords:** ANASTOMOTIC FISTULA, ESOPHAGEAL CANCER, GASTRIC CANCER, RISK FACTORS.

INTRODUCTION

Anastomotic fistulas are among the most serious postoperative complications following esophageal and gastric cancer surgery, significantly contributing to increased morbidity, prolonged hospitalization, and higher mortality rates. These fistulas result from impaired healing at the anastomotic site, leading to leakage, infection, and subsequent complications. Identifying risk factors associated with anastomotic fistula formation is crucial for optimizing surgical strategies and improving patient outcomes (1, 2).

Numerous factors have been implicated in the development of anastomotic fistulas, including patient-related parameters (age, comorbidities, nutritional status), tumor

characteristics (size, location, histopathology), and perioperative factors (neoadjuvant therapy, intraoperative technique). Despite advances in surgical techniques and perioperative management, the incidence of anastomotic fistulas remains significant, warranting further investigation into modifiable and non-modifiable risk factors (3-5).

The aim of this study is to evaluate the risk factors associated with the development of postoperative anastomotic fistulas in patients undergoing surgery for esophageal and gastric neoplasms. By comparing patients who developed fistulas with those who did not, we seek to identify key predictors that may contribute to an increased risk of fistula formation. A better understanding of these factors could support the development of preventive strategies and guide clinical decision-making in high-risk patients.

MATERIALS AND METHODS

This retrospective and observational study aimed to evaluate the efficacy and complications of esophageal stenting in the treatment of postoperative anastomotic fistulas in patients who underwent surgery for esophageal and gastric neoplasms. The analysis was conducted based on data from patients admitted to the Surgery Clinic of "Sf. Maria" Hospital in Bucharest, between 2019 and 2023. Data collection and processing were carried out during 2023-2024.

The studied population was divided into two groups:

- Study group: Patients who developed postoperative anastomotic fistulas and were treated with endoscopic esophageal stenting.
- Control group: Patients who underwent surgery for esophageal and gastric neoplasms but did not develop postoperative fistulas.

Patients aged ≥18 years with a histopathological confirmed diagnosis of esophageal or gastric neoplasm. The study group includes patients with imaging-confirmed anastomotic fistulas, while the control group consists of those without anastomotic fistulas. Patients with insufficient medical data are excluded.

The study was conducted in compliance with all national and international ethical research standards to ensure the integrity of the research process and patient protection. The study adhered to Law No. 206/2004 on good conduct in scientific research, technological development, and innovation, which defines ethical and integrity standards for research activities in Romania. The principles of the Declaration of Helsinki were implemented to uphold ethical research practices in human subjects. Fundamental ethical principles, such as confidentiality protection and respect for patient rights, were followed throughout the study.

RESULTS

Among the 110 patients included in the study, those without anastomotic fistulas had a mean age of 54.13 years (median: 54 years), with a standard deviation of 5.10 years. Patients who developed anastomotic fistulas had a higher mean age of 59.71 years (median: 58 years) and a larger standard deviation of 9.88 years. The t-test for the difference in means showed a statistically significant difference ($p < .001$) between the two groups. The mean difference was -5.58 years, with a standard error of 1.50 years.

Of the 42 female patients, 19 (45.24%) developed anastomotic fistulas, whereas 23 (54.76%) did not. Among the 68 male patients, 36 (52.94%) developed fistulas,

while 32 (47.06%) did not. The calculated odds ratio of 1.362 suggests a slightly higher risk for male patients; however, the 95% confidence intervals (0.629-2.947) include the value of 1, and Fisher's exact test ($p = 0.556$) indicates that the difference is not statistically significant.

Among the 43 smokers, 40 (93.02%) developed anastomotic fistulas, compared to only 15 cases (22.39%) among the 67 non-smokers. The odds ratio of 46.222 indicates a significantly increased risk of fistula development in smokers. The confidence intervals (95% CI: 12.518-170.680) and Fisher's exact test ($p < 0.001$) confirms statistical significance.

Regarding alcohol consumption, 33 of the 34 alcohol consumers (97.06%) developed fistulas, compared to only 22 cases (28.95%) among the 76 non-consumers. The odds ratio of 81 suggests an extremely high risk associated with alcohol consumption. Confidence intervals (95% CI: 10.425-629.371) and Fisher's exact test ($p < 0.001$) confirm the statistical significance.

Patients with smaller tumors (2 cm) developed anastomotic fistulas in only 26.09% of cases, compared to 94.44% of those with 4 cm tumors. The proportion of fistula development in intermediate-sized tumors (3 cm) was 45.59%. The chi-square test ($\chi^2 = 21.013$, $p < 0.001$) indicates a statistically significant association between tumor size and fistula occurrence.

The highest proportion of anastomotic fistulas was observed in patients with tumors located in the gastric mid-corporeal region, where 23 out of 38 patients (60.53%) developed fistulas. Subcardial tumors (Siewert III) were associated with a lower proportion of fistulas (37.93%). Junctional esophagogastric tumors (Siewert II), lower esophageal tumors (Siewert I), and mid-esophageal tumors exhibited similar fistula proportions (46.15%, 50%, and 50%, respectively). However, these differences were not statistically significant ($\chi^2 = 3.451$, $df = 4$, $p = 0.485$).

Patients with adenocarcinoma developed anastomotic fistulas in 53.49% of cases (46 out of 86), compared to 37.50% (9 out of 24) in those with squamous cell carcinoma. However, statistical analysis showed that the differences between histopathological types were not statistically significant ($\chi^2 = 1.919$, $p = 0.166$).

A progressive increase in the risk of anastomotic fistulas was observed with advancing TNM stage. No fistulas were detected in stage 1 patients, whereas the incidence was 35.71% in stage 2, 92.86% in stage 3, and 100% in stage 4. The significant association between TNM stage and fistula occurrence was confirmed ($\chi^2 = 38.286$, $p < 0.001$).

Among the 36 patients with arterial hypertension, 33 (91.67%) developed anastomotic fistulas, compared to only 22 out of 74 patients (29.73%) without hypertension. The odds ratio of 26 indicates an extremely high risk, confirmed by significant confidence intervals (7.209-93.768) and Fisher's exact test ($p < 0.001$).

Similarly, 14 out of 16 diabetic patients (87.50%) developed fistulas, compared to 41 out of 94 non-diabetic patients (43.62%). The odds ratio of 9.049 indicates an increased risk, with statistical significance ($p = 0.002$).

Preoperative radiotherapy was associated with a significantly increased risk of anastomotic fistulas, with an odds ratio of 3.514 ($p = 0.003$). In contrast, preoperative chemotherapy was associated with a slightly lower risk (OR = 0.588), but this difference was not statistically significant ($p =$

0.556).

Patients who developed anastomotic fistulas had significantly higher levels of serum creatinine (1.04 ± 0.15 mg/dL *vs.* 0.91 ± 0.14 mg/dL, $p < 0.001$), blood glucose (98.56 ± 25.65 mg/dL *vs.* 88.55 ± 13.70 mg/dL, $p = 0.012$), and lower levels of serum albumin (3.52 ± 0.17 g/dL *vs.* 3.72 ± 0.14 g/dL, $p < 0.001$). Furthermore, hemoglobin levels were significantly lower in patients who developed fistulas (10.49 ± 1.43 g/dL *vs.* 12.78 ± 0.45 g/dL, $p < 0.001$).

Patients with anastomotic fistulas had significantly lower body mass index (BMI) values compared to those without fistulas (22.77 ± 1.87 *vs.* 24.11 ± 1.42, $p < 0.001$), suggesting a relationship between lower BMI and an increased risk of fistula development (tab. I).

TABLE I.

Relationships between risk factors and the risk of fistula development

Risk Factor	No Fistula (Mean ± SD/%)	Fistula (Mean ± SD/%)	p-value
Age	54.13 ± 5.10 yrs.	59.71 ± 9.88 yrs.	< 0.001
Smoking	22.39%	93.02%	< 0.001
Alcohol Consumption	28.95%	97.06%	< 0.001
Tumor Size	2.0 cm (26.09%)	4.0 cm (94.44%)	< 0.001
TNM Stage	Stage 1 (0%)	Stage 4 (100%)	< 0.001
Hypertension	29.73%	91.67%	< 0.001
Diabetes Mellitus	43.62%	87.50%	0.002
Radiotherapy	37.88%	68.18%	0.003
Chemotherapy	61.54%	38.46%	0.556
Serum Creatinine	0.91 ± 0.14	1.04 ± 0.15	< 0.001
Blood Glucose	88.55 ± 13.70	98.56 ± 25.65	0.012
Serum Albumin	3.72 ± 0.14	3.52 ± 0.17	< 0.001
Hemoglobin	12.78 ± 0.45	10.49 ± 1.43	< 0.001
BMI	24.11 ± 1.42	22.77 ± 1.87	< 0.001

DISCUSSION

Anastomotic fistulas represent a major and frequent complication in gastroesophageal surgery, significantly impacting postoperative outcomes. In this study, the predominant localization of fistulas at the esophagojejunal anastomosis (61.82%) aligns with the existing literature, which reports a higher incidence in this segment due to technical complexity and vascularization challenges. Although esophagogastric fistulas (38.18%) were less frequent, they remain a significant cause of morbidity.

Contrast-enhanced CT scan was the primary diagnostic modality, used in 85.45% of cases, highlighting its role as the gold standard for detecting anastomotic fistulas due to its high sensitivity and specificity. The mean time to fistula onset was 7.44 days postoperatively, consistent with literature reports identifying this period as the critical window for fistula detection,

driven by inflammatory and scarring processes. The concentration of cases within the 7-8-day period suggests that mechanical tension and peak inflammatory responses contribute to anastomotic dehiscence at this stage (4, 5).

Advanced age was significantly associated with a higher risk of fistula formation, with affected patients having a mean age of 59.71 years compared to 54.13 years in the control group. Literature findings confirm that older patients are at greater risk due to slower tissue regeneration, multiple comorbidities, and reduced tissue resilience. These findings underscore the need for personalized management strategies in elderly patients, including optimized surgical techniques, closer postoperative monitoring, and targeted preventive measures (6, 7).

A slightly higher prevalence of fistulas was observed in male patients (52.94%) compared to female patients (45.24%). However, statistical analysis did not indicate a significant difference, suggesting that sex is not a major determinant of fistula formation (7).

The study found a strong association between smoking and anastomotic fistula formation, likely due to impaired wound healing, reduced tissue vascularization, collagen disruption, and increased inflammation and infection risk (8).

An even stronger correlation was observed with alcohol consumption, with an odds ratio of 81.000, emphasizing its detrimental effect on tissue regeneration. Alcohol consumption is known to inhibit angiogenesis, impair immune function, and exacerbate local inflammatory responses, thereby increasing the risk of fistula formation. These findings highlight the importance of preoperative cessation programs for smoking and alcohol consumption in high-risk patients (9).

A significant correlation was identified between tumor size and the risk of anastomotic fistulas. Larger tumors (>3 cm) were associated with higher fistula rates, likely due to vascular compromise, difficulty in achieving a tension-free anastomosis, and increased risk of dehiscence. In contrast, smaller tumors ($\leq$ 2 cm) had a significantly lower risk, reinforcing the hypothesis that tumor burden influences postoperative anastomotic integrity (10).

While gastric mid-body tumors (60.53%) were associated with a higher proportion of fistulas, statistical analysis did not identify a significant correlation between tumor location and fistula formation. Similarly, although adenocarcinoma (53.49%) had a higher rate of anastomotic fistulas than squamous cell carcinoma (37.50%), the difference was not statistically significant, suggesting that histopathology was not a decisive factor in fistula development in this cohort (11).

A progressive increase in fistula risk was observed with advancing TNM stage, with significant associations across T, N, and M components (12).

- T3-T4 tumors were associated with higher fistula rates, reflecting extensive local invasion and compromised tissue integrity, consistent with literature indicating that advanced local tumors increase anastomotic failure risk.
- All patients with distant metastases (M1) developed anastomotic fistulas, suggesting that systemic disease burden and poor overall physiological status contribute to impaired wound healing. These findings emphasize the importance of accurate preoperative staging and multidisciplinary interventions, including nutritional support

and perioperative optimization, to mitigate complication rates in advanced-stage patients.

A strong correlation was observed between hypertension and anastomotic fistula formation, indicating that compromised microvascular circulation and increased anastomotic tension may contribute to dehiscence (13,14).

Similarly, diabetes mellitus was associated with a higher fistula risk, likely due to microvascular dysfunction, chronic inflammation, and delayed wound healing. Given that diabetic patients are more prone to infections, strict perioperative glycemic control and wound surveillance are essential in reducing complications.

Preoperative radiotherapy was significantly associated with an increased risk of anastomotic fistulas, likely due to vascular damage and impaired tissue healing. Literature findings confirm that high radiation doses can compromise anastomotic integrity, particularly in advanced tumors (15).

In contrast, preoperative chemotherapy was not significantly associated with an increased fistula risk, suggesting that its impact on anastomotic healing is less pronounced than that of radiotherapy.

Altered biological parameters at hospital admission were strongly correlated with fistula development.

- Increased serum creatinine levels indicate renal dysfunction, which may impair tissue regeneration and stress response management.
- Hyperglycemia was associated with a higher risk of fistula formation, emphasizing the need for strict perioperative glucose control.
- Hypoalbuminemia, indicative of malnutrition, was a strong predictor of postoperative complications, reinforcing the importance of preoperative nutritional optimization.
- Anemia, reflected by lower hemoglobin levels, suggested tissue hypoxia and impaired healing capacity, highlighting the need for preoperative hematologic correction.

Patients with a lower BMI were more prone to anastomotic fistulas, likely due to malnutrition, reduced muscle mass, and lower energy reserves, all of which negatively impact wound healing. These findings emphasize the importance of preoperative nutritional assessment and correction, particularly in underweight patients. Nutritional interventions, including protein supplementation and enteral/parenteral support, may help reduce the risk of anastomotic fistulas and improve postoperative outcomes.

CONCLUSIONS

This study identifies multiple risk factors associated with the development of anastomotic fistulas after esophageal and gastric cancer surgery. Advanced age, smoking, alcohol consumption, large tumor size, advanced TNM stage, hypertension, diabetes, hypoalbuminemia, anemia, and preoperative radiotherapy were all strongly correlated with an increased risk of fistula formation. Understanding these risk factors is essential for identifying high-risk patients and implementing targeted perioperative interventions to improve surgical outcomes.

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NAVIGATING THE PRESSURES: ADDRESSING OCCUPATIONAL STRESS AND BURNOUT AMONG ANESTHESIOLOGISTS AND ICU PROFESSIONALS

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NAVIGATING THE PRESSURES: ADDRESSING OCCUPATIONAL STRESS AND BURNOUT AMONG ANESTHESIOLOGISTS AND ICU PROFESSIONALS (Abstract): Occupational stress and burnout have become critical issues in healthcare, particularly among anesthesiologists and Intensive Care Unit (ICU) professionals. By addressing these issues, we aim to foster a healthier work environment that can sustain the vital contributions of anesthesiologists and ICU personnel. **Materials and methods:** A cross-sectional survey was conducted among 49 anesthesiologists and intensive care unit (ICU) physicians working in several hospitals in the City of Iași, Romania. The survey included the *Perceived Stress Scale* (PSS), the *Maslach Burnout Inventory* (MBI), and additional demographic questions regarding job seniority, age, sex, and marital or long-term partnership status. **Results:** underscores the central role of stress management in preventing emotional exhaustion. Although the regression did not identify age, sex, or marital status as statistically significant predictors, the data trends still point to meaningful subgroup differences. These results align with previous research emphasizing the psychological toll of sustained high-pressure clinical environments where women may be more prone to emotional exhaustion, and younger male professionals may face higher risks of depersonalization. **Conclusions:** This study underlines the need for targeted interventions aimed at managing perceived stress as a method of preventing professional burnout. Promoting mental health, work-life balance, and organizational support should become institutional priorities, especially in critical areas such as anesthesia and intensive care. **Keywords**: OCCUPATIONAL STRESS, BURNOUT, ANESTHESIOLOGISTS.

INTRODUCTION

Occupational stress and burnout have become critical issues in healthcare, particularly among anesthesiologists and Intensive Care Unit (ICU) professionals. These highly specialized fields demand not only extensive knowledge and technical proficiency but also the capacity to perform under intense pressure, often with life-or-death consequences. The unique challenges faced by anesthesiologists and ICU workers, such as long hours, high patient acuity, and the emotional toll of critical care, contribute significantly to their stress levels (1, 2).

In recent years, the conversation around mental health in the medical community has gained momentum, revealing alarming rates of burnout among healthcare providers (3).

For anesthesiologists and ICU staff, the combination of high-stakes environments and constant vigilance can lead to chronic stress and burnout, adversely affecting their well-being and potentially compromising patient care (4, 5). Understanding the factors that contribute to occupational stress and identifying strategies to mitigate burnout are essential for ensuring the health and effectiveness of these crucial healthcare workers.

This article delves into the root causes of occupational stress and burnout among anesthesiologists and ICU professionals. It examines the psychological and physical demands of their roles, explores the impact of workplace stressors, and discusses evidence-based interventions to support their mental health. By addressing these issues, we aim to foster a healthier work environment that can sustain the vital contributions of anesthesiologists and ICU personnel.

FACTORS CONTRIBUTING TO OCCUPATIONAL STRESS AND BURNOUT

1. High workload and long hours:

Anesthesiologists: Often work long shifts and are on call for emergencies. The nature of their job requires constant vigilance and precision (6).

ICU Physicians: Manage critically ill patients, which involves complex decision-making, frequent emergencies, and long hours (7).

2. Emotional demands:

Both specialties deal with life-and-death situations regularly, leading to emotional exhaustion (1).

Interactions with patients' families during critical times add to emotional stress (8).

3. High responsibility and risks

Anesthesiologists and ICU doctors are responsible for patients during critical phases, where errors can have severe consequences (5).

The need to stay updated with medical advancements and protocols increases mental load (9).

4. Working environment

The ICU is a high-stress environment with alarms, equipment, and constant activity (6).

Operating rooms for anesthesiologists are high-pressure settings, requiring intense concentration (7).

5. Administrative and bureaucratic pressures

Documentation, compliance with regulations, and administrative duties add to the workload (10).

Managing insurance and patient care coordination can be burdensome (11).

MATERIALS AND METHODS

Study Design

A cross-sectional survey was conducted among anesthesiologists and intensive care unit (ICU) physicians working in several hospitals in the city of Iași. The survey included the *Perceived Stress Scale* (PSS), the *Maslach Burnout Inventory* (MBI), and additional demographic questions regarding job seniority, age, sex, and marital or long-term partnership status (12-14). In Romania, anesthesiologists also manage intensive care. For simplicity, the term anesthesiologist is used throughout this article (15).

Data Collection

Data were collected through a printed questionnaire distributed over a three-week period. Participation was voluntary, and responses were anonymized to ensure confidentiality. Ethical approval for this study was obtained from the Research Ethics

Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iași (Approval No. 438/28.04.2024).

Scales and inventories

Perceived Stress Scale (PSS): A widely used psychological instrument for measuring the perception of stress. Originally developed in 1983, it remains a valid and reliable tool to assess how different situations affect personal feelings and stress perception over the past month (10).

Maslach Burnout Inventory (MBI): This tool assesses three core dimensions of burnout:

- Emotional Exhaustion (feeling emotionally drained and fatigued),
- Depersonalization (developing a detached or cynical attitude toward patients),
- Personal Accomplishment (feelings of competence and successful achievement at work) (1).

RESULTS

Data from 49 respondents who completed the Perceived Stress Scale (PSS) and the Maslach Burnout Inventory (MBI) were analyzed. The purpose of the analysis was to identify statistically significant differences between demographic subgroups and to determine the predictors of emotional exhaustion.

Descriptive Statistics

- Average age: 40.2 years (range: 28-63)
- PSS Score (perceived stress): mean = 20.1, range = 4-35
- Emotional Exhaustion (MBI-EE): mean = 24.8, range = 6-50
- Depersonalization (MBI-DP): mean = 10.9, range = 0-26
- Personal Accomplishment (MBI-PA): mean = 25.2, range = 10-41

Differences in Perceived Stress Between Sexes

The independent t-test for PSS scores between men and women showed no statistically significant difference: $t(47) = -0.88$, $p = 0.39$. Thus, no significant difference was found in perceived stress levels between sexes.

Differences in Emotional Exhaustion Across Age Groups

The ANOVA test indicated a trend toward statistical significance between age groups in emotional exhaustion scores: $F(2, 46) = 2.44$, $p = 0.098$. Although not significant at $p < 0.05$, this result suggests a possible age-related influence that warrants further exploration in larger samples.

Correlation Between Age and Depersonalization

The Pearson correlation coefficient between age and depersonalization score was $r = -0.028$, $p = 0.85$, indicating no significant relationship.

Multiple Regression: Predicting Emotional Exhaustion

The linear regression model included the following predictors: age, sex, PSS score, and marital status. Results showed that the only statistically significant predictor of emotional exhaustion was the PSS score ($p < 0.001$). Sex, age, and marital status did not have a significant influence in the model. This suggests that perceived stress is a key determinant of emotional burnout.

This finding underscores the central role of stress management in preventing

emotional exhaustion. Although the regression did not identify age, sex, or marital status as statistically significant predictors, the data trends still point to meaningful subgroup differences worth exploring in future research.

The findings of this study indicate that perceived stress is the primary factor associated with emotional exhaustion, the core dimension of burnout. Although no statistically significant differences were identified between sexes or age groups regarding PSS or MBI scores, observed trends suggest that women tend to score higher on emotional exhaustion, while younger male physicians are more at risk for depersonalization.

These results align with existing literature that highlights increased vulnerability among younger or overburdened healthcare personnel (2, 3).

The lack of influence of marital status corroborates other studies suggesting that emotional balance is not necessarily determined by this factor, but rather by effective social support and individual coping strategies (13).

DISCUSSION

The findings of this study confirm the relevance of perceived stress as a key driver of emotional exhaustion in anesthesiologists and intensive care professionals. Although no statistically significant differences were observed between sexes or age groups in the Perceived Stress Scale (PSS) or Maslach Burnout Inventory (MBI) scores, the analysis showed a strong association between perceived stress and emotional burnout, as evidenced by the regression model where PSS emerged as the only significant predictor ($p < 0.001$).

These results align with previous research emphasizing the psychological toll of sustained high-pressure clinical environments (2)(3). While the t-test and ANOVA did not reveal statistically significant group differences, the observed trends suggest that women may be more prone to emotional exhaustion, and younger male professionals may face higher risks of depersonalization. These trends are consistent with studies reporting increased burnout vulnerability among early-career physicians and female healthcare workers balancing professional and social expectations (4)(5).

The absence of a statistically significant correlation between age and depersonalization ($r = -0.028$, $p = 0.85$) indicates that emotional distancing may not necessarily be a function of age, but perhaps of work-related coping strategies or organizational culture. Similarly, marital or partnership status showed no predictive power in the regression analysis, suggesting that emotional resilience may be more influenced by individual or systemic factors rather than personal relationships (13).

Taken together, these findings support the need for targeted interventions focused not only on reducing work-related stress but also on promoting psychological resources and institutional safeguards. Burnout prevention should become a core component of workforce well-being programs, especially in specialties where clinical intensity and emotional demand are persistently high.

CONCLUSIONS

The analysis highlights that although there are no statistically significant differences between sexes or age groups regarding PSS and MBI scores, perceived stress remains a major predictor of emotional exhaustion.

This study underlines the need for targeted interventions aimed at managing perceived stress as a method of preventing professional burnout. Promoting mental health, work-life balance, and organizational support should become institutional priorities, especially in critical areas such as anesthesia and intensive care. Future studies should include larger samples and longitudinal analyses to track the progression of burnout over time.

CONFLICT OF INTEREST AND FUNDING

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STUDY ON THE IMPACT OF DIODE LASER ON POSTOPERATIVE EVOLUTION IN ORAL SURGERY

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STUDY ON THE IMPACT OF DIODE LASER ON POSTOPERATIVE EVOLUTION IN ORAL SURGERY (Abstract): **Aim of study** was to compare the clinical postoperative evolution in the treatment of gingival hyperplasia by using diode laser and scalpel surgery. **Materials and methods**: A total of 40 patients with gingival hyperplasia, selected from the Clinical Learning Base of the Faculty of Dental Medicine, Grigore T. Popa University of Medicine and Pharmacy Iasi, were included in study. Participants were divided in test group (laser-assisted surgery, n = 20) and control group (scalpel surgery, n = 20). The laser surgery was performed with Epic (BioLase) (940 nm). Postoperative evolution was assessed at 24 hours, 3 days, and 7 days. The postoperative evolution was assessed by the progression of the postoperative pain intensity (Visual Analogue Scale) and healing time (days). **Results:** For gingivectomy performed with diode laser, mean VAS scores decreased from 2.60 ± 0.38 days at 24 h to 0.21 ± 0.22 at 7 days ($p < 0.001$) versus 5.20 ± 0.77 days (at 24 h) to 1.20 ± 0.64 (7 days) in the scalpel group ($p < 0.001$). Mean healing times were significantly shorter after laser therapy (3.20 ± 0.77 days) compared to scalpel surgery (8.40 ± 2.09). **Conclusions:** Laser-assisted surgery lead to faster healing time and accelerates the decrease of postoperative pain intensity during first 7 days posttreatment, compared to traditional surgical approach. **Keywords:** LASER, DIODE, GINGIVECTOMY, VAS, HEALING.

INTRODUCTION

The laser has become increasingly integrated into fields such as science, communications, astronomy, engineering, and medicine. A laser can be defined as a device that produces light energy through an optical amplification process, according to the stimulated emission of electromagnetic radiation (1). The benefits of lasers in oral surgery include the absence of harmful heat to oral tissues, bacteriostatic and bactericidal action, reduced bleeding time and elimination of the suturing of the postoperative wound (2, 3). The active component of this common dental laser is a solid-state semiconductor made from aluminum and gallium, emitting an optical wavelength generally ranging from 810 to 980 nm (4, 5). Diode laser combine ablative action with biostimulant effect (6-8). Localized biostimulator with diode lasers increases the release of endorphins and reduces the activity of inflammatory mediators up to a depth of 1 cm, while also facilitating cellular replication and thus promoting bone growth (9, 10). Localized biostimulation with diode lasers increases the release of endorphins and reduces the activity of inflammatory mediators

up to a depth of 1 cm, while also facilitating cellular replication and thus promoting bone growth (9). Diode lasers reduce the use of anesthesia required for the surgical procedures leading to greater patient compliance with less discomfort, improved overall acceptance of the procedure (10). Low-Level Laser Therapy (LLLT), also known as "soft laser therapy," has been used in medicine for over three decades. Low-level lasers emit short-wavelength red or infrared light which can penetrate to a depth of 3 mm to 15 mm in both soft and hard tissues (11, 12). LLLT can influence inflammatory processes and reduce acute inflammatory pain by decreasing, in a dose-dependent manner, levels of prostaglandin E2, IL1-beta, TNF-alpha, oxidative stress, edema, and by reducing the frequency of nociceptor activation in peripheral nerve signals (13). LLLT facilitates wound healing by accelerating fibroblast proliferation, ATP synthesis, and collagen synthesis (14-17). Laser and LED light induce a PBM effect used to accelerate healing by increasing cell viability through the stimulation of ATP synthesis in mitochondria and cell membrane photoreceptors (18-20).

The aim of this study was to compare the evolution of the clinical postoperative parameters between patients treated by diode laser-assisted and patients treated by conventional (scalpel) surgery in the treatment of gingival hyperplasia.

MATERIALS AND METHODS

In this prospective study, 40 patients (28 males; 22 females; mean age 42.5 ± 12; range 20-65 years) were selected from Clinical Base "Kogalniceanu M" of Dental Medicine Faculty, Grigore T. Popa University of Medicine and Pharmacy Iasi. The research was performed according to Code of Ethics of the World Medical Association regarding the studies performed on human subjects. Before entering in study, the patients signed a written consent. The inclusion and exclusion criteria for patients included in study are presented further.

Inclusion criteria: anterior region; age between 18-50 years; plaque-induced inflammatory gingival hyperplasia; orthodontic treatments with gingival hyperplasia; systemically healthy patients.

Exclusion criteria: gingival enlargement due to systemic predisposing factors; pregnancy and/or lactation; systemic diseases requiring antibiotic prophylaxis and/or anti-inflammatory medications; acute or untreated periodontal disease; diabetes.

The diode laser surgical procedures were used in test group (n=20). The conventional surgical procedures (scalpel) were used in control group (n=20). The parameters of diode laser EPIC (BioLase, USA) (940 nm) were as follows: 1W, pulse mode, contact, 300 µm fiber; LLLT: 0,2W power, 35J/cm^2). The results were compared with control group (n=20).

The postoperative evolution was assessed by using VAS (Visual Analogue Scale) indices and healing time (days). These parameters were recorded at 24 hours, 3 days and 7 days. VAS indices were measured by a scale from 0 to 100, where 0 indicates absent pain and 100 indicates the most severe pain. Each patient was asked to select the score that represented the pain experienced at the time of evaluation (24 hours, 3 days, and 7 days postoperatively). The chosen value was recorded as the patient's VAS score. The pain intensity was set according to VAS scores: 0-1 (no pain); 2-3 (mild pain); 4-6 (moderate pain). Healing time was considered when patients perceives the treated

gingival area as "healed," with minimal discomfort

Statistical analysis. Descriptive and analytical statistics were applied. The mean, standard deviation (SD), median was used to describe numerical data. The frequency and proportion of nominal data were presented; the comparison between categorial variables were made using the Chi-squared test. The variables to analyze were checked against the normal distribution using the Kolmogorov-Smirnov Test. Mann-Whitney test was used to compare them between the two groups, and Friedman test was used to compare outcome data within each group. Statistical significance was defined as a p-value less than or equal to 0.05. *SPSS version 29.0* was used to conduct all statistical analyses (Chicago, IL, USA Inc.).

RESULTS

At 24 hours, the laser group showed much lower pain scores (mean 2.60 ± 0.38) compared with the scalpel group (mean 5.20 ± 0.77), with a highly significant difference ($p < 0.001$). After 3 days, the laser group again reported reduced pain (mean 1.20 ± 0.39) relative to the scalpel group (mean 4.40 ± 0.77) ($p < 0.001$). At 7 days, pain in the laser group was minimal (mean 0.21 ± 0.22), while the scalpel group still showed notable discomfort (mean 1.20 ± 0.64), with the significant statistical difference ($p < 0.001$). For both techniques, pain decreased significantly over time ($p < 0.001$) (tab. I).

TABLE I.

Postoperative pain intensity evolution (laser *vs*, scalpel)

VAS	Laser		Scalpel		Mann-Whitney Test p-value
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
24 h	2.60 ± 0.385	2.57 (2.34 ÷ 2.89)	5.20 ± 0.767	5.15 (4.68 ÷ 5.76)	< 0.001**
3 days	1.20 ± 0.387	1.21 (0.85 ÷ 1.39)	4.40 ± 0.774	4.41 (3.71 ÷ 4.78)	< 0.001**
7 days	0.21 ± 0.224	0.16 (0.00 ÷0.44)	1.20 ± 0.636	1.24 (0.71÷ 1.78)	< 0.001**
Friedman test	p < 0.001**		p < 0.001**		

At 24 hours, most patients in the laser group reported only mild pain (90.0%), with 10.0% having no pain at all. In contrast, the scalpel group showed predominantly moderate pain (90.0%), and only 10.0% reported mild pain. The difference between groups was highly significant ($p < 0.001$). After 3 days, 95.0% of patients in the laser group reported no pain, while the scalpel group continued to show higher discomfort levels, with 70.0% experiencing moderate pain and 30.0% mild pain ($p < 0.001$). At 7 days, all patients in the laser group were pain-free (100.0%), compared with 90.0% in the scalpel group; 10.0% of scalpel cases still reported mild pain. At 7 days, the difference between groups was not significant ($p = 0.487$) (tab. II).

The laser group healed in an average of 3.20 ± 0.77 days (median 3.00), while the scalpel group required 8.40 ± 2.09 days on average (median 9.00). The difference between the two surgical techniques was highly significant ($p < 0.001$) (tab. III).

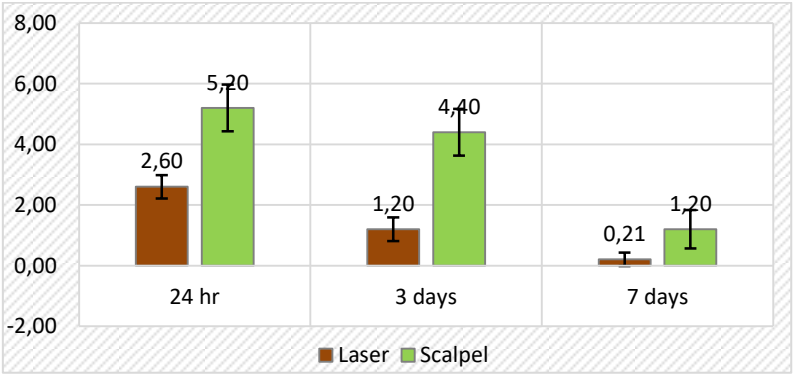

Fig. 1. Mean VAS indices at 24hr, 3 days, 7 days (laser vs scalpel)

TABLE II.

The qualitative study of postoperative pain intensity

VAS		Group				Total		Pearson Chi-squared p-value
		Laser		Scalpel				
		N	%	N	%	N	%	
24 hrs.	no pain	2	10.0%			2	05.0%	< 0.001**
	mild pain	18	90.0%	2	10.0%	20	50.0%	
	moderate pain			18	90.0%	18	45.0%	
3 days	no pain	19	95.0%			19	47.5%	< 0.001**
	mild pain	1	05.0%	6	30.0%	7	17.5%	
	moderate pain			14	70.0%	14	35.0%	
7 days	no pain	20	100.0%	18	90.0%	38	95.0%	0.487
	mild pain			2	10.0%	2	05.0%	
TOTAL		20	100.0%	20	100.0%	40	100.0%	

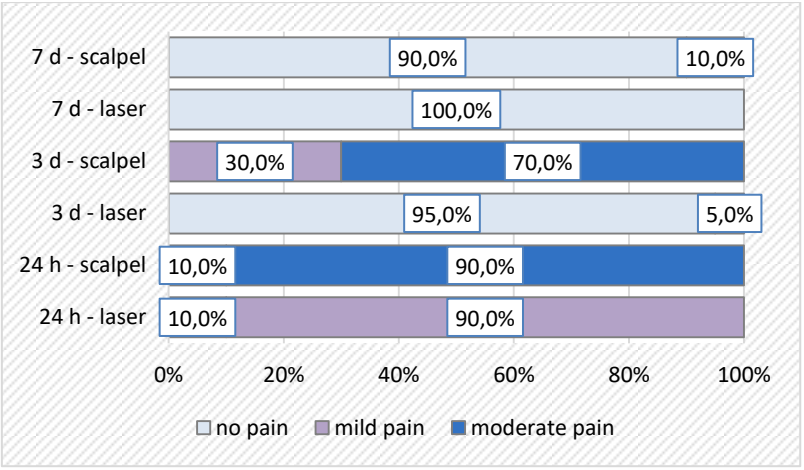

Fig. 2. Mean VAS indices at 24hr, 3 days, 7 days (laser *vs.* scalpel)

TABLE III.
Healing time within the tested samples

	Laser		Scalpel		Mann-Whitney Test p-value
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)	
Healing days	3.20 ± 0.768	3.00 (3.00 ÷ 3.00)	8.40 ± 2.088	9.00 (6.00 ÷ 9.75)	< 0.001**

DISCUSSION

Pre-prosthetic surgical procedures are required to optimize the prosthetic field in complex oral rehabilitation. Surgical interventions using scalpel are considered gold standard technique due to its ease of use and economic benefits. However, surgical lasers have the advantage of precision, patient comfort, and faster healing (21, 22). Gingivectomies are among the surgical procedures that can benefit significantly from the use of diode laser (23).

Our study demonstrates the clinical advantages of laser-assisted soft tissue surgery such as gingivectomy and frenectomy when compared with scalpel techniques. Our results in diode laser-assisted gingivectomy align with current literature (23, 24). In gingivectomy, diode laser use resulted in minimal discomfort from the first postoperative day (VAS 2.6), with a rapid decline by day 3 (VAS 1.2) and near-complete resolution by day 7 (VAS 0.6). Patients submitted to scalpel surgery had significantly higher VAS indices at each corresponding interval. Diode laser gingivectomy required a mean of only 3.2 days for satisfactory clinical healing, while traditional scalpel procedures required significantly more time (8.4 days). These differences may be explained by the reduced tissue trauma, improved coagulation, and decreased postoperative inflammation associated with laser interaction (25). In laser group, clinical functional healing represents a rapid and almost complete epithelial recovery, while deeper tissue healing is histologically incomplete but clinically favorable. The absence of mechanical damage and minimal thermal spread in diode laser assisted surgery favorize the recontouring of the soft tissues with the precision of a scalpel.

Complete gingival remodeling occurs in the next 2-3 weeks, while the final stabilization of the gingival contour is achieved within 4-6 weeks (1, 24).

In our study the ablation stage was followed by low-level laser therapy. LLLT favors the additional decrease of postoperative pain intensity and healing time according to research groups (26-28). A systematic review evaluated the effect of LLLT as an adjunct to periodontal surgery in the management of postoperative pain and healing time. It was reported a significant decrease in the mean analgesic intake during the first week in the LLLT group ($\leq$ 500 mW, $\geq$ 5 J/cm^2) when compared with control (29).

These results add to the increasing body of evidence that diode laser gingivectomy not only provides clinical benefits in terms of postoperative comfort and healing times, but also in terms of patient satisfaction. The shorter healing times and lower pain scores in laser groups have implications for patients' quality of life, leading to earlier return to normal activities, reduced intake of analgesics and fewer postoperative complications. Shorter recovery and less pain can be clinically significant in terms of patient compliance, particularly when the dental treatment plans are of

multiple stages such as periodontal or prosthetic treatments.

The limited management of postoperative cases is correlated to less need for periodontal dressing and reduced hemorrhage.

In addition, finite soft tissue ablation with low collateral damage could contribute to the earlier healing profile as observed in our study.

However, histological and immunohistochemical analysis showed no significant differences regarding inflammatory cell infiltrate in laser-treated tissues compared with scalpel surgical procedures (30, 31).

Despite specific training and higher initial investment required for diode lasers, the clinical benefits encourage their use in oral surgery (32).

CONCLUSIONS

Oral surgery performed with diode laser (940 nm) resulted in significantly lower postoperative pain compared with the scalpel technique. The diode laser offered reduced discomfort at 24 hours and 3 days, along with a markedly shorter healing period than the conventional surgical approach.

CONFLICTS OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding.

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LONGITUDINAL ANALYSIS OF MATERNAL AND CHILD HEALTH INDICATORS IN BACĂU COUNTY, ROMANIA (2021-2024)

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LONGITUDINAL ANALYSIS OF MATERNAL AND CHILD HEALTH INDICATORS IN BACĂU COUNTY, ROMANIA (2021-2024) (Abstract): This longitudinal observational study assessed the evolution of maternal and child health indicators in Bacau County, Romania, between 2021 and 2024, using annual administrative data collected from hospitals and medical practices involved in maternal and child health services. **Materials and methods**: The analysis focused on service coverage, year-to-year variations, and discontinuities in key program-related interventions. Descriptive statistical analyses were performed using SPSS version 28 and Microsoft Excel. **Results**: During the study period, improvements were observed in several components, particularly neonatal screening activities and nutritional support for low-birth-weight newborns. Coverage of Rh immunological prophylaxis among Rh-negative pregnant women increased alongside better orientation of services toward vulnerable population groups. Nevertheless, persistent challenges were identified in postnatal follow-up of preterm infants and in ensuring equitable access to infant formula. Additionally, a declining birth rate was noted, with potential implications for resource allocation and service planning. Overall, the findings indicate heterogeneous longitudinal trends in maternal and child health indicators, reflecting variable continuity and performance across service components. **Conclusions:** These results underline the need for targeted adjustments to enhance the consistency and quality of maternal and child health interventions at the local level. **Keywords**: MATERNAL HEALTH, CHILD HEALTH, LONGITUDINAL ANALYSIS, HEALTH INDICATORS, ROMANIA.

INTRODUCTION

The promotion and protection of women's and children's health represent a core public health priority at both national and international levels. These population groups require continuous, targeted interventions to ensure equitable access to healthcare services, prevent disease, and support healthy growth and development. Maternal and child health programmes contribute directly to reducing maternal and infant mortality, preventing chronic conditions, promoting family planning, and improving overall population health outcomes (1-4).

The COVID-19 pandemic exerted additional pressure on healthcare systems in Romania, revealing pre-existing structural vulnerabilities and emphasizing the importance of realistic assessments of public health programme implementation. In this context, maternal and child health interven-

tions have remained essential for addressing health inequalities and maintaining continuity of preventive and curative services. Such interventions typically follow a hierarchical approach, prioritizing primary prevention and health education, followed by early diagnosis and timely treatment, in line with international maternal and child health frameworks (5-8).

Evidence from the literature indicates that the implementation of maternal and child health programmes is frequently uneven, particularly in rural areas and among vulnerable population groups, where access to healthcare services remains limited (9). In Romania, inconsistencies in neonatal screening coverage and in the application of nutritional and immunological protocols have been reported, resulting in notable gaps when compared with European benchmarks (10-12). Urban-rural disparities, shortages of healthcare professionals, and infrastructural constraints further affect the effectiveness and equity of service delivery (13). These challenges underline the need for coordinated action among family physicians, hospital-based services, public health authorities, and community-level actors to ensure continuity and coherence of care.

At the strategic level, maternal and child health priorities in Romania are aligned with World Health Organization (WHO) recommendations and are embedded in national policy frameworks, including the National Health Strategy, the National Strategy on Equal Opportunities and Treatment between Women and Men (2022-2027), the National Cancer Control Plan, and the National Mental Health Strategy (14,15). Core objectives include reducing maternal and infant mortality, promoting reproductive health and family planning, monitoring child development, and addressing inequalities in access to health services (16-18). Within this framework, longitudinal analyses at the local level are essential for understanding temporal trends and identifying areas requiring improvement.

This study was motivated by the need to assess the performance and continuity of maternal and child health interventions in settings characterized by increased population vulnerability. In the context of disruptions generated by the COVID-19 pandemic and the subsequent recovery of the Romanian healthcare system, evaluating the local implementation of key maternal and child health components provides relevant insight into system resilience and service delivery at the county level.

The aim of this research was to conduct a longitudinal analysis of maternal and child health indicators in Bacău County over the period 2021-2024, using administrative data associated with program-related services. The specific objectives were to evaluate temporal changes in service coverage, to identify discontinuities or functional challenges across major intervention components, and to analyze trends in the number of beneficiaries by subcomponent in order to describe population coverage patterns during the study period.

MATERIALS AND METHODS

A longitudinal observational study was conducted to assess maternal and child health indicators associated with the implementation of program-related services in Bacău County, Romania. The analysis covered the period 2021-2024 and included data from three public hospitals-Bacău

County Emergency Hospital, Moinești Municipal Emergency Hospital, and St. Hierarch Dr. Luca Onești Municipal Hospital-as well as from 11 family medicine practices involved in maternal and child health service delivery at the county level.

All medical units participating in the implementation of maternal and child health interventions during the study period were considered eligible for inclusion. No exclusion criteria were applied, and all validated data available from the participating institutions were included in the analysis.

Data was collected through document review of primary and secondary sources, including official reports and datasets provided by the participating hospitals, maternal and child health statistics obtained from the healthcare units included in the study, legislative acts, national strategic documents, and databases of the National Institute of Public Health (INSP). The main variables of interest included the number of beneficiaries by intervention component, annual variations, coverage rates, and indicators of discontinuity in service provision. Calculated measures comprised absolute numbers of beneficiaries, base indices, and dynamic chain indices expressed as percentages.

Statistical analysis was descriptive reflecting the observational design of the study. Descriptive statistics, including absolute values, means, and frequencies, were generated using IBM *SPSS Statistics version 28* and *Microsoft Excel*. Comparative analyses were performed to describe temporal dynamics over the 2021-2024 period, while qualitative information from official documents was interpreted using thematic analysis. This approach allowed the identification of major longitudinal trends, urban-rural differences, and contextual factors influencing service continuity and performance.

The study was conducted in accordance with applicable legislation and ethical standards, following the Standards of Research Ethics in Medical Sciences (19) and the principles of the Declaration of Helsinki (20). Ethical approval was obtained from the Ethics Committees of Bacău County Emergency Hospital (Decision No. 30695/28.12.2022), Moinești Municipal Emergency Hospital (07.12.2022), and St. Hierarch Dr. Luca Onești Municipal Hospital (Decision No. 21613/23.12.2022), as well as from the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy Iași (Decision No. 255/17.01.2023). The study forms part of a doctoral research project conducted at the Doctoral School of the Grigore T. Popa University of Medicine and Pharmacy Iași (Doctoral contract No. 1916 / 29.10.2021),

RESULTS

The application of maternal and child health program-related services in Bacău County generated relevant findings regarding population coverage and the longitudinal evolution of key maternal and child health indicators. The analysis highlighted heterogeneous temporal trends across intervention components, reflecting both improvements in service delivery and the persistence of discontinuities during the study period. These results provide a descriptive overview of program performance at the county level between 2021 and 2024.

Number of newborns

The number of newborns recorded in hospitals across Bacău County showed a continuous decline over the 2021-2024

period. Compared with the baseline year 2021, the total number of live births decreased by approximately 30% by 2024. As shown in (*tab. I*), the number of newborns declined from 5,961 in 2021 to 5,819 in 2022 (97.6% of the baseline), followed by a marked reduction to 4,466 in 2023 (74.9%) and a further decrease to 4,148 in 2024 (69.6%). The most pronounced decrease occurred between 2022 and 2023, while the reduction observed between 2023 and 2024 was more moderate.

Preterm births

The evolution of preterm births recorded in hospitals across Bacău County showed a fluctuating pattern during the 2021-2024 period (fig. 1). Between 2021 and 2023, the number of preterm births decreased by approximately 30%, followed by an increase of about 13% in 2024 compared with the previous year. Overall, the data indicates a non-linear temporal trend, characterized by a decline during the first part of the study period and a subsequent increase in the final year.

TABLE I.

Number of newborns in hospitals in Bacau county (2021-2024)

(Source: Hospital administrative data, National Health Program PN XIII, 2021-2024)

Year	Number of newborns	Dynamic base indices (%)
2021	5.961	100.0
2022	5.819	97.6
2023	4.466	74.9
2024	4.148	69.6

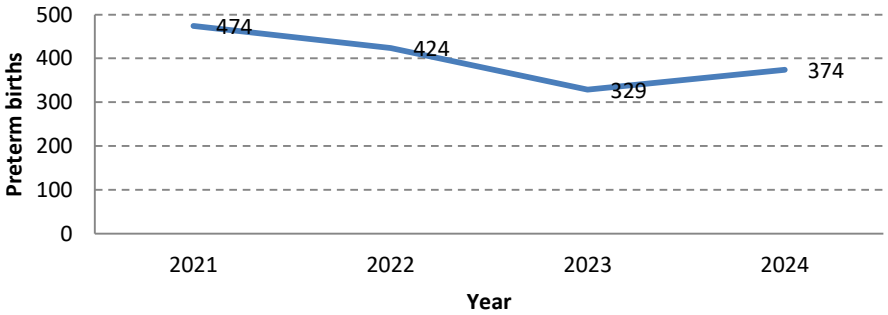

Fig. 1. Preterm births in Bacău County, 2021-2024.

Prevention of hearing loss through newborn screening

Coverage of newborn hearing screening increased substantially between 2021 and 2024, rising from 45.03% to 74.61%, which represents a relative increase of 65.7% compared with the baseline year (fig. 2). The upward trend was consistent throughout the study period and was most pronounced in 2024.

The absolute number of newborns screened for hearing loss varied year to year. In 2022, an increase of 10.39% compared with 2021 was observed, followed by a decrease of 15.22% in 2023. In 2024, the number of screened newborns increased by 39.65% compared with 2023, representing the highest value recorded during the study period.

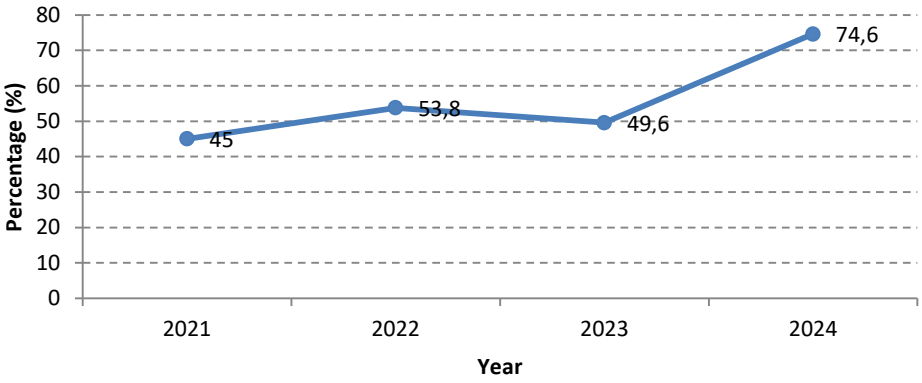

Fig. 2. Hearing loss screening coverage in Bacău County, 2021-2024.

Prevention of retinopathy of prematurity

Between 2021 and 2024, the coverage rate of screening for retinopathy of prematurity increased from 86.05% to 100% and remained at this level during the final years of the study period. Over the same interval, the number of preterm newborns eligible for screening increased from 86 cases in 2021 to 127 cases in 2024, with a corresponding increase in the number of beneficiaries, resulting in complete screening coverage during 2022-2024.

In contrast, the proportion of preterm newborns monitored after initial screening decreased progressively over the study period, declining from 93.02% in 2021 to 70.08% in 2024, indicating variability in continuity of post-screening follow-up.

Malnutrition prophylaxis in infants aged 0-12 months without access to breast milk

During the 2021-2024 period, the proportion of infants aged 0-12 months benefiting from malnutrition prophylaxis through infant formula decreased from 4.38% to 2.60%. In absolute terms, the number of beneficiaries declined from 232 cases in 2021 to 127 cases in 2024 (fig. 3). This reduction occurred gradually over the study period, in parallel with the decrease in the number of live births.

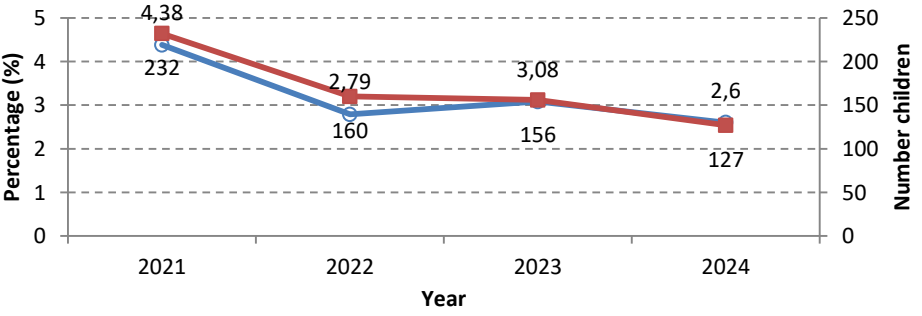

Fig. 3. Number and proportion of infants aged 0-12 months receiving malnutrition prophylaxis with infant formula in Bacău County, 2021-2024.

Malnutrition prophylaxis in low-birth-weight infants

Among low-birth-weight infants, the proportion of beneficiaries receiving nutritional prophylaxis increased from 83.92% in 2021 to 95.89% in 2024, in the context of a relatively stable number of eligible cases. This finding reflects a progressive increase in coverage over the study period.

Prophylaxis of Rh isoimmunization syndrome

Coverage with anti-D immunoglobulin among Rh-negative pregnant women showed moderate variation between 2021 and 2024. Coverage increased to 75.40% in 2023, followed by a decrease to 70.45% in 2024. The absolute number of beneficiaries followed a similar pattern, reaching a peak of 233 cases in 2023 and decreasing to 217 cases in 2024.

DISCUSSION

The findings of this longitudinal analysis indicate a heterogeneous pattern of performance across maternal and child health services at the county level. While several core interventions showed measurable improvement during the study period, persistent discontinuities and inequalities pointed to an ongoing gap between regulatory frameworks and their consistent operationalization. This pattern is consistent with the concept of the "implementation gap" described in public health literature, referring to the discrepancy between formally established policies and their effective translation into routine practice (15).

A clear illustration of this gap is provided by the prophylaxis of Rh isoimmunization through the administration of anti-D immunoglobulin. Although this intervention is well regulated and standardized at the national level, the observed variability in coverage suggests vulnerabilities in supply chains, reporting mechanisms, and service coordination. Similar challenges have been reported in other European health systems, where regional differences in access to anti-D immunoglobulin persist despite uniform clinical guidelines (16). International evidence further highlights that incomplete documentation and limited traceability of administration episodes continue to constrain effective monitoring of this intervention (17). From a clinical perspective, inadequate prophylaxis exposes Rh-negative pregnant women and their newborns to the risk of hemolytic disease, a preventable condition associated with increased neonatal morbidity (18).

The upward trends observed in newborn hearing screening and retinopathy of prematurity (ROP) screening coverage reflect progressive alignment of local services with European standards and improvements in technical capacity. These findings support the effectiveness of neonatal screening as a public health intervention for early detection of conditions with potentially irreversible consequences. However, the decline identified in post-screening follow-up among preterm infants highlights a critical vulnerability in the continuum of care. The literature consistently emphasizes that screening effectiveness depends not only on early identification but also on functional referral and follow-up pathways that ensure diagnostic confirmation and timely intervention, in accordance with ethical standards for research and clinical practice (19, 20). In the absence of such mechanisms, the overall public health impact of screening programs remains limited. The observed patterns suggest that organizational factors, including fragmented coor-

dination between maternity services, neonatology units, specialized outpatient care, and primary care, play a central role in loss to follow-up, a phenomenon also reported in other European settings (23).

Nutritional interventions display differentiated trends. The increasing coverage of nutritional prophylaxis among low-birthweight infants indicates effective identification and enrollment of a high-risk population group, demonstrating that targeted interventions can achieve high coverage when supported by clear eligibility criteria and coordinated delivery. In contrast, the declining proportion of infants receiving formula milk in the absence of breastfeeding presents a more complex interpretation. While this trend may be consistent with successful promotion of breastfeeding practices in line with international recommendations, it may also reflect persistent access barriers for families unable to breastfeed, particularly in socioeconomically disadvantaged or rural contexts. International evidence underscores the importance of maintaining regulated and equitable access to safe breast milk substitutes to avoid reinforcing nutritional inequalities (15). These findings highlight the need to interpret infant nutrition indicators within a broader equity-oriented framework.

The variability observed across service components and population groups reflects broader territorial inequalities in healthcare delivery. International studies consistently report lower coverage and reduced continuity of care in rural or underserved areas, even in the presence of centralized national policies (21,22). The results from Bacău County suggest that insufficient coordination structures and limited integration between institutional levels may fragment care pathways, reducing overall program effectiveness. Evidence from Southeastern Europe supports the relevance of adaptive governance approaches that emphasize coordination across national, county, and community levels in addressing local barriers to implementation and follow-up (23).

The sustained decline in the number of live births observed during the study period must be interpreted within a broader demographic context. Eurostat data document a long-term decrease in fertility rates and a rise in maternal age across Romania and the European Union (24). These demographic shifts create a dual challenge for maternal and child health services, combining reduced case volumes with increased clinical complexity associated with higher-risk pregnancies. The European Health Report emphasizes the necessity for health systems to integrate demographic realities into service planning and risk management frameworks (25).

This study has several limitations. It relies on administrative data, which may be affected by reporting inconsistencies or incomplete documentation, and it does not capture qualitative dimensions such as provider perspectives or organizational culture. The analysis reflects the specific context of Bacău County and may not be fully generalizable to other regions. In addition, the four-year observation period limits long-term inference, and external legislative, financial, or epidemiological factors may have influenced the observed trends.

CONCLUSIONS

The longitudinal evaluation of maternal and child health services in Bacău County over the 2021-2024 period indicates a heterogeneous pattern of performance across program components. Notable progress was observed in neonatal screening activities,

particularly in hearing screening and retinopathy of prematurity prevention, reflecting improved coverage and consolidation of early detection mechanisms with potential long-term clinical benefit.

Nutritional interventions demonstrated differentiated trends. Coverage among low-birth-weight infants increased steadily, indicating effective identification and inclusion of a high-risk group, whereas the declining proportion of infants receiving formula in the absence of breastfeeding highlights the importance of continuous monitoring to ensure that nutritional vulnerabilities are not overlooked.

Immunoprophylaxis against Rh isoimmunization showed variable coverage over time, suggesting that, despite functional implementation, full and stable protection of the target population has not yet been achieved. In addition, the decreasing continuity of post-screening follow-up among preterm infants represents a critical limitation, emphasizing that improvements in screening coverage must be accompanied by sustained monitoring to achieve their intended health impact.

The persistent decline in the number of live births observed during the study period further underscores the need for adaptive service planning in the context of demographic change, characterized by reduced case volumes and increased clinical complexity. Overall, the findings highlight both the consolidation of key preventive interventions and the persistence of organizational and continuity-related challenges, shaping a nuanced picture of maternal and child health service performance at the county level.

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CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest.

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ASSOCIATION BETW1EEN LIFESTYLE FACTORS AND OSTEOPOROSIS: A CROSS-SECTIONAL STUDY IN A ROMANIAN POPULATION

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ASSOCIATION BETWEEN LIFESTYLE FACTORS AND OSTEOPOROSIS: A CROSS-SECTIONAL STUDY IN A ROMANIAN POPULATION (Abstract): Osteoporosis is a major public health problem characterized by reduced bone mass and increased fracture risk. Lifestyle factors play an important role in its development and prevention. This study examines the relationship between lifestyle factors and osteoporosis in an adult Romanian population, providing recommendations for prevention through adequate calcium and vitamin D intake and the promotion of a healthy lifestyle. **Aim:** To evaluate the association between lifestyle habits and the presence of osteoporosis. **Materials and methods:** A cross-sectional study was conducted on 160 participants using an online questionnaire. Variables analyzed included age, sex, BMI, smoking status, physical activity, and supplement use. Statistical analysis was performed using chi-square tests, point-biserial correlations, and multivariate logistic regression ($p<0.05$). **Results:** The prevalence of osteoporosis was 13.12%. Age was the only factor significantly associated with osteoporosis ($r=0.453$; $p < 0.001$) and remained an independent predictor in logistic regression (OR=1.15; 95% CI: 1.08-1.21; $p<0.001$). No significant associations were found for sex ($p=0.19$), BMI, smoking, or physical activity ($p>0.05$). Supplement use was statistically significant ($p<0.001$), but likely influenced by reverse causality. **Conclusions:** The study highlight the importance of screening and early prevention, contributing to a better understanding of osteoporosis epidemiology in Romania. Lifestyle habits significantly influence osteoporosis risk. Public health interventions focusing on prevention, education, and modifiable risk factors are essential for reducing the burden of osteoporosis. **Keywords:** BONE DENSITY, OSTEOPOROSIS, BONE HEALTH, MENOPAUSE.

INTRODUCTION

Bone health depends on patients' diet and lifestyle. Dietary intake of calcium, vitamin D, and proteins are essential factors for maintaining bone strength throughout life, as well as for reducing bone density loss, a phenomenon specific to aging.

The risk of osteoporotic fractures results from a combination of factors that act mainly by reducing bone mineral density, but also from factors that are completely or partially independent of it. Numerous factors are associated with osteoporotic fractures, such as: reduced peak bone mass, hormonal factors, use of certain medications (e.g., glucocorticoids), smoking, low levels of physical activity, low dietary intake of calcium and vitamin D, race, small body constitution, as well as a personal or family history of osteoporosis or other associated conditions (1).

Age is a major determining factor of os-

teoporosis, with a marked increase in risk after the age of 61. Other factors have also been identified, such as higher BMI, which appears to have a protective effect due to mechanical loading and higher estrogen levels from adipose tissue; a vegetarian diet, which is associated with an increased risk of osteoporosis; and living environment-urban residents showed a slightly lower risk compared to those in rural areas, although the difference was not statistically significant. Men have a lower risk compared to women, which can be explained by hormonal factors and body composition (2).

Osteoporosis is a major public health problem, associated with bone fragility and an increased risk of fractures, and maintaining an adequate intake of calcium and vitamin D represents the foundation of prevention and treatment interventions (3).

MATERIALS AND METHODS

The study aimed to evaluate the association between lifestyle habits and bone health status. A questionnaire was developed, including questions related to the assessment of bone health, the presence of bone disorders, patients' lifestyle habits, and family history. The questionnaire was approved by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy in Iași, Romania and was administered online, with respondents' confidentiality ensured.

This cross-sectional study aimed to evaluate the association between lifestyle factors and osteoporosis in a Romanian adult population. Data were collected using a structured questionnaire that included items on demographic characteristics, bone health status, lifestyle habits (diet, physical activity, smoking, sun exposure), supplement use, and family history of osteoporosis.

Statistical analysis was performed using SPSS version 30 and JASP software. Descriptive statistics were calculated for all variables. Associations between categorical variables were assessed using the chi-square test, while relationships between continuous and dichotomous variables were evaluated using point-biserial correlation coefficients.

To identify independent predictors of osteoporosis, a multivariate logistic regression analysis was conducted. Osteoporosis diagnosis was included as the dependent variable, while age, sex, BMI (Body Mass Index), smoking status, physical activity, and dietary supplement use were entered as independent variables. Odds ratios (OR) with 95% confidence intervals (CI) were calculated. A p-value < 0.05 was considered statistically significant.

RESULTS

A total of 160 participants were included in the analysis. Osteoporosis diagnosis was self-reported and recorded as a dichotomous variable (yes/no). Independent variables included age (continuous), sex (male/female), body mass index (BMI, continuous), smoking status (yes/no), physical activity (categorical), and dietary supplement use (yes/no).

Data regarding patients' age (21-84 years), gender, body mass index (BMI) (17.6-38.2 kg/m^2), and living environment are presented in table I.

Within the group of respondents, retirees predominate (43.10%), followed by teachers (20.0%). 160 participants completed the questionnaire, of whom 21 (13.12%) reported a diagnosis of osteoporosis, while 139 (86.88%) reported no such diagnosis.

TABLE I.
Characteristics of the respondent group

	Number	Mean ± SD
Age	160	51.72 ± 14.82
BMI	160	26.98 ± 4.43
Gender: Female Male	135 25	84.4% 15.6%
Living environment: Urban Rural	128 32	80% 20%

Descriptive and bivariate analyses showed that most variables, including sex, BMI, lifestyle factors, and place of residence, were not significantly associated with osteoporosis. Age was the only variable demonstrating a statistically significant positive association with osteoporosis, as indicated by point-biserial correlation analysis (rp.bis = .453, $p < 0.001$).

To further evaluate independent associations, a multivariate logistic regression analysis was performed. The results showed that age was significantly associated with osteoporosis (OR = 1.15, 95% CI: 1.08–1.21, $p < 0.001$), indicating that the risk of osteoporosis increases with advancing age. No statistically significant associations were found for sex ($p = 0.19$), BMI ($p > 0.05$), smoking status ($p = 0.37$), or physical activity ($p > 0.05$).

Dietary supplement use was significantly associated with osteoporosis (OR ≈ 0.00, 95% CI: very wide, $p < 0.001$). However, this association is likely explained by reverse causation, as individuals diagnosed with osteoporosis are more likely to use supplements following medical advice.

Out of the 160 respondents, 21 (13.12%) reported having been diagnosed with osteo-porosis, while 139 (86.88%) reported no such diagnosis. Among those without a diagnosis, most had not undergone medical evaluation for osteoporosis (tab. II).

TABLE II.
Association between osteoporosis diagnosis and biological gender

	No	Yes	Woman	Men
Diagnosis	139 (86,9%)	21 (13,1%)	20 (14,81%)	1 (4%)

Regarding the association between an osteoporosis diagnosis and biological sex, based on the questionnaire responses, it was observed that the majority of respondents had not been diagnosed with osteoporosis, and among those who were diagnosed, most were women. The small number of respondents does not allow the results to be generalized to the overall population, and for many patients, according to their own statements, bone mineral density assessment is not a routine evaluation. Therefore, in the analyzed sample, no association was observed between biological sex and the presence or absence of osteoporosis (CC = .116, $\chi^2(1) = 2.16$, $p = .141$),

and the difference between the two sexes was not statistically significant (d = .108, 95% CI [.018, .206]).

Although, from a descriptive perspective, most individuals diagnosed with osteoporosis were women and it might appear that there is a statistically significant association, the statistical analysis did not identify a statistically significant association between biological sex and the presence of osteoporosis in the analyzed sample ($\chi^2(1) = 2.16$, $p = .141 > .05$). The observed difference may be explained by the small sample size and the low number of diagnosed cases.

Of the 21 respondents who reported having been diagnosed with osteoporosis, 3 live in rural areas (14.28%) and 18 (75.72%) in urban areas. The higher proportion of patients diagnosed with osteoporosis in urban areas does not necessarily indicate a higher risk associated with the place of residence, but may be correlated with easier access to medical evaluation services for patients living in urban areas of Romania compared to those in rural areas.

Place of residence was not associated with the presence or absence of osteoporosis (CC = .055, $\chi^2(1) = 0.49$, $p = .482$), even though 11.3% of those diagnosed with osteoporosis live in urban areas and only 1.9% live in rural areas. The difference between the two proportions of individuals from urban and rural areas was not statistically significant (d = .047, 95% CI [−.070, .164]). Although, at a descriptive level, there appears to be a difference between urban and rural environments among individuals with osteoporosis, the inferential analysis conducted on the subgroup of 21 participants does not indicate a statistically significant association.

The high p-value (p = .482), the very small contingency coefficient (CC = .055), and the confidence interval including zero suggest that the observed difference is likely the result of random variation, amplified by the small sample size. Thus, place of residence was not significantly associated with the presence or absence of osteoporosis, $\chi^2(1) = 0.49$, $p = .482$, CC = .055. Although, descriptively, a higher proportion of individuals diagnosed with osteoporosis comes from urban areas compared to rural areas, this difference is not supported by inferential analysis. The difference between the two proportions is not statistically significant and this result can be explained, at least in part, by the small size of the analyzed subgroup (n = 160), which limits the statistical power of the test and makes the observed percentage differences susceptible to random variation.

Figure 1 highlights the relationship between body mass index (BMI) and the presence or absence of osteoporosis. The scatterplot and trend line show a slight downward tendency, but without a clear separation between the two categories, suggesting no obvious association between BMI and osteoporotic status.

Regarding the association between the presence/absence of osteoporosis (a dichotomous variable-i.e., a variable that can take only two mutually exclusive values, usually coded as 0 and 1) and body mass index (BMI, a continuous variable), the null hypothesis was tested. The null hypothesis is a statistical statement assuming no relationship, difference, or effect between the analyzed variables. In statistical testing, the null hypothesis is accepted until the data provide sufficient evidence to reject it.

The point-biserial correlation between BMI and osteoporosis was not statistically significant in the analyzed group, and our

data do not provide sufficient evidence to reject the null hypothesis (rp.bis = –0.036, df = 158, p = .650, 95% CI [–0.190, 0.120]).

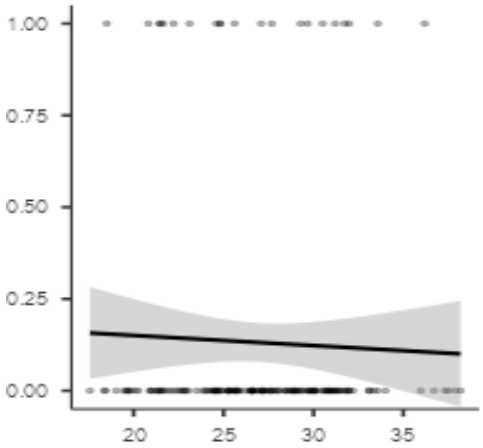

Fig. 1. Association between the presence/absence of osteoporosis and Body Mass Index (On the X-axis are the BMI values, on the Y-axis is the binary osteoporosis variable (0 = absence, 1 = presence). The line represents the estimated probability of osteoporosis, which decreases from about 15% at BMI ≈ 20 to about 10% at BMI ≈ 35, and the gray area indicates the confidence interval of the estimate.)

To evaluate the association between the presence/absence of osteoporosis (dichotomous variable) and BMI (continuous variable), the point-biserial correlation coefficient was used. The null hypothesis assumed no association between the two variables, i.e., a correlation coefficient of zero (H0: rp.bis = 0). The analysis indicated a very weak and statistically non-significant correlation between BMI and osteoporosis (rp.bis = –0.036, df = 158, p = .650, 95% CI [–0.190, 0.120]). Therefore, the data do not provide sufficient evidence to reject the null hypothesis, suggesting the absence of a significant association between BMI and the presence of osteoporosis in the analyzed group.

The same analytical method was used to examine the association between the presence/absence of osteoporosis (dichotomous variable) and participants' age (continuous variable), using the point-biserial correlation coefficient. The null hypothesis assumed no association between age and osteoporosis (H0: rp.bis = 0). The point-biserial correlation coefficient between age and osteoporosis was found to be statistically significantly greater than zero at the population level. The results indicated a statistically significant, positive correlation of moderate-to-high strength between age and the presence of osteoporosis (rp.bis = .453, df = 158, p < .001, 95% CI [.343, .553]). Consequently, the null hypothesis was rejected, providing statistical evidence that the risk of developing osteoporosis increases with age. Age accounts for approximately 20.53% of the variance in the presence/absence of osteoporosis.

Fig. 2 illustrates the relationship between age and the presence/absence of osteoporosis, showing an upward trend in

the likelihood of osteoporosis occurrence as age increases, as suggested by the trend line, without implying a deterministic relationship at the individual level.

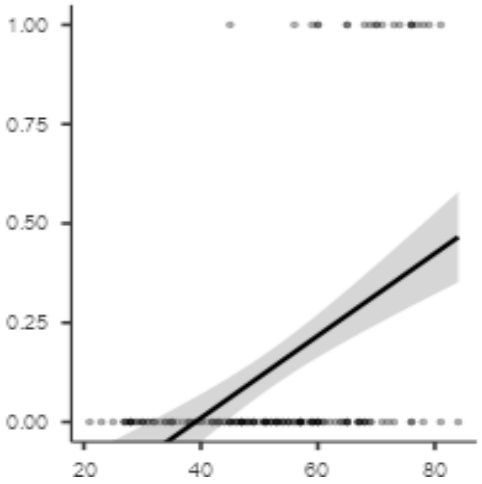

Fig. 2. Association between the presence/absence of osteoporosis and age (On the X-axis, the participants' age is shown, and on the Y-axis, the binary variable for osteoporosis (0 = absence, 1 = presence). The black line indicates the estimated probability of osteoporosis as a function of age, which increases from nearly 0% around ages 35–40 to about 45% at age 80. The gray area represents the confidence interval of the estimate.)

Our data indicate that the null hypothesis can be rejected; therefore, there is evidence to state that the risk of developing osteoporosis increases with age (rp.bis = .453, df = 158, p < .001, 95% CI [.100, .343]), with a strong effect, as approximately 20.53% of the variance in the presence and absence of osteoporosis is explained by age.

Regarding the association between a family history of osteoporosis and the presence or absence of an osteoporosis diagnosis, our data do not indicate a statistically significant association different from zero at the population level (CC = .028, $\chi^2(1)$ = 0.13, p = .721). Among the 21 respondents who reported being diagnosed with osteoporosis, 14 (66.66%) had no family members diagnosed with the condition, while 7 (33.34%) reported having relatives diagnosed with osteoporosis. The difference between the two proportions of individuals with and without a family history of osteoporosis is not statistically significant (d = .021, 95% CI [-.096, .138]).

Analysis of the association between the presence or absence of osteoporosis and the age at menopause onset shows a marginal significance (rp.bis = .153, df = 81, p = .084, 95% CI [-.030, .100]). The null hypothesis H0: The point-biserial correlation coefficient between age at menopause onset and osteoporosis is statistically significantly greater than zero at the population level cannot be rejected. However, it is possible that the effect manifests at the population level, as the analysis has low power, being conducted on 81 cases.

Regarding the relationship between the

age at menopause onset and the presence/absence of osteoporosis, a trend is observed suggesting an increasing probability of osteoporosis with later menopause onset, although this does not imply a definite causal relationship (fig. 3).

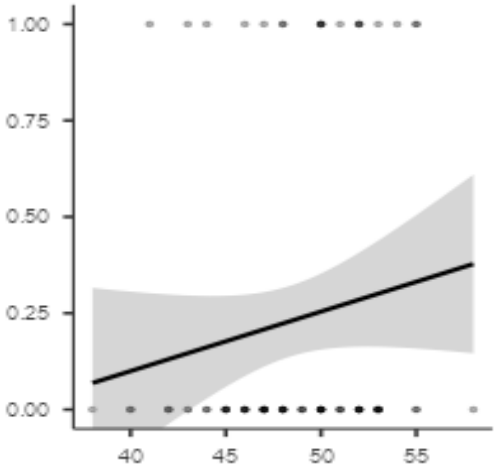

Fig. 3. Association between the presence/absence of osteoporosis and the menopause onset age (On the X-axis is the age at onset of menopause, and on the Y-axis the binary variable of osteoporosis (0 = absence, 1 = presence). The black line indicates the estimated probability of osteoporosis, which increases from about 8–10% for early menopause (~40 years) to about 35% for late menopause (~55–57 years). The gray area represents the confidence interval of the estimate.)

Our data seem to associate the presence of osteoporosis with older ages at menopause onset; however, this observation does not result from statistical analysis. The analysis of the association between the presence/absence of osteoporosis and the age at menopause onset indicates a weak positive trend, but it is not statistically significant, as the p-value is not less than 0.05 ($r_{p\beta}$ = .153, df = 81, p = .084). The 95% confidence interval includes zero, suggesting that the observed association may be due to random variation in the sample.

Consequently, the null hypothesis, which states that there is no association between age at menopause onset and the presence of osteoporosis, cannot be rejected. Although the direction of the coefficient suggests that osteoporosis tends to be associated with later menopause onset, this trend is not statistically supported in the present sample.

The lack of statistical significance may be influenced by the low power of the analysis, due to the relatively small sample size (N = 81). Therefore, the results should be interpreted with caution, and further studies with larger samples are needed to clarify the nature of this relationship.

Regarding fractures, 69.4% reported no fractures. Among those who did, 10.6% occurred in the forearm, 8.1% in the hip, 3.1% in the ribs, 2.5% in the spine, and 1.3% in the pelvis.

Analyzing the association between the

presence or absence of fractures and dairy consumption, our data do not indicate a statistically significant association different from zero at the population level (CC = .001, $\chi^2(1) = 0.00$, p = .991).

Among subjects who reported regular dairy consumption, 30.58% had a history of fractures, while among those who do not consume dairy, 30.66% had fractures. The difference between the two groups was not significant. Considering that osteoporosis and fracture risk are influenced by multiple factors, we cannot assert that dairy consumption alone determines fracture risk in the study group. To avoid misinterpretation of the results and to clarify the causal link between low dairy consumption and fracture risk, it is necessary to increase the number of subjects included in the study and to evaluate the cause of fractures while excluding accidental traumatic fractures.

DISCUSSION

Overall, the multivariate analysis confirmed that age remains the main independent factor associated with osteoporosis in the studied population, while other lifestyle variables did not show significant independent effects.

A decrease in bone density is a pathological phenomenon more frequently observed in women than in men. Regardless of sex, the prevalence of osteoporosis increases significantly with age, being observed in 50% of men and 80% of women over the age of 65 (1, 4).

The data presented in this study highlight the need for much more careful assessment of bone density in patients, as in our study many of the 160 individuals who completed the questionnaire had not undergone a bone density evaluation. Such evaluation is necessary because other studies have shown that one in two women and one in four men will experience, during their lifetime, a fracture caused by osteoporosis (2, 5).

The environment in which patients live can influence the rate of osteoporosis diagnosis. For instance, a study conducted in Spain, which included postmenopausal women showed a higher incidence of osteoporosis among women from rural areas. Specifically, 36.5% of rural women were diagnosed with osteoporosis, compared to 28.7% of urban women. Women from rural areas were generally older, shorter, and had a higher BMI compared to those from urban areas (6).

Similarly, a study conducted in China observed that among menopausal women, 26.83% of those living in urban areas and 32.53% of those in rural areas had osteoporosis. Studies on the general population reported a prevalence of osteoporosis of 10.33% in rural areas and 5.52% in urban areas (7).

The prevalence of osteoporosis depends on BMI and is inversely proportional to it. Multivariate analyses (which consider multiple factors simultaneously), adjusted for factors associated with osteoporosis, showed that underweight individuals had a significantly higher risk of osteoporosis compared to those with normal weight. In contrast, overweight and obese individuals had a lower risk of osteoporosis compared to those with normal weight (8, 9).

Underweight individuals with a BMI < 20 kg/m² had high risk for fracture compared with those with a BMI of 25 kg/m² (10).

A study conducted in China reported a prevalence of osteoporosis of 4% among men aged 50–59 years, 7.2% in those aged 60–69 years, 15.1% in the 70–79 age

group, and 26.7% in those over 80. For the same age groups, women showed a prevalence of 15.2%, 36.5%, 62.7%, and 85.8%, respectively. The onset age of osteoporosis was observed as follows: femoral neck osteoporosis began at 55 years in women and at 60 years in men (11).

Aging and the onset of osteoporosis are closely related (11). The overall decline in bone strength is due to reductions in both trabecular and cortical bone density, thinning of cortical bone, and a marked increase in its porosity. Loss of spongy (trabecular) bone mass in humans begins around the age of 30, while cortical bone density starts to decrease after the age of 50. In women, bone loss accelerates after menopause, so estrogen deficiency negatively affects bone mass and accelerates the deterioration of the skeletal system with age (12).

Osteoporosis has a high degree of heritability, but genetic factors alone are not sufficient to explain fracture occurrence in offspring, especially in adulthood (13).

The Geelong Osteoporosis Study conducted in Australia showed that a mother's fracture was associated with a higher likelihood of early fractures in daughters. Conversely, a father's fracture was associated with an increased risk of early fractures in sons, but not in daughters. Therefore, parental fracture history appears to be associated with the risk of early fractures in children in a sex-specific manner (14).

A questionnaire-based study conducted in the United States, including 8,073 women over the age of 20, showed a significant correlation between family history of osteoporosis and osteoporosis risk. The prevalence of family history was 34.8% among women with osteoporosis and 18.5% among those without the condition. Women who reported that their mother had osteoporosis had a higher risk than those who did not report this (15).

Studies on families and twins indicate that 50–85% of variations in bone mineral density are genetically determined. To reduce fracture risk, treatment for women with a family history of fractures can be initiated at a less strict bone density threshold, potentially improving patient compliance with subsequent interventions (16-18).

Large-scale genomic studies have identified several genetic variants that may contribute to the determination of bone mass and fracture risk, each having a moderate effect (19).

The process of bone density reduction begins one year before menopause, continues intensely for three more years, then slows slightly but continues at a moderate pace for another 4–8 years. On average, bone density decreases by approximately 10% during the menopausal period. In some women, loss can reach up to 20% over the 5–7 years surrounding menopause (20).

A study conducted in Sweden between 1977 and 2011 showed that at the age of 77, 56% of women with early menopause had osteoporosis, compared to only 30% of those with late menopause, indicating an almost twofold increased risk (1.83 times higher) of osteoporosis for women with early menopause. Women with early menopause had a higher incidence of fractures compared to those with late menopause, corresponding to a 1.68 times higher risk of experiencing such fractures (21, 22).

Milk and dairy represent an important source of protein, calcium, and other essential nutrients, and their consumption should not be discouraged in at-risk populations (23). According to a study from Sweden, consumption of fermented milk products

(yogurt, kefir) was associated with a lower risk of fractures, and when combined with fruit intake, it increased the rate of antioxidant protection (24).

The Age, Gene/Environment Susceptibility-Reykjavik (AGES-Reykjavik) study in Iceland reported a positive association between milk consumption and volumetric bone density at baseline, with an adjusted sex- and age-specific difference of 8.95 ± 2.5 mg/cm³ between the highest and lowest milk consumption categories (25). The regular lifelong milk consumption, from adolescence to old age, is associated with higher bone density in later life (26).

CONCLUSIONS

In this Romanian adult population, age was the primary factor associated with osteoporosis, while most lifestyle factors assessed through self-reported data were not significantly related to the condition. The findings emphasize the importance of age-based screening and clinical evaluation for osteoporosis. Larger population-based studies using objective measurements of bone mineral density are needed to better clarify the contribution of lifestyle factors to osteoporosis risk in this population.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interests, and they received no specific funding regarding this scientific research.

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PSYCHOLOGICAL PROFILE OF FRESHMAN PHARMACY STUDENTS

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PSYCHOLOGICAL PROFILE OF FRESHMAN PHARMACY STUDENTS (Abstract): In the last decades, the role of pharmacists has changed, and new tasks are required for the care of patients. Good communication skills, rapid adjustment to new techniques and research abilities must be developed during academic formation. **Aim**: the goal of the study is to shape the psychological profile of freshman pharmacy students. **Material and methods**: 106 freshman pharmacy students were asked to fill in a socio-demographic data sheet and to answer the items of four instruments: *Maslach Burnout Inventory* (measuring burnout), *Toronto Alexithymia Scale* (measuring the level of alexithymia), *Big Five Inventory* (identifying personality traits) and *Beck Depression Inventory* (evaluating depression). The statistical analysis of data was done using *SPSS Statistics 23.0.0* for MAC.OSX. **Results**: the results identified an average level of emotional exhaustion, a low level of depersonalization and a high level of personal achievement. The scores for alexithymia prove that a possible alexithymia and a mild level of depression was identified for freshman pharmacy students. Comparative results and correlation analysis are presented. **Conclusions**: the results are important for the pharmacy students to identify their personality traits and for the academic staff to adjust the educational curricula to cover the needs for professional development.
Keywords: PHARMACY STUDENT, DEPRESSION, PERSONALITY TRAITS, ALEXITHYMIA, BURNOUT, PSYCHOLOGICAL PROFILE.

The role of pharmacists has changed over the years, becoming more focused toward patient-centered care. This new targeted orientation has been sustained by the extended need of community pharmacy and by the inclusion of the chronic patient and family in the medical decisions. So, both community and hospital pharmacists were pressed to better adjust to the patient's profile and to develop more strategies to cope with stress related to the new job tasks. More abilities are now needed to develop a good relationship with patients. Also, better strategies should be applied to cope with stress related to job.

Not many studies can be found in the scientific literature about the pharmacist's psychological profile and its relationship with work. The need for this kind of research is great, because some studies proved that pharmacists were disappointed about their job performances and accused the lack of research knowledge, training, mentorship and support for their profes-

sional development and practice (1). Few studies suggest that there is a possible interaction between pharmacists' personality traits and pharmacists' performance (2) and their organizational culture profile (3).

The need for the quality of care increased over the years, abandoning the need for the traditional role in the community or hospitals. The new role demands the pharmacist to be a part of the medical team and to be in charge with hospitalized patients. In the community, pharmacists play an important role together with the family physicians. Preparing themselves to properly integrate into the community and into the medical team will assure a high level of work satisfaction for pharmacists and will also improve the quality of care for their patients. For these reasons, professional training during academic years or continuing education must consider the psychological profile of pharmacists and the possibility to improve and develop new skills for them.

Our study wants to identify the presence of some personality traits among freshman pharmacy students at the beginning of their education for this profession.

MATERIAL AND METHODS

The present research was conducted at the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi (June-September 2017), all the participants being students of this institution.

The study protocol of this cross-sectional, self-administered questionnaire was approved by the institutional ethical committee and informed consent was obtained from the participants prior to the beginning of the study.

The students from Pharmacy Faculty had to fill in a socio-demographic data sheet (registering socio-demographic characteristics, academic results, family data about siblings and parents' level of education or the presence of a chronic disease) and to answer to the items of four psychological instruments, to identify the personality traits and alexithymia and to evaluate the level of depression and burnout.

1. Burnout among medical students was assessed using the *Maslach Burnout Inventory* (MBI), the most frequently used questionnaire for measuring burnout. The MBI has 3 subscales to evaluate each domain of burnout: emotional exhaustion (the feeling of being emotionally drained by one's work, Cronbach's alpha = 0.809), depersonalization (emotional coldness, cynicism, feelings of guilt, social withdrawal, Cronbach's alpha = 0.690) and personal achievement (feelings of accomplishment in one's work, Cronbach's alpha = 0.862).

2. The personality traits were evaluated with the help of *Big Five Inventory* (BFI) which consists of 44 items corresponding to the five personality factors of the Big Five model: extraversion (Cronbach's alpha = 0.839), agreeableness (Cronbach's alpha = 0.678), conscientiousness (Cronbach's alpha = 0.820), neuroticism (Cronbach's alpha = 0.714), openness to experience (Cronbach's alpha coefficient = 0.703).

3. Alexithymia, a psychological construct that refers to people's difficulty in identifying and describing emotions because they focus their attention externally was measured using the *Toronto Alexithymia Scale (TAS – 20)*, a 20-item self-administered questionnaire. TAS-20 has 3 subscales: difficulty describing feelings or emotions (Cronbach's alpha = 0.804), diffi-

culty identifying feelings or emotions (Cronbach's alpha = 0.795), and externally oriented thinking (Cronbach's alpha = 0.336), Chronbach's alpha for alexithymia total score = 0.803.

4. *BECK Depression Inventory* (*BDI* - Beck et al, 1961), one of the most used instruments for the assessment of depression contains a number of 21 items which represent the symptoms used to diagnose depression: mood, pessimism, sense of failure, lack of satisfaction, guilty feelings, sense of punishment, self-hate, self-accusations, self-punitive wishes, crying spells, irritability, social withdrawal, indecisiveness, body image, work inhibition, sleep disturbance, fatigability, loss of appetite, weight loss, somatic preoccupations and loss of libido. The answers range from 0 to 3 and the alpha Chronbach score for this inventory is 0.861.

The statistical analysis of data was done using SPSS Statistics v23.0.0 for MAC.OSX. For the comparisons we used the t-test for independent samples and one-way ANOVA and for the correlational analysis we used Pearson and Spearman correlations. The statistical difference was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Socio-demographic data. Among the 106 freshman pharmacy students, 10 are male and 96 females, aged between 18 and 42 (M = 19.92 ± 2.69), enrolled in the 1st year of study. The sex distribution is according to the specificity of the profession (more women than men work in the pharmaceutical area). A percent of 31.4% are singleton, 47.6% have one sibling and 11.4% have 2 siblings and 68 % of students live in an urban.

Regarding academic results, the admission grades of subjects ranged between 5.40 and 9.94 (M = 8.10 ± 1.16**).**

Considering the level of education of both parents, almost half of the students declared that their mothers (45.2%) and fathers (46.7%) have at least a high school degree. 19.3% of students have at least one parents working abroad. More than half of subjects (65.6%) live by themselves in a dorm/rented apartment.

Most of pharmacy students (95.3%) declared that they do not suffer from any chronic diseases.

Psychological results. The results obtained by our participants for the questionnaires they filled in can be observed in tab. I. The results show that regarding burnout, students obtained an average level of emotional exhaustion, a low level of depersonalization and a high level of personal achievement. The scores for alexithymia evaluated with TAS-20 prove that there is a possible alexithymia and the results for BDI show a mild level of depression among freshman pharmacy students.

These results show that regarding burnout, the subjects have an average level of emotional exhaustion, a low level of depersonalization and a high level of personal achievement. The scores for alexithymia evaluated with TAS-20 are proving that there is a possible alexithymia while the score obtain at BDI show a mild level of depression among the pharmacy students.

The blend of personality traits (like openness, conscientiousness) are often related to job performance, work efficiency, less errors and good communication skills, while neuroticism and introversion act like barriers in the ability to complete a task correctly (4, 5).

TABLE I
Results obtained for MBI, BFI, TAS-20 and BDI

Instruments	Dimensions	M ± standard deviation
MBI	emotional exhaustion	**17.66 ± 9.20**
	depersonalization	**2.89 ± 4.04**
	personal achievement	**25.33 ± 12.03**
BFI	extraversion	3.58 ± 0.73
	neuroticism	3.78 ± 0.52
	openness	3.49 ± 0.67
	conscientiousness	3.10 ± 0.64
	agreeableness	3.45 ± 0.53
TAS-20	difficulty describing feelings	15.23 ± 4.64
	difficulty identifying feelings	19.46 ± 5.80
	externally oriented thinking	17.89 ± 3.99
	total score for alexithymia	**52.59 ± 11.09**
BDI	Mood	0.37 ± 0.60
	Pessimism	0.36 ± 0.48
	Sense of failure	0.32 ± 0.56
	Lack of satisfaction	0.55 ± 0.69
	Guilty feelings	0.37± 0.65
	Sense of punishment	0.61 ± 0.88
	Self-hate	0.28 ± 0.45
	Self-accusations	0.83 ± 0.99
	Self-punitive wishes	0.09 ± 0.38
	Crying-spells	0.39 ± 0.76
	Irritability	0.64 ± 0.86
	Social withdrawal	0.39 ± 0.62
	Indecisiveness	0.65 ± 0.72
	Body image	0.34 ± 0.85
	Work inhibition	0.46 ± 0.63
	Sleep disturbance	0.65 ± 0.55
	Fatigability	0.82 ± 0.71
	Loss of appetite	0.57 ± 0.83
	Weight loss	0.31 ± 0.54
	Somatic preoccupations	0.53 ± 0.69
	Loss of libido	0.20 ± 0.55
	Total score BDI	**10.08 ± 7.40**

Correlational analysis. To perform the correlational analysis, we tested the normality of our data distribution with the Kolmogorov-Smirnov test for all the variables we investigated. The results we obtained showed that the scores for agreeableness, conscientiousness, neuroticism, emotional exhaustion, openness, difficulty

describing feelings, externally oriented thinking and total score for alexithymia have a normal distribution while extraversion, depersonalization, personal achievement, difficulty identifying feelings and the total score for BDI are not distributed normally. That is why we used Pearson correlations for the variables that have a normal distribution and Spearman correlations for the ones that are not distributed normally.

TABLE II

Correlational analysis considering BFI, TAS-20 and MBI dimensions

Variables	Extra-version	Agreeableness	Conscientiousness	Neuroticism	Openness
Emotional Exhaustion	**R = -.213*** **p = .029**	R = -.016 p = .870	R = -.175 p = .073	R = .158 p = .105	R = .028 p = .788
Depersonalization	**R = -.222*** **p = .022**	R = -.177 p = .070	R = -.068 p = .491	R = .024 p = .811	R = -.071 p = .470
Personal Achievement	R = .161 p = .098	**R = .229*** **p = .018**	**R = .231*** **p = .017**	R = -.155 p = .112	R = .042 p = .668
Difficulty describing feelings	**R = -.340**** **p = .000**	R = .041 p = .676	R = -.076 p = .436	**R = .222*** **p = .022**	R = -.072 p = .461
Difficulty identifying feelings	**R = -.263**** **p = .000**	R = -.039 p = .689	**R = -.218*** **p = .025**	**R = .403**** **p = .000**	R = -.073 p = .459
Externally oriented thinking	**R = -.337**** **p = .000**	R = -.117 p = .234	R = -.153 p = .117	**R = .240*** **p = .013**	**R = -.345**** **p = .000**
Total score for alexithymia	R = -.174 p = .074	R = -.051 p = .605	**R = -.214*** **p = .028**	**R = .383**** **p = .000**	**R = -.199*** **p = .041**
Total score BDI	**R = -.348**** **p = .000**	**R = -.201*** **p = .039**	**R = -.326**** **p = .001**	**R = .497**** **p = .000**	R = -.056 p = .571

The results we obtained show that personality traits strongly correlate with a lot of the variables we investigated in this study:

- extraversion negatively correlates with emotional exhaustion, depersonalization, difficulty describing feelings, difficulty identifying feelings, externally oriented thinking, and the total score for depression, meaning that the more present this personality trait is in a person's personality structure, the lower are the scores for those variables.
- agreeableness negatively correlates with the total score for depression and positively correlates with personal achievement, so we can say that the more agreeable a person is, the more accomplished and the less depressed they will feel.
- conscientiousness negatively correlates with difficulty identifying feelings, the total score of alexithymia and the total score for depression and positively correlates with personal achievement, so the more conscientious a person is, the more personal accomplished they will feel, and they will experience less difficulties in identifying feelings.
- neuroticism positively correlates with difficulty describing feelings, difficulty identifying feelings, externally oriented thinking, the total score of alexithymia and the total score for depression, which means that the more present neuroticism will be in a person's personality structure, the more difficulties they will have to describe and

identify feeling and the more depressed they will feel.

- openness correlates with externally oriented thinking and the total score of alexithymia, proving that the more open a person will be, less difficulties they will have in being in touch with their feelings.

Table III presents the correlations between the burnout dimensions: emotional exhaustion, depersonalization and personal achievement and the alexithymia factors: difficulty describing feelings, difficulty identifying feelings, externally orientated thinking and the total score for alexithymia and the correlations between the total score for Beck Depression Inventory and the burnout dimensions and the alexithymia factors and alexithymia total score.

TABLE III
Correlational analysis between variables

Variables	Emotional Exhaustion	Depersonalization	Personal Achievement	Total Score BDI
Difficulty describing feelings	R = .264** p = .006	R = .088 p = .371	R = - .060 p = .541	R = .285** p = .003
Difficulty identifying feelings	R = .244* p = .012	R = .157 p = .689	R = .004 p = .967	R = .479** p = .000
Externally oriented thinking	R = .243* p = .012	R = .151 p = .123	R = -.028 p = .773	R = .157, p = .107
Total score for alexithymia	R = .337** p = .000	R = .174 p = .074	R = -.036 p = .716	R = .438** p = .000

The results show that emotional exhaustion positively correlates with all alexithymia dimensions and the alexithymia total score and with the self-assessed level of depression, meaning that the more emotional exhausted a person feels, the more difficulties they will have in recognizing their emotions and, also, the more depressed they will feel.

The total score for BDI positively corelates with the first two factors of alexithymia: difficulty describing feelings and difficulty identifying feelings and with the total score for alexithymia, meaning that the more depressed a person feels, the more difficulties they will have with being in touch with their emotions.

It is important that these results are taken into consideration during academic formation, results proving that high scores for alexithymia are strongly related with high levels of emotional exhaustion and depression. For the students presenting these traits, the professional training must include strategies to inform and teach them how to cope with professional stress and how to prevent depression.

Comparative analysis. To perform the comparative analysis, we used Independent Samples T-Test for the variables that have 2 levels and for the parents' level of education, and because this variable has more than two levels, we used for the comparisons, One-Way ANOVA.

Variables like gender, accommodation (if they live in a dorm or with rent), chronic disease (if they suffer or not from a chronic disease) produced no differences in the burnout factors emotional exhaustion, de-

personalization, personal achievement neither when it comes to the alexithymia factors, difficulty describing feelings, difficulty identifying feelings and externally oriented thinking or the alexithymia total score and they did not produce any difference regarding the level of self-reported depression.

Socio-demographic characteristics. Environment (urban/rural) produced no differences in emotional exhaustion, depersonalization, difficulty describing feelings, difficulty identifying feelings, externally oriented thinking, total score for alexithymia, total score BDI. The only statistically significant difference we obtained was regarding the level of personal achievement t (102) = -2.272, p = 0.025, the students that live in a rural area feeling more personally accomplished than the students who live in an urban area (Murb = 23.59, Mrur = 29.34).

Parents' level of education. In order to identify if the parents' level of education impacts student's results obtained on all four test, we made a comparative analysis. Most of the results revealed no influence on students' scores. We found that there are significant differences considering:

- the personal achievement dimension of burnout, students whose mothers have a university degree felt more personally accomplished than the students whose mothers have a college degree (Mcoll = 19.28, Muniv = 27.75, F(2,101) = 3.795, p =.026),
- difficulty identifying feelings dimension of alexithymia, the students whose fathers have a university degree having more difficulties in identifying feelings than those students whose fathers have a college degree (Mcoll = 22.04, Muniv = 18.32, F(2,102) = 3.008, p = .054).

The results of this study are important, firstly because they are identifying the psychological profile of freshman pharmacy students, this being the first study of this kind in the country. Secondly, the results are sustained by the scientific literature proving that knowing the personality traits is important for the academic formation, for the continuing professional development for pharmacists and for their quality of professional life and for the quality of care. Understanding the importance of personality traits for pharmacists' practice will provide insights for the development of professional training and support programs tailored specifically for this group (6).

Regression analysis. The analysis of correlations emphasized the influence of certain personality factors on burnout dimensions. To identify the most effective model in predicting burnout, we used multiple linear regression by the hierarchic method. The results are presented in tab. IV.

The results show that for the emotional exhaustion criterion the first three predictive models were significant. The strongest predictive model is the first one that has extraversion as predictor which explains 4.5% of the variance of the criterion emotional exhaustion. This predictor (p = 0.015, b = -2.957, beta = -0.235) has a significant influence on emotional exhaustion, their effect on the criterion being a negative one. The result proves that the more extraversion students show, the less amount of the emotional exhaustion they will experience.

Regarding the depersonalization criterion, the first four predictive models were statistically significant, but the best predictive model is the second model, with extraversion and agreeableness as predictors.

This model explains 6.6% of the variance of depersonalization. Among the 2 predictors of this model, only the second one, agreeableness (p = 0.028, b = -1.680, beta = -0.218), has a significant influence on the criterion, its effect on depersonalization being a negative one. This result proves that the more agreeableness is present in a person's personality structure, the lower their depersonalization scores will be.

TABLE IV
Regression analysis

	Models		Emotional exhaustion	Depersonalization	Personal accomplishment
Step 1	Extraversion	R^2adjusted	0.046	0.031	0.019
		ΔR^2	0.055	0.040	0.028
		F	**6.094***	**4.316***	3.023
Step 2	Extraversion, agreeableness	R^2adjusted	0.039	0.066	0.048
		ΔR^2	0.002	0.044	0.038
		F	**3.156***	**4.728***	**3.635***
Step 3	Extraversion, agreeableness, conscientiousness	R^2adjusted	0.047	0.064	0.058
		ΔR^2	0.017	0.006	0.019
		F	**2.728***	**3.384***	**3.144***
Step 4	Extraversion, agreeableness, conscientiousness, neuroticism	R^2adjusted	0.044	0.058	0.050
		ΔR^2	0.006	0.003	0.002
		F	2.214	**2.615***	**2.392***
Step 5	Extraversion, agreeableness, conscientiousness, neuroticism, openness	R^2adjusted	0.049	0.049	0.045
		ΔR^2	0.014	0.000	0.004
		F	2.087	2.077	1.986

*** p<.001, ** p<.01, *p<.05

For the personal accomplishment criterion only the second, third and fourth predictive models were statistically significant, but the best predictive model is model 3, that has as predictors extraversion, agreeableness and conscientiousness, which explain 5.8% of the variance of personal achievement. Among the 3 predictors of this model, none of them has a significant influence on personal accomplishment.

The importance of the study and some limitations

This study is important because it covers some missing data from the international scientific literature regarding the psychological profile of students studying pharmacy. This research also has some limitations: Firstly, there is a small number of male subjects, so no comparative analysis could be done. Secondly, this study is not a cross-sectional one and differences between students from all years of study were not emphasized. The third limitation is because one of the variable studied (burnout) is more common in the last years of study and not in freshman students. Considering these limitations, some results cannot be generalized.

CONCLUSIONS

Because personality traits are related to job performance and work satisfaction, the results are important for shaping training programs for pharmacy students during their academic years to develop their abilities and to help them adjust to the professional needs. The findings are important for both students and teachers. The awareness of the importance of personality for personal and professional outcomes could improve the path of the development of pharmacists, starting with the very first year of academic education.

ACKNOWLEDGMENTS

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NEWS

MALIGNANT POTENTIAL OF SMALL GASTROINTESTINAL TUMORS

Gastrointestinal stromal tumors (GISTs) are sarcomas of the gastrointestinal tract with variable clinical behavior, pathological features and molecular mechanisms. While tumor size is an important prognostic factor, data regarding the malignant potential of small GISTs (<2 cm in size) has yet to be evaluated. The aim of the study was to asses the risk of malignancy in regards to small tumor size. A study was conducted as a review of a population-based cohort of GISTs with known mutational status. In the cohort more than 70% of GISTs were incidental findings, with over a quarter of the tumors less than 2 cm. Among these, 66% had typical C-KIT mutation, either in exon 9 or 11, none had PDGFRA mutation and one third were classifiable as 'wild-type' GISTs. Mutation index was very low (< 5 mitosis/HPF) and the survivability of these patients remained high. Small GISTs are often incidental findings, with more than one in four less than 2 cm in size. The prognosis of these tumors is excellent. Additional data is needed to properly assess the risk of malignancy to avoid overt undertreatment (Søreide K. Cancer biology of small gastrointestinal stromal tumors (<2 cm): What is the risk of malignancy? *Eur J Surg Oncol* 2017; 43(7): 1344-1349).

Irene Alexandra Cianga Spiridon

LATE DIAGNOSED PHACE SYNDROME BY ANEURYSMAL SUBARACHNOID HEMORRHAGE. CASE REPORT

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LATE DIAGNOSED PHACE SYNDROME BY ANEURYSMAL SUBARACHNOID HEMORRHAGE. CASE REPORT: PHACE syndrome is a neurocutaneous disorder coined in 1996, but the clear criteria of diagnostic were established in 2009. Even though the age of the diagnostic for the disease is during childhood, we present the case of a patient with PHACE syndrome with late diagnostic, at the age of 59-year-old by aneurysmal subarachnoid hemorrhage. The particularity of this case is represented by the paucity of the diagnostic criteria, respectively the presence of facial hemangioma and the dysplasia of the cerebral arteries at the level of Willis's circle, associated with two aneurysms: ruptured aneurysm of the right posterior communicating artery and unruptured aneurysm of junction P1-P2 of the left posterior cerebral artery. The aim of the article is to height line the importance of early diagnostic of PHACE syndrome and the prevention of complications. **Keywords:** PHACE, SYNDROME, CEREBRAL VASCULAR DYSPLASIA, SACCULAR ANEURYSM.

PHACE syndrome is a neurocutaneous syndrome coined in 1996 by the American professor of dermatology Frieden (1), and referred to the association: posterior fossa anomalies (P), hemangiomas of the face and scalp (H), arterial lesions (A), cardiovascular abnormalities (C) and eye's anomalies (E). When they are associated also supraumbilical raphe (S) or sternal clefting (S), it is called PHACES syndrome (2, 3).

PHACE syndrome appears mainly in women in 90% (1), suggesting that it could represent an X-chromosome-linked dominant condition with lethal potential in the male population. Anyway, familial cases have not been reported (4). Siegel *et al*. (5) made the first step in showing a potential genetic basis of PHACE syndrome, reporting the presence of a genomic copy number variation in patients having this syndrome. Till date, the pathogenesis of this syndrome is unknown (1).

CASE REPORT

We present the case of a patient aged 59, who was admitted at the Clinic of Neu-

rosurgery "Prof. Dr. Nicolae Oblu" Emergency Clinical Hospital, Iasi, for the appearance of an intracranial hypertension syndrome dominated by severe headache. The examination underlined the presence of a facial hemangioma at the level of the left upper and lower eyelid, chin, the left cheek, respecting the alar nasal sulcus (figure 1). At the level of the left cheek, there was a scar in shape of heart ("heart" pseudo-sign) due to a free skin graft, which is mentioned in the personal medical history of the patient when she was 11 years old (fig.1). Computed tomography angiography (CTA) of the head and 3D reconstructions diagnosed a subarachnoid hemorrhage (SAH) (fig.2. A), cerebral vascular dysplasia (fig. 3), as well as a ruptured aneurysm of right posterior communicating artery (PCoA) (fig. 2. B and 3. A.), and a unruptured aneurysm of junction P1-P2 of the left posterior cerebral artery (PCA) (figure 3. B). The cerebral angiography confirmed the presence of the vascular dysplasia (figure 4.A), as well as the aneurysms (figure 4. B). It was not noticed the presence of other anomalies of the eyes or internal organs.

Fig. 1. Facial hemangioma in the distribution of segments 2 and 3. It is noticed the absence of cutaneous distribution of the trigeminal nerve, as do the "port wine stains" in Sturge Weber syndrome. It is also noticed the presence of a free skin graft with aspect of "heart".

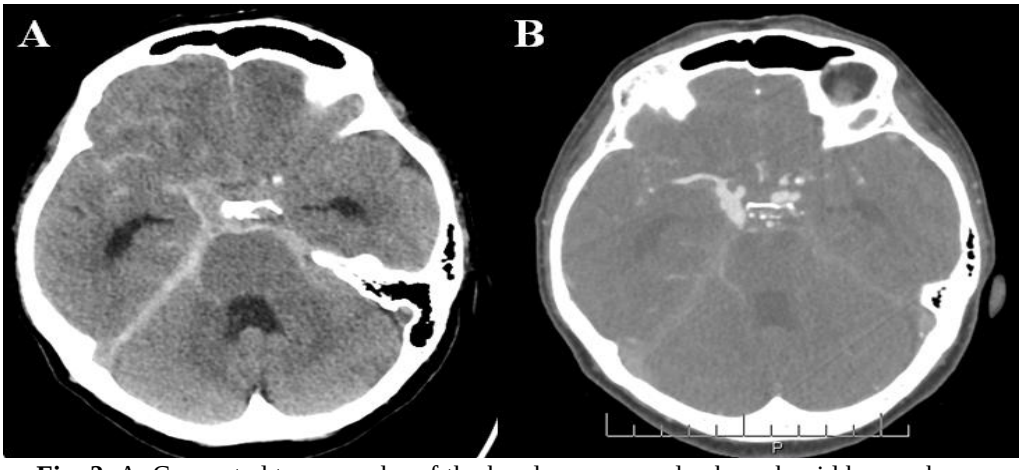

Fig. 2. A. Computed tomography of the head: aneurysmal subarachnoid hemorrhage; B. CTA: vascular dysplasia and right PCoA aneurysm.

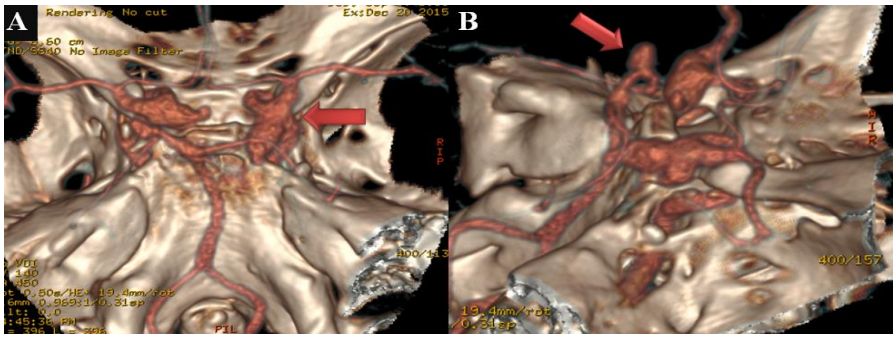

Fig. 3. A, B. CTA of the head with 3-dimensional reconstruction of the intracranial vessels showing dysplasia with right PCoA aneurysm (3. A, red arrow) and P1-P2 junction aneurysm (left PCA) (3. B, red arrow).

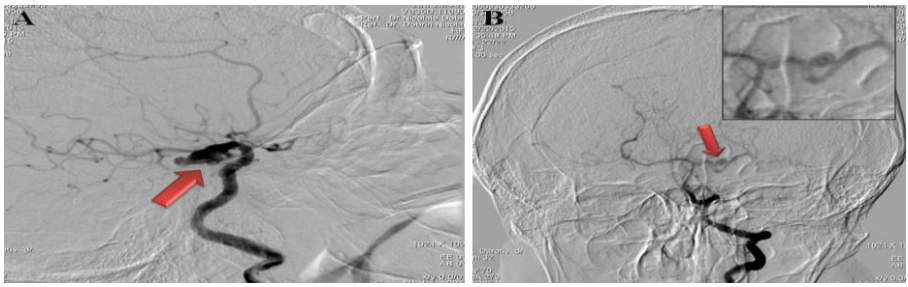

Fig. 4. Cerebral angiography: A. Lateral right view with vascular dysplasia (red arrow); B. Left vertebral artery view with P1-P2 junction aneurysm from left PCA (Dr. N.Dobrin's collection).

DISCUSSION

Criteria of diagnostic. Pascual-Castroviejo (6) was the one who proved for the first time the relationship between infantile hemangiomas and malformations of the cervical and cerebral arteries in a disease that he called "cutaneous hemangioma-vascular complex syndrome". In 1996, Frieden (1) suggest the acronym PHACES. The clear criteria of diagnostic for this syndrome were established in 2009 by the American Academy of Pediatrics, who classified them into two categories: 1. PHACE syndrome and 2. possible PHACE syndrome. Also, it has established the major and minor criteria that were determinate by following these organ systems: structural brain, cerebrovascular, cardiovascular, ventral or midline. Thus, definitive PHACES syndrome requires the presence of: facial hemangioma > 5 cm in diameter + 1 major criteria or 2 minor criteria; possible PHACES syndrome requires the presence of: facial hemangioma > 5 cm + 1 minor criteria, or hemangioma of the neck or upper torso + 1 major criteria or 2 minor criteria, or no hemangioma + 2 major criteria (7) (tab I). On considering these criteria, our patient fit into definitive PHACE syndrome, with the following valid criteria: facial hemangioma > 5 cm in diameter and dysplasia of large cerebral arteries, associated with two cerebral aneurysms.

The cerebrovascular abnormalities in PHACE syndrome. These anomalies are not only the most frequent extracutaneous man-

ifestation, but also the biggest potential source of morbidity, which can lead to the appearance of progressive vessel stenosis with acute ischemic stroke (4, 8). But the highest risk for the acute ischemic stroke is during infancy, between birth and 18 months, period that corresponds also to the cu hemangioma proliferative phase (4). This phase is followed by slow regression (9, 10). In the case of our patient, the cerebral vessels were not stenotic, and the onset was late, at the age of 59, by rupture of aneurysm of right PCoA. Even though within the syndrome isolated venous and cerebral sinus anomalies were reported, the arterial anomalies are the most frequent (7). They can be divided into four subcategories: 1: *dysplasia* (looping, kinking, ectasia or fusiform dilatation); 2: *narrowing* (agenesis, acquired stenosis or occlusion or developmental hypoplasia); 3: *aberrant course or origin of arteries* and 4: *persistence of embryonic anastomose*, and among then arterial dysplasia is the most frequent (7), present also in the case of our patient.

TABLE I.
Major and minor criteria for diagnostic of PHACE syndrome (4)

ORGAN SYSTEM	MAJOR CRITERIA	MINOR CRITERIA
Cerebrovascular	Anomaly of major cerebral arteries ▪ Dysplasia of the large cerebral arteries ▪ Arterial stenosis or occlusion with or without moyamoya collaterals ▪ Absence or moderate to severe hypoplasia of the large cerebral arteries ▪ Aberrant origin or course of the large cerebral arteries ▪ Persistent trigeminal artery ▪ Saccular aneurysms of any cerebral arteries	Persistent embryonic artery other than trigeminal artery ▪ Proatlantal intersegmental artery (types 1 and 2) ▪ Primitive hypoglossal artery ▪ Primitive otic artery
Structural brain	Posterior fossa anomaly ▪ Dandy-Walker complex or unilateral/bilateral cerebellar hypoplasia/dysplasia	Enhancing extra-axial lesion with features consistent with intracranial hemangioma ▪ Midline anomaly ▪ Neuronal migration disorder
Cardiovascular	Aortic arch anomaly ▪ Coarctation of aorta ▪ Aneurysm ▪ Aberrant origin of the subclavian artery with or without a vascular ring	Ventricular septal defect ▪ Right aortic arch (double aortic arch)
Ocular	Posterior segment abnormality ▪ Persistent fetal vasculature (persistent hyperplastic primary vitreous) ▪ Retinal vascular anomalies ▪ Morning Glory disc anomaly ▪ Optic nerve hypoplasia ▪ Peripapillary staphyloma ▪ Coloboma	Anterior segment abnormality ▪ Sclerocornea ▪ Cataract ▪ Coloboma ▪ Microphthalmia
Ventral or midline	Sternal Defect ▪ Sternal cleft ▪ Supraumbilical raphe ▪ Sternal defects	Hypopituitarism ▪ Ectopic thyroid

The facial hemangioma in PHACE syndrome. The studies of facial hemangioma patterns have identified four primary facial segments: segment 1 (frontotemporal region), segment 2 (the maxillary region respecting the nasomedial sulcus), segment 3 (the mandible, chin and lower lip) and segment 4 (the medial frontal scalp, nasal bridge and philtrum) (11). In the case presented, the facial hemangioma was developed at the level of segments 2 and 3. Also, it seems that there is a relation within this syndrome, between the regional distribution of the facial hemangioma and the locations of arterial lesions (12).

Pathophysiology of PHACE syndrome. As for the physiopathology of the disease, based on the observations, Heyer *et al*. (12) have reached the conclusion that the initial vascular changes in PHACE start during the fifth week of embryogenesis, and infantile hemangioma and the cerebral vasculopathy of PHACE seem to be related temporally to an insult that appears in early embryogenesis. To understand the pathophysiological relation between facial hemangioma and cerebral arteries malformations, Burrows *et al.* (13) hypothesized the existence of vascular growth factors or other vascular modulators produced by the hemangioma itself, which would lead to the appearance of the cerebral arteriopathy. These factors that can be secreted during embryonic period of hemangioma formation or during the proliferative phase of hemangioma were well documented (12).

CONCLUSIONS

In the case of our patient, the presence of only a facial hemangioma and the absence of the other cardiovascular, cerebral, ocular or median line anomalies as well as sternal cleft, sternal defects or supraumbilical raphe, allowed the late diagnostic of the cerebral vasculopathy associated with aneurysm rupture. The early diagnostic of the PHACE syndrome is important in following and prevention of the possible complications.

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NEWS

ADVANCES IN ANTIBODY–DRUG CONJUGATES (ADCS) TECHNOLOGY

Antibody–drug conjugates (ADCs) are a potent class of anticancer therapeutics that comprise a high-affinity antibody (Ab) and cytotoxic payload coupled via a suitable linker for selective tumor cell killing. The first FDA-approved ADC was Mylotarg (gemtuzumab ozogamicin) but it was withdrawn from market, due to the acute adverse effects and morbidity in patients, attributed to acid-labile weak hydrazone linkers and nonspecific antigen expression in healthy cells. The limitations and failures of first-generation ADCs were eliminated in second-generation ADCs with the discovery of the breast cancer-targeting ADC, Kadcyla (ado-trastuzumab emtansine) and Adcetris (brentuximab vedotin) (for treating hematological cancer). Despite the improvement in cytotoxic payloads and the introduction of stable linkers, second-generation ADCs have significant limitations in terms of their heterogeneous DAR, resulting from stochastic coupling strategies between the Ab and drug. Efforts are continuing to develop more efficient cytotoxic payloads in third-generation ADCs so that a smaller amount of drug is needed to achieve the desired therapeutic effect and to eliminate drug resistant tumor cells. The development of PBDs, derivatives of tricyclic antibiotics, has attracted interest over other DNA alkylating agents. PBDs are potent compounds, some of which have subpicomolar IC50 and do not show cross-resistance with other chemotherapeutics, such as cisplatin. Four PBD-containing ADCs are currently in clinical trials: SC16LD6.5 is in Phase II trials for small cell lung carcinoma; SGN-CD33A is in Phase II trials for AML; SGN-CD70A is used to treat patients with CD70-positive cancer, and ADCT-301 is in Phase I trials for CD25 lymphoma (Sau S, Alsaab HO, Kashaw SK, Tatiparti K, Iyer AK. Advances in antibody-drug conjugates: a new era of targeted cancer therapy. *Drug Discov Today.* 2017 Jun 13. pii: S1359-6446(17)30078-8. doi: 10.1016/j.drudis. 2017.05.011. [Epub ahead of print]

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NEGATIVE PRESSURE THERAPY IN THE SURGICAL TREATMENT OF DIABETIC FOOT

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NEGATIVE PRESSURE THERAPY IN THE SURGICAL TREATMENT OF DIABETIC FOOT (Abstract) **Aim:** To assess the role of negative pressure therapy in the treatment of diabetic foot by the evaluation of the time required for healing and the functional outcomes of patients. **Material and methods**: Prospective study conducted between September 2014 and February 2017 including 31 patients with diabetic foot clinically monitored following NPWT. Vacuum-assisted closure devices (VAC™ -Hartman) were used for applying negative pressure to the wound according to the specified settings (negative pressure, time of use of a kit) in accordance with patients' outcome. The following were monitored: changes in wound size, their bacterial load, and duration of treatment. **Results:** Healing was obtained in all cases, with an average hospital stay of 27.3 days and 8 days of therapy application. Negative bacterial wound culture results were obtained after an average of 6.45 days by the simultaneous application of negative pressure and antibiotic treatment according to the antibiogram. Skin grafts were necessary to close the defect in 4 cases. **Conclusions:** After the basic wound treatment, auxiliary methods such as negative pressure contribute to healing. In patients with diabetic foot who required surgical intervention, the use of negative pressure therapy yielded a significant benefit in the preservation of the affected limb, after minimal excision. The results obtained throughout our experience recommend the use of NPWT technique as indication for abdominal wall surgery in closing abdominal wall defects, compartment syndrome and surgical site infection after prosthetic mesh. **Keywords:** NEGATIVE PRESSURE, DIABETIC FOOT, TREATMENT.

The first experience with the use of negative pressure wound therapy (NPWT) was reported in 1987 when it was used in the treatment of soft tissue injuries and septic wounds.

Numerous articles were published in the early 1990s and the spectrum of indications was expanded for chronic wounds (leg ulcers, decubitus ulcers). In the early 2000s, a significant extension of indications of this technique applicability was noted in severe dermatological syndromes,

problematic vascular surgery wounds and an increasing use in plastic surgery. In 1993, Fleischmann described a method of wound exposure to sub atmospheric pressure to obtain debridement and wound healing in patients with open fractures. The procedure was improved in Germany by Muller, who applied it to 300 patients with infected wounds, and in 1998 Kovacs et al. described using this technique in the treatment of chronic leg ulcers.

By the application of NPWT in the surgical field in the last 20 years, an innovative approach to treat surgical wound infections was promoted. Due to its benefits, this therapy is currently being introduced widely, becoming the method of choice in some conditions, with favorable results in the treatment of "diabetic foot" wounds, minimizing the surgical gestures and preserving the affected limb.

The overall incidence of amputation ranges from 2.8 to 43.9/100,000 in the general population and from 0.46 to 13.7/1000 patients diagnosed with diabetes (1,2). The proportion of diabetes-related amputations of the total non-traumatic amputations ranges from 40% to 80%, that is a 10-30 times higher frequency than in the general population (1).

The objectives of wound healing are represented by minimizing blood loss, replacement of defects with new tissue (granulation tissue followed by scar tissue) and restoration of an intact epithelial barrier as quick as possible.

Thus, auxiliary therapies, such as ensuring local hyperbaric oxygen atmosphere, administration of growth factors, skin substitutes, grafts of cultivated keratinocytes, electrical stimulation and local drainage, with or without flushing by sealing the wound in negative pressure, may facilitate local healing (2). Increasing blood flow in the circulation and compromised or damaged tissue oxygenation enhance resistance to infection.

Successful wound healing, spontaneous or after surgery is correlated with the bacterial load of the affected tissue which must be less than 10^5 bacteria per gram of tissue. Bacterial accumulations greater than 10^5 interfere with wound healing. The study aims to demonstrate the usefulness of the method and that its clinical use increases the benefits to both patients and medical institution. The objectives of the study were to assess the healing time after negative pressure application, the extent of the surgical procedure and the functional outcomes of the patient.

MATERIAL AND METHODS

The prospective study was conducted on a sample of 31 patients admitted to the Second Surgery Clinic of the “Sf. Spiridon” County Clinical Emergency Hospital from Iasi. The study was conducted between September 2014 and February 2017. Data related to patient demographics, history of the underlying disease, complications and markers of inflammation were used. All wounds were monitored by wound size measurements and photographed to document wound progression.

Laboratory investigations prior to NPWT have included laboratory tests (CBC, markers of infection), X-rays, collection of samples from wound or secretions for antibiogram prior to surgical procedures. The above-mentioned parameters were monitored throughout the treatment period, in accordance with recording data on the duration of NPW, number of surgical debridement at every wound dressing change and used negative pressure values.

Patients were monitored until the wound has completely healed.

Prior to the application of the VAC™ therapy device, the appropriate surgical debridement of all wounds was ensured, followed by wound cleansing through jet lavage irrigation (H_2O_2, betadine and saline).

NPWT dressings have been shaped to fit the wounds, which are then sealed with a semi-permeable film from the kit.

Pressure level was set individually for each patient based on their needs, using continuous suction initially, and then intermittent suction, in the 40 to 110 mmHg range.

Pressure settings depended on the local wound conditions and the pathological conditions of the patient were considered.

Dressing change was performed every 48-72 hours by the treating physician.

RESULTS

The number of admitted patients per study year varied (2014-3 patients, 2015-9 patients, 2016- 18 patients, 1 patient 2017). Demographic data revealed that 18 of the 31study patients were male and 13 females, aged between 38 and 82 years, mean age 65.35 years (67 years in men and 63.07 in women). Sixty-five percent were rural patients.

Sixty-eight percent (21 patients) presented to the Emergency Department and 32% (10 patients) were admitted for various chronic complaints.

The complications of the underlying disease (diabetes mellitus) recorded in the study patients were peripheral neuropathy, vasculopathy, neuropathy, and diabetic retinopathy.

The recorded comorbidities were hypertension, severe anemia and peripheral vascular disease.

Depending on the extent of the surgical procedure underwent by the patients on admission, these were classified as: major and minor. The major ones consisted in the amputation of the leg (6%) and of the thigh (6%), and the minor ones in necrectomy (13%), amputations of fingers with metatarsal head resection (23%); incision, drainage and debridement (29%); anterior foot amputation (23%).

Healing was obtained in all cases to a period of hospitalization that ranged from 5 to 49 days and an average of 27.3 days. The period in which negative pressure therapy was applied in the studied group was between 3 and 16 days.

Dressings were applied for an average of 8.12 days, and dressing changes varied between 48 and 72 hours, with an average of 63 hours.

Wound size at baseline ranged from 7.5 cm^2 to 69 cm^2 with an average of 25.50 cm^2, their closure being achieved after the application of therapy. Antibiogram samples were collected prior to the initiation of treatment and until the closure of the defects, and came back negative after an average of 6.45 days.

The negative pressure used ranged from a minimum of 40 mmHg to a maximum of 110 mmHg, average use 71.61 mmHg, depending on the local wound conditions and considering the pathological characteristics of the patient.

Bridge Technology (multiple aspiration drains) was used in two study patients. In all cases continuous suction was used initially, the device being then set to intermittent suction. In 3 patient's aspiration drainage was associated with local antibiotic-containing instillations (according to the antibiogram), the favorable results being

confirmed by antibiogram. In one case two shifts of the kit were performed and in two cases it took three shifts until the wound could be closed.

DISCUSSION

Loss of sensation in the legs specific to neuropathy combined with poor monitoring can cause any injuries to go unnoticed, to infect, or, in severe cases, amputation (finger or toe) to be the only option left.

The number of diabetes-related amputations has increased successively in 2006-2010, so that 5,827 cases were reported in 2010, 41.32% greater than in 2006 (3).

After the introduction of this method in the treatment of diabetic foot in our clinic a significant reduction of major surgeries by 12% (bellow-knee and thigh amputations) compared to the results obtained by the conventional treatment methods used in 34% of the patients. Of the 311 diabetic patients admitted to our clinic over a 2-year period pre NPWT implementation, 102 patients underwent major surgeries.

Several studies have demonstrated the benefits of NPWT in the treatment of infected wounds, showing superior efficacy compared to conventional dressings (4).

In our study, NPWT was used for a mean duration of 8 day until wound closure or grafting, with an average hospital stay of 27 days, results like those reported by McCallon (4) in cases of diabetic foot, where satisfactory healing was obtained in half the time compared to the control saline gauze group.

The mechanisms of action of NPWT may be quantified for generating micro- and macro deformation to the wound. The reduction in wound size due to the flattening of the pores of the material used and the action of centripetal forces exerted on the entire surface of the wound by the applied sponge determines its macro deformation.

The cells are subjected to various mechanical forces, including the hydrostatic pressure of the extracellular fluid, stretching and compression transmitted over the entire cell array; all these joining the force of gravity determine micro deformations to the wound (5).

Analysis of the above-mentioned elements has shown that the tissue near the sponge structure is subject to compressive forces. In general, the regular but highly variable microenvironment created during this therapy is causing micro deformation. Micro deformations are the morphological result of the integrated mechanics, the shape of the cells being an important determinant of their function (6,7). Furthermore, it is known that cells can adapt to physical stress and thus the functions of some cells can be initiated by physical and dynamic changes.

Angiogenesis is initiated by cell bed micro deformation that causes local hypoxia and thus leads to an increase in the vascular endothelial growth factor (VEGF). The temporary reduction of blood flow to the edges of the wound stimulates angiogenesis through hypoxia-inducible factor (HIF) -1alfa -> VEGF with an increase in density of microcirculation (8-10).

Depending on the underlying pathology, chronic wounds and swelling are often concomitant, the accumulated excess fluids being accepted as a contraventional healing factor by the compressive effect exerted locally on cells and tissues. Applying negative pressure in these situations reduces extracellular fluid accumulation resulting in better blood perfusion by reducing compression on the microvasculature (11).

Toxins, bacteria and exudate may be removed from the wound site along with the fluids (12).

The dressing is impermeable to proteins and microorganisms, thus reducing the risk of wound contamination, and has limited permeability to water vapor and other gases, thus helping to maintain a moist and stable wound environment.

The lack of a wound bed which could allow cell extension and adhesion prevents the development of isometric tension within the cell, thus leading to its spheronization and finally to apoptosis, situation that was not found in chronic wound bed (6).

Before applying NPWT patients were subjected to excisional debridement requiring dressing change twice a day, under surgical stress, maintaining systemic inflammatory response syndrome (SIRS) and an increased risk of septic complications, while the NPWT system allowed a few days between procedures, thereby increasing patient comfort and decreasing hospital costs (time in the operating room, workload, materials) (13).

Microbiological surveillance of the wound after initiation of NPWT was shown to significantly improve the course, with insignificant reduction in the incidence of wound contamination. Clinically, necrosis occurred in a limited number of cases, and the obvious remission of SIRS and hypercatabolic syndrome was observed (14).

Negativizing of antibiogram was obtained in all study patients after an average use of NPWT of 6.45 days, results like those reported in the literature; studies documented decreased bacterial load of the wound in about 20% of patients 4 days after the initiation of NPWT and in 60% of patients 8 days after the initiation of treatment. Studies by Morykwas and Argenta, Banwell et al., and Morykwas et al. have reported increased bacterial clearance in the infected wound by using NPWT.

CONCLUSIONS

In our study, we observed that correct placement of the interface material with adequate wound debridement is essential for the success of NPWT. The application of negative pressure reduces extracellular accumulation of fluid, leading to a better blood perfusion by lowering the compression to the microvasculature and the dressing having limited permeability to water vapor and other gases helps maintain a moist and stable wound environment.

We recommend the use of intermittent suction technique whenever possible because in addition to the physical wound bed changes that lead to an increased bacterial clearance at this level, it also facilitates the association with local instillation of antibiotic. The use of negative pressure not adapted to local conditions can lead to wound bed ischemia.

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NEWS

NEW BIOMARKER CANDIDATE IN BREAST CANCER

Researchers from Turkey, Germany, and USA evaluated the role of HLJ1 gene (DNAJB4) in breast cancer progression. This gene belongs to the DNAJ (HSP40) family of heat shocks proteins (HSPs) known as tumour suppressor genes in colon and gastric cancers. It was analysed the HLJ1 *in vivo* and *in vitro* expression and its genetic and epigenetic alterations. HLJ1 expression was found to be reduced or lost in breast cancer cell lines (SK-BR-3, MDA-MB-231, ZR-75-1). In patients with breast cancer, the expression of HLJ1 was less frequent in groups with invasive carcinoma compared with groups with ductal carcinoma *in situ*, benign conditions and normal tissue. Regarding the methylation, the CpG island located in the 5'-flanking region of the HLJ1 gene was found to be methylated in breast cancer cell lines. HLJ1 expression was restored in the ZR-75-1 cell line by DNA demethylating agent 5-Aza-2'-deoxycytidine (5-AzadC) and histone deacetylase inhibitor trichostatin A. Epigenomic mechanisms such as CpG methylation and histone deacetylation might contribute to downregulation of HLJ1 expression. Authors concluded that HLJ1 could be a possible tumour suppressor candidate and potential biomarker for breast cancer. Future epigenomic, functional and clinical studies are needed to assess its potential for biomarker and its role in personalised oncological diagnostic. (Acun T, Doberstein N, Habermann JK, et al. HLJ1 (DNAJB4) Gene is a novel biomarker candidate in breast cancer. *OMICS.* 2017; 21(5):257-265.).

Alina Manole

FECAL MICROBIOTA TRANSPLANTATION IN RECURRENT NAP1/B1/027 CLOSTRIDIUM DIFFICILE INFECTION RESISTANT TO VANCOMYCIN AND METRONIDAZOLE IN A PATIENT WITH ULCERATIVE COLITIS: A CASE REPORT

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FECAL MICROBIOTA TRANSPLANTATION IN RECURRENT NAP1/B1/027 CLOSTRIDIUM DIFFICILE INFECTION RESISTANT TO VANCOMYCIN AND METRONIDAZOLE IN A PATIENT WITH ULCERATIVE COLITIS: A CASE REPORT (Abstract): Most of the studies showed that inflammatory bowel diseases (IBD) patients inflammatory bowel diseases with *Clostridium difficile* infection (CDI) have more of the whole range of short- and long-term worst outcomes than those without CDI. Initial infection with the BI/NAP1/027 epidemic clone was found to be a significant risk factor for relapse. However, current literature is suggesting increasingly that for patients with infections that fail to resolve with traditional antibiotic regimens, FMT's average cure rate of > 90%. We report a case of a 40-year-old man, diagnosed with ulcerative colitis (UC) in 2012 who presented in our clinic for 20 watery stools per day with mucus and blood, hypogastric pain, pyrexia and chills. Rectosigmoidoscopy and histopathological examination diagnosed active lesions of ulcerative colitis with *Clostridium difficile* (*C. difficile*) toxins A/B enzyme immunoassays (EIA) testing initially negative. The patient was non-responder at day 10 of intravenous (iv) corticotherapy and received induction therapy with Infliximab 5mg/kg. EIA testing for *C. difficile* was repeated at day 12 of hospitalization with positive results for toxins A/B, and associated oral therapy with vancomycin and metronidazole was initiated without clinical response in day 7, reasons for what intravenously therapy with tigeciclyne was started with good response. Patient was discharged after 10 days of tigeciclyne, but came back twice for two relapses of *C. difficile* colitis treated successfully with tigeciclyne, reasons for what fecal transplantation was performed in Matei Bals Institute in Bucharest which induced remission of both CDI and UC. **Keywords**: ULCERATIVE COLITIS, *CLOSTRIDIUM DIFFICILE* VANCOMYCIN AND METRONIDAZOLE REZISTANT, TYGECYCLINE, FECAL MICROBIOTA TRANSPLANTATION.

Most of the studies showed that IBD patients with CDI have more of the whole range of short- and long-term worst outcomes than those without CDI. Patients with ulcerative colitis have more severe outcomes than those with Crohn's disease (1). Rates and severity of *Clostridium difficile* infection (CDI) in hospitals in North America and Europe have increased since 2000 and correlate with dissemination of an epidemic strain characterized by higher than usual toxin A and B production, the presence of a

third toxin, binary toxin, and high-level resistance to antibiotics. The strain, which is restriction endonuclease analysis group BI, pulse-field gel electrophoresis type NAP1, and polymerase chain reaction ribotype027, is designated BI/NAP1/027(2, 3, 4). One of the most problematic aspects of CDI is the propensity of recurrence in 15% to 35% of patients who initially respond to antimicrobial therapy. In addition, recurrent CDI is difficult to treat and contributes to significant morbidity and mortality and increased health care expenditures (5). Initial infection with the BI/NAP1/027 epidemic clone was found to be a significant risk factor for relapse (6).

CASE REPORT

A 40-year-old man was admitted in our department in May 2014 for 20 watery stools per day with mucus and blood, hypogastric pain, pyrexia and chills. His medical history includes chronic hepatitis B infection in treatment with entecavir 0.5mg per day and UC diagnosed in 2012, in treatment with azathioprine at a dose of 150 mg per day (2,5 mg/kg). He confessed cessation of smoking for one year, previously being a chronic smoker of 20 cigarettes per day. Should be mentioned in his medical history multiple flares of UC since diagnosis due to low-compliance to medication, also self-discontinuation of azathioprine 2 weeks before hospital admission because of lack of money. At clinical examination, the patient was apyretic, underweight with a BMI of 17.5kg/m^2 with skin and mucosal pallor, tachycardia and with tenderness in lower abdomen with palpation. Laboratory tests showed moderate leukocytosis, important increase in C-reactive protein levels and hypochromic microcytic anemia. Stool test were negative for infections. *Clostridium difficile (C. difficile)* toxins A/B enzyme immunoassays (EIA) tested negative.

Rectosigmoidoscopy revealed absent vascular pattern, edema, erythema, friability of the mucosa and erosions (fig. 1).

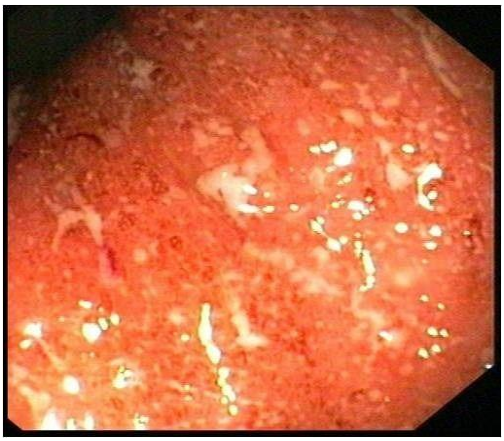

Fig. 1. Colonoscopy image showing continuous lesions of edema, erythema and friability on 60 cm of the colonic mucosa explored.

Histopathological examination showed diffuse chronic mucosal inflammation, cryptitis, regenerating epithelium with erosion and superficial fibrino-leukocytic exudate, HE, 200x (fig. 2).

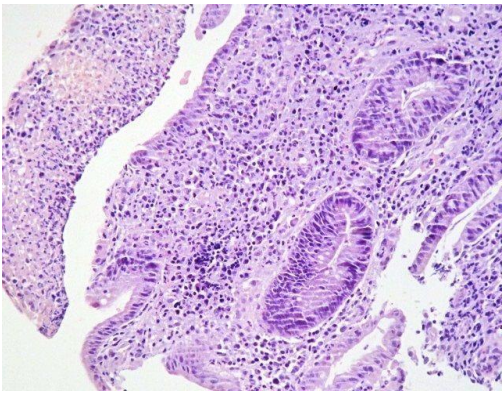

Fig. 2. Examination of biopsy specimens of inflamed colonic mucosa.

The severity of UC flare was framed in a Mayo score of 12 points. The patient received intravenous (i.v.) corticotherapy with lack of response at day 10. First induction dose of infliximab 5 mg/kg was administered with the maintenance of oral corticotherapy. EIA testing for *C. difficile* was repeated at day 12 of hospitalization with positive results for toxins A/B. We decided to stop further administration of infliximab and start tapering corticotherapy until *C. difficile* eradication. We initiated oral vancomycin 1,000 mg per day in association with oral Metronidazole 1,500 mg per day and *Saccharomyces boulardii* capsules 500 mg per day. After three days, the patient developed digestive intolerance with vomiting and the regimen was changed to iv metronidazole 1,500 mg/day. At day ten of treatment the patient had a decrease in the number of stools (9 per day, with no blood but with the presence of pus), still with persistent abdominal pain. Blood tests showed no modification regarding inflammatory panel with increased values in serological inflammatory markers, severe hypochromic microcytic anemia that required blood transfusions and severe hypoalbuminemia. Fecal cytology showed the presence of leukocytes.

Nucleic acid amplification test (NAAT) for *C. difficile* toxin genes was positive for hipervirulent NAP1/B1/027 strain. Oral vancomycin was continued with increased dosage 2,000 mg per day (500 mg every 6 hours) in association with vancomycin enemas 500 mg four times per day. After ten days of previous regimen due to lack of response we added tigecycline 50 mg every 12 hours intra-venously for 16 days with clinical response consisted in normalization in the frequency and consistency of stools. The patient was discharged from hospital with the recommendation to continue vancomycin in a tapering regimen 250mg four times daily with halving doses weekly until 125 mg every other day for 7 days in association with *Saccharomyces boulardii* capsules 500 mg per day. Oral corticotherapy tapering was continued. After one month, the patient was re-admitted to the hospital for the recurrence of watery bloody stools and severe abdominal pain. We performed EIA retesting for *C. difficile* which was positive for both toxins A and B and fecal cytology which showed the presence of leukocytes. Patient received a second course of intravenous tygecicline 50 mg every 12 hours with favorable evolution and the normalization of stools at day 7. At two weeks follow up the patient presented with the second relapse of *C. difficile* colitis and again was treated with tygecicline in association with oral metronidazole, oral vancomycin and *Saccharomyces boulardii* capsules with favorable evolution. After this third recurrence of *C. difficile* infection patient received fecal microbiota transplantation (FMT) in Matei Bals Institute in Bucharest with a donor from the personal of the Hospital, through a naso-gastric tube. One month after, the patient had a fourth recurrence of *C. difficile* colitis meeting the same pattern as the previous and we performed a second procedure of FMT (changing the donor with one person from the patient's village). At further follow-ups for a period of 2 years the patient was in clinical remission of IBD with the normalization of clinical examination, weight gain and with normal range for serological inflammatory markers, on maintenance therapy with oral 5-ASA 3 g/day.

DISCUSSION

The endoscopic finding of pseudo membranes found in 50% of *C. difficile* infected patients (per data from last decade) is less common (13%) in IBD patients with CDI (7) and our case had also an endoscopic aspect of ulcerative colitis flare. A strong pre-test suspicion for CDI (as in our case watery diarrhea, severe hypoalbuminemia, severe anemia, severe acute inflammatory syndrome), empiric therapy for CDI should be considered regardless of the laboratory testing result, as the negative predictive values for CDI are insufficiently high to exclude disease in these patients (8). In our patient, the clinical presentation of *C. difficile* colitis mimic an underlying IBD flare and the first test for toxins in the stool was negative, and that made us to escalate the immunosuppressive therapy that lead to a very severe evolution of this patient. Toxins A+B EIA testing commonly used as first diagnosis test for CDI has a sensitivity of single sample testing low (72%) and may increase by 10% with second stool testing (84%). Three sample testing increase sensitivity to 86% (8). Currently, standard recommendations for treatment of mild CDI include metronidazole or vancomycin, with data suggesting that vancomycin is more efficacious than metronidazole in severe CDI (1). This is the first Romanian report of a case of *C. difficile* colitis with the BI/NAP1/027 strain in a patient with UC, community acquired. Rafila et al (9) report in their study that PCR ribotype 027 was found to be the most dominant type in CDI in Bucharest, accounting for 68% of the Romanian isolates during 2011-2012, 84% healthcare associated and 16% community acquired. Now we report resistance of this strain to metronidazole or vancomycin and a good response to tigeciclyne therapy, which practically saved the life of this patient. We think that Infliximab worsened the evolution of CDI in our patient, our point of view differs from Seicean A et al (10).

CONCLUSIONS

Our case emphasizes the need for FMT use as a promising rescue therapy for refractory IBD and CDI. FMT was indicated because of repeated relapses of *C. difficile* colitis, as suggested by current data from the literature. FMT practically cured both diseases, with the condition of a good quality donor and the brief protocol of patient preparation before and during FMT.

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NEWS

INTRADUCTAL TUBULOPAPILLARY NEOPLASM OF THE PANCREAS: AN UPDATE FROM A PATHOLOGIST'S PERSPECTIVE

The intraductal neoplasms of the pancreas are defined by the current 4th edition of the World Health Organization (WHO) classification as macroscopic epithelial neoplasms with ductal differentiation that grow in the pancreatic ductal system. The 2 entities included in this category are intraductal papillary mucinous neoplasm (IPMN) and intraductal tubulopapillary neoplasm (ITPN). Before the current WHO classification, intraductal neoplasms of the pancreas were classified into 3 groups: IPMN, pancreatic intraepithelial neoplasia (PanIN), and intraductal tubular neoplasm. Both IPMN and ITPN can be either cystic or solid-mass forming. The distinction between the intraductal neoplasms and PanIN is that PanIN is microscopic. On the contrary, by definition, both IPMN and ITPN are grossly and radiographically detectable lesions with an arbitrary minimal size criterion of 1 cm. As these neoplams are considered to be precursor lesions to carcinomas, surgery and close clinical follow-up is generally recommended. Morphologically, they can mimic some subtypes of IPMN. However, molecular studies revealed that they are distinct entities. Further studies are needed to elucidate the clinical, pathologic, and molecular features of ITPN, which could be used as guidance for the management of patients (Rooney SL and Shi J. Intraductal Tubulopapillary Neoplasm of the Pancreas: An Update from a Pathologist's Perspective. *Archives of Pathology & Laboratory Medicine* 2016; 140 (10): 1068-1073).

Doina Butcovan

UNEXPECTED CORONARY THROMBOSIS INDUCED BY ANTIPHOSPHOLIPID SYNDROME (HUGHES SYNDROME): CASE REPORT

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UNEXPECTED CORONARY THROMBOSIS INDUCED BY ANTIPHOSPHOLIPID SYNDROME (HUGHES SYNDROME) - CASE REPORT (Abstract): Antiphospholipid syndrome (APS), one of the most common states of acquired hypercoagulability, is diagnosed by the persistent presence of antiphospholipid antibodies and recurrent episodes of vascular thrombosis. We present the case of a 39-year-old man late-presenting for cardiac rehabilitation treatment after primary percutaneous coronary intervention (PCI) performed for anteroseptal myocardial infarction. He was a nonsmoker, with no prior personal history of other cardiovascular diseases (CVD) or cardiometabolic syndrome. The 60% thrombotic occlusion of the left anterior descending artery (LAD) leading to the acute cardiac event was the only abnormality that was found. The only etiological explanation was the late measurement and the positive tests for antiphospholipid antibodies. In young patients with no history of thrombotic disorder, such as cancer, cardiovascular or metabolic diseases, the unexpected onset of myocardial infarction by thrombotic coronary occlusion can be attributed to silent, undiagnosed autoimmune condition.
Keywords: ANTIPHOSPHOLIPID SYNDROME, MYOCARDIAL INFARCTION, ANTIPHOSPHOLIPID ANTIBODIES, AUTOIMMIUNE DISEASES.

Antiphospholipid syndrome (APS), also known as Hughes syndrome (1), is an autoimmune disorder and represents an acquired hypercoagulable state. It is characterized by vascular thromboses or pregnancy morbidity, such as fetal loss, and it occurs in patients who have persistent antiphospholipid (aPL) antibodies (2). As aPLs have been a hallmark feature of APS for almost half a century, aPLs detection is the first-line approach for diagnosing the APS. Per 2006 revised APS classification criteria (3), the diagnosis of APS requires the persistent presence of at least one of the following aPLs, including lupus anticoagulant (LA), immunoglobulin G (IgG) and/or immunoglobulin M (IgM) anti-cardiolipin (aCL) antibodies, and IgG and/or IgM anti-β2 glycoprotein 1 (aβ2GP1) antibodies. Immunoglobulin A (IgA) aCL and IgA aβ2GP1 antibodies are not currently included in the laboratory criteria for APS, but are suggested as "noncriteria" antibodies for seronegative patients with clinical suspicion of APS (4, 5).

CASE REPORT

A 39-year-old male presented for cardi-

ac rehabilitation program after coronary artery revascularization surgery. The patient had positive family history of cardiovascular diseases (CVD), but no personal history of other cardiovascular conditions or cardiometabolic syndrome. He was a nonsmoker. Physical examination revealed a status of anxiety, tachycardia, and mild dyspnea. Blood test showed complete blood count within normal values, with a slightly elevated erythrocyte sedimentation rate (ESR) (normal values for men < 50 years: 0-15 mm/hour), normal levels of lipid profile, fasting blood sugar (FBS) and glycated hemoglobin (HbA1c) indicating a good short-term and long-term control of blood sugar levels. Kidney function, liver function and hydro-electrolytic balance were normal. On admission, the only abnormal findings were the biological markers for myocardial necrosis - CPK 210 U/L, CK-MB 19.3 U/L and Troponin T 2.03 ng/ml. Resting electrocardiogram (ECG) revealed ST segment elevation in anteroseptal precordial leads (V1-V4) (fig. 1). Apart from ST elevation, a pathological Q wave (0.06 seconds wide, and a depth > ¼ height of R-wave it precedes) in V1-V3 precordial leads. Heart rhythm was regular, but on sinus tachycardia of around 110 beats per minute. The normal electric heart axis (QRS axis) was preserved.

Coronarography angiography showed no injury to the left main artery (LM) and left circumflex coronary artery (LCX), but revealed a 60% thrombotic occlusion of the left anterior descending artery (LAD) (fig. 2).

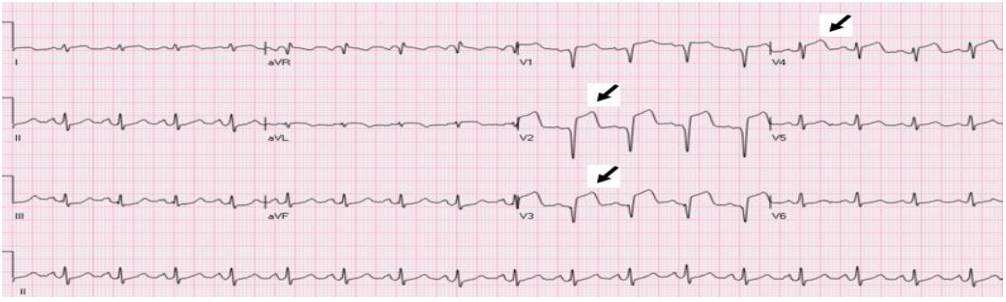

Fig. 1. ECG - anteroseptal ST segment elevation myocardial infarction

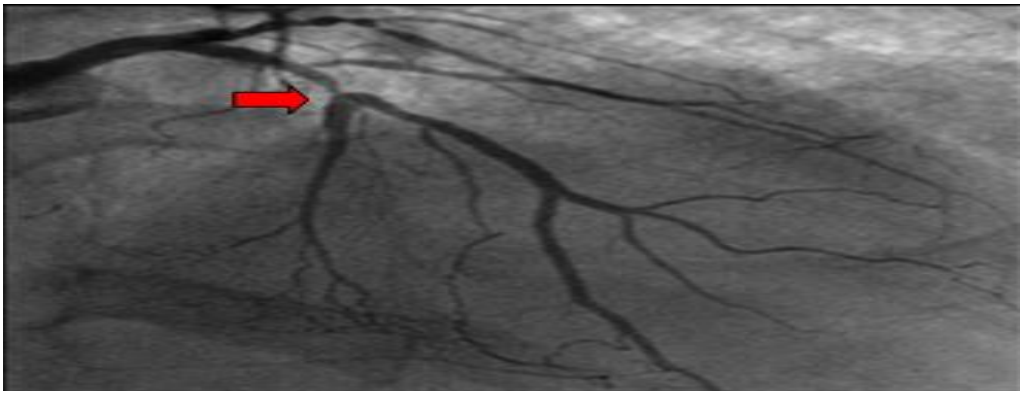

Fig. 2. Coronary image of 60% LAD obstruction

Two months later the patient presented for cardiac rehabilitation program. Clinical assessment on admission to the rehabilitation clinic showed: normal weight (body mass index 24 kg/m²), resting blood pressure (BP) of 100/55 mmHg and heart rate of 70 beats per minute. Dyspnea was the only remaining physical symptom. The 24-hour continuous BP monitoring revealed normal BP levels and normal dipper profile. The 2D transthoracic Doppler echocardiography showed mild tricuspid regurgitation, mild mitral regurgitation, hypokinesis of the basal segment of the interventricular septum, and apical akinesis of the anterior wall. Resting electrocardiogram showed chronic anteroseptal myocardial infarction (fig. 3).

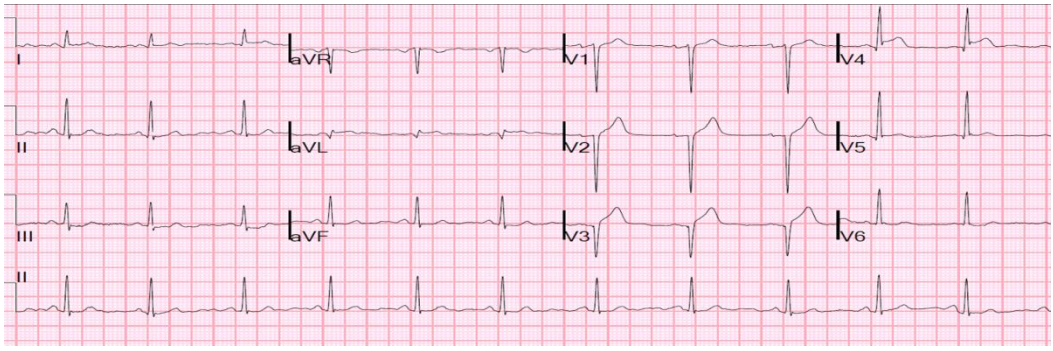

Fig. 3. ECG with appearance of chronic anteroseptal myocardial infarction

Laboratory test values during cardiac rehabilitation program showed normal red blood cell count; liver and kidney functions were normal as well, cholesterol parameters, sodium and potassium levels were within normal ranges. The immunology report revealed the presence of LA (lupus antibody) and aCL (anticardiolipin antibody) IgG 88 GPL/mL (IgG > 40 phospholipid units (GPL)/mL), aCL Ig M 100 MPL/mL (Ig M >99 phospholipid units (MPL)/mL).

DISCUSSION

Antiphospholipid syndrome comprises a heterogeneous group of autoimmune diseases. These conditions are characterized by recurrent arterial or venous thrombosis, or both, and/or pregnancy morbidity, as well as by the presence of aPL antibodies. APS is classified as primary or APS associated to other diseases. Primary APS (PAPS) is defined by the presence of aPL antibodies, but no evidence of any underlying systemic autoimmune disorder (6).

In our case, a relatively young patient with no personal risk factors for myocardial infarction, we thought that an autoimmune disease could be responsible for the coronary thrombosis. The positive antiphospholipid antibodies supported the diagnosis of antiphospholipid antibodies syndrome.

The clinical diagnosis of APS is supported by the laboratory findings, as the main clinical features of thrombosis and pregnancy morbidity also occur in many other diseases. In clinical settings, the standard enzyme-linked immunosorbent assay (ELISA) gives a large variation in antibody positivity, because there are no standardized kits. Studies conducted on the compared to commercial ELISAs showed similar performance and results between laboratories in detecting IgG/IgM aCL and IgG/IgM aβ2GP1 autoantibodies (7-12).

As for cardiac manifestations in APS, they may range from pulmonary hypertension, intracardiac thrombosis, to dilated cardiomyopathy, coronary artery disease and valvular heart disease (13-15). Although coronary artery disease and restenosis following percutaneous coronary intervention (PCI) are less common, the risk of their occurrence is increased in APS patients compared to other conditions. The pathophysiological explanation for the fact that APS patients are predisposed to stent thrombosis resides on APS predilection for thrombotic events. In addition, unresponsiveness to aspirin and clopidogrel can predict the risk of stent thrombosis (16).

As in adults and elderly, in young adults' atherosclerosis is the main cause of coronary events. Other causes of coronary heart disease are related in about 20% of cases to nonatherosclerotic factors - such as autoimmune diseases, connective tissue disorders, or coronary abnormalities. However, cases of systemic lupus erythematosus (SLE) and antiphospholipid syndrome (APS) may present different onset clinical manifestations. In SLE, there are no age-based differences regarding the prevalence of myocardial infarction; conversely, this major coronary event is unusual in APS and it might appear during the progression of the antiphospholipid syndrome.

The phenotypes of vascular damage in APS are characterized by the strong interactions between circulating aPL and cell surface molecules of target cells, primarily endothelial cells and platelets. The molecular basis of APS is still largely unknown. Endothelial cells produce nitric oxide, factor that promotes vascular health by regulating the physiological processes (thrombosis, endothelial-leukocyte interaction, vascular cell migration), and by modulating the vascular tone.

In all young patients with cerebral infarction, myocardial infarction, pulmonary embolism, recurrent miscarriages, and unexplained low platelet count, the high likelihood of antiphospholipid antibody syndrome should be considered. Among the form of APS associated to other diseases are various systemic autoimmune syndromes, especially systemic lupus erythematosus (SLE) (17).

CONCLUSIONS

This case highlights the importance of considering in the emergency department the prothrombotic states, such as SLE and APS, in adult patients with no prior cardiovascular risk factors presenting with acute myocardial infarction due to unexplained intracoronary thrombosis.

Early diagnosis of catastrophic APS and aggressive therapies are essential to help such patients from succumbing to this potentially fatal condition.

This syndrome should be suspected more frequently in all young patients than in adult patients, especially if they do not present any cardiovascular diseases and develop myocardial infarction.

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EFFICIENCY OF TREATMENT FOR FEMALE SUI (STRESS URINARY INCONTINENCE) USING THE TVT-O® (TENSION-FREE VAGINAL TAPE OBTURATOR) TECHNIQUE

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EFFICIENCY OF TREATMENT FOR FEMALE STRESS URINARY INCONTINENCE USING THE TENSION-FREE VAGINAL TAPE OBTURATOR TECHNIQUE (Abstract): **Aims**: The modern *Tension-Free Vaginal Tape Obturator* (TVT-O®) technique is used worldwide to treat *Stress Urinary Incontinence* **(**SUI). The subject of the paper is timely and with a major practical potential. The paper presents an ample retrospective study on a series of patients hospitalized at the Second Clinic of Obstetrics-Gynecology of "Cuza-Vodă" Obstetrics and Gynecology Clinical Hospital from Iasi, for a period of 3 years. In the period January 2010–January 2012, 164 female patients were subjected to surgical procedures to treat type II SUI, inserting TVT-O® tapes. **Material and methods**: Patients were selected based on anamnesis, clinical examination and positive Bonney's test. When genital prolapse was associated with SUI, simultaneous with strip insertion, other interventions were practiced, too. Cases were followed in the immediate postoperative period, at 2 months, 6 months and 1 year, in order to analyze the efficiency of the method. 164 female patients aged between 38 and 65 were included in the study, of which 60 cases had isolated SUI and in 104 of the cases SUI was associated with genital prolapse. **Results**: In 85% of the cases, the healing rate was satisfactory, and in 15% of the cases, it was partially satisfactory, keeping the percentage at 6 months and 1 year. In respect of complications at 16 female patients, SUI was improved, but persistent, in 14 cases the mictional necessity was described as absent before intervention; in these cases, we used oxybutynin, in 4 of them-tape sectioning and in 4 cases the exteriorization of the tape was relieved after 2 months, performing the secondary vaginal suture. The simple operatory technique, the reduced hospitalization period, the short convalescence and the immediate results support the TVT-O® procedure in the treatment of SUI. Postoperative complications are rare and do not compromise results. The main goal of *"Sling"* interventions is the creation of a reliable closure mechanism, supporting the passive urinary retention by urethral compression. **Conclusions**: Our point of view with regard to the controversy of using TVT-O® tapes is that the technique does not have to be abandoned, being extremely efficient in academic centers with ultra-specialized staff.
Keywords: STRESS URINARY INCONTINENCE, TVT-O®, TAPE, MICTIONAL IMPERIOSITY, DETRUSOR DYSSYNERGIA.

Urinary incontinence (UI) is a condition that seems to affect up to a quarter of the women over 45 years-old, causing discomfort or even a high level of severity.

Over time, UI was considered a consequence of the anatomic changes determined by births or great physical effort, being seen as a symptom of pelvic organ prolapse. UI is produced by the association of anatomic causes with a series of factors that determine disorders of the bladder and urethra. Thus, we can speak of an anatomic type of UI or a "functional" UI, and a mixed UI. In conclusion, we can distinguish SUI (stress urinary incontinence) caused by morpho-functional changes caused especially by parturition ("anatomic" UI) and "functional" UI based on the presence of involuntary contractions of the detrusor in the bladder filling phase. By definition, SUI is described as an involuntary urine loss generated by physical effort in the absence of the contraction of the detrusor muscle. It emerges during intra-abdominal pressure increase, when intra-bladder pressure increase and exceeds urethral closing pressure, having an intermittent character.

Though urinary incontinence does not endanger the patient's life, it is a state that strongly affects physical, psychological and social wellbeing, thus affecting the quality of life.

Though using the TVT (tension-free vaginal tape) technique, the cure rates have been satisfactory, in 2001, Delorme et al. (1) proposed a trans obturator approach with the purpose of reducing significantly intraoperative complications.

Different surgical and non-surgical techniques have been adopted in the treatment of urinary incontinence for the past years, and the management principles have changed along with the introduction of new and less invasive techniques.

Starting 1913, when Howard Kelly et al. (2) described his first anterior plication stitch until today, several authors have proposed over 200 surgical approaches for the treatment of stress urinary incontinence. Although different treatment schemes have been proposed, the ones based on surgery remain the main treatment.

The Marshall Krantz procedure and the Burch urethropexy were the first surgical techniques using suprapubic approaches to elevate the paraurethral tissues to the Cooper's ligament or the pubic periosteum (3).

Though these techniques were considered in the past the golden standard, with high cure rates during the first postoperative year in comparison to the Kelly procedure, many physicians tried to bring a surplus to these techniques in order to reduce the hospitalization period, morbidity and postoperative complications (4).

Petros and Ulmstein, through the integral theory of female urinary incontinence by means of mid-urethral support stirred an actual medical revolution, with this theory leading to the TVT technique development towards the end of 1990, and a few years later Delorme E. proposed the TOT (transobturator tape) method.

The advantage of this procedure is the fact that it excludes the need for intraoperative cystoscopy and avoids the retro pubic area, thus excluding the risk of bowel perforation or vessel lesion.

The TVT procedure resembles the TOT, with a different technique to insert the tape, but no difference has been reported regarding the cure rate. The techniques can be compared as regards the patients' satisfaction a year after surgery. Several studies reveal the fact that the TOT can reduce significant postoperative complications (5).

MATERIAL AND METHODS

The collection, processing and interpretation of the information necessary for the study led to knowledge on the particularities of the stress urinary incontinence cases hospitalized in the 2nd Clinic of Obstetrics and Gynecology of "Cuza-Vodă" Obstetrics and Gynecology Clinical Hospital from Iasi, between January 2010 and January 2013, by using the data recorded in the observation charts and the computerized system. This study is a retrospective analysis on all cases of operated stress urinary incontinence type II, in which TVT-O® tapes were inserted.

Between 2010 and 2013, 164 cases of SUI were diagnosed and investigated, and patients were selected based on their anamnesis, clinical examination and positive Bonney test (6).

Stress urinary incontinence was classified depending on the degree of stress causing it, namely: 1st degree – incontinence emerges only under high stress, 2nd degree – incontinence emerges under moderate stress, such as walking down and up the stairs, 3rd degree – incontinence emerges under low stress such as orthostatic, the patient being continent in clinostatism. The Bonney test is performed by the exploring physician with the middle finger and forefinger placed on either side of the urethra, ascending the urethra-bladder junction without compressing it. This test enables the differentiation between stress urinary incontinence and the detrusor dissinergy and it is positive when urine loss disappears during the test performance.

The data collected as a study material and considered refer to: the total number of cases of urinary incontinence type II investigated, diagnosed and operated, the annual distribution of the disease cases, the repartition of cases according to age groups, personal physiologic history (menstrual status, parity) and pathological history, the duration of the surgical intervention, the duration of hospitalization, the postoperative antibiotic treatment, postoperative complications.

The insertion of the TVT-O® (*tension free vaginal tape obturator*) tape was a sole operation or associated with other interventions for the correction of the associated genital prolapse. The efficiency of this technique was evaluated immediately after surgery, after 2 month, 6 months, and 12 months.

The study included operated patients with SUI type II who underwent the insertion of TVT-O® tape and who came for postoperative check-up after 2, 6, and 12 months, and excluded patients who did not come back for postoperative check-ups (31.7% of the total).

RESULTS

Following the processing and evaluation of the 164 cases of stress urinary incontinence operated, recorded in the 2nd Clinic of Obstetrics and Gynecology of "Cuza-Vodă" Obstetrics and Gynecology Clinical Hospital from Iasi, between 2010 and 2013, we should mention that from the point of view of the temporal variable, a maximum number of cases was in 2012, with 66 cases recorded (40.24% of the total), and the fewest were recorded in 2010.

Our study revealed that the average age of the patients diagnosed with SUI type II is 53 years, and the patients included in the study were between 38 and 65 years old.

Most hospitalizations (78.3% of the total) were persons over 50 years-old, which is associated with the "enrichment" of the morbid status with age.

The estrogens deficit generates a decrease in the capacity of proximal closure of the urethra, microbial colonization manifest-

ed clinically by dysuria, polakiuria, and urethral atrophy leads to the shortening of the urethra, the disappearance of the bladder-urethral angle and the emergence of an ectropion of the urethra mucous membrane, eventually favoring stress urinary incontinence. From the point of view of the menstrual status, 59.75% of the total women included in the study were menopausal and 40.24% of the total had genital activity.

During labor, direct lesions are generated to the soft pelvic tissues and partial denervation of the pelvic floor. The dilacerations of the connections between vagina, urethra and bladder produced by excessive distension, together with the production of cortisol and progesterone during pregnancy lead to the laxity of the fascial supporting structures and therefore cause the decrease in intraurethral pressure and an increase in the mobility of the urethra. Our study did not include any nulliparous patients, 46 women (28.05%) of the cases were primarae and 118 women (71.95%) were multiparae. Obesity and the high body mass index represent a risk factor for SUI, and patients can improve incontinence by losing weight. A comparison was made between the TOT efficiency and safety from the weight point of view (weight excess and normal weight). Patients were divided depending on their body mass index into 3 batches: batch X with normal weight (BMI<25 kg/m^2), batch Y with overweight (BMI 25-30 kg/m^2) and batch Z with obesity (BMI>30 kg/m^2). The study revealed a BMI between 19 and 48kg/m^2, with an average of 28.5 kg/m^2. Of the 164 patients, batch X with BMI<25 kg/m^2 represents 26.8% of the total (44 patients), batch Y with BMI 25-30 kg/m^2 includes 79 patients (48.2% of the total), and batch Z includes women with BMI>30 kg/m^2, namely 41 patients (25%) (tab. I, fig.1).

TABLE I

Distribution in batches depending on the BMI

	Batch		
	X	**Y**	**Z**
BMI (kg/m^2)	<25	25-30	>30
No. of patients	44	79	41

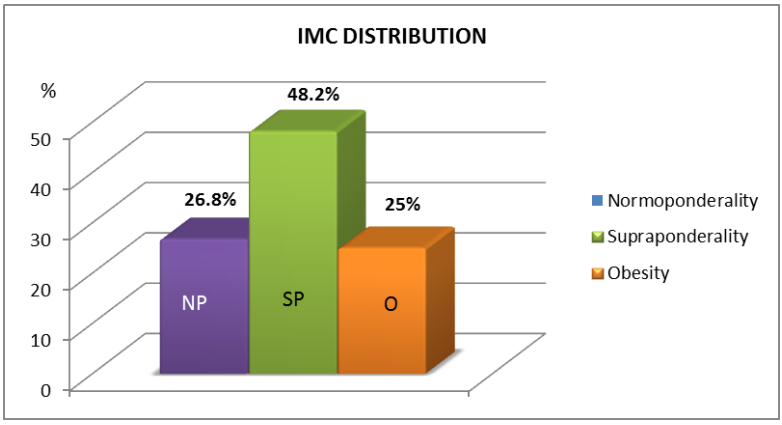

Fig. 1. Distribution of cases in the three batches: X, Y and Z

From the point of view of the associated conditions, 48 patients (29.3% of the total number of patients included in the study) had associated pathology: 5 patients had angina pectoris, 12 suffered from high blood pressure, 22 had chronic venous insufficiency, 15 had dyslipidemia, 3 had hypothyroid and 2 had left renal lithiasis (fig. 2).

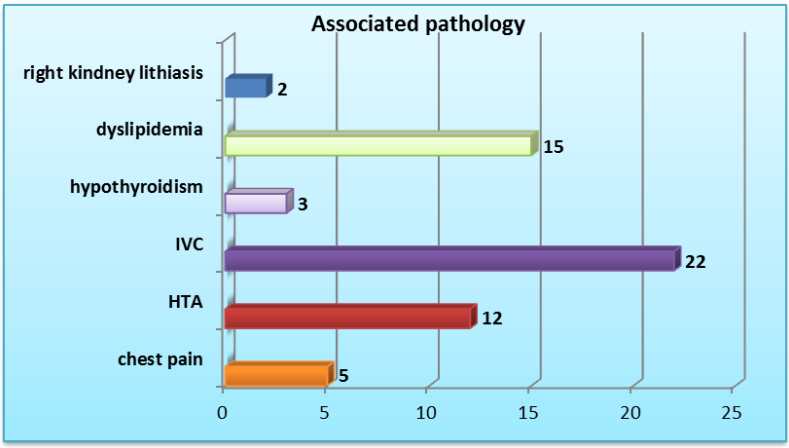

Fig. 2. Distribution depending on the associated pathology

We found isolated SUI in 60 cases, genital prolapsed associated with UI in 63.41%. Of the 104 patients, rectocele was associated in 89 (85.58%), cystocele 11 (10.58%), and both cystocele and rectocele in 4 patients (3.84%) (fig. 3).

The duration of surgery varied between 20 minutes and 1 hour and 20 minutes, with an average of 35 minutes.

The insertion of the TVT-O® tape was a sole operation in 37.8% of the cases, namely in 62 patients, and in 62.2%, namely 102 patients, anterior colpoperineorraphy was associated with posterior colpoperineoraphy, myorhaphy anal levators and right labial cystectomy (3 cases).

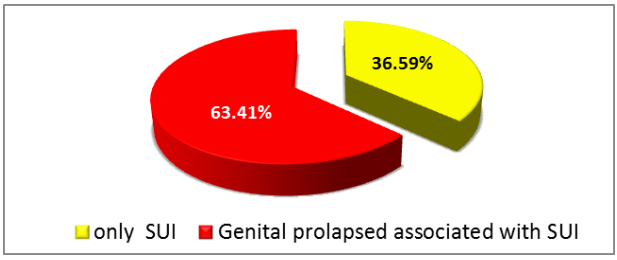

Fig. 3. Stress urinary incontinence associated or not with genital prolapsed

From the anesthetic point of view, general anesthesia was performed with orotracheal intubation in 25 (15.24%) patients' general anesthesia using the larynx mask in 17 (10.37%) and rachianesthesia in 64 (74.4%) patients.

The average hospitalization time was 3.75 days, between 2 and 7 days.

In our study, the cure rate was 85% satisfactory and 15% partially satisfactory, after 2 months as well as after 6 months and 12 months.

52 (31.7%) of the 164 patients did not come for postoperative check-ups, therefore only 112 patients – 68.3% - were monitored immediately, after 2 months, 6 months and 12 months. As regards complications, 16 (14.28%) had improved but persistent SUI, 14 (12.5%) experienced previously inexistent imperious need to urinate (prosthesis positioned too tightly), which were treated with Oxybutynin or by sectioning the prosthesis in 4 cases, and in other 4 cases of prosthesis exteriorization in the check-up after 2 months after surgery, for which a secondary vaginal suture was performed.

The bladder voiding dysfunction after surgery for incontinence is a possible complication of all procedures treating SUI. The avoiding dysfunction term indicates the term of obstructive uropathie until the full urinary retention and includes irritative symptoms such as hyperactivity of the detrusor or imperious need to urinate de novo.

The impossibility of storing urine is determined mainly by the hyperactivity of the detrusor muscle. There are basic theories of this hyper reactivity that include hypersensitivity acquired through the parasympathetic denervation produced by the obstruction of the urinary bladder, denervation produced by surgical dissection regardless of the voiding obstruction. The voiding impossibility is given mainly by the obstructive voiding of the urinary bladder determined by the increased pressure generated by urethral compression. The sutures placed too medially and therefore too close to the urethra can lead to urethral deviation or periurethral scars.

Among postoperative complications, there were three cases of allergic reaction to Ciprinol, in which the antibiotic was replaced with Gentamicin based on prior testing, and 1 case of urine loss upon suppression of the urinary probe, which was corrected with Oxybutynin (Driptane) the second postoperative day.

DISCUSSION

In order to establish the individual statistical risk for every woman of developing SUI, a rigorous evaluation of all health problems that she can have (or has) is necessary. Other factors, such as obesity, parity, hormonal status, can be risk factors for SUI.

A large number of long-term studies are necessary to establish the efficacy of the transobturator tape in comparison to the TVT technique. Trying to define the cure rates of this surgical procedure, Novara et al. say in a systematic analysis of literature that patients treated by the TOT technique had slightly lower rates of complications than the ones treated by the TVT method, with a significantly low risk of vagina perforations.

Our study revealed that the TVT-O® technique was as safe and efficient in treating SUI, regardless of the body mass index. After surgery, life quality improved the same way for each patient, regardless of the batch to which she belonged, depending on weight. There were no significant differences as regards complications. Our study revealed a BMI between 19 and 48 kg/m^2, with an average of 28.5 kg/m^2. The 164 patients were divided into 3 batches, X, Y and Z, respectively, with normal weight, overweight and obese, with the highest percentage in batch Y, with a BMI

between 25 and 30 kg/m^2, representing overweight.

Most hospitalizations were of persons over 50, which is associated with the "enrichment" of the morbid status that comes with age. Our study revealed that the average age of patients diagnosed with SUI type II is 53 years-old.

56.76% of the 164 women included in the study were menopausal and 40.24% cases had genital activity.

Adding the TVT-O® intervention during the anterior prolapse correction seems to convey higher durability to the correction resulting in the long run in a lower rate of the urinary symptoms and a better life quality in comparison to isolated anterior colporraphy. Isolated SUI was found in 60 cases, genital prolapsed associated to SUI in 63.41% of the total number of patients.

Moreover, in subsequent postoperative consultations, no patient had increased body mass index and no patient was susceptible of recurrent SUI, which contradicted the literature results of studies on incontinence surgery in obese patients (7).

In comparison to the literature data, patients were monitored for 29 months and the cure rate was 78.8%, in what concerns our study, the cure rate is slightly higher-85%- but the monitoring period in our study was 12 months.

The absence of intraoperative complications is in accordance with the literature data. As for postoperative complications, specialized literature reports 11.2%, and in our study postoperative complications were 25% (8).

Abdel-Fattah et al. (9) did a secondary analysis, in a randomized study on women who had any type of surgical intervention for SUI and showed that TOT had a good success rate (he referred to a success rate and a cure rate of 69.6 and 76.5% respectively, but there were no significant differences in the cure rates for the insertion of the tape through the obturator hole from the outside to inside and from the inside to outside).

Based on their report regarding the data available in the two randomized clinical studies, Novara et al. (10) could not demonstrate any significant difference between TVT-O and TOT.

In a randomized multi-centric study done in 2007, Porena et al. (11): 148 women, of which 73 underwent TVT and 75 underwent TOT, concluding that both surgical approaches were as safe, without significant ongoing differences of the intra- and postoperative complications during the 2-year monitoring period.

Another element to be discussed is the urodynamic tests. As regards our study, preoperative urodynamic exploration was not performed, which is a disadvantage, because this exploration could help in selecting cases more efficiently and in increasing the patients' satisfaction degree.

CONCLUSIONS

Our study data demonstrate that the TVT-O® procedure is safe and effective and that the body mass index did not influence the result during 12 month (in which the patients included in the study were monitored) and does not have contraindications for continent surgery.

The treatment for SUI has undergone revolutionary improvement for the past 15 years. The transobturator techniques either from the inside to outside or from the outside to inside have been proposed this past decade precisely to minimize complications.

As regards the cure rate, both proce-

dures –TOT and TVT-O®– improved women's quality of life and have almost equal cure rates.

The TVT-O® surgical technique is a minimally invasive procedure characterized by simplicity, reduced hospitalization, short convalescence period, and immediate results. Intraoperative complications are absent and postoperative complications are few and solving them does not compromise the continence results.

The main purpose of the *"Sling"* intervention is to create a certain closing mechanism helping in the passive retention of urine by compressing the urethra.

Important randomized clinical studies with a longer monitoring period related to the said techniques are necessary to establish exactly the efficacy and safety of the said minimally invasive technique.

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BILATERAL BREAST CANCER: DIAGNOSIS AND PROGNOSIS

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BILATERAL BREAST CANCER: DIAGNOSIS AND PROGNOSIS (Abstract): **Aim**: To assess bilateral breast cancer patients, initially diagnosed with stage II unilateral breast cancer. **Material and Methods**: 113 patients with stage 0-II breast cancer diagnosed between 1983 and 2011 were assessed. Of these, 8 patients had bilateral breast cancer: 7 patients with metachronous bilateral breast cancer and 1 patient with synchronous breast cancer. Breast ultrasound, mammography, computed tomography and magnetic resonance imaging were used to diagnose recurrence, loco regional and distant metastasis. **Results**: Age at diagnosis ranged from 37 to 59 years, with a maximum age incidence in the 4th decade (age between: 31-40 years). The average time interval between the two breast cancers was 8.125 years. The most common histological type was invasive ductal carcinoma. All eight patients with bilateral breast cancer had at least one type of recurrence/metastasis, mostly in the liver, and statistically the pleuro-pulmonary and liver metastases were the most frequent causes of death. **Conclusions**: Patients in the 4th decade diagnosed with unilateral breast cancer are at risk of developing bilateral breast cancer. In metachronous breast cancer, the time interval between the detection of the second breast cancer and death is directly proportional to the time interval between the two breast cancers. **Keywords**: BILATERAL BREAST CANCER, METASTASES, DEATH.

Primary bilateral breast cancer is rare, with a reported incidence ranging between 0.5 – 12% (1, 2). Synchronous form is less common than the metachronous one, with a ratio of 1:4 (3). The second breast cancer in the contralateral breast is more frequently diagnosed today than in the past due to the implementation of the national follow-up protocol after surgical treatment.

Bilateral breast cancer is a particular type characterized most often by a genetic component, occurs in a hormonal context and is characterized by an aggressive development (1, 4, 5).

MATERIAL AND METHODS

We retrospectively analyzed 113 patients with stage 0-II breast cancer, treated with radical or breast-conserving surgery. They were diagnosed with mammary neoplasia between January 1983 - December 2011, and deaths from breast cancer were recorded between January 2005 - December 2012. After diagnosis, patiens were referred to the

Oncology Clinics of the Iasi „Sf. Spiridon" University Hospital or Iasi Regional Cancer Institute, where they were assessed according to the national follow-up protocols. The imaging tools included standard mammography, breast ultrasound, computed tomography and magnetic resonance imaging, which allowed the detection of locoregional recurrences or distant metastases.

For statistical data analysis we used SPSS 16.00 for Windows to determine the frequency, multiple regression, enter method, regression coefficient, Pearson correlation (r), relative risk (RR) assessment, and odds ratio (OR), Chi-Square Tests, ANOVA test, test of significance (significant at $p<0.05$) and confidence interval (CI).

RESULTS

Of the 113 cases included in the study, 8 (7%) patients had bilateral breast cancer. Of these 7 (87.5%) patients had metachronous bilateral breast cancer and 1 (12.5%) synchronous breast cancer. In patients with metachronous breast cancer the interval between the two cancers was variable, as follows: 3 years in 1 patient, 5 years in 2 patients, 6 years in 2 patients, 15 years in 1 patient and 24 years in 1 patient.

In the study group the average time interval between the two breast cancers was 8.125 years (tab. I).

TABLE I

Case distribution against time interval

Breast Cancer type	No. of cases	Minimum time interval between breast neoplasms (yrs.)	Maximum time interval between breast neoplasms (yrs.)	Average time interval between breast neoplasms (yrs.)
Bilateral	8	0	24	8.1250

Age at the time of diagnosis of the first breast cancer wranged from 37 to 59 years, with a maximum frequency in the 4th decade (4 cases) (fig. 1).

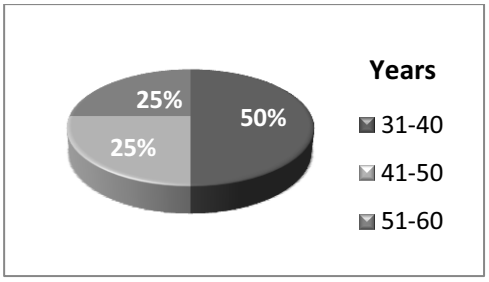

Fig. 1. Case distribution according to age at the time of diagnosis of the first breast cancer.

All patients were initially diagnosed with stage II breast cancer in one breast. Controlateral breast cancer was diagnosed as:

- stage I, in 5 patients, (breast cancer in the second breast occurred during the first six years after the first diagnosis of cancer);
- stage II, in 2 two patients (breast cancer in the second breast occurred 15 to 24 years after the first diagnosis of cancer);
- stage II, in one patient with synchrounous breast cancer.

Surgical procedures included bilateral radical mastectomy (3 patients), radical mastectomy in one breast and breast-conserving surgery in the other breast (3 patients), bilateral breast-conserving surgery (2 patients). Histopathology revealed that the most frequent type was invasive ductal carcinoma (5 cases), while intraduc-

tal carcinoma and invasive lobular carcinoma were found in one case each. One patient had invasive ductal carcinoma associated with ductal carcinoma in situ. It was noted that in a majority of cases (5 cases) the right and left breast tumors shared the same histological type, but in 3 cases the right and left breast tumors were of different histological types. Immunohistochemistry revealed that the tumors were in 5 (62.5%) patients' hormone receptor-positive and Her-2/neu protein-negative, in 2 (25%) patients hormone receptor-negative and Her-2/neu protein-negative, and in 1 (12.5%) patient hormone receptor-positive and Her-2/neu protein-positive.

All 8 patients with bilateral breast cancer had at least one type of recurrence/metastasis. Three patients had only one type of metastasis; two patients had two types of metastasis; one patient presented three types of metastasis and two patients four types of metastasis. Liver metastases were the most common (5 patients) and pleural metastases the less common (1 patient) (tab. II).

TABLE II

Case distribution by type of recurrence/metastasis

Metastasis	Local	Lymph nodes	Bone	Lung	Pleura	Liver	Skin
No. of cases (%)	3 (37.5)	2 (25)	3 (37.5)	2 (25)	1 (12.5)	5 (62.5)	2 (25)

The relative risk and odds ratio (OR) of any metastasis to determine other types of metatstasis were evaluated. When OR>1, the data are statistically significant. Although liver metastases were the most common, they were not a risk for the occurrence of other types of metastasis, being the sole type of metastasis in two patients. Only local recurrence and lymph node metatstases presented a risk for the occurrence of other types of distant metastases. Significant associations were found between local recurrences and lymph node recurrence (OR = 15.333) and lymph node recurrence and skin metastases (OR = 7.636).

The time interval between first diagnosis and death was variable: 5 years (2 patients), 6 years (2 patients), 7 years (1 patient), 9 years (1 patient) and 25 years (1 patient).

The time interval between the diagnosis of the second cancer and death was: 1 year (2 patients), 2 years (2 patients), 3 years (1 patient), 4 years (1 patient), 5 years (1 patient) and 8 years (1 patient).

In our study group, all 8 patients had bilateral breast cancer and breast cancer as a cause of cancer death. The Pearson correlation coefficient (r) was claculated to detect the association between cause of death and bilateral breast cancer or type of secondary metastases that could cause death. Pearson correlation coefficient is used to measure the strength of a linear association between two variables, where the value r = 1 means a perfect positive correlation, value r = -1 means a perfect negataive correlation, and 0 is no correlation.

In our case, it showed a significant negative correlation between causeof death and bilateral breast cancer (r = -0.246), between cause of death and lung metastasis (r = -0.562), pleural metastasis (r = -0.394) and liver metastasis (r = -0.506).

DISCUSSION

In our study the incidence of bilateral breast cancer was of 7%, similar to that reported in the literature (6), with the pre-

dominance of bilateral metachronous cancer.

A peculiarity of bilateral breast cancer in our study was the age at the first cancer diagnosis. While the literature shows an increased frequency of unilateral breast cancer in the fifth decade of life (51-60 years), in our study the patients had their first breast cancer diagnosed most commonly in the fourth decade (31-40 years) (7, 8). One particular aspect is that in most patients the breast cancer in the second breast was lower stage than in the first one (9). Thus, in all patients included in the study, the first malignancy was diagnosed in stage II and the second malignancy in stage I (5 cases). This was possible when all steps in the breast cancer imaging protocol were followed. This protocol provided a close follow-up of patients during the first 5 years after surgery: usually breast ultrasound at six months and annual mammograms. In the other two metachronous breast cancer cases, the contralateral cancer was diagnosed in stage II (more than 10 years after the first diagnosis). In these patients with stage II controlateral disease, the diagnosis wasmade during the annual breast cancer follow-up imaging. It is known that bilateral breast cancer at a young age can have a hereditary origin, and these patients may have a genetic predisposition for other type of cancer (10-14). Although our study group included patients who had another type of cancer (pancreas, stomach, colon, cervix, bladder, ovary, lymphoma, tongue), none of the patients with bilateral breast cancer had any other type of cancer.

Histology showed that invasive ductal carcinoma was the most common hystologic type in both breast tumors, and combination histologies were uncommon (one case). Although the literature shows that the immunohistochemical characteristics of bilateral breast tumors are different (15), in our study the immunohistochemical data were similar for the two breast tumors.

Bilateral breast cancer patients had various types of single- or multiple-organ recurrences/metastases, but it was statistically demonstrated that liver, lung or pleural metastases are causes of death.

In most cases with metachronous bilateral breast cancer it was found that the time interval between the second breast cancer detection and death is directly proportional to the time interval between the two breast cancer diagnoses. Thus, it was noted that when bilateral breast cancer was diagnosed after a long time (10-15 years), patient death occurs after a longer time interval (4 to 5 years) as compared to the patientsin which the time interval between the two cancers is shorter (1-5 years), when patient death occurs withinless than 4 years after the second breast cancer was diagnosed. In patients with bilateral breast cancer, metastases from the second breast cancer were the major cause of death.

CONCLUSIONS

This study revealed that patients diagnosed with unilateral breast cancer during the fourth decade of life (31-40 years) are at risk of developing a controlateral breast cancer.

In patients with bilateral breast cancer, early diagnosis depends on breast imaging according to a well-established protocol during and after the first five postoperative years.

In metachronous breast cancer the time interval between the detection of the second breast cancer and death is directly proportional to the time between the two breast cancer diagnoses.

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SEVERE AMIODARONE-INDUCED BRADICARDIA CONCEALES SICK SINUS SYNDROME: CASE REPORT

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SEVERE AMIODARONE-INDUCED BRADICARDIA CONCEALS SICK SINUS SYNDROME: CASE REPORT (Abstract). Sinus node dysfunction is one of the most common arrhythmias in elderly patients; it is usually associated with intermittent and variable symptoms, thus making it difficult to diagnose. We present the case of an elderly female patient with a personal history of atrial fibrillation treated for the last three years with amiodarone; she was admitted to the Geriatric Clinic for non-specific symptoms with onset two months previously for which she had already sought care in different medical services. Clinical examination showed severe bradycardia; ECG and Holter ECG on admission confirmed severe bradycardia, with a heart rate between 29 and 50 beats/min (bpm). Given her long-term treatment with amiodarone we looked for and found hyperthyroidism; the endocrine examination led to the diagnosis of mixed type Amiodarone-induced thyrotoxicosis and initiation of corticosteroid and antithyroid treatment. The evolution of cardiac arrhythmia was monitored with the help of several Holter ECGs performed after amiodarone washout and return to the euthyroid state, which revealed a tachycardia-bradycardia syndrome initially masked by the side effects of the unsupervised therapy with amiodarone, and properly treated by the implantation of a pacemaker. **Keywords**: SINUS NODE DYSFUNCTION, TACHYCARDIA-BRADYCARDIA SYNDROME, HYPERTHYROIDISM, AMIODARONE.

There are several normal age-related changes of the cardiovascular system, one of the most important affecting the conduction system with increasing fibrosis around the sinus node and loss of specialized cells and fibers. These changes, associated with a decreased response to sympathetic agonists would lead to a decreased peak heart rate and a significant decrease in heart rate variability, resulting in bradycardia, which may be further exacerbated by medication and ischemia (1). Older patients often have delayed conduction in the atrio-ventricular (AV) node, which would generate different types of AV blocks, that are usually without symptoms and could progress to complete AV block (2).

These significant changes in the anatomy and function of the conduction system of the aging heart explain the increased frequency of sick sinus syndrome, a group of arrhythmias presumably caused by a malfunction of the sinus node, including sinus arrest, sinus node exit block, sinus bradycardia and tach-

ycardia-bradycardia syndrome.

The manifestations of this disease are diverse, intermittent and often difficult to surprise on casual ECG (3), reason for which the epidemiological data are limited and the incidence of this disease in the general population remains unclear (4, 5). Syncope and/or fatigue are the cardinal symptoms in the bradycardia forms, and palpitations in the tachycardia forms, but it is not uncommon (especially in the elderly) that symptoms to be limited to dyspnea, reduced exercise capacity or mild cognitive impairment (sometimes interpreted as the onset of dementia syndrome) (6). In this context, Holter ECG monitoring remains the investigation of choice, identifying not only the accurate diagnosis but also the right moment for the implantation of pacemaker.

When confronted with fast atrial fibrillation, controlling the ventricular rate should be of the outmost importance, and the favorite antiarrhythmic drug is amiodarone (5). This trend led to a massive use of this drug in elderly patients, which was accompanied by a constantly increasing number of unwanted side effects, most frequently occurring at thyroid level. The lack of periodic and systematic control can be accompanied by serious clinical situations, such as severe bradycardia, that can be masked by nonspecific symptoms and thus easily overlooked and undertreated.

CASE PRESENTATION

We present the case of an 86 year old female patient admitted to the Geriatric Clinic for several non-specific symptoms, such as: dry and irritative cough, vertigo and generalized itching with nocturnal exacerbation. The onset of these symptoms was insidious about two months prior to this hospitalization, the patient having already sought care in different medical services. On admission she presented: facial erythema, scratching lesions on the posterior chest wall, rhythmic heart rate of 46 beats/min, and bilateral upper limb resting tremor. Her medical history revealed an episode of fast atrial fibrillation that had occurred three years earlier and was successfully treated with amiodarone, although the biochemical thyroid function tests indicated at that moment subclinical hyperthyroidism, with TSH concentration at the lower limit of normal range but with fT4 and fT3concentrations within the normal range.

Our diagnosis was severe bradycardia, most probably due to thyroid dysfunction secondary to the long-term, unsupervised treatment with amiodarone. The biochemical tests revealed very low serum levels of TSH hormone (TSH = 0.0l pmol/l), high levels of fT4 (fT4 = 30.8 µUI/ L), and normal levels of fT3. ECG on admission confirmed sinus bradycardia (Figure 1); however, the 24-hour ECG monitoring showed sinus bradycardia alternating with idioventricular rhythm; the average heart rate was 35 bpm, range 29 to 50 bpm; the absence of any cardio-cerebral symptoms allowed the postponing of pacemaker implantation.

The endocrine examination confirmed the diagnosis of mixed type amiodarone-induced thyrotoxicosis, and a treatment with corticosteroids and antithyroid drugs was initiated.

The clinical course was favorable, as the patient reported the disappearance of dry cough and prurigo 48 hours after the discontinuation of amiodarone and the heart rate slowly ascended (up to 50 bpm). A 24-hour ECG monitoring 1 month after the discontinuation of amiodarone treatment showed an average heart rate of 45 bpm, range 39 to 100 bpm, which led us to suspect an underlying, unsuspected bradycardia-tachycardia

syndrome. A third 24-hour ECG monitoring was done after 6 weeks of thyroid treatment, when thyroid function had already returned to normal; hence, we considered that this one would reflect the correct cardiac status of our patient'. The result was very much the same as the previous one, thus confirming the diagnosis of bradycardia-tachycardia syndrome, for which a pacemaker was implanted.

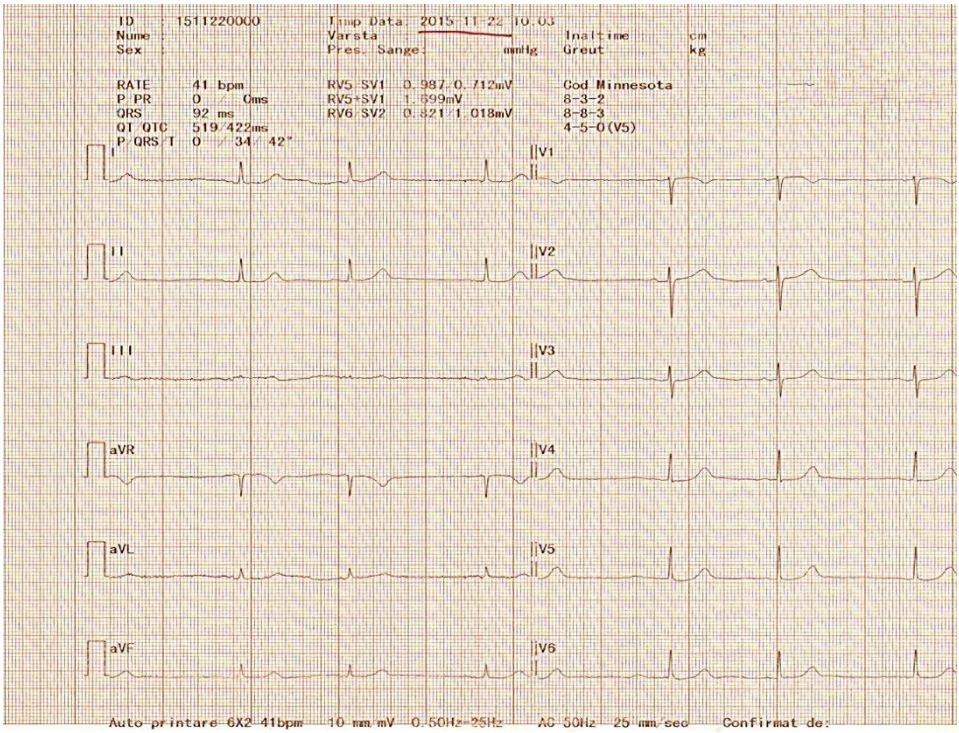

Fig. 1. ECG aspect on admission

DISCUSSION

The normal aging process of the heart includes fibrosis and loss of specialized cells and fibers of the conductive system, which would favor an increased incidence of sick node dysfunction. This condition can take many forms, including bradycardia- tachycardia syndrome. The diagnosis of this form can be difficult, as random ECG is more often normal. The bradycardia episodes are usually without any symptoms; the tachycardia episodes are associated with palpitations and the patient is wrongly diagnosed with only tachyarrhythmia and would receive an antiarrhythmic treatment, usually amiodarone. This drug should be carefully monitored, not only for the well-known side-effects, but also for the negative effect on the bradycardia episodes.

Numerous studies (7, 8, 9) show that low doses of amiodarone can be used safely in patients with fast arrhythmias and normal systolic function. However, the length of the treatment with amiodarone proved to be an independent risk factor in the prediction of adverse effects (4), mainly changes at the cardiovascular (im-

portant bradycardia), thyroid (hyper- and hypothyroidism), pulmonary and hepatic level (9, 10).

Still, the absence of cardiac symptoms (syncope, fatigue) and hyperthyroidism led to a delay in the diagnosis. This case also presented some of the less known side effects of amiodarone, that include nervous system disorders (paresthesia, pain and muscle weakness, tremor, walking and moving disorders, headache, vertigo), gastrointestinal disorders (nausea, taste alteration, constipation, lack of appetite) or skin disorders (itching, petechiae) (8) that can be easily interpreted as a concomitant chronic or aging-related disease.

CONCLUSIONS

Our case emphasizes the need for sustained monitoring of treatment with amiodarone; the multiple side effects (cardiovascular and thyroid) associated with the progression to a higher-degree AV block led to severe bradycardia, fortunately without cerebral and cardiac consequences.

The therapeutic decision, especially in tachycardia-bradycardia syndrome should be carefully considered; the patients that require antiarrhythmic therapy should be rigorously monitored, mainly those treated with amiodarone, and the 24-hour ECG monitoring should be the safest way for identifying the excessive secondary "bradicardization".

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POSTOPERATIVE ARTERIAL RELAXATION AND INTRAOPERATIVE VENOUS DILATION AS INTERCONNECTED PROGNOSTIC FACTORS FOR THE MATURATION OF ARTERIOVENOUS FISTULAS

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POSTOPERATIVE ARTERIAL RELAXATION AND INTRAOPERATIVE VENOUS DILATION AS INTERCONNECTED PROGNOSTIC FACTORS FOR THE MATURATION OF ARTERIOVENOUS FISTULAS (Abstract) **Aim**: To investigate if immediate arterial distention can be used as a predictive factor for the development of a good fistula. **Material and methods**: Over a 5-months period (January- May 2015) all the patients who underwent an arteriovenous fistula between the radial artery and the cephalic vein of the forearm at the Second Surgical Clinic of the Iasi Regional Cancer Institute and were willing to participate were enrolled in the study. The diameters of the vessels were measured 1 hour and 8 weeks after surgery. **Results:** We found statistically significant differences for all measured diameter variations between the calcified artery and normal artery groups ($p<0.001$ for the arterial distention at 1 hour and 8 weeks after surgery and $p=0.002$ for the venous distention 8 weeks after surgery). A linear regression also showed that the degree of arterial distention immediately after surgery and the venous distention 8 weeks after surgery were statistically correlated. **Conclusions**: Arterial distention immediately after surgery and therefore the lack of it due to the presence of arterial calcifications can be used to predict whether or not a good fistula can be achieved at a 1% statistical significance level. **Keywords**: HAEMODIALYSIS ACCESS, ARTERIOVENOUS FISTULA, PREDICTIVE FACTORS OF FISTULA MATURATION, ARTERIAL RELAXATION

INTRODUCTION

The purpose of any arteriovenous anastomosis for hemodialysis access is the development of a functional fistula which is as close to the ideal as possible. As a definition, an adequate fistula is located at a depth of 6 mm from the skin surface, has a minimum diameter of 6 mm and can accommodate a blood flow higher than 600 ml/minute (1). A major limiting factor in achieving such a fistula is the caliber of the vessels to be anastomosed, not necessarily from a technical point of view but because there are certain limits below which the development of an adequate fistula is impossible. Parmar et al. (2) showed that 45.5% of the arteriovenous fistulas created by using an artery with a diameter < 1.5 mm had failed to mature within 12 weeks, compared to an artery > 1.5 mm where

maturation was reached in all cases. They also found statistically significant difference in blood flow rates if the artery before surgery was <1.5 mm (347 ml/minute) or >1.5 mm (521 ml/min). Regarding the vein diameter, Mendes et al. (3) reported that 16% of fistulas were adequate with a vein diameter of 2 mm or less, compared to 76% of those larger than 2 mm. Fila et al. (4) showed that when the initial vein diameter was less than 2 mm only 60.2 % of the fistulas reached maturation. In this group of functional fistulas the intraoperative vein dilation leads to an average 75% increase while in the group of non-functional fistulas the average intraoperative distention of the vein was only 48 %, showing that vein elasticity is also of great importance (4).

MATERIAL AND METHODS

The aim of the study was to evaluate if arterial relaxation immediately after surgery, arterial relaxation within the first 8 postoperative weeks and intraoperative vein dilation correlate with vein distention 8 weeks after surgery, as a measure of fistula maturation.

Between January and May 2015, a number of 19 patients underwent an arteriovenous fistula between the radial artery and the cephalic vein of the forearm at the Second Surgical Clinic of the Iasi Regional Cancer Institute.

Of the 19 study patients 12 (63.2 %) were women and 7 (36.8 %) men. The mean age of the patients was 55 ± 13.63 years, range 24-80 years. Eight patients (42.11%) had diabetes. Twelve patients (63.2%) were included in the group of patients with a normal, elastic artery and 7 (36.8%) in the group with calcified artery.

Inclusion criteria: patients with end-stage chronic kidney disease in which an arteriovenous fistula between the radial artery and the cephalic vein of the forearm was created. Exclusion criteria: patients with fistulas other than one between the radial artery and the cephalic vein of forearm; patients unwilling to participate in the study.

The patients enrolled in the study were assigned to 2 study groups according to the macroscopic appearance of the arterial wall during surgery: the first group included diabetic patients with calcified arteries that were practically non-distensible, transformed into a rigid tube; the second group included patients with a macroscopically normal artery.

Before surgery the blood pressure was measured in both arms and vein diameter was measured using ultrasound (5) at a point located 4 cm above the anastomosis so as not to compromise the wound dressing when measuring the vein after surgery. The initial measurement of vein diameter was performed without a tourniquet in order to assess its ability to distend. The diameter of the artery was also measured at 4 cm above the anastomosis. The measurements were taken preoperatively, one hour postoperatively, and 8 weeks after surgery (during the first dialysis appointment). Each measurement was performed 3 times and the average of these measurements was recorded.

All of the patients underwent an arteriovenous anastomosis in a side-to-end fashion between the radial artery and the cephalic vein of the forearm. The diameter of the venous side of the anastomosis was increased by up to 50% by using a longitudinal incision in the venous wall. That was the reason why in all cases the anastomosis diameter was 50% larger than the vein diameter before surgery. The vein was

dilated by injecting an isotonic saline solution while digitally compressing the vein. After surgery, all patients were advised to perform flexion exercises for 8 weeks.

The statistical analysis of the data was performed using IBM *SPSS* version 17.0 for Windows. The statistical tests performed were the student t-test in order to check for statistically significant differences between groups and linear regression in order to assess whether or not immediate arterial and venous dilation can be used to predict the fistula outcome. For this study, a 5% significance level was considered satisfactory.

RESULTS

We compared the percentage of arterial and venous distention immediately after surgery and at 8 weeks after surgery and the percentage of the vein dilation at 8 weeks after surgery, between the patients with a macroscopically calcified radial artery and those with a macroscopically normal artery, as assessed during surgery.

The mean arterial distention 1 hour after surgery in calcified artery patients was 2.28 ± 3.23% compared with 37.52 ± 12.75% in normal artery patients. When assessed at 8 weeks after the procedure, the mean arterial distention in the calcified artery group was 9.36 ± 9.36 %, while in the normal artery group it was 97.25 ± 47.18 %.

As to vein distention we found that intraoperative distention in the calcified artery group was 202.24 ± 105.69 %, while in the normal artery patients it was 117.99 ± 112.19%. The venous distention 8 weeks after surgery in the calcified artery group was 31.85 ± 5.44 % and 95 ± 44.11% in the normal artery group.

We then analyzed the differences between these diameter variations from a statistical point of view using an independent samples t-student test. We found that the difference between the calcified artery and normal artery groups was statistically significant for all diameter variations with $p<0.001$ for arterial distention at 1 hour and 8 weeks after surgery, and $p=0.002$ for venous distention 8 weeks after surgery.

As a final step in our analysis we performed a linear regression analysis of arterial distention 1 hour after surgery and venous dilation 8 weeks after surgery and we came to the conclusion that arterial distention immediately after surgery and therefore the presence of arterial calcifications can be used to predict whether or not a good fistula can be achieve at a 1% statistical significance level ($p<0.001$, r-square 0.72). However, when comparing intraoperative venous distention and venous distention 8 weeks postoperatively we found that intraoperative venous dilation is a less effective predictor of fistula outcome ($p=0.021$, r-square 0.24).

DISCUSSION

Determining whether a fistula is adequately matured or not is very important and hemodialysis nurses can do that. In more and more studies their experience has been considered the best tool for fistula assessment, before even measuring the flow through the fistula. The clinical criteria followed are: a superficial vein, with a diameter adequate for the puncture, with a uniform thrill and a length of minimum 10 cm available for puncture (6).

Once the fistula is opened during the procedure, the wall of the vein withstands a higher pressure than the venous pressure which causes structural alterations of the wall. Depending on the capacity of the wall to distend the vein lumen it thickens by

hypertrophy of smooth muscle cells. Thus, the venous wall becomes resistant to repeated punctures and extrinsic compression (7).

When the vascular clamp is removed from the artery after the anastomosis is completed there is a rapid redistribution of the blood flow in the region depending on pressure gradients and flow resistance. The overall resistance downstream to the artery anastomosed to the vein decreases. The flow thus increases through a constant section of the artery and the speed of the flow and the shearing stress are also increased leading to a progressive dilation of the artery (8).

The mechanism through which the arterial wall becomes increasingly rigid and thus reduces the vascular compliance required to maintain adequate flows through the fistula are not entirely known. They may include fluid overload, arterial calcification, inflammation, activation of the sympathetic and rennin–angiotensin system, increased lipid oxidation, nitric oxide anomalies (9). The increased arterial wall rigidity has direct implications on fistula outcome because given the inadequate blood flow through the fistula hemodialysis is not effective. An example is the diabetic patient in which the accelerated atherosclerosis transforms the artery into a rigid tube unable to relax and increase the blood flow (10).

A key role in arterial wall relaxation is played by the nitric oxide. Its synthesis is increased by the alterations in local blood flow, as shown by many studies (10, 11, 12). It is also responsible for the activation of metalloproteinases in the extracellular matrix of the cells within the vascular wall. These lead to the destruction and reorganization of the cellular matrix that could explain the changes occurring in the vascular wall (13).

The parameters analyzed in our study were the intraoperative venous dilation, the postoperative arterial dilation measured 1 hour after the procedure and the arterial and venous dilations 8 weeks postoperatively. The choice of these parameters was based on physiological reasons, as we believed they all play a role in achieving an adequate venous distention: the intraoperative venous dilation is a measure of vein elasticity; the venous distention between the measurement performed 1 hour after procedure and 8 weeks after surgery is due to hemodynamic factors and vein elasticity; the arterial distention between the preoperative measurement and the measurement 1 hour after the procedure evaluates the immediate capacity of the artery to relax after the decrease in the resistance distal to the fistula; arterial distention 8 weeks after the procedure shows how well the artery has accommodated to the new hemodynamic conditions in order to increase the flow through the fistula.

In our study, in the patients with calcified arteries the differences between the diameter of the artery before the procedure and 1 hour after the procedure were, as expected, quite small, due to the fact that the calcified artery is basically a rigid tube. However, in the group of patients with macroscopically normal arteries, there was a significant increase in the diameter of the artery after surgery, but the increase was extremely variable, depending on each patient's hemodynamic condition, as shown by the large standard deviation.

The venous dilation was considered of great importance in our study because the vein size at 8 weeks after the procedure is very important in determining whether the fistula had adequately matured or not. It is for this reason that we tried to assess

whether arterial distention immediately after surgery and arterial distention 8 weeks after surgery can be used to predict adequate fistula maturation, assessed in our study by the distention of fistula between the measurement performed 1 hour after surgery and 8 weeks after surgery. We found that both the immediate and late arterial distention are a good predictor of late venous distention, and even better than the intraoperative venous distention. This shows that an artery capable to relax in order to increase the flow through the fistula is essential for an adequate maturation of the fistula.

An adequate increase in the diameter of the vein or artery alone does not independently result in a patent fistula.

If the vein is elastic and dilates well during the procedure it does not mean that it will produce a good fistula unless the artery is able to relax and increase the blood flow.

At the same time a healthy, non-calcified artery can in time progressively dilate a vein that appeared to be unable to dilate during the procedure. Arterial relaxation happens immediately in normal arteries and our study shows it can be used as a prognostic tool of adequate fistula maturation.

CONCLUSIONS

We have shown that good immediate arterial distention also means an adequate, matured fistula 8 weeks after the procedure. At the same time the immediate venous distention is also a prognostic factor of an adequate fistula and we believe that together the elasticity and compliance of the arterial and venous walls are inextricably linked in determining the outcome of a fistula.

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NEWS

SELENIUM SUPPLEMENTATION VIA BRAZIL NUT IMPROVES THYROID HORMONE LEVELS IN HEMODIALYSIS PATIENTS: A PILOT STUDY

Hemodialysis (HD) for pacients known with renal failure is associated with low levels of total and free T3 (triiodothyronine). This fact that can be explained partialy by a selenium (Se) deficit that is also a common feature for HD. Selenium is an essential micronutrient important for the thyroid hormone metabolism. This study aimed to investigate the effect of selenium supplementation via Brazil nut (rich in Se) on thyroid hormone levels on HD patients. Fourty patients with HD received for 3 months one nut daily that contained in average 58.1 mg Se. All patients presented at baseline a Se deficit, low T3. After the intervention, Se, FT4 (free thyroxine) and GPx (glutathione peroxidase) activity plasma levels arised significantly increased ($p < 0.05$), while the TSH levels were reduced but not significantly. In conclusion, by increasing Se levels an improvement in thyroid hormone levels in HD patients is obtained (Stockler-Pinto M B, Carrero J J, Carvalho L *et al* Effect of selenium supplementation via Brazil nut (Bertholletia excelsa, HBK) on thyroid hormones levels in hemodialysis patients: a pilot study. *Nutr Hosp*. 2015;32(4):1808-1812).

Emilia Ghiță

INCIDENCE OF REASONS FOR REVISION HIP SURGERY: PRELIMINARY STUDY

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INCIDENCE OF REASONS FOR REVISION HIP SURGERY: PRELIMINARY STUDY (Abstract). **Aim**: To analyze the main causes and short/mid-term clinical outcomes of revision total hip arthroplasty**. Material and Methods**: All patients treated over a period of 36 consecutive months at the Orthopedic Clinic of the Iasi Rehabilitation Hospital were analyzed. The causes for total hip arthroplasty (THA) failure were grouped into one of the six categories: aseptic loosening, infection, dislocation, component wear, periprosthetic fracture, and pain. **Results:** The rate of risk for THA failure is differently influenced by factors such as age, weight index, associated comorbidities, gender, and physical activity level. Most revisions (72.4%) were for aseptic loosening. Age group distribution showed that most patients who required revision, regardless of age group, were women. **Conclusions:** Although the risk of infection is higher in the first six weeks after surgery, early infections rarely require partial or total replacement of the prosthesis. Infections that require prosthesis revision are late and deep, but the incidence of such complications is low. Loosening is the most common cause of THA failure. **Keywords:** ARTHROPLASTY, CAUSES OF FAILURE, LOOSENING, INFECTION.

Most studies on the success rate of primary hip arthroplasty show that this surgical procedure provides early resumption of joint mobility and normal functioning of the articular and periarticular structures that control the movement of the coxofemoral joint. Even though primary THA ensures restoration of hip biomechanics, complications affecting the quality of reconstruction, which lead to THA failure and require partial or total revision may occur with time (1).

Several authors have reported success rates of about 90% for primary THA 10 years after surgery, increasing to 95% in patients over 75 years (2). As to the incidence of causes for revision articular reconstruction the reported values relatively differ from one study to another, depending on the geographical area and prevalence of risk factors (3, 4, 5).

A study aimed at assessing the indications for revision and understanding the mechanisms and timing of primary THA failure revealed that high activity level is an important risk factor especially in

younger patients (6).

The aim of this study was to analyze the main causes and short/mid-tem clinical outcomes of revision THA in the Orthopedic Clinic of the Iasi Rehabilitation Hospital.

The overall objective of this study was to identify and rank the causes of primary hip arthroplasty failure in order to optimize joint reconstruction.

The study had the following subjacent goals: to identify and analyze the reasons for primary THA failure, to evaluate the mid-term clinical outcomes of revision THA,and to compare our results with those of similar studies.

MATERIAL AND METHODS

The study protocol included:

• collection, centralization and grouping of clinical data and information on the prosthetic materials used in revision hip arthroplasty;

• statistical processing of clinical data in order to determine the level of significance of the findings regarding the reasons for revision hip arthroplasty;

• creating a database on revision hip arthroplastiesin view of evaluating and correct selection ofthe prosthesis and arthroplasty technique for a reduction in the failure rate of endoprostheses.

The retrospective observational study was conducted on a total of 29 cases operated at the Orthopedic Clinic of the Iasi Rehabilitation Hospital in the interval 2011-2014.

For this study we used information from the medical records and records of surgical procedures of a consecutive series of 29 revisions performed in 24 patients. The mean postoperative follow-up was 12 months (range 6 to 24 months).

Detailed clinical and demographic data were extracted for each patient: age at first intention hip arthroplasty, sex, diagnosis, prosthesis survival time, and the exact cause/causes for primary hip arthroplasty failure.

The primary data sources included perioperative charts, operative notes, discharge summaries and relevant radiographs.

The primary diagnosis was grouped into the following categories: osteoarthritis, inflammatory arthritis (including rheumatoid arthritis and ankylosing spondylitis), osteonecrosis, hip dysplasia, post-traumatic arthritis and a miscellaneous group of other causes.

Harris hip scores were used to quantify clinical outcomes. Patients were scheduled for clinical and radiological follow-up at 3, 6 and 12 months and annually thereafter. The mean postoperative follow-up was 1 year.

In each case, the reason for failure was classified into one of the following six categories: aseptic loosening, infection, instability, component failure, periprosthetic fracture, or pain. In 3 cases in which there were multiple reasons for failure these were equally considered in systematizing the data.

RESULTS AND DISCUSSION

To analyze the incidence of reasons for revision the information on the 29 revision total hip arthroplasties included in the study were systematized. The causes of failure were:

- component loosening (septic and aseptic) of total hip prosthesis (21 cases; 72.4 %),
- periprosthetic fractures (5 cases; 17.2% - 3 cases Vancouver type B2 and 2 cases Vancouver type B3)
- luxation of hip prosthesis (2 cases; 6.9 %),
- deterioration of the femoral stem (1

case; 3.4 %).

Of the 24 patients, two thirds were women and only one third were men.

The mean age of the study patients at the time of revision was 64 years (range 46-84 years).

Age group distribution of the study patients showed that regardless of age group most of the patients who required revision were women (fig. 1).

The analyzed data showed that in the study group aseptic loosening, with an incidence of 72.4%, is the most frequent reason for revision (fig. 2).

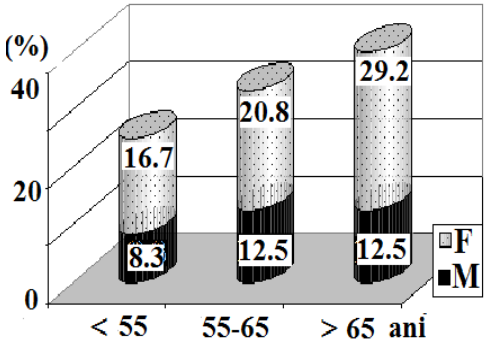


Fig. 1. Age group distribution of the study group

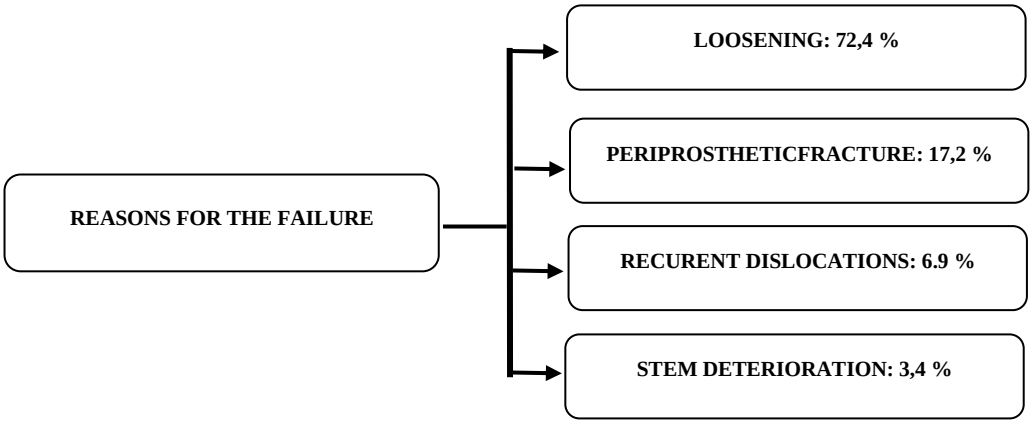


Fig. 2. Incidence of reasons for revision in the study group

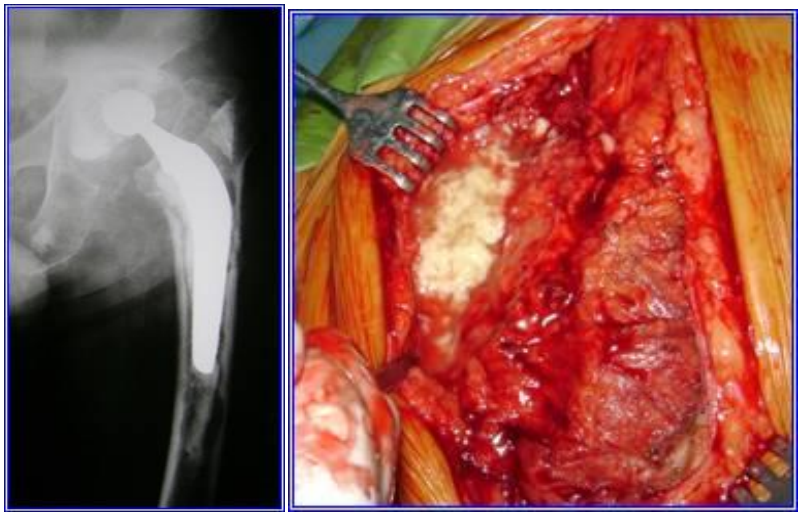
Analyzing the data on the 2.107 first intention THAs performed in France from January 1, 2010 to December 31, 2011, Delaunay et al. (7) showed that the reasons for revision in order of priority were: mechanical loosening (42%), periprosthetic fracture (12%), infection (11%) wear/ osteolysis (11%), dislocation (10%), surgical technique error (6%), implant deterioration (3%). The revision procedure used in half of the cases (49%) was all-component revision. The study also revealed that although dislocation was not the main cause for primary THA failure, it remains the most common early complication after revision.Katz et al. (8) conducted a retrospective cohort study of patients who had elective total hip replacement for osteoarthritis in the United States between July 1, 1995, and June 30,1996,and a 12 year follow-up period. The data showed that the risk of revision THA for the surviving patients was approximately 2% per year for the first eighteen months and then 1% per year for the remainder of the follow-up period. The absolute risk of death over the follow-up period (12 years) exceeded the risk of revision THA by 10 times (59% vs. 5.7%) in patients

older than 75 years at the time of primary THA and by 3 times (29% vs. 9.4%) in patients aged 65 to 75 years at the time of primary surgery.

In multivariate Cox proportional hazard models, the relative risk of revision was higher in men than in women. In patients aged 65 to 75 years at the time of primary THA the relative risk of revision was 1.23 (1.15, 1.31, CI = 95%) and in those over 75 years of 1.47; (1.37, 1.58) CI = 95%.

Corbett et al. (9) identified in the literature a number of population-based studies assessing the 10-year revision risk (2000-2010). The analysis of data in the national THA registries from England and three Scandinavian countries (Sweden, Finland and Norway) suggests revision risks stratified by age and fixation technique of 5% to 20% at ten years after surgery. Kaplan-Meier estimates showed that cemented implants had ten-year revision-free implant survival rates of 88% to 95% and the uncemented implants of 80% to 85%. In patients under 60 years at the time of primary surgery revision-free implant survival rates ranged from 72% to 86% and in those older than 60 years the rates ranged from 90% to 96% (9).

Mobilization of the hip prosthesis may be due to periprosthetic loosening or polyethylene wear. These phenomena, often due to uneven distribution of forces involved in hip biomechanics, cause unnatural tribological couples generating very small particles. The composition of such particles, polyethylene, acrylic cement, ceramic or metal, depends on the endoprosthesis type (10). Patient's immune system will recognize such particles as a foreign body and will generate an immune response. A strong response to wear particles may result in biological bone resorption (osteolysis) and loosening (fig. 3).



(Collection of Iasi Rehabilitation Hospital – Orthopedic Clinic)

Fig. 3. Hip X-ray image of a 68-year-old patient operated upon 9 years earlier with an osteolytic area and acetabular and femoral loosening on hip x-ray; intraoperative view, seropurulent discharge at the proximal femur

Loosening is more likely as the physical demand that will be placed on the joint after surgery is higher. This accounts for the higher incidence of this cause of HTA failure in younger and more physically active patients (fig. 4).

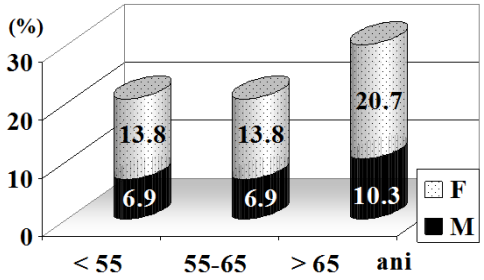


Fig. 4. Incidence of loosening by age group and sex

Although multiple dislocations are rare, some elderly, debilitated patients, or those with other locomotor deficiencies are prone to this complication. The risk of dislocation is higher during the first months after surgery. In case of repetitive dislocations revision surgery is necessary (Fig. 5).

A study by the Mayo Clinic (11) on 10,500 THAs reported a 3 times greater risk of dislocation with the posterolateral approach (4.3%) compared to the anterior (1.8%) and anterolateral (1.7%) approaches.

Dudda et al. (12) found a 6 times greater risk of dislocation with the posterior approach compared with the anterolateral approach or lateral approach with trochanteric osteotomy.

Fig. 5. Upper left hip prosthesis dislocation *(Collection of Iasi Rehabilitation Hospital – Orthopedic Clinic)*

CONCLUSIONS

In our study, data analysis shows that loosening is the most common reason for revision exceeding by one order of magnitude any of the other immediate or late complications.

The rate of the risk of revision THA differ with respect to the mechanism of failure and is influenced differently by such factors as age, weight index, associated co morbidities, gender, physical activity level.

Revision surgery for recurrent dislocations requires repositioning or even replacing of the prosthetic components.

Infection after THA can occur at any time after surgery. Although the risk of infection is higher in the first six weeks after surgery, immediate infections rarely require partial or total replacement of the prosthesis. Infections that require prosthesis revision are late and deep, but the incidence of such complications is low.

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NEWS

ANTIMICROBIAL ROLE OF FILAGGRIN-2 PROTEIN

Filaggrin-2 (FLG2) is a water-insoluble 248 kDa S100 fused-type protein found in the upper epidermis and eccrine sweat glands. A study by Hansmann *et al.* tested the antimicrobial activity of filaggrin-2 against *Pseudomonas aeruginosa* and other Pseudomonads and found that recombinant FLG2 C-terminal protein fragments have potent antimicrobial activity against *P. aeruginosa* and after cultivation of *P. aeruginosa* on stratum corneum, antimicrobially active FLG2 fragments are released from insoluble material by the bacteria themselves. These FLG2 C-terminal fragments have an alternative mode of action different from most other antimicrobial peptides, as they do not induce pore formation, but membrane blebbing. According to Hansmann et al., FLG2 fragments interfere with bacterial replication, inhibit their growth on skin surfaces and contribute to the skin's antimicrobial defense, explaining why *Pseudomonas* infections of healthy skin are very rare. Also, the absence of filaggrin 2 at certain body surfaces and in the case of burned skin seems to be responsible for the higher susceptibility to these infections. The study concluded that FLG2 C-terminal fragments could represent new *Pseudomonas*-targeting antimicrobials (Hansmann B, Schröder JM, Gerstel U. Skin-Derived C-Terminal Filaggrin-2 Fragments Are Pseudomonas aeruginosa-Directed Antimicrobials Targeting Bacterial Replication. PLoS Pathog. 2015;11(9):e1005159. doi: 10.1371/journal.ppat.1005159).

Teodora Vremera

THE IMPORTANCE OF CLINICAL AND INSTRUMENTAL DIAGNOSTIC IN THE MAMMARY GLAND CANCER

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THE IMPORTANCE OF CLINICAL AND INSTRUMENTAL DIAGNOSTIC IN THE MAMMARY GLAND CANCER (abstract): Breast cancer is the most common oncology disease in women and is one of the major public health issues. Worldwide, is the second leading cause of cancer death in women and cancer research is a priority in all the laboratories of the world, in terms of uncovering the appearance causes of the malignant process, understanding the mechanisms of development, but most of all, the discovery of early diagnostic methods and effective treatment. Ignorance, fear of diagnosis, lack of health education and of efficient programmes for prevention and screening could cause diagnosis of the disease to be detected in the majority of cases in advanced stages, when treatment remains only palliative and very costly, in this cases the patient's suffering being immense. In this way, regarding the clinical diagnosis in stage I mammary gland cancer, in the 496 stage I MGC patients, during the primary clinical investigation the diagnosis of stage I MGC was established only in 165 (33.3%) patients, and in 232 (46,8%) patients the diagnosis of suspicion MGC was obtained. Also, in terms of instrumental diagnosis, such as mammography, ultrasonography in mammary gland cancer stage I, it seems that in accordance with literature data the pathological process features assessment in the mammary gland is problematic especially in young age. Thus, it seems that MGC represents a polymorphic and pathogenic disease and it cannot be admitted that all subgroups of patients will obtain identical results from one tactic of treatment determined for all the patients with MGC. In this way, the concept of MGC both clinical and patho morphological, combines different cell clones depending on its microstructure and biology. As a result, the evolution of the disease, the prognosis and the effectiveness of the treatment may vary in different patients at the same stage, depending on the degree of malignancy of the tumor, its histopathological structure, the degree of expression of molecular markers identification and also immune resistance. **Keywords:** MAMMARY GLAND CANCER, BIOLOGICAL RELEVANCE, DIAGNOSTIC.

Breast cancer is the most common oncology disease in women and is one of the major public health issues. Worldwide, is the second leading cause of cancer death in women and cancer research is a priority in all the laboratories of the world, in terms of

uncovering the appearance causes of the malignant process, understanding the mechanisms of development, but most of all, the discovery of early diagnostic methods and effective treatment (Devi et al., 2000).

Ignorance, fear of diagnosis, lack of health education and of efficient programmes for prevention and screening could cause diagnosis of the disease to be detected in the majority of cases in advanced stages, when treatment remains only palliative and very costly, in this cases the patient's suffering being immense.

This is also very important since mammary gland cancer (MGC) is one of the most common malignant tumors in women. In this way, in 2008 its morbidity was extremely increased especially in the Eastern Europe, where it was 38% in Ukraine, in Moldova - 25,4 %, while in Russia it was 38,2% and in Romania it was around 39.8%. Also, in the economically developed countries it represents up to 15-20 % of all cases of death by cancer in women and 3-5 % of all deaths (Fitsgibbons et al., 2001).

It is generally believed that conservative surgical treatment for the mammary gland represents a surgical operation by which the tumor with the adjacent mammary glandular tissue is removed with the evidation of the axillary and subclavicular lymph nodes, but keeping the organ (the so-called organ saving surgery).

The contemporary stage in surgery is characterized by an increasing share of organ saving operations in MGC stage I. Also, the most published sources indicate that the best results after organ saving operations were obtained in stage I of MGC (Hamilton et al., 2000; Vitucci et al., 2000). Moreover, the frequency of organ saving operations during treatment of MGC depends, in the first place, on the proportion of registered patients with the early stages of the MGC.

Moreover, due to various causes (e.g. suspicious multicentre tumor growth, affecting central quadrant, and suspicious metastatic involvement of axillary lymph nodes) the share of mastectomies in the surgical treatment of MGC remains high. Also, taking into account the fact that the average age of patients with MGC has decreased in the recent years, the organ saving surgical interventions and keeping of the mammary gland have become more and more significant, especially in reducing the mental trauma of the patients with MGC (Goldhirsch et al., 2007).

So far, there is no firm decision regarding the selection of the optimal order in the stages of treatment. In this way, these could represent very important aspects which could contribute to an increase in the survival of MGC patients.

Thus, an important aspect of the present study referred to the selection for the prognostic decisive factors in the biological development variance of the tumoral process for the elaboration of optimal treatment schemes in stage I MGC.

MATERIAL AND METHODS

The material of this study includes clinical and laboratory data analysis, the informational value of various investigation and treatment methods on 680 patients, selected between 1996 and 2004.

The patients included in the study were divided into 3 groups: the retrospective group, the prospective group and the control group.

In this way, the **retrospective group** included 496 stages I MGC patients, diagnosed and treated during 1996-2004. The

retrospective group was divided into 2 groups according to the type of clinical development of tumoral process after treatment: the 1st group including 419 patients without progress for 5 years after the treatment, and the 2nd group including 77 patients with the progressing tumoral process after treatment.

On the other side, the **prospective group** included 66 stages I MGC patients, diagnosed and treated between 2007-2008. For the patients in the prospective group there were assessed the molecular expressions of the HER2/neu molecular markers, CEA tumoral markers, CA-15.3, hormonal receptors RE and RP, hormonal homeostasis and immunological indices.

Finally, **the control group** comprised of 118 patients, from which 56 patients with Ha, Ilb, or lila stage MGC, and 62 patients with benign diseases.

In the control group the immunological and homeostasis hormonal parameters were studied for comparison with the stage I MGC.

A special inquiry was developed for this study, which included 150 parameters regarding special body or tumor features, the informational of various methods of investigation and the results of different treatment variants applied.

Data analysis

The decisive prognostic factors determination was performed by discriminate analysis. By applying discriminate analysis - within the groups of the retrospective lot the decisive prognostic factors of the clinical evolution variance were determined in the stage I MGC patients.

RESULTS

In this way, regarding **the clinical diagnosis in stage I mammary gland cancer,** in the 496 stage I MGC patients, during the primary clinical investigation the diagnosis of stage I MGC was established only in 165 (33.3%) patients, and in 232 (46,8%) patients the diagnosis of suspicion MGC was obtained. In other cases, the pathological process in the mammary gland was found as benign disease - fibro-adenoma (6.0%), fibrocystic disease nodules in 8.7% or other variations - in 5.2% cases (tab. I).

TABLE I

The variance of the clinical diagnosis variants in stage I MGC as depending by age

No	Clinical diagnosis	The age of patients						Total	
		< 35 years old		35 - 49 years old		> 50 years old			
		31 patients		224 patients		241 patients		496 patients	
		No. abs.	%	No. abs.	%	No. abs.	%	No. abs	%
1	Cancer	6	19.4	61	27.2	98	40.7	165	33,3
2	Cancer susp.	10	32.3	107	47.8	115	47.7	232	46.8
3	MFC	3	9.7	33	14.7	7	2,9	43	8.7
4	FA	11	35.5	14	6.3	5	2.1	30	6.0
5	Other*	1	3.2	9	4.0	12	5.0	26	5.2

Also, in terms of instrumental diagnosis – mammography, ultrasonography in mammary gland cancer stage I, it seems that in accordance with literature data the patholog-

ical process features assessment in the mammary gland is problematic in young age, especially due to the proliferating changes in the mammary glandular tissue adjacent to the tumor which contribute to the lessening of the clinical picture, including the palpable one, which decreases the possibility of accurate clinical assessment regarding the character of the pathological process in the mammary gland.

Additionally, by analyzing the frequency of correct clinical diagnosis of stage I MGC during the clinical primary investigation by age, we found the following: for the patients up to 35 years, the correct diagnosis of MGC was established only in 19.4% cases, while for the patients aged between 35-49 years old in 27.2% cases.

Moreover, in the patients aged over 50 years old, when the adjacent background is presented by adipose metaplasia of mammary glandular tissue, which allows a correct assessment of the size, density, surface and character of the tumor, the frequency of accurate diagnosis assessment was the highest-40,7% cases. Therefore, during menopause, for the glandular tissue metaplasia, based only on clinical data the correct diagnosis of stage I MGC was established in less than half of cases.

Also, analysis for the frequency of the correct diagnosis setting at the first clinical investigation in relation to the tumor dimensions found that in tumors with a diameter < 0.5 cm the clinical diagnosis was correct only in 11.6% cases, while from 0.6 – l.0 cm the frequency of correct MGC diagnosis setting essentially increased up to 41.6% cases and in tumors l.l-2.0 cm in 38.4% cases (tab. II).

TABLE II

Clinical diagnosis variants in stage I MGC, as depending of the tumor dimensions

No	Clinical diagnosis	The dimensions of the tumors						Total	
		< 0.5 cm		0.6-l.0 cm		l.l-2.0 cm			
		43 patients		166 patients		219 patients		428 patients	
		No. abs.	%	No abs.	%	No abs.	%	No abs	%
1	Cancer	5	11.6	69	41.6	84	38.4	158	36.9
2	Cancer suspicion	17	39.5	72	43.4	112	51.1	201	47.0
3	MFC	10	23.3	14	8.4	10	4.6	34	7.9
4	FA	6	14.0	4	2.4	6	2.7	16	3.7
5	Other*	5	11.6	7	4.2	7	3.2	19	4.5

In addition, the instrumental investigation, mammography in particular, is considered a diagnostic method with higher possibilities than the clinical ones and it is recommended as a method for the preclinical detection of patients with suspected mammary glands cancer, inclusively within the framework of prophylactic investigations for women after age 35, when the adipose metaplasia of breast glandular tissue occurs.

Also, within the complex investigations mammography was performed in 287 (57.9%) patients out of whom the correct diagnosis of mammary gland cancer was established for 70 cases.

In this way, by analyzing the frequency of the mammography correct diagnosis of

stage I mammary gland cancer based on patients age, the correct diagnosis of stage I mammary gland cancer for the patients under 35 years of age was 50%; for the age group between 35-44 years was 66.9% cases, and for patients older than the age of 50 was of 74.1% cases.

Additionally, through the analysis of the mammographic findings, depending on the tumor size, the following results were obtained: in tumors with size TIa: < 0.5 cm the correct mammographic conclusion of cancer of the mammary gland was established 4 times more frequently compared to clinical examination corresponding to 52.2% respectively 11.6% cases.

Also, for tumor size T Ib: 0.6-1.0 cm the mammographic correct diagnosis was established in 70.7% cases, while the correct diagnosis registered a even higher frequency for tumor sizes Tic: 1.1 - 2.0 cm, which means 76.2% of the cases (tab. IV).

TABLE III

Mammographic findings in stage I MGC, as determined by the patients age

No	Mammographic conclusions	The age of patients						Total	
		< 35 years old		35 - 49 years old		> 50 years old			
		8 investigated patients		136 investigated patients		143 investigated patients		287 investigated patients	
		No. abs.	%	No abs.	%	No abs.	%	No abs	%
1	Cancer	4	50,0	91	66,9	106	74,1	201	70,0
2	FAM	1	12,5	36	26,5	29	20,3	66	23,0
3	FA	3	37,5	9	6,6	8	5,6	20	7,0

$\mathbf{X^2} = 2.96$ $\mathbf{p} > 0.05$

TABLE IV

Mammographic findings in stage I MGC patients, after to the tumor diameter.

Mammographic conclusions	The dimensions of the tumor						Total	
	< 0.5 cm.		0.6-l.0 cm.		l.l-2.0 cm.			
	No. abs.	%	No. abs.	%	No. abs.	%	No. abs.	%
Cancer	12	52,2	70	70,7	96	76,2	178	71,8
MFC	9	39,1	19	19,2	24	19,0	52	21,0
FA	0	0	9	9,1	5	4,0	14	5,6
Solitary chistum	2	8,7	1	1,0	1	0,8	4	1,6
Total investigations	23	100	99	100	126	100	248	100

Also, we did analyze these results from the point of view of the age, as well as for the size of the tumor.

In this way, as in patients up to 35 years and, in particular to 30 years, the mammographic investigation may be contraindicated due to glandular breast tissue without signs of pronounced metaplasia, the method of instrumental diagnosis in 85 patients was the mammary glands UGS. Thus, the correct conclusion of MGC presence was established in only 40 of the cases, which does not essentially improve the frequency of correct diagnosis, when compared to

clinical diagnosis.

Moreover, in patients up to 35 years the correct MGC conclusion was 27.3%, while between 35-49 years it was 39.5% and over 50 years it was 44.4% cases. Also, the MGC was suspected in 9.4% of the USG investigated patients, while 38.8% of the cases were concluded by the MFC and 7.1% by FA, 4.7% cases through solitary cyst presence.

Therefore, independently of age, the correct diagnosis of MGC by MG investigation was more informative than by USG investigation (tab. V).

TABLE V

Ultrasound findings in patients with stage I MGC, as depending to age.

USG Conclusions	The age of the patients						Total	
	< 35 years old		35 -49 years old		> 50 years old			
	11 patients investigated		38 patients investigated		36 patients investigated		85 patients investigated	
	No. abs.	%	No. abs.	%	No. abs.	%	No. abs.	%
Cancer	3	27.3	15	39.5	16	44.4	34	40.0
Cancer suspicion	2	18.2	1	2.6	5	13.9	8	9.4
MFC	4	36.4	17	44,7	12	33.3	33	38.8
FA	2	18.2	2	5.3	2	5.6	6	7.1
Solitary chistum	0	0	3	7.9	1	2.8	4	4.7

In addition, with regards to the tumor size, the MGC ultrasonographic diagnosis for tumor size < 0.5 cm proved to be correct in 25% cases with an informative value 2 times lower than that provided by mammographic investigation for tumors with the same dimensions, but 2 times more frequent by than clinical examination for tumor size of 0.5 - l.0 cm, where the diagnosis was correct in 37.8% cases and for tumor diameter between 1.1-2.0 cm - 46.7% cases (tab. VI).

TABLE VI

Ultrasonographic assessment for stage I MGC patients, as depending to the size of the tumor

USG Conclusions	Tumoral dimensions							
	< 0.5 cm		0.6-1.0 cm		1.1-2.0 cm		Total	
	8 patients investigated		37 patients investigated		30 patients investigated		75 patients investigated	
	No. abs.	%	No. abs.	%	No. abs.	%	No. abs	%
Cancer	2	25.0	14	37.8	14	46.7	30	40.0
Cancer suspicion	1	12.5	4	10.8	2	6.7	7	9.3
MFC	3	37.5	15	40.5	11	36.7	29	38.7
FA	0	0	3	8.1	2	6.7	5	6.7
Solitary chistum	2	25.0	1	2.7	1	3.3	4	5.3

DISCUSSION

Our results presented in this study, as seen through the 5-year survival, within each stage of the MGC are determined, firstly, by the type of applied treatment. This is very important, since the variant of applied treatment, the possibility for the administration of a neoadjuvant treatment depend on the informational value of the applied diagnostic methods.

Additionally, in the extended forms of MGC, ultrasound and mammography semiotics does not raise any difficulties and the correct conclusions could reach up to 92-98% cases.

Also, early forms of MGC semiotics in these two instrumental methods are rather poorly represented, which decreases the frequency of correct conclusions to 20.75% (Charafe-Jauffret et al., 2009). At the same time, the correct conclusions in the USG and MG investigations along with the clinical picture allow us to determine the exact place for the aspiration biopsy for cytological confirmation of the tumor process.

Additionally, another very important aspect of the present study referred to the selection for the prognostic decisive factors in the biological development variance of the tumoral process for the elaboration of optimal treatment schemes in stage I MGC.

In this way, it is generally accepted that the actual methods of MGC treatment which include the surgical component, radiotherapy, chemotherapy (CT) and hormone therapy (HT) could ensure a 90 - 95% rate of over 5 years survival in stage I of MGC (Leitner et al., 1995).

At the same time, the selection of the surgical intervention volume for the radiotherapy and chemotherapy place in the treatment of stage I MGC cannot be considered definitively determined. In this way, in several works of actuality the authors demonstrate that early stages MGC patients can be successfully cured only by surgical intervention (Baselga et al., 2009). Other researchers regard as mandatory the inclusion of radiotherapy and chemotherapy in the treatment schemes of early stages MGC patients (Colleoni et al., 2004).

Moreover, some authors considers applying radiotherapy only in patients which were subjected to organ saving surgery (Spooner et al., 1997), while others commented that in the cases of older patients aged over 55 years with tumors with sizes up to 2.0 cm, the mammary gland sectoral resection is effective without radiotherapy (Fisher et al., 2009).

For these reasons it is only generally recommended to perform chemotherapy in combination with radiotherapy for the action against the tumor pre-clinical metastases, especially since The Oncology Centers have not yet accumulated a sufficient number of randomized studies, in order to provide a meta-analysis sustained answer about the concrete treatment scheme for these patients.

In addition, establishing immunological hormonal homeostasis peculiarities depending on the degree of expression of molecular markers can be used to clarify the mechanisms of unfavorable clinical course in tumors with different degrees of expression of the RE, RP hormonal receptors and the HER2/neu molecular marker.

Moreover, within the international conferences in San-Gallen in 2005, 2007, 2009 on mammary gland cancer treatment, the criteria for the application of radiotherapy and chemotherapy in the MGC were discussed (McCloud et al., 2009).

In this way, as criteria for determining the treatment tactics of stage I MGC pa-

tients are considered the factors that characterize the tumor – the molecular markers indices, molecular receptors indices, tumor size and must be individually assessed.

Thus, in patients with tumor diameter < 1.0 cm and negative lymph nodes only the surgical treatment is recommended.

Also, although the literature data indicate increased frequency of hormonal disorders in MGC patients, the hormone therapy is yet limited to the indication of the anti-estrogens or progestins. In this way, it is necessary that hormonal therapy also include hormonal homeostasis regulation, since MGC patients may present hypothyroidism, hyperprolactinemia and other hormonal imbalances with various causes.

Also, it is necessary to take account of the immunological status particularities, because immunological status influences the results of treatment.

In addition, in the literature there are concrete data are about the fact that in MGC (including stage I), the degree of expression of molecular markers affects clinical development.

In this way, compared with stages II to IV, in stage I MGC, the expression of RE and RP is more pronounced (Rosen et al., 2009). This can be explained by the fact that, alongside the tumoral progression, the degree of tumor differentiation decreases.

Thus, this could represent an inverse relationship, as the low degree of differentiation contributes to high rates of tumor growth, which is why at the addressing point, these have a higher local or local/regional spread (Hughes et al., 2001).

CONCLUSIONS

MGC represents a polymorphic and pathogenic disease and it cannot be admitted that all subgroups of patients will obtain identical results from one tactic of treatment determined for all the patients with MGC. Thus, the concept of MGC both clinical and patho morphological, combines different cell clones depending on its microstructure and biology. As a result, the evolution of the disease, the prognosis and the effectiveness of the treatment may vary in different patients at the same stage, depending on the degree of malignancy of the tumor, its histopathological structure, the degree of expression of molecular markers identification and also immunoresistance.

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NEWS

CARDIAC PARAGANGLIOMAS

Extra-adrenal paragangliomas (PGLs) are uncommon neuroendocrine tumors. These tumors can originate from either the sympathetic or parasympathetic paraganglia. **Parasympathetic** PGLs are usually found in the head and neck region and do not release catecholamines. They are also referred to as nonchromaffin PGLs or chemodectomas in the literature. **Sympathetic** PGL, a synonym of pheochromocytoma, accounts for 0.1% to 0.2% of all patients with hypertension. This tumor is situated mostly along the sympathetic trunk in the abdomen, and exerts similar clinical and histopathological features as its adrenal counterpart. Less than 2% of PGLs are located in the chest, in which the cardiac PGLs (CPGLs) are extremely rare. The majority of these reports are single case studies or small series. CPGLs may develop catecholamine-related symptoms or respiratory obstruction symptoms. The tumors in most cases were found during imaging studies. It is rather difficult to clarify the accurate anatomical location via routine preoperative images such as computed tomography (CT) and echocardiography.In this review, in all reported cases were discussed clinical presentations, imaging findings, pathology, location, therapy, outcomes, and treatment patterns of CPGLs. The study found that most CPGLs were functional, preferably located in left retro-pericardium, and mostly fed by right coronary artery and left circumflex coronary artery. The combination of MIBG, MRI, and coronary angiography could well outline the lesions. Complete resection was the most effective strategy to cure this disease (Wang JG, Han J, Jiang T, and Li YJ. Cardiac Paragangliomas. *J Card Surg* 2015; 30: 55–60).

Doina Butcovan

PYODERMA VEGETANS DEVELOPED ON CHRONIC LEG ULCER

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PYODERMA VEGETANS DEVELOPED ON CHRONIC LEG ULCER (Abstract): *Pyoderma vegetans* is a rare disorder that more commonly affects middle-aged persons, with a male predilection. It is characterized by vegetating lesions that coalescence into a plaque with eroded surface, covered by purulent discharge and crusts. The etiology of this disease is not known with certainty, but it is often associated with bacterial infections in immunocompromised patients. We report the case of a 73-year-old men who presented to the Iasi Dermatology Clinic with a large, irregular, relatively well-defined dermohypodermic ulcer, with infiltrated sclerosing borders, accompanied by pain, with the floor covered in the Northern part by a proliferative, vegetative bleeding area, and the rest by a yellowish secretion and cellular debris, located on the left leg. Bacteriological examination of ulcer secretion identified *Pseudomonas aeruginosa*. Anatomopathological examination confirmed the development of *Pyoderma vegetans* on chronic leg ulcer. Under specific treatment for chronic leg ulcer and eradication of infectious focus the outcome was favorable both in terms of trophic ulcer scarring and *Pyoderma vegetans* healing. **Keywords**: PYODERMA VEGETANS, CHRONIC LEG ULCER.

Pyoderma vegetans is a rare condition, first described by Hallopeau in 1898 (1); it is more common in middle-aged persons and there is a male predilection (5). *Pyoderma vegetans* is clinically characterized by vegetating lesions that coalescence into a plaque with eroded surface, covered by purulent discharge and crusts (2). It can occur at any level, but there is a predilection for limbs and infected loss of substance, traumatic or of any other nature (2, 3). The etiology of this disease is not known, but is often associated with bacterial infections in immunocompromised individuals (3, 4, 5, 6). *Pyoderma vegetans* has been associated with ulcerative colitis (7, 8), T-cell lymphoma (9), and alcoholism with malnutrition (10), HIV infection (11), chronic myeloid leukemia (12) and lupus nephritis (13).

CASE REPORT

We present the clinical case of a 73-year-old male patient residing in a rural area, who presented to the Iasi Dermatology Clinic with a large, irregular, relatively

well-defined trophic ulcer, with infiltrated sclerosing borders, intensely vegetative, bleeding area in the Northern part, located on the left leg. The patient had both a family history (mother) and personal history of chronic venous insufficiency.

The patient was known with chronic venous insufficiency and superinfected chronic left leg ulcer since age 48 years old, with a fluctuating course (ulcer decreased in size or increased in size and depth), and therapeutically neglected. The patient said he noticed during the last year an intense proliferation of the floor of ulcer, bleeding and pus discharge.

Dermatological physical examination revealed on the left leg a large dermohypodermic ulcer (15 X 10 cm), irregular, relatively well-defined, with infiltrated sclerous borders, painful, the floor covered in the Northern part by a proliferative, vegetative, bleeding portion, and the rest by an yellowish secretion and cellular debris (fig. 1).

Fig. 1. Trophic ulcer with a vegetative Northern area

The ulcer was surrounded by a painful erythemato-edematous placard, imprecisely defined, inflammatory signs present, covered here and there by crusts. Asymptomatic erythematous macules, violet-colored and hyperpigmented, isolated and coalesced into plaques, that did not disappear on vitro pressure were present on both legs.

Laboratory findings were: inflammatory syndrome, mild hyperglycemia and presence of *Pseudomonas aeruginosa* in ulcer discharges. Venous Doppler ultrasound did not detect any signs of superficial and deep vein thrombosis in the lower limbs. Skin biopsy revealed a fragment with deep ulceration, covered by necrotic debris with leukocytes and granulation tissue, epidermis at ulcer edges is uneven with papillomatous thickening and subjacent dermis with abundant polymorphous inflammatory infiltrate.

The findings of local physical examination and laboratory investigations corroborated the following positive diagnoses: chronic venous insufficiency CEAP class VI, trophic left leg ulcer superinfected with *Pseudomona aeruginosa*, *Pyoderma vegetans* on the left leg, periulcer cellulitis, stasis dermatitis.

Treatment consisted in surgical debridement by scalpel excision of the slough followed by systemic drug therapy (antibiotic according to antibiogram, phlebotropic and vasodilator agents) and topical drug therapy (periulcer dermo-corticoid, nitration of vegetative lesions with 1% silver nitrate crystals for the beginning and then, silver nitrate ointments as daily dressings) in combination with rigorous local hygiene with water and dermatological soap and avoidance of prolonged standing.

Under proper treatment, the course was favorable with an approximately 50% ulcer closure within 30 days, flattening and progressive disappearance of *Pyoderma vegetans* lesions after the infectious focus was cleared up (one-month bacterial examination was negative), and periulcer erythema-

tous placard gradually diminished to disappearance (fig. 2, 3).

Fig. 2. Ulcer appearance after 30 days of treatment

Fig. 3. Ulcer appearance after 4 months of treatment

DISCUSSION

Venous ulcer is the most severe manifestation of chronic venous insufficiency, being a substance loss secondary to trophic skin changes due to venous stasis (14). It affects the lower third of the leg, with a predilection for the left leg (47%). It is associated with ochre and purpuric dermatitis, sclerodermiform dermo-hypodermimitis, eczematiform dermoepidermitis, and more rarely with white atrophy and thrombophlebitis (14). The main complication of venous ulcer is a secondary bacterial infection (2). Often, bacteriological examination of ulcer discharge reveals gram-positive bacteria, such as *Staphylococcus aureus* in about 56% of cases, and gram-negative bacilli, such as *Pseudomonas aeruginosa* and *Proteus* (3).

It was suggested that *Pyoderma vegetans* is a response to massive bacterial colonization or epidermal invasion in an immunocompromised patient (15). The most common microbial agents isolated are staphylococci and beta-hemolytic streptococci. Other suspected agents are *Klebsiella spp*, *Bacteroides spp*, enterococci, *Pseudomonas aeruginosa* and *Corynebacterium spp* (5, 16). A case associated with *Trichophyton mentagrophytes* has also been reported.

Some authors believe that the microbiological agent is not primarily responsible for the disease and the infection develops secondary to an immune dysfunction. In support of this theory, in some of cases serum immunoglobulin levels were decreased and phagocytic dysfunction was observed (5, 16, 17).

The association of chronic venous ulcer with and *Pyoderma vegetans* is rare.

The diagnosis of the disease depends primarily on the exclusion of other diseases that can present as chronic vegetative lesions, including squamous cell carcinoma, Kaposi disease, achromic malignant melanoma, tuberculosis vegetans, pyoderma gangrenosum, and pemphigus vegetans (4, 5, 8). The histopathological characteristics of *Pyoderma vegetans* are pseudoepitheliomatous hyperplasia, and the presence of numerous abscesses both in the hyperplastic epidermis and dermis. The abscesses may contain neutrophils or eosinophils (4, 5).

Pyoderma vegetans lesions can heal spontaneously, but usually recur and become chronic. There are no standard treatment modalities. The literature emphasizes the importance of treating the underlying

disease (5). Thus, the treatment of *Pyoderma vegetans* occurring on a chronic leg ulcer is primarily based on venous ulcer therapy.

The three phases in the management of venous ulcer are: cleansing and debridement, granulation phase, and epithelization and healing. Debridement is commonly performed to remove slough, eschar and bacterial biofilms from the ulcer bed with the aim of increasing wound healing. The most common commonly used are: surgical debridement using scalpel, scissors or curette, which is the most rapid and precise, but can be painful, and autolytic or enzymatic debridement, which are slow and can be facilitated by the use of moisture-donating dressings or protease preparations, respectively. Granulation tissue must be kept permanently moist. It is used a hydroactive wound dressing that provides a moist wound environment for optimal healing, thus being highly effective in the granulation phase. During the epithelialization phase dressings are applied to make sure that moisture is retained over a wound. Hydroactive ointment dressing, hydrogel dressing, or hydrocolloid dressing (designed for already epithelized wounds) are the best choices (3, 14).

No standardized treatment plan for *Pyoderma vegetans* is available, although antibiotic therapy has been frequently used with variable results. Good results have been reported in the literature when administering the following associations: intravenous ceftriaxone and topical aluminum acetate soaks, ampicillin and x-ray radiation, ciprofloxacin and fluocortolone, and etretinate and systemic corticosteroid therapy (4, 5).

CONCLUSIONS

In the reported case, the diagnosis of *Pyoderma vegetans* is both clinical and bacteriological, and anatomopathological. The outcome was favorable with both chronic leg ulcer and *Pyoderma vegetans* healing.

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NEWS

FUSARIUM MYCOTOXINS DIETARY INTAKE THROUGH BEER CONSUMPTION IN EUROPE

Mycotoxin contamination of food and beverages represents a serious world-wide problem. Mycotoxins are secondary metabolites naturally produced by various species of fungi, including *Fusarium* genus. Deoxynivalenol is the most widely detected mycotoxin in the cereals used in beer industry. Mycotoxins are thermally stable and present a good water solubility, therefore the contamination of beer might occur in any step of the brewing process. Mycotoxins are incriminated for their hepatotoxic, carcinogenic, nephrotoxic, neurotoxic and immunosuppressive effects. Although deoxynivalenol was not classified by the IARC as being carcinogen to humans, it is still responsible for a series of deleterious effects for the organism such as: weight loss, endocrine dysfunctions and immune alterations. Rodríguez-Carrasco *et al*. determined the content of 14 *Fusarium* toxins and their metabolites in a total of 154 beer samples produced in Europe, in order to estimate the dietary intake of these mycotoxins. For this purpose, they developed an analytical method which consisted in a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extraction technique followed by gas chromatography-tandem mass spectrometry analysis; the analytical method was validated. Only two mycotoxins were found in a high number of beer samples: deoxynivalenol in 59.7% of samples and HT-2 toxin in 9.1 % of samples. However, their average concentrations (17.2 μg/L for deoxynivalenol and 2.8 μg/L for HT-2) represent only 5% of the deoxynivalenol PMTDI (provisional maximum tolerable dietary intake) and 7-12% of the HT-2 TDI (tolerable dietary intake) for European population. The authors concluded that a moderate consumption of beer does not pose any health risk for the Europeans. Nevertheless, beer consumption represents a significant mycotoxin source for heavy beer drinkers (Rodríguez-Carrasco Y, Fattore M, Albrizio S, Berrada H, Manes J. Occurrence of *Fusarium* mycotoxins and their dietary intake through beer consumption by the European population. *Food Chem* 2015; 178: 149-155)

Constantin Ciobanu

HISTOPATHOLOGICAL DIAGNOSIS CRITERIA IN ENDOMETRIAL HYPERPLASIA

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HISTOPATHOLOGICAL DIAGNOSIS CRITERIA IN ENDOMETRIAL HYPERPLASIA (Abstract**):** Endometrial hyperplasia is a condition of the abnormal proliferative lesions of the endometrium. **Aim**: To highlight the value of histopathological examinations with assessment of the ways to obtain endometrial biopsies and the appreciation of the contribution of different histopathological techniques that can be used to establish a correct and complete diagnosis. **Material and methods:** The study was conducted in a group of 50 patients diagnosed with endometrial hyperplasia. In all cases the bioptic curettage was performed using a curette to obtain more abundant material for the histopathological examination. **Results**: Histopathological diagnosis guides the therapeutic attitude and has an important prognostic value. The study group was characterized by histoarchitectonic heterogeneity, presented even in the same case. Histoarchitectonic and cytological changes differentiate endometrial hyperplasia from physiological proliferative endometrium. **Conclusions**: The diagnosis of certainty between the four types of endometrial proliferation established by the WHO can only be done through the histopathological examination. **Keywords:** HISTOPATHOLOGICAL EXAMINATION, ENDOMETRIUM, HYPERPLASIA.

In the female genital apparatus, the endometrium undergoes cyclic proliferation, differentiation, degeneration and regeneration, processes regulated by the hypothalamic-pituitary-ovarian axis. The disruption of this cyclical development leads to the occurrence of dysfunctional uterine bleeding, which often has endometrial hyperplasia as morphological substrate.

The endometrial cycle undergoes physiological changes with age. Perimenopause and post menopause affects the endometrial cycle, female gonads and have a systemic impact. Hormonal imbalances also lead to morphological and functional changes in secondary sexual characteristics (1). In this context, the "aging face" phenomenon is accelerated due to the faster loss of subcutaneous supportive adipose layer and a decrease in quality of fibrous connective structures.

Endometrial hyperplasia represents abnormal proliferative lesions of the endometrium, leading to increased mucosal thickness, alterations in glandular architecture and changes in the gland-to-stroma ratio

(2). The lesion is hormone-dependent, and as a result it is reversible, but can progress to an invasive carcinoma in the presence of genetic changes that cause a monoclonal glandular proliferation (3). A category of patients with endometrial proliferation consists of those with a congenital malformation of the uterus. This type of malformation correlates with the concomitant existence of one or more malformations at other levels.

There are two types of hyperplasia, an atypical one, closely related to adenocarcinoma and a nonatypical one, which is little linked with the carcinomatous lesion (4). The notion of simple endometrial hyperplasia refers to nonatypical hyperplasia without glandular architectural abnormalities, whereas complex glandular hyperplasia defines nonatypical hyperplasia with abnormalities of glandular architecture (5). The presence of cytological atypia is the most important factor related to progression to cancer, especially in postmenopausal women (6).

The high degree of morphological variability of endometrial proliferation, even within the same tissue sample, is responsible for the difficulty in defining and establishing clinical and morphological diagnostic criteria. All forms of hyperplasia present certain morphological features showing an increase in gland-to-stroma ratio, irregularities in the shape and the size of the gland.

The macroscopic examination usually reveals an enlarged uterus and, on the hysterectomy specimens the endometrium is pale, diffuse thickened or polyploid, with a smooth, velvety or granular surface (7).

The microscopic examination highlights architectural alterations, characterized by glandular complexity and the amount of stroma that separates the glands, distinguishing simple and complex forms of hyperplasia, regardless of the presence of atypia.

Hyperplasia without atypia represents excessive proliferative response to uncompensated estrogen stimulation. The endometrium responds in a diffuse way with a balanced increase of both glands and stroma (10). *Simple hyperplasia* usually involves the entire endometrium with a diffuse thickening of the endometrium. Microscopically appears glandular proliferation with irregular shapes and sizes, separated by abundant stroma. *Complex hyperplasia* presents a disordered glandular proliferation, to the detriment of the stroma. The endometrial glands are agglomerated with an increase in architectural complexity separated by a relatively reduced stroma, the gland-to-stroma-ratio is 3/1 (8).

Atypical hyperplasia is distinguished from the precedent by cytological atypia of the glandular epithelium, represented by the loss of axial polarity, nuclear pleomorphism that often has irregular contours, prominent nuclei and distinct or dense chromatin. Atypia appears almost always focal.

MATERIAL AND METHODS

The study was conducted in a group of 50 patients aged 24 to 70 years, diagnosed with endometrial hyperplasia admitted to the Iasi "Cuza-Voda" Hospital. All patients underwent comprehensive clinical and laboratory investigations, and the certainty diagnosis was based on the histopathological examination of bioptic curettage or surgical specimens.

The routine general physical examination has been supplemented in many cases with specialized examinations (gastrointes-

tinal, cardiovascular, renal, etc.). In all cases the bioptic curettage was performed with the curette to obtain more abundant material, the practice demonstrating that the aspiration method is a less favorable opinion. The specimens were embedded in paraffin, then special stains were made: hemalums-eosin, tri-color van Gieson, periodic acid-Schiff (PAS) coloring.

RESULTS

The histopathological examination of the collected fragments aims to identify the nuclear changes occurring at the cytological level and to assess the degree of their diffusion.

Approximately 2/3 of the studied cases were identified with simple endometrial hyperplasia. Young women, up to 40 years of age, had heterogeneous histopathological aspects with simple hyperplasia zones with average glandular density; the glands retain their regular contour and circular lumens. In the same cases, it appeared on sections glands with irregularly contoured, even with a corrugated lumen (fig. 1).

Cases which has a high-density gland have an uneven and narrow lumen (fig. 2).

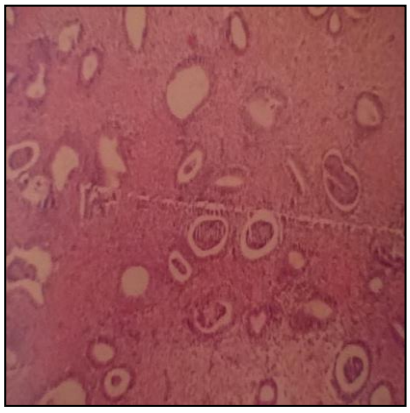

Fig. 1. Simple hyperplasia, regular contour glands and medium frequency; HE 20x

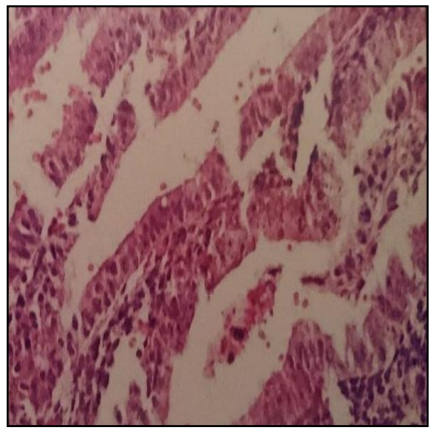

Fig. 2. Endometrium with simple hyperplasia; HE 40x

The endometrial epithelium appears simple, prismatic, tall, with fusiform nuclei perpendicular to the basal membrane (fig. 3). These glands may have endoluminal papillary proliferations.

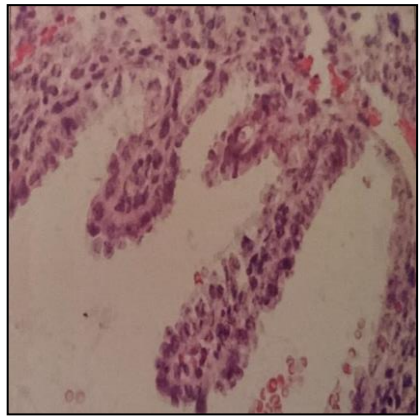

Fig. 3. Endometrium with dilated gland and papillary proliferation; HE 40x

The surface epithelium presents a decrease in height inversely proportional to age. The epithelia of older people have a homogeneous appearance, starting from glands with irregular shape and wide lumen in the premenopausal and going to areas

with regular appearance and narrow lumen in older people. In this situation, intraluminal proliferation persists.

One third of the studied cases had complex hyperplasia. This type of hyperplasia has a characteristic appearance of the glands, arranged back to back and separated by a minimal thickness stroma. Their lumens are extremely irregular due to the significant endoluminal papillary proliferation (fig. 4).

Fig. 4. Complex endometrial hypertrophy, with rare intraluminal proliferation; HE 40x

The glands also exhibit many cystic dilations that give the appearance of heterogeneity. Characteristic of this type of endometrial proliferation is the existence of glandular branches and even intergranular communication.

In both forms of hyperplasia, there is a degree of stromal fibrosis directly proportional to the age of the patient.

DISCUSSION

The importance of this study is revealed by the fact that it is unanimously accepted (9) that simple endometrial hyperplasia may progress to atypical endometrial hyperplasia, the latter being considered precancerous status. In the classic diagnosis of endometrial hyperplasia, the gland-to-stroma ratio is used.

Histopathological diagnosis guides the therapeutic attitude and has an important prognostic value. The study group is characterized by a histoarchitectonic heterogeneity. Characteristic from this point of view are the variety of shapes and glandular diameters and the intraglandular ramifications. The diagnosis of certainty of simple endometrial hyperplasia by the gland-to-stroma ratio should ideally be made between days 10-14 of the physiological endometrial cycle.

Another histopathological criterion of differentiation of the two types of hyperplasia is related to the structure of the gland, which in simple hyperplasia must be tubular, right and without branches. Variations in contour and lumen diameter occur in both types of hyperplasia.

Complex endometrial hyperplasia is histopathological diagnosed, primarily by the loss of individuality of the flanders, with the appearance of intergranular branches and communications. The type of atypical complex proliferation is marked by the existence of papillary proliferations of the glandular epithelium. Also, the epithelium appears arranged in a pseudo-stratified or even stratified form. Complex hyperplasia may present elongated cells with “cane-like” nuclei arranged in narrow bundles. Between stromal cells may be present clear cells, these being lipid-containing macrophages (10).

The presence of nuclear atypia by increase in nuclear volume and change in the nuclear/cytoplasmic ratio with budding of the core, thickening of its membrane, and the coagulation of chromatin are criteria of proliferative atypia. The nucleus migrates to the geometric center of the cell, and the cytoplasm acquires a basophilic appearance. These are malignant or premalignant changes and call into question the differen-

tial diagnosis between endometrial carcinoma and well-differentiated epidermoid carcinoma (11, 12, 13, 14). The histopathological differentiation criteria of the two possible diagnoses refer to the fact that in endometrial carcinoma papillary proliferations are extremely pronounced, with atypical cells and mitosis, and the glands get a cribriform aspect

In neither of these two situations a secretory glandular epithelium was found, no matter the age of the patient.

Another important aspect we watched was the perilesional vascularization. As in other neighboring neoplasms, such as rectal cancer, peritumoral vascular branches acquire characteristic, proliferative aspects of paraneoplastic neovasculogenic (15). These aspects have been noticed by using the PAS reaction to reveal the basal glandular membrane.

CONCLUSIONS

Endometrial hyperplasia is a non-invasive, non-physiological, estrogen-dependent endometrial proliferation. In our study results that histoarchitectonic and cytological changes differentiate endometrial hyperplasia from the physiological proliferative endometrium. The diagnosis of certainty between the four types of endometrial proliferation established by the WHO can only be done through the histopathological examination.

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NEWS

HYPERCHOLESTEROLEMIA – GENETIC DISEASE

Hypercholesterolemia is a common disorder which was reported in 39% of the worlds' adult population in 2008. Most of the patients with familial hypercholesterolemia (FH) have a mutation in the LDLR gene which is dominantly inherited. Mutations in the APOB and PCSK9 genes accounts for a smaller percentage of autosomal dominant FH. This review explored the recent evidence of association of the genetic variants with hypercholesterolemia and the 3 lipid traits: total cholesterol (TC), HDL cholesterol (HDL-C) and LDL-C and the biological effects of these genes in lipid homeostasis. The focus is mainly on the genes with a recognized or a potential role in lipid metabolism for which the association with the serum lipid traits; TC, LDL-C and HDL-C was observed in candidate gene association studies, genome-wide association studies (GWAS), genetic linkage studies and other studies with a case–control design. These include the genes encoding apolipoproteins, lipoprotein receptors and receptor related proteins, lipid transporters and lipid transfer proteins, the enzymes and proteins with a regulatory role in lipoprotein metabolism as well as the proteins that regulate the expression of those genes. The function of these genes in lipid metabolism and the association of single nucleotide variants (SNVs) in these loci with serum lipid traits, are not yet clearly identified. In summary, GWAS and meta-analyses have identified over 190 SNVs associated with serum TC, LDL-C and HDL-C levels in about 60 genes. Fourteen non-synonymous coding variants were identified in 10 genes that accounts for 7% of the variants in lipid related genes described in GWAS. Significant challenges remain. The known genes account for only a proportion of the cases and a substantial amount of missing heritability still exists (Paththinige CS, Sirisena ND, Dissanayake VHW. Genetic determinants of inherited susceptibility to hypercholesterolemia – a comprehensive literature review. *Lipids in Health and Disease* (2017); 16:103).

Alexandra Maștaleru

NAPKIN PSORIASIS - CASE REPORT

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NAPKIN PSORIASIS- CASE REPORT (Abstract): Psoriasis is a chronic inflammatory disease that can affect up to 1% of children. Genetic (family history of psoriasis) and environmental factors (bacterial or viral infections, stress, and trauma) are frequently involved in its occurrence. Napkin psoriasis is a particular form of psoriasis affecting mainly children younger than 2 years of age and can be classified together with other diseases under diaper rash. We present the case of a 4-month-old infant, born at term, naturally, weight and height within the normal range, who was brought to the Dermatology Clinic for the occurrence of erythematosquamous lesions in the anogenital area, buttocks and upper third of the thighs, with subsequent dissemination of lesions. The onset of symptoms began a few days after a respiratory tract infection. Initially he received treatment with systemic antibiotic and topical corticosteroid and antibiotic with unfavorable outcome. Laboratory tests revealed iron-deficiency anemia, leukocytosis, thrombocytosis, accelerated ESR, marked hepatic cytolysis, hyperphosphatemia and nasal carriage of Staphylococcus aureus. A systemic antihistamine and nonspecific desensitization treatment was administered. Topical treatment consisted in the removal of predisposing factors and irritants (diaper, urine) by rigorous hygiene, application of topical non-fluorinated corticosteroid and use of emollients, with favorable course of the lesions. The peculiarity of the case is that the diagnosis of psoriasis was based on history, physical examination and laboratory tests, in the absence of a pathology examination to confirm the diagnosis. Pathology examination could not be performed due to patient's age as biopsy required general anesthesia. **Keywords**: PSORIASIS IN CHILDREN, NAPKIN PSORIASIS, DIAPER RASH

Psoriasis is a chronic inflammatory disease affecting the growth and differentiation of the epidermis, with polygenic predisposition and various triggering factors (infections, medicines, physical or mental trauma, stress) (1). It evolves through successive bouts with a high individual variability in frequency. Although less common in children than in adults, the frequency of psoriasis at early ages remains quite high.

In approximately one third of patients the onset is during the first 20 years of life (2). In 25-45% of patients the disease starts before age 16 years, 10% before age 10 and 2% before age 2 (2). Studies conducted in Denmark and India reported onset between age 6 and 10 in most patients, whereas according to studies conducted in Australia and the Middle East the onset was before age 4. Congenital psoriasis with onset at birth or during the first days of life is very rare. Plaque psoriasis is the most common form both in adults and in children (68.6% of cases). However, initial lesions in children may be fewer and less scaly and thus difficult to diagnose in some cases. In a study conducted in China guttate psoriasis was found in 28.9% of patients, erythrodermal psoriasis in 1.4%, and palmoplantar pustular psoriasis in 1.1% of children (3). There are differences regarding sex distribution between various reports. Some authors believe that it is more common in women (4, 5), as in adults (6), others believe that gender distribution is relatively equal in children (7, 8, 9). Psoriasis may occur in newborns and infants and is difficult to diagnose due to the particular clinical features. Newborns and infants mainly develop the so-called napkin psoriasis or diaper psoriasis characterized by anogenital lesions, the diaper area, as an intensely erythematous placard, slightly scaly, dry, and well-defined. According to data reported by Morris *et al.* (8) this form of psoriasis is most common in children under two years of age.

CASE PRESENTATION

We present the case of a 4-month-old male infant residing in a rural area admitted to the Dermatology Clinic of the Iasi "Sf. Spiridon" Emergency Hospital for the emergence of a placard erythemato-squamous in the anogenital area, buttocks and upper third of the thighs. From patient's personal history we found that he was born at term, naturally, birth weight 3000 g, height 50 cm, APGAR score 9, with favorable postnatal development, breast-fed for one month then formula-fed, vaccinated according to the immunization schedule, but the prophylaxis of rickets with vitamin D3 incorrectly administered. From the previous medical history we mention acute nitrite poisoning at age 2 months.

The skin disease started approximately 2 weeks before admission as a symmetrical erythematosquamous placard with well-defined margins in the anogenital area, buttocks and upper third of the thighs (fig.1) with local discomfort at diaper changing and washing the genitals expressed by crying and restless. The rash appeared a few days after the onset of respiratory symptoms (rhinorrhea, initially watery then mucopurulent, cough, low-grade fever, and change in bowel habits). Shortly after, plaques and placards with the same features appeared at distance (armpits, elbow crease bilaterally, anterior and posterior neck, scalp, anterior and posterior thorax, periocular and retroauricular regions). Home treatment was initiated by the family physician, consisting in antibiotics, calcium gluconate and topical dermocorticosteroids and antibiotic ointment for 5 days with unfavorable outcome. Because skin lesions persisted the mother decided to seek specialist advice at the Dermatology Clinic.

On admission the infant was afebrile, eutrophic, weighed 7,500 g, was 62 cm tall, and his general health status was slightly impaired. General physical examination at admission revealed a superficial physiological generalized microadenopathy, pale tint to the skin and mucous membranes, tempo-

ro-occipital craniotabes, anterior fontanelle 3/4 cm in diameter, with sagittal dehiscence, normotensive, closed posterior fontanelle, seromucous rhinorrhea, rare nonproductive cough, symmetric rib cage excursions, RR = 40/min, systolic murmur grade II-III / 6 to the base of the heart and apex, HR = 130 min. On local examination the following were seen: erythemato-squamous plaques and placards, well-delimited, covered by pearly-white thick, multi-layered scales, Auspitz signs present at Brocq methodic scaling, located in the anogenital area, buttocks, inner thighs, axillary region, elbow crest bilaterally, peri-ocular region bilaterally, lower third of the abdomen, and posterior thorax (fig. 2, 3).

Fig. 1. Erythematosquamous placard in the anogenital area, buttocks and upper third of the thighs

Fig. 2, 3. Erythematosquamous plaques and placards located in the anogenital area, buttocks, inner thighs, trunk, face, limbs

Laboratory findings:

- Iron-deficiency anemia (serum iron = 23mg/dL).
- leukocytosis (26,190 / mm^3).
- marked hepatic cytolysis (ALAT = 266U/L, ASAT = 133U/L), drug-induced.
- hyperphosphatemia (P = 6.28 mg/dL), and high ALP (760UI/l), in the context of

deficiency rickets.

- thrombocytosis (T = 723,000 mm^3).
- VSH acceleration (17mm/h, 43mm/2h).
- nasal exudate: Staphylococcus aureus carriage.

Although the diagnosis of psoriasis is confirmed by pathological examination, this could not be performed due to patient's age as biopsy required general anesthesia. Typical clinical appearance of the lesions, history and laboratory tests were enough to make the diagnosis of napkin psoriasis.

Systemic treatment with antihistamines and nonspecific desensitization treatment, removal of local predisposing factors and irritants (diaper, urine) by rigorous hygiene, application of topical nonfluorinated dermocorticosteroids gradually tapered to avoid rebound phenomenon and use of emollient wash gels, bath emulsion and face and body creams were initiated. The course was obviously favorable since treatment day 2 with significant improvement of skin lesions (fig. 4), the baby being much quieter. At discharge the patient was referred to a pediatrician for the assessment and treatment of respiratory disease, anemia, deficiency rickets and the other changes detected by laboratory tests.

Fig. 4. Improved appearance of lesions after 2 days of treatment

DISCUSSION

The clinical forms of pediatric psoriasis are the same as in adults: plaque, guttate, pustular, erythrodermic, reverse, arthropathic. Usually children have a family history of this condition, according to Morris *et al.* (8) 71% of patients having a family history. Genetic and environmental factors are incriminated in the development of psoriasis in children. The lesions are clinically similar to those in adults: well-delimited erythematosquamous plaques and placards, positive Auspitz sign, but initial lesions may be less scaly and less infiltrated than in adults. Another difference is that in children the lesions are usually symptomatic, itchy, and the prevalent locations are the scalp, extensor surface of the limbs, trunk, face and ears. A particular form is located in the diaper area, being reported in 45% of children aged less than two years.

The ocular involvement is noticed in one third of the patients with psoriasis. Dry eye, conjunctivitis, episcleritis and anterior uveitis are the most frequent findings. No ocular signs have been noticed in our patient.

In newborns and infants diaper area is a unique topographic region in terms of skin lesions, as multiple diseases prevalently occur at this level, all called diaper rash. According to some authors diaper rash can be classified into three categories (10): diseases caused directly or indirectly by the wearing of diapers (candidal intertrigo, irritant contact dermatitis), lesions located elsewhere, but can be exaggerated in the groin area due to the irritating effects of wearing a diaper (atopic dermatitis, seborrheic dermatitis, psoriasis), and lesions that appear in the diaper area irrespective of diaper use (bullous impetigo, Langerhans cell histiocytosis, *acro-dermatitis entero-*

pathica, congenital syphilis, scabies). Often the positive diagnosis is based only on history and physical examination, the clinician being faced with difficulty in making a correct diagnosis. In the case of recalcitrant diaper dermatitis on the groin, unresponsive to treatment, a possible diagnosis of psoriasis should be suspected.

CONCLUSIONS

The occurrence of psoriasis in children is possible, but few cases have been reported in infants. Although it is recognized that the onset of psoriasis in children is more common in families with psoriasis, in the here presented case the genetic component could not be demonstrated. Although initially difficult to make a certain diagnosis, through careful and thorough history (with the help of the mother), clinical and laboratory assessment we concluded that our patient had a form of psoriasis- napkin psoriasis. Key factors in triggering psoriasis are bacterial, viral or fungal infections. In our patient the incorrectly treated respiratory infection led to impaired liver function and favored the appearance of psoriasis. Treatment of psoriasis in children requires special attention and close cooperation between the dermatologist, pediatrician and parent, this triad being the key to successful treatment. The diagnosis of psoriasis is based on clinical criteria, no specific laboratory tests being available except for pathology examination, which in newborns and infants is difficult to perform as it requires general anesthesia. These young patients should be followed-up closely, parents should be informed about the disease and its possible progression; in some cases it progresses to pustular psoriasis, and in 15% of cases the children will develop other forms of psoriasis in later life.

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MAIN NEUROENDOCRINE FEATURES, DIAGNOSIS AND THERAPEUTIC POSSIBILITIES IN THE CHRONIC FATIGUE SYNDROME, AN UNDERDIAGNOSED ENTITY

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MAIN NEUROENDOCRINE FEATURES, DIAGNOSIS AND THERAPEUTIC POSSIBILITIES IN THE CRONIC FATIGUE SYNDROME, AN UNDERDIAGNOSED ENTITY (Abstract): Chronic fatigue syndrome is characterized by severe, persistent fatigue which is not relieved by rest and is not associated to other medical conditions. Other common symptoms are including concentration and memory impairment, muscle and multiple joints pain, extreme exhaustion after physical or mental exertions, irritable bowel syndrome-like symptoms and depression, anxiety, mood swings and panic attacks. Etiology of the syndrome is not yet clear, post-viral and stress hypotheses were not verified. Diagnosis is confirmed in case of new onset of severe fatigue, for six consecutive months or more; fatigue is leading to significant reduction of the activity levels and is accompanied by other four or more of the specific associated symptoms, which are also lasting for six months or longer. The management of the disease is based on cognitive behavioral therapy, graded exercise therapy and pacing; medication plays a minor role in therapy. The occupational status is severely affected, more than half of the cases being unable to work. Full recovery rate is in average of about 5 %. **Keywords:** CHRONIC FATIGUE SYNDROME (CFS), HYPOTHALAMIC-PITUITARY-ADRENAL AXIS (HPA axis), HYPOCORTISOLISM

Chronic fatigue syndrome (CFS) is the term used to define a significantly disabling medical condition, characterized by persistent fatigue and other troubling symptoms which are lasting at least six months in adults and three months in children or adolescents. The fatigue is not due to exertion, is not relieved by rest and is not tributary to other medical conditions (1). Other terms used to designate the syndrome are myalgic encephalomyelitis or post-viral fatigue syndrome. Although genetic, infectious and psychological hypotheses were proposed, the etiology of CFS is not known and data are supporting the idea that the syndrome should have multiple causes (2).

In 1934, an out-break of a then so-called atypical poliomyelitis took place at the Los Angeles County Hospital; numerous doctors and nurses were affected and the disease resembled a lot with what is now described as chronic fatigue syndrome. In 1955, another out-break at the Royal Free Hospital in London was called benign myalgic encephalomyelitis. The term of chronic fatigue syndrome was first introduced in 1987 (3).

NEUROENDOCRINE FEATURES, POSSIBLE ETIOLOGY

Main endocrinological research findings in chronic fatigue syndrome are mild hypocortisolism, a blunted adrenocortico- tropin (ACTH) response to stressors and enhanced negative feed-back sensitivity to glucocorticoids. Yet, there is no specific or uniform dysfunction of the HPA axis in CFS and approximately half of the relevant studies did not show evidence of hypocortisolism, or of an abnormal response to various types of challenge tests.

Prospective studies suggest that HPA axis is not a factor involved in the pathogenesis of the early stages of CFS, but it is believed that it might play a role in perpetuation and enhance of the symptoms later on, in the course of the disease (4).

A 2011 meta-analysis evidenced a mild but statistically significant hypocortisolism in chronic fatigue syndrome; hypocortisolism being associated with a weaker response to cognitive behavioral therapy (5).

At the level of the central nervous system, a hyperserotonergic state and hypoactivity of the HPA axis are characteristic, but still there is no answer if these abnormalities are a cause or a consequence of the syndrome. Alterations in serotonin signaling can develop physiologic and behavioral changes in these subjects (3).

Polymorphism in genes encoding serotonin pathways may show genetic predisposition in the pathophysiology of CFS. Gene-related dysfunction of HPA axis which regulates the stress response might be involved in CFS (6).

A prospective study found that after viral and non-viral infections, some of the cases met the criteria for CFS, concluding that post-infective fatigue syndrome is a valid model for the investigation of one possible pathway to CFS. Nevertheless, prevalence and roles of both infection and stress in the etiology of the syndrome are not known (7).

All racial and ethnic groups are affected by the disease; lower income groups of population show a slightly higher risk to develop chronic fatigue syndrome. Between 60 and 85 % of cases of CFS are women but it seems that the prevalence among men is underreported. The disease is occurring more frequently between the ages of 40 and 59; CFS is developing more rarely among children and adolescents than among adults. Blood relatives of people with chronic fatigue syndrome seem to be more predisposed to develop the disease than the rest of the population (7, 8). National health organizations estimated that more than one million Americans and a quarter of a million people in the UK have chronic fatigue syndrome; even more, it is estimated that approximately 80 % of the existing cases are undiagnosed (8).

DIAGNOSIS

The onset of CFS is abrupt, frequently accompanied by a flu-like illness, while a large number of cases begin with several months of severe adverse stress (9).

The three most important diagnosis criteria are: a new onset of severe fatigue for six consecutive months or more, not related to effort, not substantially relieved by rest and which is not determined by other medical conditions; the fatigue is the cause of significant reduction of the previous activity levels. These criteria are associated with four or more of the following symptoms, lasting six months or longer: post-exertion malaise, physical or mental effort bringing on extreme, prolonged exhaustion and sickness; impaired memory or concentra-

tion, unrefreshing sleep; muscular pain, multiple joints pain, headaches of a new type or more severe, recurrent sore throat, cervical or axillary tender lymph nodes.

Other common symptoms are: brain fog, difficulty in maintaining orthostatic condition (dizziness, balance problems or fainting); irritable bowel syndrome-like symptoms, such as bloating, stomach pain, constipation, diarrhea and nausea; chills and night sweats; visual disturbances (sensitivity to light, blurring, eye pain); allergies or sensitivities to foods, odors, chemicals, medications or noise; depression, anxiety, panic attacks, mood swings. Cognitive symptoms are especially deficits of attention, memory and reaction time. Processing speed of complex and simple information is impaired (10, 11).

A common diagnosis of CFS is characterizing a disease in which functional capacity of the affected subjects is varying a lot. While there are cases which had relatively normal lives, there are others who are totally bed-ridden and are unable to take care for themselves. The majority of the persons with CFS diagnosis are drastically reducing work, school and family activities, for long time periods. Severity and disability of the syndrome are the same in both genders (11, 12).

Many cases are suffering from disabling chronic pain. Important reduction in the level of physical activity and in the complexity of the conducted activities, were noticed. The impairment is comparable to that found in late-stage AIDS, lupus, rheumatoid arthritis, chronic obstructive pulmonary disease and end-stage renal disease (13). CFS affects the functional capacity and the well-being of an individual, more than major medical conditions such as multiple sclerosis, congestive heart failure and type II diabetes mellitus (14).

Since there are no characteristic laboratory abnormalities to diagnose chronic fatigue syndrome, the recommended tests are used to exclude other causes for typical symptoms. Conditions which must be excluded are hypothyroidism, anemia, diabetes, psychiatric disorders – major depression, anorexia nervosa, bulimia, bipolar disorder, alcohol and other substances abuse. Multiple chemical sensitivity, Gulf War syndrome and post-polio syndrome have symptoms similar to those of CFS (14).

THERAPEUTIC POSSIBILITIES

Most often, even under therapy, a full recovery from chronic fatigue syndrome is rare. Cognitive behavioral therapy, graded exercise therapy, as well as pacing have been moderately effective for many cases. These therapeutic methods are useful in monitoring and self-consciousness, in managing the symptoms and adapting the level of activity to the possibilities of the subject (15). Since the syndrome is polymorphous and shows different manifestations in different subjects, the therapy should be adapted to the needs of each individual.

Medication plays a minor role in management of the syndrome. Antidepressants and immune-modulators are used; the role of antidepressants remains controversial (16, 17).

PROGNOSIS

Evolutions with remission and relapse, which make more difficult the management of the syndrome, are frequent. In remission, the extending of the activities may easily worsen the symptoms, with relapse.

Occupational disability is important, since more than half of the cases are unable to work, while almost two-thirds of the cas-

es are limiting their work; less than a fifth of the cases are working full-time (11).

The average full recovery rate in people with chronic fatigue syndrome is of 5 % (range 0-31 %). In the conducted studies, an improvement with consequently increase of the quality of life, is reported in 39.5 % (18).

Yet, the etiology, pathophysiology, nomenclature and diagnostic criteria of the chronic fatigue syndrome are a matter of controversy among specialists (18).

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ATYPICAL PRESENTATION OF SYSTEMIC LUPUS ERYTHEMATOSUS: PAROTITIS AND SECONDARY SJOGREN'S SYNDROME CASE REPORT

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ATYPICAL PRESENTATION OF SYSTEMIC LUPUS ERYTHEMATOSUS: PAROTITIS AND SECONDARY SJOGREN'S SYNDROME (A CASE REPORT) (Abstract): Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by auto antibodies directed against self-antigens, immune complex formation and immune deregulations and may affect joints, skin, kidneys, heart, lungs, nervous system, and immune system. The onset can be variable and the symptoms can occur for many years. Parotitis as the initial manifestation of systemic lupus erythematosus (SLE) is a rare condition and can be associated with Sjogren's syndrome. In this article we present the case of a young patient who was diagnosed with Sjogren's syndrome retrospectively, after she met the criteria for SLE. **Keywords**: SYSTEMIC LUPUSERYTHEMATOSUS, PAROTITIS, SJOGREN'S SYNDROME.

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by auto antibodies directed against self-antigens, immune complex formation and immune deregulations and may affect joints, skin, kidneys, heart, lungs, nervous system, and immune system (1). The onset can be variable and the symptoms can occur for many years (2). Parotitis as the initial manifestation of systemic lupus erythematosus (SLE) (3) is a rare condition (4) and can be associated with Sjogren's syndrome (5).

The Systemic Lupus International Collaborating Clinics group revised and validated the ACR SLE classification criteria in 2012 (6). According to the revision, a person is defined as having SLE if any 4 or more of the 11 criteria are present, respectively or simultaneously, during any interval of observation. Parotitis associated with Sjogren's syndrome is a rare condition in childhood (7, 8).

Sjogren's syndrome may be a primary disorder or may be secondary to other autoimmune disorders, such as systemic lupus erythematosus (9). Thus, it is required to be excluded a number of underlying causes of that disease (most of them of infectious origin). The natural history of SLE is unpredictable (10). Two of the most common complications are lupus nephritis (11) and cerebral damage.

We present the case of a young patient

with SLE in which the initial manifestation was parotitis and Sjogren's syndrome.

CASE PRESENTATION

We report the case of a 10-year old girl who presented with bilateral parotid swelling, symmetrical, non-traumatic and non-thrombocytopenic purpura on the lower limbs, accentuated by physical effort and orthostatic position, without fever (fig.1). Her symptoms have progressed gradually over the past 12 months.

The patient's past medical history is only remarkable for parotitis diagnostic, one year ago. She also accused intermittent arthritis, ocular manifestations consisting of itching and pain, recurrent epistaxis and photosensitivity. She has no other family history of medical illnesses, particularly heart disease or connective-tissue disease.

Fig. 1. Bilateral parotid swelling

The patient's physical examination reveals a normal nutritional state (BMI=21 kg/m2), "butterfly rash", bilateral swelling parotid, gingivostomatitis, an arterial blood pressure of 105/65 mmHg, a heart rate of 72 beats per minute, a respiratory frequency of 20 per minute, symmetrical purpura on the lower limbs; clinical examination did not reveal any tenderness of the abdomen, no hepatomegaly or splenomegaly and no arthritis.

Traumatic and toxic causes have been excluded, clinically and anamnestically. Multiple bacterial and viral infectious agents have been associated with the development of parotitis. We therefore determined serologies for HIV 1 and 2, cytomegalovirus (CMV) and Epstein-Barr (EBV) viruses' infection and IgG fractions were positive for CMV and EBV. Mumps was excluded anamnestically (disease was present in her medical history) and the clinical examination did not correspond to a condition manifested by acute symptoms such fever, shivering, myalgia, pain and local skin changes. Tuberculosis was excluded by negative history for TB contact, negative tuberculin intradermal reaction (IDR) and normal chest X-ray. Other bacterial infections were excluded. Mikulicz syndrome was also ruled out by clinical appearance of the parotid glands, absence of both lacrymal and submaxillary glands'swelling and lack of other syndromes (chronic lymphocytic leukemia, sarcoidosis). Maxillofacial consultation excluded sialolithiasis and reported gingivostomatitis, tooth cavities and dry mouth (fig. 2).

Fig. 2. Gingivostomatitis

Ophtalmological examination has found xerophtalmia and dry eyes. The diagnostic of Sjogren's syndrome could not be confirmed by biopsy.

Laboratory tests showed other pathological associated issues which pleaded for an autoimmune disease: an important inflammatory syndrome (erythrocyte sedimentation rate - ESR of 131 mm per hour), C-reactive protein (CRP) was positive in all determinations, a very high value of total serum proteins (122 g/l), hypergammaglobulinemia (51.7%), mild normochromic, normocytic anemia (haemoglobin - Hb of 9 g/dL), plasmocytic expansion on bone marrow biopsy. Bence-Jones protein was not determined, but multiple myeloma is usually met in adults over 40 years. Other serologies like p-ANCA and c-ANCA antibodies were negative. Liver function tests revealed mild hepatocytolysis (ASAT 45 U/L, ALAT 63 U/L), kidney function (urea, creatinine, clearance of creatinine) was normal. The abdominal ultrasound examination revealed no pathological findings.

Our case meets 5 of the 11 required ACR criteria for SLE: "butterfly rash", arthritis, photosensitivity, immunological changes such as anti-nuclear antibody titer (ANA) over 32 (positive titer over 1), anti-deoxyribonucleic acid antibodies (dsDNA) positive titers. These findings are classical for SLE. We believe those findings to be consistent with lupus associated with Sjogren's syndrome.

We also excluded other viral infections (hepatitis B, C) and we evaluated the status of lupus-target organs (heart – the cardiac ultrasound exam was normally, kidney – function and ultrasound exam) for initiating the therapy with corticosteroids. Cortisone therapy included methylprednisolone pulses, three consecutive administrations (30 mg/kg/day), following the oral medication.

Clinical and paraclinical evolution was slowly favorable with remission of arthralgia, decreased parotid swelling, improvement of biological inflammatory syndrome (ESR decreased at 70 mm/h), anemia (Hb of 11.4 g/dl). But a number of adverse effects and complications of immunosuppression were present: over-weight (8 kg), stretch marks, a prolonged evolution of gingivostomatitis, despite local treatment and oral hygiene, which could lead to a series of infectious complications such maxillary sinusitis, chronic rhinitis, conjunctivitis and urinary infections. She had no pancreatic involvement during evolution, the pancreatic ultrasound and levels of pancreatic enzymes were normal.

After two years of evolution the patient showed renal impairment: persistent hematuria and proteinuria, low levels of C'3 fraction of complement. It required renal biopsy for staging of lupus nephritis and so she was transferred to the Nephrology Clinic.

DISCUSSION

Primary Sjogren's syndrome occurs in the absence of another underlying rheumatic or immune disorder, while secondary Sjogren's can be associated with other diseases, such as systemic lupus erythematosus (SLE), scleroderma, rheumatoid arthritis (RA).

Thus, there is an overlap of Sjogren's syndrome with many other immune mediated diseases and it is sometimes difficult to determine whether a clinical manifestation is a primary consequence of Sjogren's syndrome or is due to one of its overlapping disorders.

Almost half of patients with Sjogren's syndrome also present with cutaneous find-

ings, such as dry skin (xeroderma), palpable and nonpalpable purpura, and/or urticaria (12).

Primary disease is a rare condition in childhood; only 145 cases of primary disease have been described in the international pediatric literature (13, 14). The majority of patients with Sjogren's are in conjunction with other autoimmune diseases, which are considered secondary to Sjogren's syndrome.

Individuals with secondary Sjogren's syndrome may have systemic lupus erythematosus, scleroderma, and primary biliary cirrhosis; therefore it takes a whole battery of tests and investigations for these patients to exclude other disorders.

Also, long-term, Sjogren's syndrome can lead to complications that affect teeth, kidneys, eyes, lungs and joints and even to lymphoma (15). Another rare manifestation in lupus is cerebral vasculitis.

In the literature there have been documented only few cases of atypical onset of Sjogren's syndrome (16).

In two of these cases the age of diagnosis was 10 years, similar to the age of our case.

In our experience and evidence with 40 cases of systemic lupus erythematosus this is the only case with associated Sjogren's syndrome.

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NEWS

AMNIOTIC MEMBRANE AND FINGERTIP INJURIES

A group of researchers from Department of Orthopaedic Surgery, Shiraz University of Medical Sciences conducted a study that evaluated the efficacy of amniotic membranes (AM) as a biologic wound dressing material for coverage of fingertip injuries, which are the most common hand injury, represent management challenges for hand surgeons. The study included 30 patients with full-thickness zone 1 fingertip skin loss. The patients were divided into 2 groups using the block randomisation method. In the first group, a skin graft was used for coverage and in the second group, AM was used. All patients were operated on by the same hand surgeon between February 2012 to October 2012. The minimum follow-up period was 6 months. Two point discrimination (T.P.D), light touch, healing time, days lost from work and infection rate were evaluated and recorded. Fingertips in both groups were assessed. T.P.D, light touch and days lost from work were significantly lower in the AM group than in the skin graft group. Healing time was lower in the skin graft group. In the both groups, no infection was detected. Patients of both groups were satisfied of their treatment and healing progress. The authors showed the effectiveness and safety of AM for the treatment of fingertip amputation, which can produce better sensation and functional outcomes than skin graft transplantations. (Fijan A1, Hashemi A2, Namazi H. A novel use of amniotic membrane for fingertip injuries. *J Wound Care.* 2014 May;23(5):255-8. PMID: 24810309).

Simona Tucaliuc

BIPOLAR DISORDER, NOT SO RARE DIAGNOSIS: SUBTYPES OF DIFFERENT DEGREES OF SEVERITY, DIAGNOSIS, THERAPY

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BIPOLAR DISORDER, NOT SO RARE DIAGNOSIS: SUBTYPES OF DIFFERENT DEGREES OF SEVERITY, DIAGNOSIS, THERAPY (Abstract): Bipolar disorder is manifesting as a mood disorder, typically showing episodes of mania, alternating with depressive episodes. The subtypes are including bipolar I disorder (one or several manic episodes) and bipolar II disorder (hypomanic episodes and one or several major depressive episodes). Nevertheless, sub-threshold diagnosis criteria may include another 5.1, up to 6.4 % of the population as having a bipolar spectrum disorder diagnosis. Anyone who received the diagnosis is not considered cured afterwards (just in remission). Diagnosis is considering the symptoms of mania, hypomania and depression. Therapy is based on lithium, anticonvulsants, for the manic symptoms, lamotrigine for the depressive episodes and antipsychotics. Under medication, most of the affected subjects are living a normal life; to a certain degree, medication may also prevent the relapses. **Keywords:** BIPOLAR DISORDER, MANIA, HYPOMANIA, MAJOR DEPRESSIVE EPISODES.

Bipolar disorder is considered a mental illness manifested as a mood disorder, also known as bipolar affective disorder, manic-depressive disorder, or manic depression. Typically, subjects with bipolar disorder show episodes of mania alternating with periods of depression. Approximately 4 % of the general population is affected by bipolar disorder. Prevalence is the same in men and in women and in different cultures and ethnic groups.

Variations in moods and energetic level were observed as part of the human personality, since ancient times. Terms like melancholia and mania have origins in Ancient Greek language. At the beginning of the 19th century, a French psychiatrist Jean-Etienne Dominique Esquirol described lypemania, an affective monomania and became the first to contour the modern depression (1).

In 1854 Jules Baillarger detailed to the French Imperial Academy of Medicine a biphasic mental illness, manifested with periodic mood changes between mania and depression; he used the term of "dual-form insanity", named afterwards by Jean-Pierre Fairet "circular insanity"(2). The concept was further developed by the German psychiatrist Emil Kraepelin, who used the concept of cyclothymia and he studied the evolution of the untreated bipolar cases. Adolf Meyer introduced the concept of bipolar disorder, as a result of genetic factors influenced by social and psychological factors.

In 1957, the German Psychiatrist Karl Leonhard was the first to define the subtypes of bipolar disorder, he also introduced the concept of bipolar and unipolar, to characterize the forms with exclusively depressive episodes.

EPIDEMIOLOGY

Approximately 4 % of the population has one of the bipolar disorder types at a certain period in their life. Further analysis of data from the USA is showing that 0.8 % of the population experience at least an unique manic episode (the diagnosis criterion for bipolar I) and 0.5 % have a hypomanic episode (the diagnosis criterion for bipolar II or cyclothymia).

When sub-threshold diagnostic criteria were included (such as one or two symptoms over a short time-period), another 5.1 % up to 6.4 % were considered as having a bipolar spectrum disorder (3). A recent analysis of data from US National Comorbidity Survey showed that 1 % of the population had lifetime prevalence criteria for bipolar I, 1.1 % for bipolar II and 2.4 % for sub-threshold symptoms. Data are similar in both sexes and in different cultures and ethnic groups. However, the severity of the disease may vary widely across the globe. Late adolescence and early adulthood are peak years for the onset of bipolar disorder (4).

Bipolar disorder can cause suicidal ideation that leads to suicidal attempts. One out of three patients with bipolar disorder reports past attempts of suicide, or succeeds it. The annual average suicide rate is 0.4 %, 10 to 20 times higher than in the general population. The standardized mortality ratio from suicide in bipolar disorder is reported to be between 18 and 25 (5).

SUBTYPES

There is no clear consensus in what concerns how many types of bipolar disorder do exist. The concept of bipolar disorder is referring to a spectrum of disorders; the newest classification includes three specific subtypes and a non-specified form.

Bipolar I disorder is characterized by one or several manic episodes. Further is registered if there were several episodes and the type of the most recent episode. A depressive or hypomanic episode is not required for diagnosis, but it often occurs.

Bipolar II disorder is characterized by the missing of the manic episodes; instead are appearing one or several hypomanic episodes and one or more major depressive episodes. Hypomanic episodes do not show as severe manifestations as mania does, and usually do not impair drastically social and/or professional life and do not manifest psychotic symptoms. This can make bipolar II disorder harder to diagnose; hypomanic episodes may look like periods of successful achievements, being less reported, unlike distressful depressive episodes.

Cyclothymia is described like a history of hypomanic episodes, alternating with episodes of depression, which do not meet diagnosis criteria for major depressive episodes. A low degree of mood cycling does exist, which is observed as a personality trait and is interfering with functioning of the individual.

Bipolar disorder NOS (not otherwise specified) is the diagnosis established when the disorder is not meeting the criteria for a specific subtype (6).

DIAGNOSIS

Signs and symptoms of bipolar disorder include abnormally elevated (manic or hypomanic) mood estates which impair

normal functions of daily life. Numerous cases experience also depressive episodes, although not all. A test suitable for the diagnosis does not exist, and the diagnosis may be difficult to establish, even for the psychiatric specialists. It is also hard to differentiate the depression found in the bipolar disorder, from the pure unipolar depression. An earlier age of onset is often predicting depression as the first few episodes. Since bipolar diagnosis needs a manic or hypomanic episode, many bipolar patients are first diagnosed and treated as having major depression (7).

Mania is the characteristic feature of the bipolar disorder, being a period of elevated or irritable mood, which can take the form of euphoria and is lasting at least one week. Patients usually have an increase in energy and a decreased need for sleep (most of them need just three or four hours of sleep per night). Some can go days without sleeping. Usual symptoms are pressured speech with the feeling of racing thoughts. Attention is affected, and the individual is easily distracted. Rational capacities may be impaired and the subjects may be engaged in risky behaviors, which otherwise are not characteristic for them. They may experience alcohol, cocaine or other stimulants, or sleeping pills abuse. The behavior may be aggressive, intolerant or intrusive. They may feel unstoppable, or ”chosen” and „in a special mission”, or may have another delusional ideas. Hyper sexuality may appear. At extreme, a manic estate can manifest psychosis, a break with reality, the process of thinking being affected together with the mood; sometimes this may lead to violent behaviors. Manic states may induce severe anxiety and irritability, or euphoria and grandiose ideas. Specific rating scales can measure the severity of the manic symptoms. Sleep disturbances, psychomotor and appetite changes may announce the onset of a manic episode, two or three weeks before (8).

Hypomania is a mild to moderate state of elevated mood, manifested by optimism, pressure of speech and activity and a reduced need for sleep. Unlike mania, most of the times hypomania does not interfere with normal functioning of the subject. People with hypomania are usually more productive, while manic subjects show attention troubles, which are impairing daily activities. Hypomanic subjects do not have delusions or hallucinations. Because it generally generates a state of well-being, hypomania is rarely reported as a pathologic condition. Only when depression is accompanying it, or if the mood changes are uncontrollable, hypomania may lead to search for specialized assistance. Untreated, a hypomanic episode may last from a few days to several weeks. Usually, symptoms may last a few weeks, up to a few months (9).

The depressive phase of bipolar disorder appears as persistent feeling of sadness, anxiety, guilt, anger, isolation or hopelessness. Disturbances of sleep and appetite are common, as well as: fatigue and lack of interest for enjoyable activities, decrease of concentration capacity, apathy, depersonalization, loss of interest in sexual activity, social anxiety, irritability, lack of motivation and morbid, suicidal thoughts. In severe cases psychosis may appear, known as severe bipolar depression with psychotic features. Psychotic symptoms are represented by usually unpleasant delusions or more rare, hallucinations. A severe depressive episode may last from two weeks, up to more than six months, if untreated (10).

Mixed affective episode is a condition in which symptoms of mania and depression occur simultaneously. Classical examples are weeping during mania and racing

thoughts during a depression phase. Mixed episodes are the most dangerous of the bipolar phases, since during this; the risk of suicide attempts greatly increases (5).

The later diagnosis of bipolar disorder may be preceded by different pathologic conditions developed during childhood, such as mood abnormalities, major depressive condition and ADHD (attention deficit hyperactivity disorder) (11).

Bipolar disorder should be differentiated from other mental disorders which do manifest similar symptoms, like schizophrenia, attention deficit hyperactivity disorder (ADHD) and borderline personality (12).

Bipolar disorder diagnosis is officially defined such that anyone who has a history of hypomania and depression receives this diagnosis, whatever their functioning and vulnerability are. This is considered „an ethical and methodological issue", as long as no one is considered as being recovered from bipolar disorder, only in remission, according to official criteria. This situation is delicate, since brief hypomanic episodes are widespread in population and not associated with any dysfunction (13).

THERAPY

Several medications are used in the treatment of bipolar disorder. Lithium is effective in the therapy of acute manic episodes and in preventing manic relapses; it is also decreasing the risk of suicide and self-harm in bipolar disorder (14).

Sodium valproate, which was initially used as anticonvulsant, is now an usually prescribed drug, effective in treating manic episodes (15).

Lamotrigine is to a certain degree, effective in the treatment of the bipolar depression (16). According to the severity of the disease, anticonvulsants can be used in combination with lithium, or alone.

Antipsychotics are used in the therapy of mania, although the long term effects are not clear. Olanzapine is used for prevention of relapses, even if it is not as effective as lithium.

Antidepressants showed no higher efficacy than that of the mood stabilizers. Benzodiazepines are used as adjuvants to medications.

Psychotherapy is targeting the alleviation of main symptoms, recognition of relapse risk and maintenance of remission. Psycho education and cognitive behavioral therapy are efficient in preventing relapses (17).

RECOVERY AND RECURRENCE

Studies showed that 50 % of hospitalized (which means most severe cases) had full recovery in six weeks, and 98 % in two years. In two years, 72 % had symptomatic recovery (lack of all symptoms) and 43 % functional recovery (prior occupational and living status). Nevertheless, 40 % experienced a new episode within two years of syndrome recovery and 19 % evolved without recovery (18).

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NEWS

ORAL APIXABAN FOR THE TREATMENT OF ACUTE VENOUS THROMBOEMBOLISM

Apixaban, an orally administered factor Xa inhibitor, may be, according to a recent study, an alternative to the standard treatment of venous thromboembolism. In this study, double-blind, randomized, was compared apixaban (at a dose of 10 mg x 2/day 7 days followed by 5 mg x 2/day 6 months) with conventional therapy (subcutaneous enoxaparin followed by warfarin) in 5395 patients diagnosed with acute venous thromboembolism. Apixaban proved noninferior to conventional therapy ($p < 0.001$). Major bleeding occurred in 0.6% of patients receiving apixaban and 1.8% of those who received conventional treatment. Rates of other adverse effects were similar in the 2 groups. The results of next studies will provide data on the safety and efficiency of administration to selected groups of underweight patients with cancer or with a creatinine clearance below 50ml/min. (Agnelli G, Buller HR, Cohen A, et al., Oral Apixaban for the treatment of acute venous thromboembolism, *N Engl J Med* 2013; 369: 799-808)

Corina Dima-Cozma

CURRENT STATUS IN VITAMIN D AND REGULATORY T CELLS-IMMUNOLOGICAL IMPLICATIONS

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CURRENT STATUS IN VITAMIN D AND REGULATORY T CELLS (IMMUNOLOGICAL IMPLICATIONS) (Abstract): There has been a continuous effort to understand possible non-Ca metabolism roles of vitamin D, including its role in the immune system and, in particular, in T cell-medicated immunity. Vitamin D receptor is found in significant concentrations in the T lymphocyte and macrophage populations, when we refer to immune system, and pretty much in any human tissue and cells. Until the eighties, *no one* had imagined that vitamin D might play a role in the functioning of the immune system. Today we accepted that the normal immune system harbors a regulatory T cell (Treg) population specialized for immune suppression. Currently, the most commonly known regulatory T-cell lineage is called $CD4^{+}CD25^{high}FoxP3^{+}$ regulatory T cells. Several autoimmune disorders have been linked to a deficiency in vitamin D_3. In some autoimmune diseases, including multiple sclerosis (MS), a compromised Treg function is believed to be critically involved in the disease process. Vitamin D insufficiency has ramifications not only for bone health, but also in other non-skeletal areas of vitamin D function, such as immune cells, muscle cells and, perhaps, adipocytes. As a final conclusion, further researches in the field of vitamin D, Tregs, immunity (inflammatory processes, rejection, autoimmune diseases, etc.), either *in vitro* on cell cultures or *in vivo* using lab animals or volunteers are still necessary. **Keywords:** VITAMIN D, REGULATORY T CELLS (TREG), AUTOIMMUNE DISEASES, VITAMIN D RECEPTOR (VDR), FORK-HEAD BOX P3 (FOXP3), ADIPOGENESIS, ADIPOCYTE

In recent years there has been an effort to understand possible non-Ca metabolism roles of vitamin D, including its role in the immune system and, in particular, on T cell-medicated immunity. Vitamin D receptor is found in significant concentrations in the T lymphocyte and macrophage populations, when we refer to immune system, and pretty much in any human tissue and cells. However, its highest concentration is in the immature immune cells of the thymus and the mature CD8 T lymphocytes. Until 1980, *no one* had imagined that vitamin D might play a role in the functioning of the immune system. The function of vitamin D was largely considered to be in the area of calcium, phosphorus, and bone metabolism. It prevents rickets in children,

osteomalacia in adults, and hypocalcemic tetany. Continued pursuit of the metabolism of vitamin D resulted in the understanding that vitamin D must first be hydroxylated in the liver to form 25-hydroxyvitamin D_3 ($25(OH)D_3$), the major circulating form of the vitamin (1). This form of vitamin D was subsequently found to be metabolically inactive and must be further converted to a final active form, 1□,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (2, 3). The attempt to understand how the active form of vitamin D carries out its functions led to the discovery of vitamin D receptor (VDR) in 1974 and 1975 (4, 5). It became clear that this $1,25(OH)_2D_3$ (radiolabelled) is localized almost entirely in the nucleus in a specific fashion in target tissues. This localization was also found in other tissues not previously considered targets (6, 7). For example, keratinocytes of skin, islet cells of the pancreas, lymphocytes, and promyelocytes, adipocytes, etc. showed specific nuclear localization of $1,25(OH)_2D_3$ and the presence of VDR. The discovery of VDR in these tissues resulted in the idea that vitamin D hormone had functions beyond calcium and phosphorus metabolism, which prompted investigations into the noncalcemic actions of vitamin D (8). Noting VDR in promyelocytes, Abe et al. and Tanaka et al. (9, 10). demonstrated that vitamin D hormone can suppress proliferation of promyelocytes and cause their differentiation into the monocyte. Similar effects of vitamin D hormone on several cancerous cell lines ensued (11). A role of vitamin D hormone in cellular differentiation thus became known.

It is now widely accepted that the normal immune system harbors a regulatory T cell (Treg) population specialized for immune suppression (12). Tregs exert functions in the induction and maintenance of immune tolerance (13-16). The family of Tregs is represented by a heterogeneous cell population that includes adaptive and naturally occurring Tregs (13-16). According to the current paradigm, adaptive Tregs develop from naïve T cells in the periphery and can produce IL-10 and transforming growth factor β (TGF β), whereas naturally occurring Tregs originate in the thymus as $CD4^+CD25^+$ cells and perform their suppressive functions through cell-to-cell contacts and membrane-bound TGF β and cytotoxic T-lymphocyte antigen-4 (CTLA-4) (13-16). The transcription factor FoxP3 is necessary for the development of this subpopulation of $CD25^+$ Tregs, being their characteristic marker (17).

Vitamin D and Tregs interactions and possible pathways

Broadly speaking, Tregs have the capability to suppress the activity of the immune system and to regulate self-tolerance. However, it is not possible to unambiguously define Tregs as a homogeneous group, since T cells of different phenotypes have been shown to exhibit immune regulating potential (18). Currently, the most commonly known regulatory T-cell lineage is called $CD4^+CD25^{high}FoxP3^+$ regulatory T cells. The fork-head box p3 (FoxP3) transcription factor is the cell lineage–determining master transcription factor of this T-cell subtype (18). Generation of Tregs is shown to depend on the cytokine transforming growth factor (TGF)-β (19). A new population of Tregs has been described lately that is under the regulation of interleukin IL-35 (TR_{35}) (18). Although the first report showed them to be $FoxP3^+$, a more recent study suggests that T_{R35} cells

are independent of FoxP3 (20). Because not much information exists regarding the influences of dietary products on generation or function of this population, within this report, Tregs are defined as $CD4^+CD25^+$ $FoxP3^+$ T cells unless otherwise stated (18).

Vitamin D_3 can be generated in the skin through processes using ultraviolet B (UVB)-mediated photolysis of a cholesterol derivative and a spontaneous isomerization step. Vitamin D_3 needs to be enzymatically active to its hormonal form! Because endogenous production of Vitamin D depends on UVB exposure, the concentration is highly influenced by environmental factors such as seasonal sunshine length and latitude, behavioral factors such as time and surface area of exposed skin, skin pigmentation and age.

Vitamin D_3 is also taken up through diet. These factors lead to great variations in its circulating form and calcidiol, spurring an ongoing discussion about "natural," "normal" and "desired" levels of Vitamin D and its derivatives. A daily intake of 100 µg (4,000 IU) is suggested as a safe dose, elevating the $25(OH)D_3$ levels to desired concentrations in most humans (21). This number is higher than the recommended nutrient intake of between 5 and 15 µg/day (200–600 IU) by WHO (World Health Organization).

This section deals with the role of Vitamin D and its derivatives on the immune system, focusing on their effects on $FoxP3^+$ Treg generation and function in different diseases. UVB light exposure as well as local calcipotriol (a synthetic derivative of calcitriol) treatment can lead to vitamin D receptor (VDR)-dependent induction of $FoxP3^+$ Tregs in the skin (18). Vitamin D_3 supplementation is reported to reduce the CNS autoimmune disease EAE (experimental autoimmune encephalomyelitis) in females but not in males or ovariectomized female mice. The disease protection correlated with high local $1,25(OH)_2D_3$ (active form) levels in the inflamed spinal cord, but not in serum. It was suggested a slower degradation of $1,25(OH)_2D_3$ process in the CNS, mediated through a suppression of the enzyme CYP24A1, a 24-hydroxylase with $1,25(OH)_2D_3$–inactivating properties (18, 22, 23). The protective effect in females could be restored in ovariectomized females through estrogen supplementation (24).

An additional pathway for the treatment effect of $1,25(OH)_2D_3$ has been shown by induction of high numbers of $FoxP3^+$ Tregs in combination with IL-2 (25). In support of this, dietary administration of 1α-(OH)-vitamin D_3, an active vitamin D_3 analog, led to reduced diabetes in non-obese diabetic mice and increased $FoxP3^+$ T cells (26). Previously, it was shown that a $1,25(OH)_2D_3$ analog also had a therapeutic effect in a spontaneous autoimmune non-obese diabetic model, resulting in an increased protective $CD25^+$ Treg population (28).

A combination of $1,25(OH)_2D_3$ and the anti-inflammatory corticosteroid dexamethasone synergistically induced primarily IL-10–producing $FoxP3^-$ Tregs (28, 29). Anyway, in an experimental IBD (inflammatory bowel disease) model, $1,25(OH)_2D_3$ and dexamethasone had a FoxP3 expression–promoting effect in the gut (30). Not only was the amount of FoxP3 expression increased by $1,25(OH)_2D_3$ treatment, but the potency of $FoxP3^+$ Tregs was accentuated, even in a Th2-driven *in vivo* asthma model (31).

The $1,25(OH)_2D_3$ precursor calcidiol

[25(OH)D_3] is mainly 1α-hydroxylated in the kidney to its active form, but also it has been shown to be processed in immune cells (18). $CD3^-$ and $CD28^-$ activated T cells as well as dendritic cells (DCs) are able to produce 1,25$(OH)_2D_3$ from its precursor, but T cells could not directly produce precursor from Vitamin D under these conditions (18). To support this, it is shown that 1α-hydroxylase is highly up-regulated in activated human T cells (32). Furthermore, 1,25$(OH)_2D_3$ up regulates VDR expression, which has been shown to correlate with the protective effects of 1,25$(OH)_2D_3$ in EAE (18). Nevertheless, it is reported that 1,25$(OH)_2D_3$ induces $FoxP3^+$ Treg conversion. Was also showed that multiple sclerosis (MS) patients have lower levels of calcidiol and 1,25$(OH)_2D_3$ during active disease, suggesting that Vitamin D_3 metabolites might play a role in controlling Treg formation and the inflammatory process. This role is further underlined by findings about positive correlations of serum calcidiol and Treg function in MS patients (32, 33). Upper studies also received support in a retrospective analysis of serum samples preceding disease onset and from epidemiological studies showing an inverted correlation of sun exposure and MS prevalence (34). Although CNS-specific conversion of encephalitogenic T cells into $FoxP3^+$–induced Tregs is described (18), its correlation to local vitamin D levels is still obscure. The instability of pancreas-retrieved Tregs has been suggested (35), but it is not reported if this is correlated to levels of vitamin D derivatives in pancreas of diabetic mice. Studying the endogenous levels of vitamin D derivatives in other target tissues of immune attacks, such as in gut, joint and lung, and their role in induction of tissue-induced Tregs could be valuable.

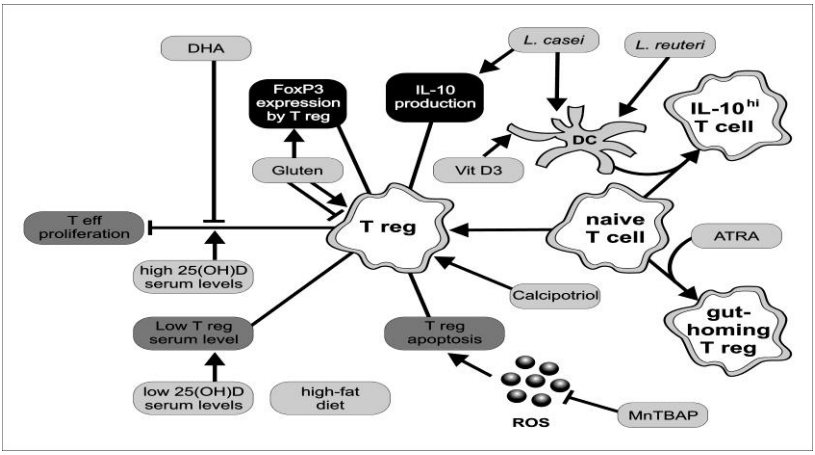

Fig. 1. Overview of dietary components and conditions (represented by dark grey boxes) influencing regulatory T cells (Tregs). The Treg-modulating effects of dietary components could be direct by mediating expansion and/or suppressive function of Tregs or indirect via modulation of DCs and their subsequent actions on Tregs. Additionally, the indirect influence of dietary components could be mediated by regulation of naive T cells to convert to Tregs or by blocking the function of inflammatory effectory T cells (Teffs). The positive anti-inflammatory effects of Tregs are mediated by expression of FoxP3 and production of IL-10 (indicated by black boxes). ROS = reactive oxygen species. $IL\text{-}10^{hi}$ = high expression of IL-10. (18 modified)

Vitamin D + Treg cells in autoimmune diseases

Several autoimmune disorders have been linked to a deficiency in vitamin D_3 (17, 36).

In some autoimmune diseases, including multiple sclerosis (MS), a compromised Treg function is believed to be critically involved in the disease process. In vitro, the biologically active metabolite of vitamin D has been shown to promote Treg development. A poor vitamin D status has been linked with MS incidence and MS disease activity (33). Moreover, high circulating levels of vitamin D have been associated with lower risk of multiple sclerosis (32).

Vitamin D is an important regulator of the immune system in general and multiple sclerosis in particular. Experimentally, invariant natural killer T (iNKT) cells have been shown to be important suppressors of autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE; an animal model of MS), as shown in literature. On the other hand, in experimental allergic asthma iNKT cells are required for disease induction and are therefore pathogenic. The active form of vitamin D (calcitriol) suppresses EAE. The development of EAE symptoms is accelerated in vitamin D deficiency. Interestingly experimental asthma is less severe in vitamin D deficiency although there is no effect of calcitriol on disease severity. The data suggest that an important target of vitamin D in EAE and asthma are the iNKT cells. Vitamin D and/or vitamin D receptor deficiency results in the impaired development of iNKT cells. Vitamin D is critical very early during development of the immune system. Low levels of vitamin D *in utero* resulted in significantly reduced numbers of iNKT cells that failed to recover when calcitriol was used to supplement neonatal or adult mice. The data suggest that one of the consequences of early vitamin D deficiency is a reduction in the numbers of iNKT cells that develop. The iNKT cells are required for the beneficial effects of calcitriol in EAE. The important role of vitamin D on iNKT cells could impact the development of human immune-mediated diseases including MS and asthma (37).

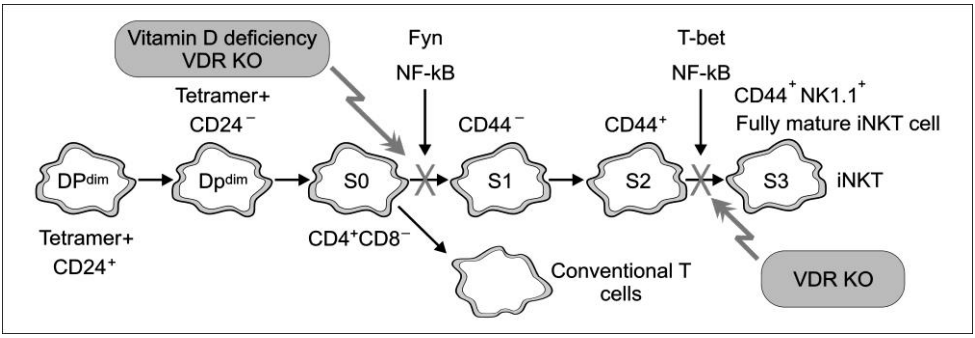

Fig. 2. Vitamin D and vitamin D receptor (VDR) targets in invariant natural killer T (iNKT) cell development (38, 39 modified).

iNKT cells develop in the thymus following several different phenotypic changes. The earliest iNKT cell precursor, DPdim expresses the invariant T-cell receptor (tetramer +) and $CD24^+$. The early iNKT cells down-regulate CD24 and diverge from the

other CD4/CD8 DP cells that go on to become conventional T-cells. Expression of two transcription factors (Fyn and NF-kB) is important in the movement of iNKT cells from stage (S) 0 to S1. Vitamin D and VDR deficiency affect the number of iNKT cells that rapidly expand and enter the S1 stage in maturation. There is no effect of vitamin D deficiency on the further maturation of iNKT cells. VDR knockout (KO) iNKT cells have an additional block in maturation at the S2 stage and fail to fully develop into mature iNKT cells. T-bet and NF-kB expression is associated with the transition of iNKT cells from S2 to S3. VDR KO iNKT cells express significantly less T-bet than there fully mature S3 wild-type counterparts (see Fig. 2) (37, 39).

Vitamin D3 is known to induce Treg cells by rendering antigen-presenting cells with immunologic tolerance (tolerogenic), its direct effect on human naturally occurring Treg cells is unclear. Some groups investigated if and how $1,25(OH)_2D_3$ can directly affect the proliferation and function of human naturally occurring Treg cells *in vitro*. So they demonstrated that these Treg cells express vitamin D receptors that were up-regulated following anti-CD3/CD28-bead stimulation. $1,25(OH)_2D_3$ inhibited proliferation of Treg cells even when exogenous interleukin-2 was provided. Treg cells were more susceptible to the inhibitory effect of $1,25(OH)_2D_3$ than conventional T cells. $1,25(OH)_2D_3$ neither affected the anergic state nor the suppressive function of Treg cells but induced a subtle increase in interleukin-10-secreting cells. The cell-division-inhibiting effect of $1,25(OH)_2D_3$ on Treg cells was also demonstrated *in vivo* by supplementing vitamin D-deficient HIV-1-infected patients with 2000 IU cholecalciferol (vitamin D_3), conform with literature. Increased serum $1,25(OH)_2D_3$ levels were associated with a drop in the number and percentage of Treg cells, which may be attributed to a decrease in the proliferating $FoxP3^+$ Treg cell population (40).

Treg cells are characterized by a $CD25^{high}$ $CD4^+$ phenotype and signature transcription factor, fork-head box protein 3 (FoxP3). They are a part of the immune system and are crucial in the regulation of immune homeostasis (40). It has been demonstrated that the regulatory function of $CD4^+$ $CD25^+$ Treg cells is hindered in autoimmunity, allergy and infectious diseases, indicating that these cells play a huge role in immune-mediated pathology. In last years, evidence has conducted researchers to the potential therapeutic application of Treg cells either in potentate their regulatory activity in inflammatory diseases such as autoimmunity, allograft rejection, graft versus allergic diseases and/or host disease also in blocking their suppressive activity related to tumor immunity or vaccine development (40).

VITAMIN D AND ADIPOGENESIS

Despite the ability to synthesize vitamin D in the skin and the availability of vitamin D in some foods, vitamin D deficiency is increasingly recognized as a widespread global nutritional problem, as shown in literature (41). Vitamin D insufficiency has ramifications not only for bone health, but also in other non-skeletal areas of vitamin D function, such as immune cells, (42) muscle cells and, perhaps, adipocytes (43, 44).

Studies conducted in Japan almost 20 years ago indicated that treatment of 3T3-L1 pre-adipocytes in culture with $1,25(OH)_2D_3$, the bioactive form of vitamin D, affected differentiation and adipocyte metabolism. (47). These early studies indi-

cated that nM concentrations of 1,25-$(OH)_2$-vitamin D could inhibit adipogenesis and reduce the accumulation of triacylglycerol by 50% compared to fully differentiated control cells. In addition, treatment of pre-adipocytes with other vitamin D metabolites, such as 24,25-$(OH)_2$-vitamin D, could also inhibit pre-adipocyte differentiation, but at higher concentrations than $1,25(OH)_2D_3$ that paralleled their reduced affinity for the vitamin D receptor. These early studies also noted that specific $1,25(OH)_2D_3$ binding was evident in pre-adipocyte 3T3-L1 cells, but there was no evidence of specific binding for $1,25(OH)_2D_3$ in mature adipocytes (42). This finding is consistent with the idea that any influence of vitamin D on adipogenesis would likely be exerted early in the pre-adipocyte to adipocyte transition when more VDR was available. Theoretically, the sensitivity of the pre-adipocyte to $1,25(OH)_2D_3$ would be increased by a greater amount of vitamin D receptor or other limiting VDR-associated proteins. More than a decade ago, it was shown that one of the early effects of $1,25(OH)_2D_3$ treatment of 3T3-L1 pre-adipocytes was an increase in vitamin D receptor mRNA expression in pre-adipocytes, which occurs within 4h of treatment in cell culture (42). Little was appreciated, however, about any direct importance of the unliganded VDR in adipogenesis, or the molecular details whereby $1,25(OH)_2D_3$ treatment inhibited differentiation of pre-adipocytes.

DISCUSSION

Summarizing all written up, we could say that definitely exist profound metabolic links via multiple metabolic and signaling pathways between vitamin D (provided as food intake or endogenous produced), its active hormonal form $1,25(OH)_2D_3$, VDR from various cells (bone, muscle, hepatocytes, epithelial cells, neurons, white cells, adipocytes), various other signaling proteins (cytoplasmic and nuclear) or enzymes, transcriptional and translational factors in nuclei, and various genes (vitamin D direct related or others).

So, various groups studied different aspects of vitamin D intake amount, in those various levels in its pathway (metabolically, signaling, etc.) and its effect over immunity (hence its relation with Tregs), beyond vitamin D effects on calcium metabolism. Various groups oriented their research over vitamin D and adipogenesis and inflammation related to adipose tissue. Numerous groups studied interrelations between vitamin D and Tregs in many autoimmune diseases, infections, allergies, organ rejection and so on.

Starting from all this work done or still in progress, we could summarize it is still necessary to study on *in vitro* and *in vivo* (on lab animals and voluntary patients) the non-calcemic effects in metabolism by vitamin D (and her derivatives) in an healthy organism or in many diseases, to either ameliorate there, the effects of that disease (or medication related disease) or as co-treatment even.

CONCLUSIONS

The work in the field of vitamin D, Tregs, immunity (inflammatory processes, rejection, autoimmune diseases, etc.) is still necessary either *in vitro* on cell cultures or *in vivo*, using lab animals or volunteers. Also, studies on the relation between vitamin D, Tregs and adipocytes are still needed, this avenue requiring a huge amount of work, to which our research group is hoping to contribute in the future.

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GENOTYPE - PHENOTYPE CORRELATION IN TRISOMY X: A RETROSPECTIVE STUDY OF A SELECTED GROUP OF 36 PATIENTS AND REVIEW OF LITERATURE

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CORRELATION GENOTYPE – PHENOTYPE IN TRISOMY X: A RETROSPECTIVE STUDY OF A SELECTED GROUP OF 36 PATIENTS AND REVIEW OF LITERATURE (Abstract): Trisomy X (47,XXX) is a gonosomal aneuploidy characterized by the presence of an extra X chromosome in a female person. Usually the diagnosis is established made postnatally by chromosome analysis in patients with suggestive clinical signs. Clinical signs vary by age. In prepubertal patients have a growth retardation associated with uncharacteristic facial dysmorphism, mild mental retardation with behavioral disorders**,** plus clinical signs of ovarian dysgenesis**,** postpubertal. **Aim:** We analyzed retrospectively the genotype - phenotype correlations for a selected group of 36 patients diagnosed with trisomy X (homogeneous or mosaic) by cytogenetic methods (X chromatin and karyotype). **Material and methods:** Analysis of the clinical data of 36 patients diagnosed with trisomy X and correlation with the results of X chromatin and karyotype. **Results**: Clinical signs detected in patients with homogeneous trisomy X 47,XXX (22.22%), mosaic 46,XX/47,XXX (16.66%) or 47,XXX/48,XXXX (5.55%) were prepubertal, growth retardation associated with dysmorphic facial (upslanted palpebral fissure, epichantus, thin lips) and postpubertal, signs of ovarian dysgenesis (secondary amenorrhea, early menopause). The phenotype of patients with different gonosomal mosaic corresponding to Turner syndrome, incorporating a cell line with trisomy X (55.55%) was variable, correlated with the type of chromosomal abnormalities detected. **Conclusions**: The results of our study are similar to those obtained in other studies and emphasizes that phenotypic variability of patients with trisomy X feature makes it difficult to genotype - phenotype correlations. **Keywords**: HOMOGENOUS AND MOSAIC TRISOMY X, X CHROMATIN, KARYOTYPE.

Trisomy X, which determines the triple X syndrome is an gonosomal aneuploidy characterized by the presence of an extra X chromosome in a female (1). This syndrome has a frequency of approximately 1/1000 female births, but only 10% of cases are diagnosed. In 90% of cases, the extra X chromosome results from a maternal nondisjunction in meiosis I, associated with increased maternal age at conception (1-3).

Prenatal diagnosis can be achieved through amniocentesis or chorionic villi biopsy. Postnatal diagnosis is achieved by chromosomal analysis of peripheral blood in patients presenting a suggestive clinical picture, variable by age. Prepubertal pa-

tients have hypotonia, delayed development (motor / language), uncharacteristic facial dysmorphism (upslanted palpebral fissure, round facies, hypertelorism, epicantus, ear anomalies), IQ at the lower limit of normal, cognitive impairment or behaviour, anxiety, and scoliosis. Postpubertal patients display the characteristic signs of gonadal dysgenesis: menstrual disorders (early menarche, irregular periods, early menopause) or reproductive disorders (infertility or repeated miscarriages) (1,2, 4-10).

Cytogenetic tests - sex chromatin test (Barr test) and chromosomal analysis (karyotype) – confirm the diagnosis of trisomy X.

The X chromatin test is positive, and evidence cells with one or two Barr bodies (11-13), the results being correlate with the karyotype. The most common chromosomal abnormality is free homogeneous trisomy 47,XXX, but in 10% of cases abnormalities are identified in mosaic 46,XX/47,XXX, 47,XXX/48,XXXX, 45,X/47,XXX or 45,X/46,XX/47,XXX (4,5).

The differential diagnosis is made with the other polysomy X, which may have clinical signs common with trisomy X but dysmorphic changes are more severe and patients have hypostature, severely affected intellect and sterility associated with disorders of sexual differentiation (1,13).

The presence of premature ovarian failure and developmental and behavioral disorders, requires differential diagnosis with fragile X syndrome and tall stature requires differential diagnosis with Marfan syndrome (1,13).

Prenatal detection of trisomy X requires an adequate genetic counseling for the patients who are to be given information about the disease, any neurodevelopmental and psychological issues problems, as well as information on the risk of recurrence in a future pregnancy, which is <1% (4,10). Also, information will be provided about the possible occurrence of premature ovarian failure and the risk of 1-5% to have offspring with trisomy X or trisomy XXY (5, 12).

The **Aim** of our retrospective study was to make a genotype - phenotype correlation for a selected group of 36 patients diagnosed with trisomy X (homogeneous or mosaic) through cytogenetic testing (X chromatin and karyotype).

MATERIAL AND METHODS

Between January 1965 and December 2012, 36 patients were diagnosed cytogenetically with homogeneous or mosaic trisomy X (5.35% of the total of 672 patients evaluated for the presence of clinical signs evocative for an ovarian dysgenesis) in Iasi Medical Genetics Centre.

Clinical evaluation of patients included anthropometric measurements, assessment of pubertal development and evidence of clinical signs suggestive for a gonadal dysgenesis.

Cytogenetic evaluation included the *X chromatin test* (Barr bodies) and the G banding *chromosome analysis* (450-550 bands/metaphase). *The X - chromatin (Barr body)* was analyzed on buccal mucosa smear with at least 300 cells being analyzed (14). The karyotype was also performed in the presence of an abnormal test (frequency less than 10% of Barr bodies or presence of 2 or 3 Barr bodies). *Chromosomal analysis* based on a short-term culture of activated T-lymphocytes (72 hours) was performed according to the Moorhead technique (1960), adapted in our laboratory. Metaphases were examined by optical microscope and selected cells were karyotyped. For each case a minimum of 16 metaphases were analyzed. In cases of detected cells with different number of chromosomes X, the number of cells analyzed was increased to 32, 64 or even 96.

RESULTS

Based on the analysis of the karyotypes register of Cytogenetics Laboratory of the University of Medicine and Pharmacy "Grigore T. Popa" Iaşi, we identified 36 patients with trisomy X (homogeneous and mosaic) (tab. I).

In most cases (69.44% to 25 patients), there was a reference diagnosis related to a gonadal dysgenesis, and in three cases (8.33%) there were purimalformative syndromes.

We note that the clinical suspicion of triple X syndrome existed only in 2 cases (5.55%). We believe that this data can be correlated with phenotypic polymorphism of trisomy X.

The age of patients at diagnosis ranged from 1 month to 42 years. In prepubertal period (0-12 years) 11 cases were diagnosed (30.55%), 12 other cases (33.33%) were diagnosed during puberty (12-16 years) and 13 cases (36.11%) were diagnosed in the post pubertal period (over age 16) (tab. I).

The X chromatin test performed on buccal mucosa smear test (Barr body) was positive in all cases, revealed the presence of 1, 2 or 3 Barr bodies, and the results were compared with the chromosomal analysis results. In cases where a cell line with X monosomy was identified, the percentage of X chromatin test was positive, but low (<10%).

The karyotype was 47,XXX in 8 patients (22.22%), 46,XX/47,XXX in 6 cases (16.66%), 47,XXX/48,XXX in 2 patients (5.55%) and revealed various gonosomal mosaics containing a cell line with trisomy X and one line with X chromosome monosomy, in 20 cases (55.55%) (tab. I).

For patients diagnosed in the prepubertal period (0-12 years) the main clinical signs were growth retardation (90.9%) and facial dysmorphism with abnormally shaped ears (72.72%), thin lips (63.63%), hypertelorism (54.54%), epichantus (54.54%) and upslanted palpebral fissure (63.63%) with just only one patient having tall stature (9.09%). Moderate mental retardation was detected in 3 patients (27.27%) - all showing the karyotype 46,XX/47,XXX; only one patient presented borderline intelligence (9.09%) and karyotype 45,X/46,XX/47,XXX, while the remaining patients presented a normal intellect. In the case of a patient with mosaic 47,XXX/48,XXXX a psychomotor retardation was detected. Two patients had psychiatric disorders, and one was diagnosed with mosaicism 46,XX/47,XXX presented behavioural disorders (narcissism and masturbation). Other abnormalities detected were: skeletal (45.45%), genitals (36.36%), cardiac (18.18%) and reno-urinary (9.09%).

In cases of patients with trisomy X, diagnosed pubertal (12-16 years) and postpubertal (over 16 years) the main clinical signs were ovarian dysgenesis: late menarche (24%), delayed puberty (28%), reduced development of breasts (40%), reduced pubic hair growth (36%), reduced / absent axillary hair growth (40%), irregular menstrual cycles (24%), primary amenorrhea (24%) and secondary amenorrhea (20%). Postpubertary, facial dysmorphism was uncharacteristic with upslanted palpebral fissure (8 cases -32%), down-slanted palpebral fissure (4 cases -16%), abnormal ear shape (4 cases, 16%) and thin lips (5 cases -20 %). Tall stature was present in 20% of cases (5 patients), and growth retardation in 32% of cases (8 patients). Psychiatric disorders were present in 4 patients (16%), one of them having a free homogeneous trisomy 47,XXX and presenting sexual identity disorders without cognitive impairment.

TABLE I

Motivation of cytogenetic analyses performed and the results of Barr test and karyotype in patients with trisomy X.

No.	Age years	Reference diagnosis	Barr test	Results of karyotype
1.	0.9	CFD	+/ 1,2 B	46,XX/47,XXX
2.	10	SPH	+/ 1,2 B	45,X/46,XX/47,XXX
3	6	SPH	+/ 1,2 B	45,X/46,XX/47,XXX
4.	29	POF	+	45,X/46,XX/47,XXX
5.	14	PD	+	45,X/46XX/47,XXX
6.	13	GD	+	45,X/47,XXX
7.	15	TS	+	45,X/46,XX/46,X,r(X)/47,XXX
8.	16	SPH	+	45,X/47,XXX
9.	12	TS	+	45,X,inv(9)(p13;q12)/47,XXX,inv(9)(p13;q12)
10.	0.1	PMS	+/ 1,2 B	45,X/46,XX/47,XXX +mar
11.	29	PA	+/ 1,2 B	47, XXX
12.	23	POF	+/ 2 B	45,X/47,XXX
13.	13	CFD	+/ 1,2 B	47,XX,+C/46,XX
14.	22	RD	+/ 1,2 B	46,X,dic(X)/45,X/46,XX/47,XXX
15.	31	POF	+/ 1,2 B	47,XXX
16.	19	SPH	+/ 1,2 B	45,X/47,XXX
17.	1.1	SPH	+/ 1,2,3 B	47,XXX/48,XXX
18.	1.7	TX	+/ 1,2,3 B	47,XXX/48,XXX,+mar
19.	16	PA	+/ 1,2 B	47,XXX
20.	0.4	PD	+/ 1,2 B	47,XXX
21.	42	TS	+/ 1,2,3 B	45,X/46,XX/47,XXX/48,XXXX
22.	0.3	PMS	+/ – 1, 2 B	46,XX/47,XXX
23.	0.7	PMS	+/ - 2 B	47,XXX
24.	9	TX	+/- 2 B	45,X/46,XX/47,XXX
25.	9	GD	+/- 2 B	47,XXX
26.	12	SPH	+/- 1,2 B	45,X/47,XXX
27.	13	GD	+/- 1,2 B	46,XX/47,XXX
28.	13	SPH	+/ – 1,2 B	45,X/46,XX/47,XXX
29.	15	GD	+/ 1,2 B	46,XX/47,XXX
30.	15	PD	+/ -2B	45,X/47,XXX
31.	18	SPH	+/-2 B	45,X/47,XXX
32.	18	PA	+/ 2 B	45,X/46,XX/47,XXX
33.	19	PA	+/ 2 B	47,XXX
34.	23	POF	+/ 2 B	47,XXX
35.	30	GD	+/ 2 B	46,XX/47,XXX
36.	39	SA	+/ 2 B	45,X/47,XXX

CFD- cranio-facial dysmorphia, SPH - staturo-ponderal hypotrophy, POF - Premature ovarian failure, PD - pubertal disorders, GD - gonadal dysgenesis, TS - Turner syndrome, PMS - plurimalformative syndrome, PA - Primary amenorrhea, RD - reproductive disorders , TX - triplo X syndrome, SA - secondary amenorrhea, B - Barr corpuscle.

DISCUSSION

The frequencies of the different types of chromosome abnormalities detected in our study compared to other studies are shown in Table II. We note that the frequency of trisomy 47,XXX (22.22%) was similar to

that detected by Robinson et al, 1982 - 18.03% (20), and the frequency of mosaic 45,X/47,XXX (25%) was similar to that detected by Sybert - 33.33% (19). For other types of mosaic trisomy X, the frequency varied in all three studies.

TABLE II

Frequency of chromosome abnormalities detected in female patients with homogeneous and mosaic trisomy X in the present study compared to other studies in the literature

Type of chromosomal abnormality	Present study	Sybert (19)	Robinson et al. (20)
Number of cases (%)	36	87	61
47,XXX	8 (22.22%)	-	11(18.03%)
46,XX/47,XXX	6 (16.66%)	-	2 (3.27%)
47,XXX/48,XXX	2 (5.55%)	-	-
45,X/46,XX/47,XXX	9 (25%)	68 (91.17%)	2 (3.27%)
45,X/47,XXX	9 (25%)	29 (33.33%)	-
45,X/47,XXX/48,XXX	1(2.77%)	-	-
45,X/46,XX/46,X,r(X)/47,XXX	1(2.77%)	-	-
45,XX,dic/45,X/46,XX/47,XXX	1(2.77%)	-	-

The characteristic phenotypic variability of trisomy X and reduced number of patients (36 cases) generated difficulties in genotype-phenotype correlations. These issues are compounded by the fact that there may be somatic mosaics undetectable in the absence of analysis of other tissues, respectively, the existence of patients with homogeneous trisomy X or cryptic mosaics undetectable in peripheral blood.

In prepubertal period the major clinical features were: growth retardation (small to medium), mild craniofacial dysmorphia and mild mental retardation associated with behavioral disorders. In the post pubertal period the elements suggestive for trisomy X were ovarian dysgenesis sometimes associated with mild mental retardation and psychiatric disorders.

Clinical signs detected in patients with homogeneous and trisomy X mosaic included in our study were similar to those presented in the literature. The only discordant element was in values of stature. Bibliographic data indicates the presence of a tall stature, detected in 80-89% of patients (1). From 8 patients with homogeneous trisomy X, 7 had normal stature and only one showed a slight decrease of stature (-1.5 SD), probably correlated with premature birth (15). Of the 6 patients with mosaic 46,XX/47,XXX, five showed a normal staturo-ponderal growth and one had tall stature (+1.81 SD). One of the 2 patients (2.85%) with polysomy X and mosaic 47,XXX/48,XXXX, presented a normal stature, ponderal hypotrophy (- 3.65 SD) and microcephaly (- 8.25 DS) associated with mild mental retardation, whereas patients diagnosed with polysomy X and

karyotype 47,XXX/48,XXX,+mar presented a tall stature (+ 3.86 SD), ponderal hypertrophy (+ 2.04 SD) and facial dysmorphism (hypertelorism, bilateral epichantus, upper lip eversion, macrostomia, dysplastic ears with preauricular tags or pits) and borderline intelligence.

Cognitive deficits associated with severe psychomotor retardation are rare in trisomy X, but 5-10% of cases may have a borderline intelligence with IQ = 85-90%, but overall IQ is lower than the first-degree relatives (1,16,17).

There are no serious mental disorders, but patients have issues with language and neuromotor development but this can be corrected with appropriate support from the family (17). In our study we detected an intellectual deficit with moderate mental retardation or borderline intelligence in 5 patients. Only 2 patients had psychiatric disorders manifested by neurasthenic syndrome and behavioural disorders (narcissism and masturbation) and one patient presented sexual identity disorders.

In patients with homogeneous polysomy X intellect is severely affected and sterility is are associated with disorders of sexual differentiation and dysmorphic manifestations (13), but this was not observed in our patients with mosaic polysomy X: 47,XXX/48,XXXX and 47,XXX/48,XXX,+mar.

The frequency of skeletal anomalies detected - 45.45% (5 cases) - was similar to that reported in the literature by Tartaglia et al, 2010 (1) - 42-65%. Only one patient (9.09%) presented urinary stasis and the frequency of reno-urinary had genital abnormalities and one of them presented an anal imperforation associated with rectovaginal fistula. The most common heart defect detected in patients with trisomy X were atrial and ventricular septal defects, pulmonary stenosis and aortic coarctation (1,18). Two of our patients had cardiac defects (atrial septal defect and mitral insufficiency, respectively, tricuspid insufficiency and mitral valve prolapse).

In 20 patients (55.55%) with gonosomal mosaicism having different cell lines with trisomy X and monosomy X, the phenotype was variable and correlated with the type and the proportion of cell lines present. Data from the literature show that 10% of patients with trisomy X have an abnormal mosaic, the most common being 46,XX/47,XXX or 47,XXX/48,XXXX or different gonosomal mosaics corresponding to Turner syndrome: 45,X/47,XXX or 45,X/46,XX/47,XXX (4.5). On the other hand, 3-4% of the patients diagnosed with Turner syndrome had a cell line with 47,XXX trisomy (1,19).

All 9 patients diagnosed with Turner syndrome and karyotype 45,X/46,XX/47,XXX had hypostature (between [-1.88] - [-3.75 DS]), normal intellect and absence of lymphedema in neonatal period. Four patients were diagnosed prepubertal, and 5 patients postpubertal, the main clinical signs in the latter being delayed puberty, primary amenorrhea and premature ovarian failure.

Sybert analyzed 17 patients with trisomy X mosaic and indicated that the average age of diagnosis in patients with trisomy X was higher than in Turner syndrome, most of the cases being detected in adulthood because of reproductive disorders. In patients with Turner syndrome having a cell line with trisomy X lymphedema was not detected and hypostature was identified only in 14% of patients. In addition, mental retardation was not identified in any of the cases with mosaic having a 47,XXX cell line (19).

In our study, in cases with karyotype 45,X/47,XXX (9 cases), neonatal lymphedema was absent and moderate hypostature was present in 7 cases. Two of the patients had borderline intellect. The diagnosis was established in the postpubertal period in 7 cases, and two patients were diagnosed in the peri pubertal period in the presence of a deficiency of secondary sexual characters.

The presence of spontaneous menarche in patients with trisomy X mosaic was detected by Sybert in 70% of cases, compared with 11% of patients with monosomy 45,X and 34% for those with mosaic 45,X/46,XX. Functional cell death is earlier than in normal women, generating secondary amenorrhea.

Often, the reproductive function is deficient, particularly correlated with primary sterility or increased frequency of spontaneous abortions (19).

In our study we identified two patients with pregnancy: one patient with mosaic 46,X,dic(X)/45,X/46,XX/47,XXX who had a stillborn baby with plurimalformative syndrome and one patient with 45,X/47, XXX mosaic whose pregnancy was terminated by miscarriage.

Unlike homogeneous trisomy X associated with deficits of learning, language and visual or auditory perception, in patients with mosaic trisomy X type 45,X/47,XXX or 45,X/46,XX/47,XXX, we I have not detected such issues, an aspect revealed by Sybert (19).

CONCLUSIONS

The results of our retrospective study, consistent with the literature, emphasize the difficulty of establishing a genotype - phenotype correlations for homogeneous and mosaic trisomy X.

Prepubertal, the diagnosis was established on the basis of growth retardation associated with uncharacteristic facial dysmorphism and mild mental retardation associated with behaviour disorders and, postpubertal, on by the presence of signs of ovarian dysgenesis associated with mild mental retardation and psychiatric disorders.

We emphasize the importance of Barr testing as a screening test in school age girls having borderline intellect or learning difficulties, that would allow early detection of trisomy X, thus enabling psychological, or psychiatric assistance, useful for social integration.

Early cytogenetic diagnosis of trisomy X would allow the female sex hormone replacement therapy, useful for improving socialization and, possibly the potential for reproduction

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PERCUTANEOUS HERBERT SCREW OSTEOSYNTHESIS IN TRANS-SCAPHOID PERILUNATE FRACTURE-DISLOCATIONS

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PERCUTANEOUS HERBERT SCREW OSTEOSYNTHESIS IN TRANS-SCAPHOID PERILUNATE FRACTURE-DISLOCATIONS (Abstract): Trans-scaphoid perilunate fracture-dislocation is the most common variety of fracture-dislocations of the wrist. Because of the carpal scaphoid fracture association, post-reduction stability is poor, often requiring surgery. Of the several surgical techniques used over time, percutaneous screw fixation for scaphoid fracture is currently preferred. **Material and methods**: Six cases of trans-scaphoid perilunate fracture-dislocation were surgically treated by percutaneous reduction of the scaphoid fracture and percutaneous insertion of Herbert screw in a proximal to distal manner. Postoperative immobilization was followed by rapid resumption of wrist mobility. Results: Four of the six patients have resumed normal, painless mobility of the wrist within 3 to 6 weeks post immobilization; in the remaining two cases there were a slight wrist stiffness attributed to persistent slight rotatory deficiency between the fractured scaphoid fragments. **Conclusions**: The percutaneous Herbert screw fixation provides a firm fixation with good compaction of scaphoid fracture, enough stability to allow minimal soft tissue and local damage, early mobilization following minimal immobilization, and thus rapid healing. **Keywords**: TRANS-SCAPHOID PERILUNATE FRACTURE-DISLOCATIONS, PERCUTANEOUS OSTEOSYNTHESIS, HERBERT SCREW.

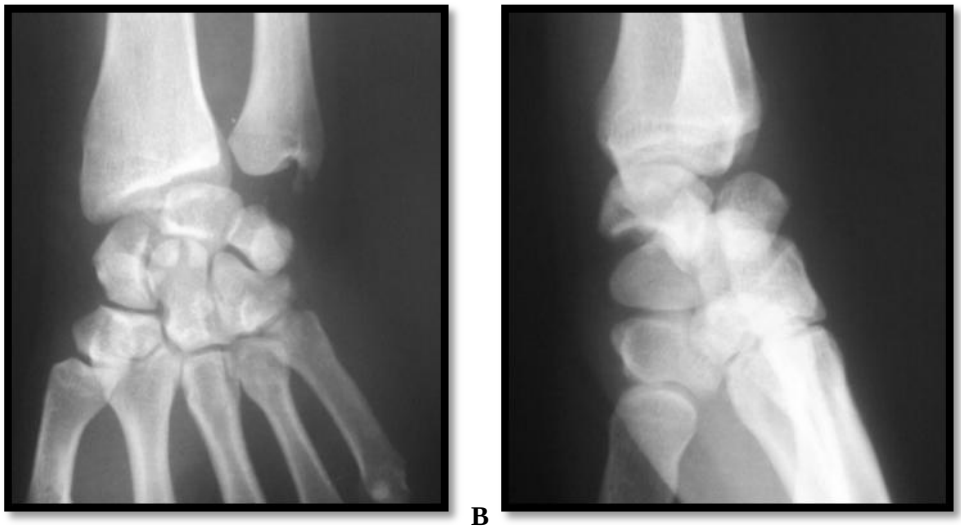
Trans-scaphoid perilunate fracture-dislocation**s** are the most common pattern of carpal fracture-dislocation (1, 2). These injuries can lead to poor functional results (3, 4) as a result of wrist arthritis; therefore, overtime, treatment was focused on anatomical fracture reduction, stable fixation and rapid mobilization.

Closed reduction and cast immobilization often result in loss of reduction (44% - 68%) and/or scaphoid nonunion (23% - 27%) (5). Therefore, open reduction and internal fixation seem to be a reasonable alternative to the previous technique, but these methods are accompanied by soft tissue damage by extensive dissection and implicitly interruption of vascular supply.

Other techniques used in these fracture-dislocations were closed reduction with percutaneous fixation of the fracture, the advantages being minimal dissection, stable fixation and early mobilization. Initially, these techniques used Kirschner pins, but as these did not provide adequate interfragmentary compression cannulated screws that were successfully used in isolated fractures of the scaphoid were preferred (6).

MATERIAL AND METHODS

In the interval August-December 2012 6 patients presented to our unit with trans-scaphoid perilunate fracture-dislocations, the fracture line separating the scaphoid proximal pole from scaphoid body. Patients were aged 19 to 34 years; the incriminated mechanism was fall exerting an axial load with hyperextension and ulnar deviation of wrist. For diagnosis we used the Mayfield classification, five cases being stage 2 (fig. 1) and one stage 4.



A **B**

Fig. 1. Wrist radiography (A - anterior-posterior and B – lateral views): trans-scaphoid perilunate fracture-dislocation Mayfield stage 2

In all cases the course of treatment consisted in emergency closed reduction of the carpal dislocation and plaster splint immobilization.

Post-reduction, radiological control showed in most cases a persistent misalignment between forearm bones and the first row of carpal bones and between the first and the second row of carpal bones observable by Gilula line interruption (fig.2). This was attributed to the imperfect reduction of the scaphoid fracture, causing dislocation instability and requiring surgery. To avoid extensive dissection, given its local vascular implications, we preferred a minimally invasive technique that consisted in percutaneous reduction of the scaphoid fracture and its fixation with a Herbert screw.

There was generally a waiting period of 4-7 days, while the hand was immobilized in brachiopalmar, proclive plaster splint; inflammatory and analgesic treatment was instituted. During this period edema had resolved facilitating our attempts at closed reduction of the scaphoid fracture.

Surgery was performed under loco-regional anesthesia (infra-clavicular plexus). Orthopedic fracture reduction was achieved and maintained with a temporary percutaneous olecranon forceps. A guide pin was introduced through the scaphoid tuberosity, then advanced to the proximal pole and exteriorized on the dorsal wrist, later serving to the introduction of the Herbert screw in proximal to distal direction (fig. 3).

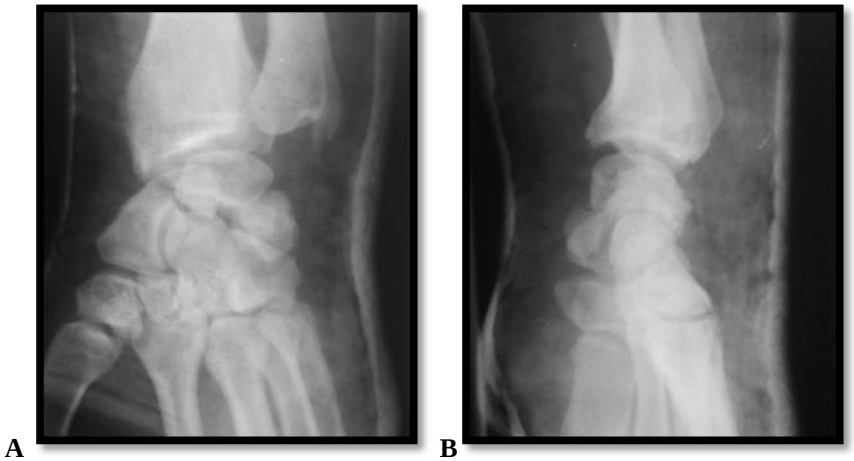


Fig. 2. Post-reduction wrist radiography: A - anterior-posterior view - reduction of the scaphoid fracture with interfragmentary diastasis and maintaining a persistent interruption of Gilula lines, B – lateral view – reduction of the trans-scaphoid perilunate fracture-dislocation.

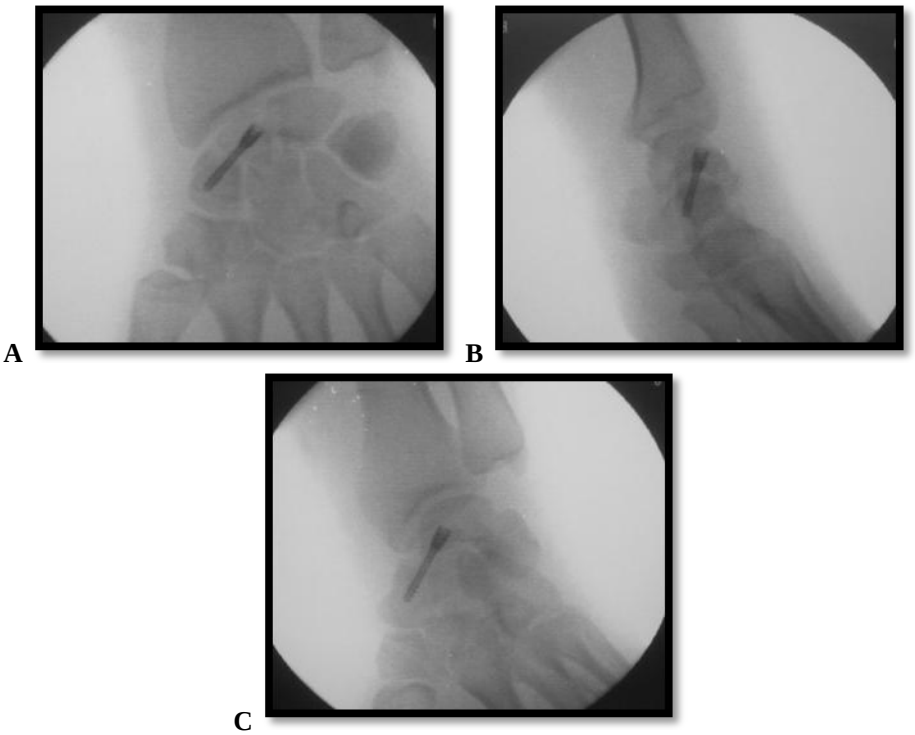


Fig. 3. Post-surgery wrist radiography: A - anterior-posterior view, B – lateral view, C – scaphoid view): good reduction of scaphoid fracture with compactness and good alignment between the carpal bones

Dislocation stability was then examined, only one of the six cases requiring additional stabilization with Kirschner pins. Post-reduction, in two of the six patients there was a slight rotator deficiency between the fractured scaphoid fragments.

Postoperative immobilization consisted of brachiopalmar plaster splint kept for 3 weeks and antebrachiopalmar plaster splint for another 3 weeks, followed by physiotherapy.

Four of the six patients have resumed normal, painless mobility of the wrist within 3 to 6 weeks post immobilization; in the remaining two cases there were a slight wrist stiffness attributed to persistent slight rotator deficiency between the fractured scaphoid fragments.

DISCUSSION

Currently, most authors believe that for best results, all dislocations or perilunate fracture-dislocations of the wrist must be reduced and rigidly fixed as soon as possible. It is sometimes necessary to delay definitive treatment because of the general condition of the patient, excessive swelling of the hand and wrist, or the need for adequate surgical care.

Hee et al. (8), in 1999, in a study on16 cases with trans-scaphoid perilunate fracture-dislocations obtained good results by open reduction and fixation with Herbert screw or Kirschner pins alone or with screw. Inoueet al (9) in a study of 29 patients treated by open reduction and internal fixation with Herbert screw observed good functional results in patients immobilized for 4 weeks postoperatively compared to those immobilized for more than 5 weeks.

Lam et al. (10) introduced in 2002 the cannulated percutaneous screw fixation of the scaphoid fracture, a difficult technique that can be an alternative to open reduction technique of recent trans-scaphoid perilunate fracture-dislocations.

Wong (11), in 2008, in a retrospective study of 21 patients treated by closed reduction of the carpus dislocation associated with closed reduction and percutaneous screw fixation of scaphoid fracture followed by closed reduction and stabilization with Kirschner pins of the carpal dislocation obtained an average Mayo score of the wrist of 80, with three excellent and two poor results, concluding that minimally invasive techniques can be considered a good reverse shuttle to open techniques.

Excellent results with minimally invasive techniques were reported by Chouet al. (12). They used in a series of 24 patients a technique involving percutaneous screw fixation of the scaphoid fracture and axial fixation with Kirschner pins of the carpal dislocation performed 7 days after injury. Clinical results were evaluated based on the range of motion, pain, functional status, and Mayo scores, the authors concluding that this technique is an appropriate treatment for acute trans-scaphoid perilunate fracture-dislocations of the carpus.

CONCLUSIONS

The percutaneous technique with Herbert screw offers a good protection of soft tissue with minimal local implications and firm fixation, good compaction of scaphoid fracture providing a rapid healing with good stability post-reduction, with minimum immobilization and possibility of early mobilization.

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NEWS

OLFACTORY DYSFUNCTION AND NEURODEGENERATIVE DISORDERS

Olfactory dysfunction is common, about 5% of the general population having anosmia and becomes more common with advancing age. Since many patients with neurodegenerative diseases - including Parkinson's or Alzheimer's - have early olfactory loss, testing for disturbances of the sense of smell is becoming important in neurology. Olfactory dysfunction is considered an early sign of neurodegenerative disorder that may facilitate diagnosis years before the onset of motor or cognitive disturbances. The use of anti-inflammatory drugs, surgery and olfactory training can help as the treatment for olfactory loss. (Hüttenbrink KB, Hummel T, Berg D, Gasser T, Hähner A. Olfactory dysfunction: common in later life and early warning of neurodegenerative disease. *Dtsch Arztebl Int*. 2013; 110(1-2): 1-7. doi: 10.3238/arztebl.2013.0001).

Carmen Pânzaru

ALZHEIMER - CERTITUDES AND HYPOTHESES

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ALZHEIMER – CERTITUDES AND HYPOTHESES (Abstract):Alzheimer's disease is a degenerative, progressive and irreversible condition, which affects cognitive functions. It was first described in 1907, by the German physician Alois Alzheimer. Although at the time, it was considered a rare disease, in 2010 in the world were estimated 35.6 million cases of dementia, most of these with the diagnosis of Alzheimer's disease. Typical neuropathologic lesions are represented by the amyloid plaques, neurofibrilar tangles and synapses and neurons losses. It was hypothesized that the amyloid protein has prion-like properties. Even from the first descriptions of the disease, atypical features were observed – the second case described by the physician Alois Alzheimer, had only plaques, the tangles were missing. About 19 % of the healthy old subjects present in the brain the same lesions as Alzheimer's cases, while in 10 % of the cases of disease, in necropsy are present only the plaques or only the tangles. These aspects are even more paradoxical, as the certain diagnosis is established only at necropsy, on anatomopathological lesions. Even so, the international diagnosis criteria, based on clinical aspects, can establish a certain, probable or a possible diagnosis. It exist an early-onset form, as well as a late-onset form of disease (which appears after 80-85 years of life); genes are involved in the genesis of the disease. A lot of money are spent to find an efficient medication in the treatment of the disease (tramiprosate – an amyloid-antagonist, Dimebon, gamma-secretase inhibitors or a vaccine – a synthetic form of the amyloid protein); for the moment the used medication may at its best only to temporary improve the symptoms. Some scientists believe that approx. 30 % of the cases are wrongly diagnosed with Alzheimer, being in fact other forms of dementia, or that we deal with several biologic processes, generating rather an Alzheimer's syndrome, meanwhile others are unsatisfied by a poor diagnosed disease and its popular receipt as a part of the normal aging process. **Keywords**: ALZHEIMER'S DISEASE, DEMENTIA, AMYLOID PLAQUES, NEUROFIBRILAR TANGLES, AMYLOID β PEPTIDES.

EPIDEMIOLOGICAL LANDMARKS, HYPOTHESES AND CONTROVERSIES

Being a degenerative, progressive and irreversible disease, which is dramatically damaging the intellectual functions, the Alzheimer's disease was first described in 1907, by the German physician Alois Alzheimer, being then considered a rare entity. Now is considered an epidemical disease, with major social impact, and which threatening is growing together with the phenomenon of global aging of the human population.

2009 World Alzheimer Report has estimated 35.6 million dementia affected individuals for 2010, and this number was estimated to double at each 20 years, thus resulting 65.7 million in 2030 and 115.4 million in 2050. Large part of this growth is attributed to the increase in number of the dementia affected people in the countries with low and medium incomes/inhabitant. In the same report, the specialists are underlying the under diagnosis of this disease, as well as the stigmatization of the affected people and of their families, all over the world, independent of the size of the country, or the level of wealth. Alzheimer's disease was diagnosed in all the countries, of any culture and race, in which the diagnosis screening was done. The level of awareness of the disease, as a severe medical condition is greatly variable, from one country to another. In low and medium developmental level countries, the degree of awareness is decreased, dementia being still considered a normal estate of the normal aging process (1).

Recognized risk factors are: the advanced age, the genotype Apo E epsilon 4, cranial trauma, cardiovascular risks (smoking, diabetes, high blood pressure, and hyper cholesterolemia), and possible metal exposure, especially to aluminum. The gene for the E apolipoprotein on the 19 chromosome is a protein, which is modulating the transport of the phospholipids, with role in the synaptic remodeling. Apo E epsilon 4 is associated with a high risk for Alzheimer's disease (3).

Classical neuropathological features

- amyloid plaques and neurofibrilar tangles, with synapses and neurons losses.

- granulovacuolardegenerescence of the hypocampus and deposition of amyloid in the vessels, are usually present as well.

- the amyloid plaques are made of 40-43 aminoacidsoligopeptides, derived through proteolysis from a much larger precursor amyloid protein fraction – APP – amyloid precursor protein. The precursor protein is coded by a single gene and is made of several sequential forms, fractions of the plasma membrane integral proteins type I. The amyloid is made of 28 aminoacids of the big extracellular aminoterminal domain of the APP and of the first 12-15 aminoacids of the intramembranary region.

- the associated microtubular tau protein, under a hyperphosphorylated, modified form, is the major protein component of the spiralated filaments in the structure of the neurotubules, thus in the structure of the neurofibrilar tangles. The normal tau protein is not glycosilated, while in the brain of the Alzheimer's patients, this protein appears under a glycosilated form. Although the abnormal phosphorylation is leading to the inhibition in the assembly of the microtubules, the glycosilation is mantaining the helical structure. Normal tau protein is involved in the axonic growth. The abnormal phosphorylated form of the tau protein is forming the spiralated filaments pairs of the neurotubules, and it appears in the neurons of the hippocampus, medial temporal lobe, parietotemporal region and in the associative frontal cortex, leading to the death of the interneurons. These zones are affected by the loss of the synapses, with the injury of the synaptic passages of the neurotransmitters (2).

- mutations at the level of three proteins – presenilin 1, presenilin 2 and the precursor protein of the β amyloid, were identified in the familial form of the Alzheimer's disease. These mutations are increasing the extracellular concentration of the β amyloid and are enhancing its brain deposition (3).

ETIOPATHOGENIC HYPOTHESES

1. Although the hypothesis which is stating that the β amyloid peptides (Aβ) are the primary causal agents of the Alzheimer's disease is supported by the large majority of the researchers, a large number of data is showing that it is rather unlikely, the Aβ peptides to be the single etiologic factor of the disease.

2. There are proofs which support the fact that factors which are unrelated to amyloidosis, including genetical factors, are important in the pathogenesis – defects of the endolysosomal metabolism, the alteration of the intracellular signal cascades, damaging in the releasement of the neurotransmitters, are all contributing to the appearance of the synaptic dysfunctions and/or neurodegenerescence and hence to the dementia of the Alzheimer's disease (4).

3. A hypothesis is launching the possibility of the development of the neurofibrilar tangles, as a result of the age-related dysfunction in the function and viability of the microglial cells. The neurodegenerescence would be from this point of view, the result of a decline of the immune functions, rather than a hyperactivity of of the immune system (5). The death of the cholinergic neurons of the Meynert basal nucleus is resulting into the deficiency of acetylcholine, characteristic for the disease; a transmitter involved in the memory process (7). The loss of the serotoninergic neurons of the median zone of the raffe and of the adrenergic neurons of the locus coeruleus is determining the serotonin and the norepinephrine deficits. The exact role of the inflammation in the pathogenesis of the disease is still controversial; although studies which evidentiate the microglial activation do exist (as a marker of the brain immune response), prednisone, as well as NSAID (non steroid alantin inflammatory drugs) were ineffective in the treatment of the disease (6).

4. New studies have even proposed a prionic hypothesis in the pathogenesis of the Alzheimer's disease. It was noticed that the peripheral deposits of beta-amyloid, from different tissues, others than the brain, may finally reach „to infect" the brain. Injection of the amyloid-containing brain tissue, obtained from old mice, in the abdomen of younger animals, had led several months later to the detection of the amyloid in the brain of younger injected animals. The studies which used transgenic mice (genetically induced to produce Aβ human amyloid) had led to the prion-like qualities of the amyloid protein hypothesis. Prions show protein structure and are transmissible, as it is happening in the Creutzfeld-Jakob disease. Yet, there are no proofs that the Alzheimer's disease or the brain amyloid angiopathia are transmissible (between mammals or humans) in the same way as the prionic diseases (8).

DIAGNOSIS – DISEASE OR SYNDROME ?

The diagnosis is established upon the clinical criteria stated by the National Institute of Neurological and Communicative Disorders and the Alzheimer's Disease and Related Diseases Association, in the USA. It is estimated that these criteria are establishing the diagnosis, with an accuracy of 90 %. On the basis of these criteria, the diagnosis is certain, probable, or possible, but the certitude of the diagnosis is obtained only through necroptic examination of the anatomo-pathologic lesions. Nevertheless, were signaled cases of subjects with minor memory troubles, but who had

typical Alzheimer's disease lesions, in necropsy.

The major clinical manifestation is represented by the memory loss, first for the recent events, but afterwards for the past events, as well. Language troubles are first manifesting by the search of the right words, to name different objects, evolving into communication disability, limited vocabulary, nominal aphasia and difficulties of understanding.Apraxia is developing (the inability to perform simple daily activities, such as combing the hair), acalculia (the inability to perform simple arithmetic calculations) visual and spatial disorientation (disorientation in what concerns the position in space of the body).

The mood troubles are including depression, anxiety, apathy, which may appear early in the development of the disease, while in the advanced stages, hallucinations are frequent, together with psychotic symptoms; aggressiveness and sexual behavioral troubles are most unpleasant for the caring persons. In the advanced or terminal stages, is appearing the frontal lobe syndrome, and the sphincters incontinence, myoclonus, convulsions, up to the vegetative estate, when all cognitive activities are disappearing (10).

Ten cardinal symptoms
of the Alzheimer's disease

- decline of the rational thinking
- difficulties in accomplishing complicate tasks
- language troubles
- time and spatial disorientation
- decline of the abstract thinking
- loss of the recent memory affecting daily tasks
- personality changes
  mood and behavior troubles
- changes in perception of the reality
  loss of the initiative

MRI examination is revealing the progressive atrophy of the hippocampus, which is an important zone of the memory processes (10). Dosages of the Aβ peptide and of the tau protein, in the cerebrospinal fluid, are considered biomarkers for the Alzheimer's disease, being estimated that their use, will increase the accuracy of the diagnosis. Decrease of the levels of the Aβ peptide and increase of the tau protein, in the cerebrospinal fluid, are the proof of the evolution of the disease, being the consequence of the deposition of the amyloid peptide into the brain, and of the excretion of tau protein by the neurons which undergo degenerescence (11).

Anatomopathologic lesions, which represent the certitude diagnosis, might be present in healthy old-aged subjects (19 % among these), as well as in subjects who developed another type of dementia. In the necroptic examination, in about 10 % of the cases of Alzheimer's disease are present only the amyloid plaques (otherwise, the second case described by Alois Alzheimer had only plaques in necropsy), or only the neurofibrilar tangles.

The book written by dr. Peter Whitehouse and Daniel George, „The Myth of Alzheimer's: What You Aren't Being Told about Today's Most Dreaded Diagnosis" (2010, http//booksandideas.com) is putting the question whether the concept itself of Alzheimer's disease is clear enough, and if there are not rather several different pathologic processes, since not even A. Alzheimer was not sure that he had described something different from the senile dementia. Dr. Whitehouse is considering that too often, this is a label, in the same way like that of dementia, and that it must be reevaluated in what measure the

Alzheimer's lesions are not a part of an individual aging process.

Early onset and late onset Alzheimer's concepts appeared as a consequence of the fact that, the majority of the Alzheimer's disease diagnoses are now established in patients over 85 years old, while the cases described by Alzheimer were relatively young – 51, respectively 54 years old. Even in the early onset forms, it is difficult to state that the pathologic process is really distinct, from that one in the senile dementia.

Based on the similarity of the brain lesions, in the sixth decade of the XX century, especially under the pressure of the Anglo-Saxon specialists, the concepts of Alzheimer's disease and that of senile dementia were unified. This contributed to the perception of the Alzheimer's disease as a separate medical condition, since the term of senile dementia had such deep negative connotations. There are differences in the symptoms of the two types of Alzheimer's disease – in young patients, the troubles of language, gesture, recognition of faces/objects are predominating, while in the case of old-aged patients, memory troubles are clearly predominating. There are studies which conclude that approximately 30 % of the Alzheimer diagnosed patients do have in fact, another type of dementia.

There are voices who state that we should talk about the „Alzheimer's syndrome", which would include several types of diseases (12, 13).

THERAPY, BETWEEN FAILURES AND PROMISES

Among the factors which decrease the risk for Alzheimer's disease, with some degree of efficiency is signaled – the brain stimulation (examples – chess, puzzles) and regular physical exercise (14, 15, 16). The therapy is targeting on one hand to reduce the risk of development of the disease, by using food supplements, NSAID (such as aspirin, ibuprofen, which unfortunately in several studies, did not prove to be efficient) and the treatment of the acetylcholine deficit, by administration of cholinesterase inhibitors and antagonists of the receptor N-methyl-d –aspartate (NMDA) on the other hand, and experimental medication, hoped to be a real revolution in the therapy of the disease.

Some studies investigated the real prevention efficiency of group B vitamins, of high doses of E and C vitamins, of the choline, omega 3 fatty acids (DHA), of the statines and NSAID; unfortunately, the results were not those which were expected (17,18,19).

Cholinesterase inhibitors (Donepezil, Rivastigmine tartrate, Galantamine) and the antagonists of the receptor – NMDA - (Memantine – Namenda) are determining only a temporary improvement of the cognitive functions, and of the behavior (20).

From the recently experimented medications, Tramiprosate, an oral administration, small molecule antagonist of the amyloid, is considered promising; this is linking the soluble amyloid β peptide (Aβ) thus interfering with the cascade effect of the amyloid, its deposition, and with the brain toxic effects of the peptide. The signaled side effects were mild – mouth dryness, slight depression and increase of the sweat secretion (21).

Dimebon is another small molecule, with oral administration, which was commercialized in Russia in the 80^{s}, for its antihistaminic properties and was dropped off, as other superior antihistaminic agents were

developed. 70 % of the Alzheimer's patients who took Dimebon for one year, have improved their estate, or at least have stabilized their Alzheimer's symptoms (22).

Gamma-secretase inhibitors – LY 450 139, were developed by the Eli Lilly company as a modifier of the disease evolution; the medication inhibit the gamma-secretase, enzyme involved in the formation of the Aβ peptide. From 51 patients who received a 100/140 mg daily dose, for 6-12 weeks, in 43 cases who finished the trial, was registered the marked decrease of the blood Aβ levels (58.2% from those who received 100 mg/day and 64.6 % from those who received 140 mg/day). Minor side effects, such as rash, fatigability and sleepiness appeared (23).

A new approach in the Alzheimer's disease was to furnish an alternative source of energy to the glucose-deprived neurons. Ketasyn (AC-1202) is providing ketonic bodies to the glucose-deprived neurons; they can be metabolized, even when glucose cannot be. AC-1202 is converted into ketonic bodies (beta-hydroxibutirate) as a glucose-alternative for the energy-compromised neurons. Good results were obtained in patients who had not the E4 variety of the apolipoprotein gene (ApoE epsilon 4) which appears in half of the Alzheimer's disease patients. AC-1202 has significantly improved the memory and the cognitive capacity in negative ApoE 4 patients. The subjects who took AC-1202 for 9 months have registered a very small progress of the disease. The side effects were minimal; sometimes appeared gastro-intestinal troubles (24).

In the case of the vaccine AN 1792, which uses a synthetically form of the amyloid's protein, some patients developed an aseptic meningo encephalitis, reason for which the trial was stopped. In 59 patients, anti Aβ antibodies had detectable levels after 4.5 years; it was observed a more reduced dependence on the caring personnel and a slower decline of the memory tests. Among the vaccinated patients who developed antibodies, 75% did not need institutionalization, being able to remain at home, compared to only 53% in the placebo group. The necroptic studies of the deceased cases, after the finalization of the trial, showed reduced deposition of amyloid in the brain. Now are conducted experiments to elaborate safer vaccines. It is unknown why not all the vaccinated cases responded to the vaccine; probably the particular features of the aging process and the individual variability of the immune system are making the difference (24).

There are numerous studies which investigate the prophylactic and curing potential of plants, used in the traditional medicine (chinese, indian, european) in Alzheimer's disease. *Ginkgo biloba* has the property to neutralize the neurotoxic effect of the amyloid peptides, largely attributed to its antioxidant action. Galantamine, an alkaloid of the Amarylidaceae family was used in the southern Russia and in Bulgaria in the treatment of the poliomyelitis; its properties of acetylcholinesterase inhibitor were signaled in the fifties and consequent studies had led to the commercialization of Galantamine. Different components from *Panax ginseng, Lyciumbarbarum, Uncaria rhyncophylla, Yokukansan* and a lot of other plants, used in the oriental traditional medicine, as well as *Salvia officinalis, Crocus sativus* (saffron), *Mellisaofficinalis*, used in the european traditional medicine, proved useful in the therapy of the memory troubles and in the improvement of the cognitive dysfunctions of Alzheimer's

disease (25) .

While some researchers are deploring the insufficient financing of the research efforts in the Alzheimer's disease (for example the winner of The Nobel Prize for medicine for 2010, Robert G. Edwards), others, like dr. Peter Whitehouse, believe that in fact Alzheimer's disease is overestimated and should be considered an aspect of the aging process.

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NEWS

MOLECULAR EPIDEMIOLOGICAL SURVEILLANCE FOR TUBERCULOSIS BY WHOLE GENOME SEQUENCING

Tuberculosis (TB) is a contagious disease caused by *Mycobacterium tuberculosis* characterized by a high rate of incidence and mortality worldwide. Although the World Health Organization (WHO) established a global program for controlling and monitoring tuberculosis, the decline in the number of cases has been slow. Thus, the TB control strategies have targeted the development of molecular techniques to monitor disease transmission. Genotyping methods analyzed a small part of the bacterial genome for specific identification of strains, but they may not be able to determine the origin a tuberculosis outbreak or how it spread in population. A longitudinal molecular epidemiological study conducted by Niemann and collaborators showed that whole genome sequencing (WGS) provides more information about transmission chain of TB than classical genotyping methods: IS6110 RFLP (restriction fragment length polymorphism) and 24-locus MIRU-VNTR (mycobacterial interspersed repetitive unit–variable number of tandem repeats). The authors a study demonstrates that WGS is able to characterize clinical isolates with high discriminatory power, at strain level. Using WGS method, the researchers have estimated that the *M. tuberculosis* has a time-dependent genomic variation with a rate at 0.4 mutations per genome per year and with a doubling time of 22 hours, or 400 generations per year. Based on the results of the study, the same authors concluded that, in the context of decreasing sequencing costs, WSG can be an effective method for TB surveillance by identification of outbreak-related transmission chains (Roetzer A, Diel R, Kohl TA et al. Whole Genome Sequencing versus Traditional Genotyping for Investigation of a Mycobacterium tuberculosis Outbreak: A Longitudinal Molecular Epidemiological Study. *PLoS Medicine*, 2013;10:e100138 DOI: 10.1371/journal. pmed.1001387).

Cătălina Luncă

FAMILIAL SYNDROMIC PAPILLARY THYROID CARCINOMA - REPORT OF TWO CASES

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FAMILIAL SYNDROMIC PAPILLARY THYROID CARCINOMA - REPORT OF TWO CASES (Abstract) **Aim**: to describe two cases of familial papillary thyroid carcinoma. **Material and methods**: patients were investigated by fine needle biopsy, MRI imaging and tumor biopsy, (first case) and histological examination of colonic and thyroid tumors (first case) and histological examination of thyroid tumor (second case). **Results and discussion**: case presentation: first case, 68 years old man had a colonic polyposis (attenuated form with only a few polyps) and a thyroid nodule. After hemicolectomy for a supposed colonic carcinoma with liver and lung metastases, histological examination revealed no malignant colonic disease. Two month later the diagnosis of invasive thyroid tumor with lymph node metastases was made, but only an open biopsy was done because tumor invasiveness demonstrated on MTI imaging. The biopsy identified a papillary thyroid carcinoma. Case 2: the son of the patient (30 years old) without known diseases was invited to be assessed for thyroid disease. Ultrasound examination discovered a large nodule with microcalcifications. Microscopic examination done after total thyroidectomy revealed a cribriform morular variant of papillary thyroid carcinoma, a variant that is known to be associated with FAP. Radioiodine ablation was made followed by suppressive thyroxine treatment. In the second case adenomatous polyposis was not found yet. In our knowledge these are the first cases of familial thyroid papillary carcinomas in our setting. Familial history allowed an earlier diagnosis and a good management of the disease in the second case. **Conclusions**: according to the literature and our first experience, screening for thyroid cancer must be done in all patients with FAP and in those with a FAP proband in the family. **Keywords**: FAMILIAL NONMEDULLARY THYROID CARCINOMA, FAMILIAL ADENOMATOUS POLYPOSIS, CRIBRIFORM, MORULLAR VARIANT, PAPILLARY THYROID CANCER.

Follicular-derived thyroid cancer represents 60-95% of all thyroid malignancies and at least 5 % of the patients have familial disease (1). Familial nonmedullary thyroid cancer (FNMTC) may be classified into two categories (1, 2, 3, 4): a. Non syndromic familial tumor syndromes with preponderance of FNMTC: familial papillary thyroid

carcinoma (FPTC) with or without oxyphilia, FPTC associated with renal papillary renal neoplasia, FNMTC type 1. b. Syndromic familial tumor syndromes in which nonmedullary thyroid carcinoma occurs with a known frequency: familial adenomatous polyposis – FAP and Gardner's syndrome, PTEN-hamartoma tumor syndrome, Carney's complex and Werner's syndrome.

Familial adenomatous polyposis (FAP) and Gardner's syndrome (a FAP variant) are produced by a loss of function germline mutation of APC (adenomatous polyposis coli) gene, a tumor suppressor gene located on the chromosome 5q21(2) . First mutation of APC gene was described in 1991 and 800 mutations were identified thereafter (5). It is an autosomal dominant disease with an incidence which varies between 1/8300-1/11300-1/37600 which may be responsible for up to 1% of colonic cancers. Classic form develops in the second decade of life and is characterized by occurrence of hundreds or thousands of adenomatous colonic polyps. Attenuated FAP shows fewer polyps, occurs later in life and carries a lower risk of colonic cancer (6). Extracolonic diseases occur in 70 % patients with FAP (5).

First case of an association between FAP and thyroid cancer was reported by Crail (quoted by Half 2009(6)). FAP-associated thyroid cancer occurs especially in women, at young age, and has a particular histology (2, 7, 8). Women with FAP have a 160 times higher risk developing thyroid cancer compared with general population (6).

FAP-associated thyroid carcinoma is a syndromic form of FNMTC and occurs with the following reported incidence: Nose V. 2012 2-12% (2), Herraiz M et al. 2007: 7 % (9), Steinhagen E. et al 2012: 6.1 % (10), Harb WJ and Sturgis EM 2009: 5%(11), Jarrar AM et al 2011: 2.6 %(12). These incidences are considered high enough to justify screening for FNMTC in all patients with FAP (Nose V 2011) by ultrasound neck examination and fine needle biopsy (FNB) even in small nodules (9).

Histological appearance of FAP-associated NMTC is similar in close relatives (13) and has a cribriform morular pattern with papillary architecture, morula formation, cribriform pattern with solid areas and spindle cells (7, 14, 15, 16). Preoperative diagnosis may be suspected on fine needle biopsy which shows columnar cell with fine to granular chromatin, nuclear grooves, papillary and acinar fragments (17). Immunohistochemistry on FNB and histological specimens allows detection of accumulation of beta-cathenin in cytoplasma and nucleus which is characteristic for APC mutation (18).

The known association between FAP and NMTC allows an earlier diagnosis by screening for thyroid cancer in FAP-confirmed patients and an earlier diagnosis of FAP in patients with cribriform morular variant of papillary thyroid carcinoma(13). Cribriform morullar variant of FAP-associated thyroid carcinoma usually has an indolent course, rare metastases and a good prognosis (2).

MATERIAL AND METHODS

Patients were investigated by fine needle biopsy, MRI imaging and tumor biopsy, (first case) and histological examination of colonic and thyroid tumors (first case) and histological examination of thyroid tumor (second case).

CASE PRESENTATION

Case no. 1. A male patient of 68 years with complains of alternative diarrhea and

constipation was submitted to colonoscopy in December 2011 in the Clinic of Gastroenterology of "St. Spiridon" Universiy Emergency Hospital. At 30 cm from anal edge a semipediculated polyp was identified and removed. In the proximity of the hepatic angle of the colon a large polypoid tumor was found and a superficial biopsy was performed. Histological examination of both specimens performed in the Laboratory of Pathology of the same hospital showed tubular adenomatous polyps with polymorphic inflammation and absence of malignant lesions (fig. 1).

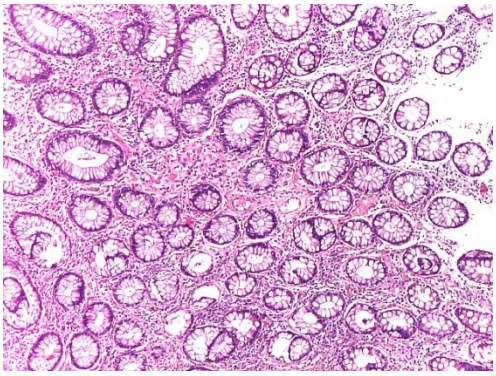

Fig. 1. Case 1. Polyp removed at 30 cm. from the anal from the edge: tubular adenomatous polyp with superficial erosions and important inflammation without signs of malignancy

The patients postponed surgery until 2 month later when he was admitted in emergency in the IstSurgery Clinic of the aforementioned hospital for diffuse abdominal pain and absent of intestinal transit for less than 24 hours. At physical examination a large, painful tumor mass was detected on the right side of the abdomen. Abdominal X-ray showed liquid and air levels suggesting an occlusive state and chest X-ray showed lung nodules with an appearance of metastases. Liver ultrasound detected multiple nodules also considered to be metastases from a colonic cancer Physical examination also identified a firm thyroid nodule of 6 cm its largest diameter, belonging to the left thyroid lobe, with preserved mobility on the neck structures and absence of compressive symptoms. The patients were operated in emergency. During surgery a colonic invagination took place due to an intraluminal tumor of 11 cm. the largest diameter, situated in the proximity of hepatic angle. Right hemicolectomy and resection of 13 cm of the terminal ileon, and ileal-transversal anastomosis were performed. Most lymph nodes in the area were carefully removed. An extensive macroscopic of the surgical specimen showed a large polypoid tumor mass, partially ulcerated which in microscopic examination had fibroblastic axes covered by ovoid cells, plasmocytes and eosinophils. All lymph nodes removed showed only histiocytosis. The patient came after 8 week and his diagnosis of colonic carcinoma with liver and lung metastases was revised by the surgeons and their attention was directed toward the thyroid nodule as a possible carcinoma. This time, the thyroid nodule was larger, both thyroid lobes strongly attached to the trachea, and bilateral lymph node enlargement was detected by clinical examination and neck ultrasound. Fine needle biopsy brought small micropapillary structures, thyroid cells with large nuclei, nucleols and cytoplasmatic intranuclear inclusions (fig. 2). The attempt to remove the tumor failed and only a large biopsy was possible. The diagnosis was papillary thyroid carcinoma (fig. 3). With the diagnosis of stage T4N1Mx papillary thyroid carcinoma, the patient was transferred to the Clinic of Endocrinology of the same hospi-

tal. MRI examination confirmed the presence o large thyroid tumor (7.2 x 4.5 x 3.5 cm), developed also between the thyroid and cricoid cartilages, infiltrating left vocal cord, the esophagus on a length of 13 cm, and muscles and fat around it. All lymph node compartments surrounding the thyroid presented enlarged lymph nodes between 1.2 and 7 cm (fig. 4). The hormonal profile of the patient was normal: TSH=2.86 μIU/ml, FT_4= 11.93 pmol/ml. In these circumstances the patient was placed on suppressive therapy with T_4 and X-ray therapy was indicated for palliation.

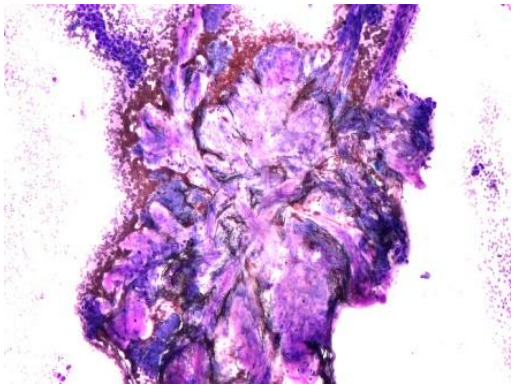

Fig. 2. Case 1. Fine needle biopsy of the thyroid nodule: tridimensional micropapillary architecture

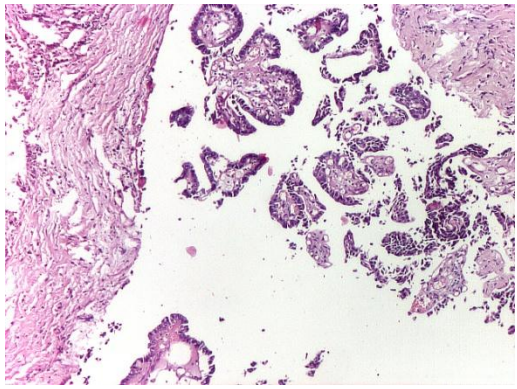

Fig. 3. Case 1. Histology of open biopsy of thyroid nodule: papillary thyroid carcinoma

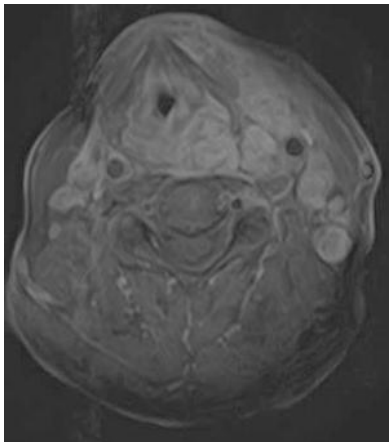

Fig. 4. Case 1 MRI examination of the neck: sections T1 axial Fat Sat + ivc: tumor extension between thyroid, cricoids and arytenoids cartilages producing reduction of laryngeal vestibulum. Large lymph nodes anterior from thyroid cartilage, bilaterally near the jugular vein and posteriorely.

Case no. 2

During the time when the first described patient stayed in the Clinic of Endocrinology, his son of 40 years old was informed that he carries a certain risk de have a thyroid disease and asked for permission to be examined for that. A physical examination, a large thyroid nodule was found in the right thyroid lobe. The patient was not aware of it before examination. Neck ultrasound identified a large inhomogeneous nodule of 3.9 x 5.3 x 2.9 cm with high vascularity and microcalcifications. Important blood contamination made the FNB specimen inadequate. The patient was operated in the Ist Surgery Clinic and a total thyroidectomy was performed. A postoperative bleeding was successfully solved.

The pathological examination described on a background of colloid goiter a multi-

centric papillary carcinoma with psammomas infiltrating the thyroid, without extension beyond the thyroid capsule. The tumor presented solid or cribriform areas and morula formation. Papillary cancer areas were found in the controlateral lobe (fig. 5 a, b). Immunostaining for $HBME_1$ was positive in 75% of tumor cells in their luminal pole, without basolateral membrane staining (fig. 5 c). 25% of cells of the cribriform and solid areas were also positive for $HBME_1$. The immunistaining with bcl_2 was positive in 100% of tumor cells (fig. 5 d). Histopathological staging of the disease was pT3Nx-G1. The pathologist conclusion was that the aforementioned characteristics are those familial forms of papillary thyroid carcinoma.

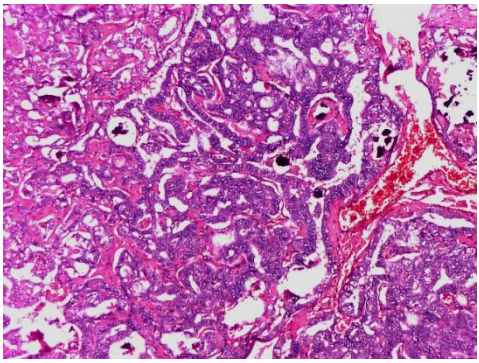

Fig. 5 a. Case 2. Papillary thyroid cancer with cribriform. Morullar pattern and a few psammomas

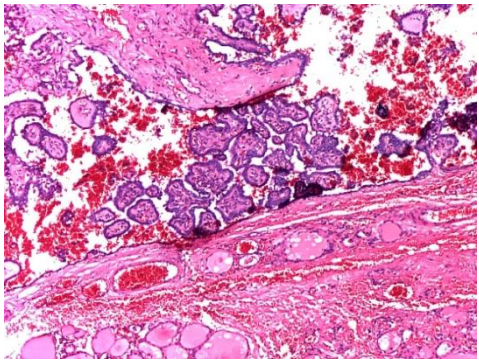

Fig. 5b. Case 2 Papillary cancer in the controlateral lobe

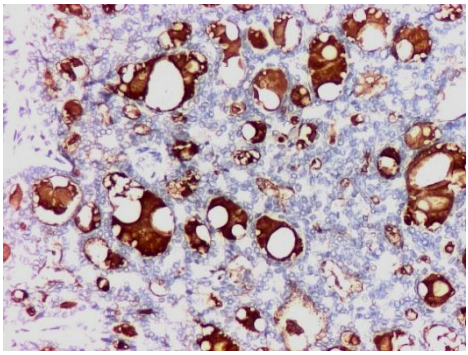

Fig. 5 c. Case 2. HBME1 immunostaining positive only in the luminal pole of tumor cells without basolateral staining

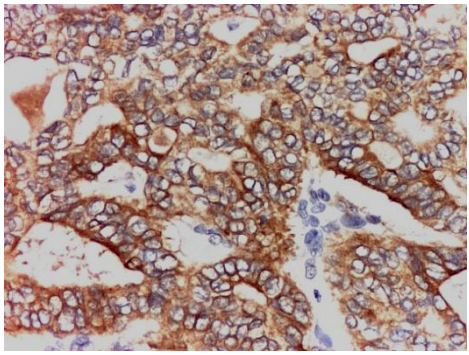

Fig. 5d. Case 2. Intense positive membrane immunostaining of tumor cells for bcl_2

The patient received a radioiodine dose of 70 mCI for ablation. Before ablation his hormonal profile showed: TSH=66.8 µIU/ml, thyroglobulin=16.5 ng/ml and antithyroglobulin antibodies: 22 IU/ml. Post ablation scan detected radioiodine uptake in the thyroid bed. After radioiodine ablation the patient receives suppressive

therapy with 150 µg of T_4. The colonoscopy performed two month showed that patient had no colonic polyposis. Careful ultrasound neck examination of his sister revealed no thyroid abnormalities.

DISCUSSION

In our knowledge, this is the first case of familial nonmedullary thyroid carcinoma (papillary carcinoma) reported in our setting. The reported cases have some typical features of FAP-associated papillary thyroid carcinoma and some particular characteristics. The proband case seemed to have an attenuated form of FAP (AFAP) with late onset of the disease and only a few polyps, proximally situation of polyps and infrequent rectal involvement as this form was defined by Half E. et al (6). The course of thyroid cancer in the proband was indolent for a very long time as mentioned for FAP-associated PTC by Cetta (16) and Ito (19), but suddenly it turned into an aggressive form with local invasion, lymph node metastases and distant lung and liver metastases as seen in the non syndromic FPTC (2, 20).

The typical histology of cribriform, morular variant of PTC, multicentricity and bilateralism (2, 15, 16) were seen in the second case. The tumor lost the affinity for HBME1 and preserves its immunostaining with bcl_2. After Nasr (21), only luminal staining for HBME1 without basolateral staining is considered to be negative for HBME1 immunostaining. These features along with presence of AFAP and papillary carcinoma in the father raise the possibility that thyroid cancer could occur before adenomatous polyposis in this particular case.

The occurrence of the disease in males (father and son) could be also considered particular features because FAP-associated thyroid cancer occurs especially or almost exclusively in women at younger age (12).

The phenomenon of "genetic anticipation" defined as "occurrence of a genetic disease at progressively younger age and with increase aggressiveness in successive generations" described by Mc Innis (1996, quoted 22) for pure, nonsyndromic familial thyroid carcinoma was seen in the second reported case. Although after a long indolent course, the diagnosis a thyroid cancer was made at the age of 68 in the father and at the age of 40 in the son.

The most important point was in our cases the earlier detection of thyroid cancer in the son, allowing an adequate management of the disease and an expected good prognosis.

CONCLUSIONS

The occurrence of a simultaneous thyroid cancer in a patient with adenomatous polyposis must be suspected at the time of diagnosis of colonic disease.

Immediate screening for thyroid cancer must be performed in all first-degree relatives of patients with FAP by neck ultrasound and FNB if possible, allowing an earlier detection and a better prognosis of the thyroid disease.

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MICROSCOPIC COLITIS AND SMALL INTESTINAL BACTERIAL OVERGROWTH – DIAGNOSIS BEHIND THE IRRITABLE BOWEL SYNDROME?

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MICROSCOPIC COLITIS AND SMALL INTESTINAL BACTERIAL OVERGROWTH – DIAGNOSIS BEHIND THE IRRITABLE BOWEL SYNDROME? (Abstract): Some patients previously diagnosed with irritable bowel syndrome (IBS) may develop microscopic colitis or small intestinal bacterial overgrowth (SIBO). **Aim:** To estimate the prevalence of microscopic colitis and SIBO in patients with IBS, to evaluate the symptoms and the efficacy of treatment. **Material and methods:** We examined patients with IBS admitted in our clinic during a three-year period. We identified patients with microscopic colitis by performing total colonoscopy with multiple biopsies from normal intestinal mucosa and those with SIBO by performing a H_2-breath test with glucose. We compared the symptoms and the effectiveness of the treatment. **Results:** Out of the 132 patients initially diagnosed with IBS 3% (n=4) had microscopic colitis and 43.9% (n=58) had SIBO. Diarrhea was the main symptom in patients with microscopic colitis and SIBO (p=0.041), while abdominal pain, abdominal bloating and flatulence were prominent in IBS patients (p=0.042; p=0.039; p=0.048). Specific treatment with rifaximin in SIBO patients negativated H_2-breath test in 70.9% cases. **Conclusions:** Patients suspected to have irritable bowel syndrome should be evaluated for microscopic colitis and SIBO. The proper diagnosis and the specific treatment may cure some difficult cases of the so called "irritable bowel syndrome". **Key words:** irritable bowel syndrome, microscopic colitis, collagenous colitis, lymphocitic colitis, small intestinal bacterial overgrowth.

With prevalence between 2.5 and 22% worldwide, irritable bowel syndrome (IBS) is a chronic functional gastrointestinal disorder with heterogenous clinical manifestations and no pathognomonic features. Differentiation of IBS from other diseases may be sometimes difficult, especially when colonoscopy looks totally normal (1, 2, 3).

Microscopic colitis (MC) became a frequent diagnosis in the last years. The symptoms are similar to the IBS ones. The mucosa appears normal during colonoscopy, but multiple biopsies are required for a diagnosis that depends on well-defined histological criteria (4, 5, 6, 7, 8).

Another disease similar in clinical manifestations with IBS is small intestinal bacterial overgrowth (SIBO). It is characterized by an increased number (≥105 CFU/mL) and/or abnormal type of bacteria in the small

bowel. The gold standard for diagnosis is the microbial culture of jejunal aspirates, but because of its invasivity H2-breath tests are currently the most important diagnostic methods in SIBO, even if their accuracy is not so high (9, 10, 11, 12).

Our study is therefore aimed to estimate the prevalence of microscopic colitis and SIBO in patients diagnosed with IBS according to Rome III criteria and to compare clinical and epidemiological features of the patients in the three groups. We have also evaluated the effectiveness of treatment with rifaximin in patients diagnosed with SIBO.

MATERIAL AND METHODS

We included in our prospective study patients with a prior diagnosis of IBS, admitted in our clinic between April 2007 and April 2010. The diagnosis of IBS was confirmed on the basis of Rome III criteria and colonoscopy was performed. Prior to inclusion in the study all participants signed an informed consent form. Our study had the approval of Elias Hospital Ethics Committee.

Based on abnormal laboratory tests and colonoscopy some patients were excluded from the study. We insisted on the history of drug consumption and the presence of an acute episode of gastroenteritis before the onset of IBS-related symptoms. Clinical evaluation form registered data about the prominent symptoms, time between the onset of symptoms and the diagnosis of IBS, MC and SIBO and the treatment applied.

During colonoscopy at least four biopsies of normal mucosa from different regions of the colon were taken. Minimal macroscopic lesions (erythema, edema or superficial ulcers of mucosa) were noticed. Characteristic histopathological changes for collagenous colitis were: subepithelial collagenous band thickening > 10 μm, increased number of inflammatory cells in lamina propria, increased intraepithelial lymphocytes and possible surface epithelial damage. Lymphocytic colitis was defined by > 20 intraepithelial lymphocytes/100 epithelial cells and an increased number of inflammatory cells in the lamina propria, associated with normal collagen band. Superficial epithelial injuries were also identified.

All patients in the study group underwent glucose breath test in order to detect SIBO. We measured H_2 in expired air before and after ingestion of 50 g of glucose dissolved in 250 ml water. Measurements were performed every 15 minutes for 120 minutes; the test was positive if one value of expired H_2 exceeded 10-12 ppm over baseline. In patients with positive glucose breath test rifaximin was administered at the daily dose of 1200 mg for 7 days and the test was repeated after two weeks. Simultaneous with the second test patients completed a new clinical form.

We noticed the following: the criteria for MC and/or SIBO in patients with IBS, the demographic features of the patients, the main clinical manifestations, the possible association of autoimmune diseases and drugs in microscopic colitis patients, the period of time between the onset of symptoms and the diagnosis of IBS, MC and SIBO and the outcome after treatment.

Demographic and clinical characteristics of patients in the study group were compared statistically by Student-t test for quantitative variables and χ^2 test for qualitative variables. Statistical hypothesis testing was performed by calculating the value of p ($p < 0.05$ considered statistically significant) and 95% confidence interval. Data were statistically analyzed using *SPPS*

version 17.0 software.

RESULTS

Figure 1 summarizes the general methodology and the results of our study.

We confirmed the diagnosis of IBS based on Rome III criteria in 132 patients. Depending on their predominant transit disorder they were classified into three types of IBS: with diarrhea (39.4%), with constipation (23.5%) and with mixed bowel changes (37.1%).

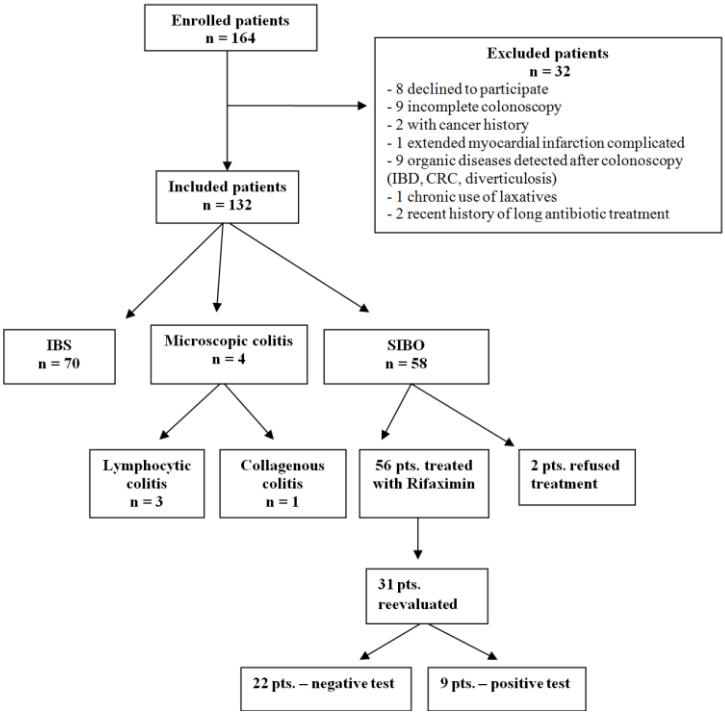

Fig. 1. Methodology and study results

The average age of patients in our study group was 46.2 ± 15.2 years, and there was a predominance of women (59.1%). In 12 patients (9.1%) we identified a history of an acute gastroenteritis. The most frequent symptoms were transit disorders (diarrhea or alternating diarrhea/constipation), bloating, flatulence and abdominal pain. Blood tests (including thyroidian hormones, antinuclear antibodies and rheumatoid factor), abdominal ultrasound and microbiological stool examination were normal in all patients. Fecal calprotectin test was positive in 15 cases (11.4%).

All 132 patients underwent lower gastrointestinal endoscopy, which detected minimal colonic mucosal changes in 10 patients (7.6%). Following detailed histopathological examination of normal mucosa biopsies, microscopic colitis was found in only four patients. The main clinical and biological characteristics of patients with

MC are listed in table I.

Based on positive results on breath test we confirmed the presence of SIBO in 58 patients (43.9%): 67.2% women and 32.8% men. The mean age was 42.5 ± 14.8 years. The most common symptoms were diarrhea and bloating, as well as in patients with IBS. Positive fecal calprotectin test was observed in only 13.8% patients.

56 patients with SIBO received rifaximin at the daily dose of 1200 mg for 7 days. Only 31 patients (55.3%) came to repeat the test after 2 weeks. In retested patients, negativity was achieved in 70.9%. For the 9 patients with positive second test a new cycle of rifaximin was prescribed and 3 patients had a negative breath test after two weeks. There were no side effects during treatment and obtaining negative breath test was followed by partial or total remission of symptoms in all patients.

TABLE I

Characteristics of patients with MC

Age/ sex	MC type	Smoker	Celiac disease	NSAIDs	PPI	Diarrhea/ nocturnal	Weight loss	Period of symptoms	Calprotectin	Normal mucosa
46/ F	LC	Yes	Yes	No	No	Yes/ no	No	2 years	Yes	Yes
58/ F	CC	Yes	No	No	No	Yes/ yes	No	6 years	Yes	Yes
65/ M	LC	No	No	No	Yes	Yes/ yes	Yes	5 months	Yes	Yes
72/ F	LC	No	No	Yes	No	Yes/ yes	Yes	7 weeks	Yes	Yes

Correlating the type of the disease with symptoms we found that diarrhea and nocturnal diarrhea were common manifestations in patients with SIBO (p=0.041) and abdominal pain was more frequent in patients with IBS (p=0.042). The following were also more common in patients with IBS: abdominal bloating (p=0.039) and flatulence (p=0.048). For other symptoms we obtained no statistically significant values (fig. 2).

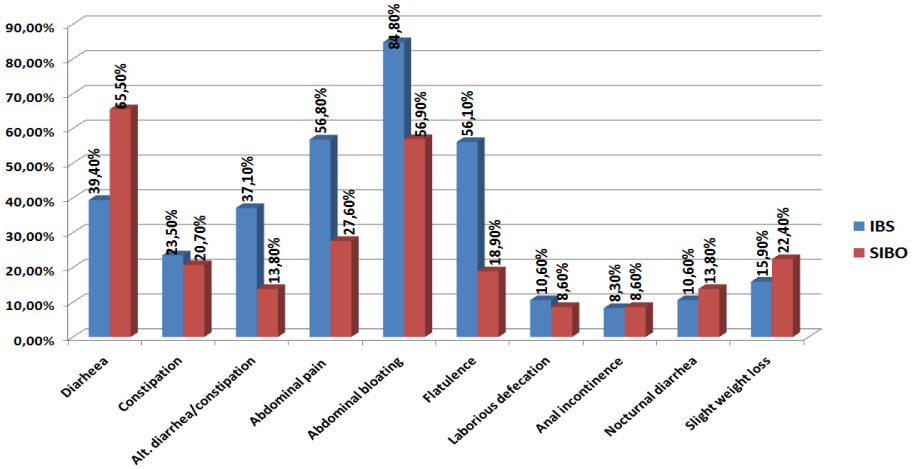

Fig. 2. Comparison of symptoms in patients with IBS and SIBO

DISCUSSION

An increasing number of papers in the literature claim that a diagnosis of IBS could hide some other entities. After successful exclusion of the most frequent causes of chronic diarrhea or persistent constipation a number of rarely identified diseases can mimic IBS. Two of them have drawn our attention: microscopic colitis and SIBO.

Microscopic colitis has become a more frequent diagnosis in the last years due to increasing awareness of physicians as well as to a greater accuracy of diagnostic methods. A study performed in Sweden on 1018 patients identified microscopic colitis in 10% of the patients who underwent colonoscopy for chronic watery non-bloody diarrhea, the percentage increasing to 20% when only patients above the age of 70 were considered (7). Another study on 30 patients previously diagnosed as having IBS reported an incidence of lymphocytic colitis of 23.5%, while other authors found among diarrhea-IBS patients an incidence for collagenic colitis of 7.2% and for lymphocytic colitis of only 2.2% (13, 14). In our group the frequency of MC was lower: 3% of the total of IBS patients and 7.7% of the diarrhea-IBS. Due to the small number of microscopic colitis, statistical interpretation was not possible, but the symptoms were similar to those described by other studies (14, 15).

An association between MC and the use of specific drugs was reported, especially NSAIDs (up to 34%), proton pump inhibitors (Lansoprazole) and ticlopidine (14, 15, 16). Of our four patients with MC, one was treated with NSAIDs and one with Lansoprazole before the onset of diarrhea, which led us to consider these drugs as causative. The association between MC and other autoimmune diseases is seen up to 40% of cases, the most frequent being rheumatoid arthritis, celiac disease, tiroiditis and diabetes. We identified in our group a single case of celiac disease in a patient with lymphocitic colitis.

Budesonide appears to be the optimal drug choice for treating microscopic colitis nowadays. Despite this fact, only one patient in our study had to be treated with budesonide, the other three improved on symptomatic treatment (loperamide).

SIBO can be identified in 45-50% of IBS patients, partly explaining the symptoms (12, 17, 18, 19). Based on glucose breath tests we were able to diagnose SIBO in 43.9% of IBS patients. The major part (65.5%) was experiencing diarrhea, while fewer experienced chronic constipation (20.7%) or mixed transit disturbances (13.8%). The second frequent complain in our group of SIBO patients was bloating (56.9%), a symptom that most authors regard as the most specific sign of alterations in the intestinal flora (12).

One week of rifaximin 1200 mg/day has led to negative results of the glucose hydrogen breath test in 70.9% of cases. Our data confirm the results of other studies which showed a positive response between 59.4% and 82.6% for SIBO patients treated with rifaximin, with or without subsequent treatment with probiotics. Repeated courses of rifaximin in initial non-responders has led to a favourable outcome in 33.3% of cases, a better response than the ones obtained with ciprofloxacin, norfloxacin, amoxicillin - clavulanate, gentamicin, cotrimoxazole, polymyxins or tetracyclin (12, 18, 19).

Symptoms in IBS, MC and SIBO pa-

tients show some degree of overlap, but also a few significant differences. Most patients experiencing diarrhea (80.76%) and especially those with nocturnal diarrhea (78.57%) have been diagnosed with MC or SIBO. Abdominal pain, bloating and flatulence were described more frequently in patients with IBS and less in MC or SIBO patients. In what calprotectin is concerned, we found positive tests more frequently in MC and SIBO (80%) and only a smaller proportion in IBS patients.

CONCLUSIONS

We can conclude that, in order to correctly identify MC or SIBO, patients presenting as IBS should undergo an initial evaluation including colonoscopy with multiple level biopsies from normal-appearing mucosa and a glucose or lactulose hydrogen breath test. The symptoms of the three described conditions are very similar. Nocturnal diarrhea and a positive stool test for calprotectin can help differentiating MC and SIBO patients from those suffering from IBS, but their specificity is far from 100%. Specific management with budesonide in MC and with rifaximin in SIBO leads to significant clinical improvement and a favorable response in difficult to treat cases of „irritable bowel syndrome".

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NEWS

EXTRACELLULAR MATRIX ADMINISTRATION AS A POTENTIAL THERAPEUTIC STRATEGY FOR PERIODONTAL LIGAMENT REGENERATION

The current strategies employed for the treatment of connective tissue disease include the application of stem cells, the use of functional molecules that can reorganize tissue integrity and cellular activities to recover connective tissue function. Approaches to the regeneration of periodontal tissue, which is the tooth-supporting connective tissue, have made some progress recently and provide a useful experimental model for the evaluation of future strategies to treat connective tissue diseases such as periodontal disease. The ultimate goal of periodontal tissue regeneration is to reconstruct the ligament structure that will sustain the required mechanical force to connect with mineralized tissues such as cementum and alveolar bone. The administration of extracellular matrix could be used as an additional therapeutic strategy to stem cell transplantation and cytokine administration in periodontal tissue regeneration therapy. Although various available tissue engineering technologies can now achieve periodontal tissue regeneration, extracellular matrix administration therapy is likely to play an essential future role in the development and regeneration of periodontal tissue and attenuate the signaling events that mediate tissue degradation. Hence, extracellular matrix administration could serve as a novel technology in periodontal tissue regeneration and also as a viable approach to alleviating connective tissue disorders such as Marfan's syndrome [Saito M, Tsuji T. Extracellular matrix administration as a potential therapeutic strategy for periodontal ligament regeneration. Expert Opin Biol Ther. 2012 Mar;12(3):299-309].

Gianina Iovan

IDENTIFICAREA MODIFICĂRILOR MASEI ŞI *TURNOVER*-ULUI OSOS LA PACIENȚII CU POLIARTRITĂ REUMATOIDĂ TRATAȚI CU CORTICOSTEROIZI ÎN SCOPUL ELABORĂRII UNEI ATITUDINI TERAPEUTICE OPTIME

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IDENTIFICATION OF BONE MASS AND BONE *TURNOVER* IN PATIENTS WITH RHEUMATOID ARTHRITIS TREATED WITH CORTICOSTEROIDS IN ORDER TO ELABORATE AN OPTIMAL THERAPEUTIC ATTITUDE (**Abstract**). Corticosteroids (CS) are currently used in Rheumatoid Arthritis (RA) in conjunction with either synthetic remissive or biologic drugs. **Aim**: In our study we used have focused on bone mineral density assessment (BMD) in RA patients with and without low doses of CS in order to elaborate an optimal therapeutic approach. **Material and methods**: prospective observational study on 55 consecutive patients with RA (1987, ACR diagnostic criteria) classified in two groups based on CS use: group A - 23 RA receiving CS and subgroup B – 32 RA without CS. All patients have been evaluated according to a predefined protocol including demographics, clinical, biological and therapeutic RA characteristics, BMD and T-score assessment by DXA (Hologique QDR) (1994, WHO classification). Subgroup analysis was done in SPSS-12 software, $p<0.05$. **Results**: No significant differences in demographics and RA related parameters ($p>0.05$) have been demonstrated between subgroups. However, significant changes in BMD and T-score have been reported in RA receiving CS as follows ($p<0.05$): up to 74% cases with osteoporosis, 13% with fracture and 8.7% with osteopenia (A) *versus* 31.3% with osteoporosis, 28.1% with fracture and 15.6% with osteopenia (B). Moreover, 90% of RA under 7.5 mg CS daily and all receiving >10mg daily presented with osteoporosis; also, osteoporosis has been demonstrated all postmenopausal RA in group A (75%) and only 68% of group B (76%). **Conclusions**: concomitant CS use in RA, even low doses, is commonly associated with low BMD, irrespective of other risk factors. **Key words**: BONE MASS, BONE *TURNOVER*, RHEUMATOID ARTHRITIS, CORTICO-STEROIDS

Poliartrita reumatoidă (PR), reumatismul inflamator cu largă frecvență, afectează aproximativ 1% din populația generală, fiind recunoscută ca o boală severă, autoîntreținută şi progresivă, care induce leziuni osteo-articulare, cu deficit funcțional şi pierderea capacității de muncă, cu o mortalitate prematură considerabilă, reducând semnificativ speranța de viață a bolnavilor cu această afecțiune (1, 2, 3).

În patogenia PR este greu de definit o succesiune riguroasă a evenimentelor, de-

oarece celulele şi sistemele biologice umorale acţionează complex, simultan şi au un mare grad de interdependenţă. Ele se desfăşoară în două planuri, în structura sinovialei şi în cavitatea articulară, între cele două compartimente existând multiple influenţe reciproce (4). Se poate aprecia că celulele oferă suportul evoluţiei cronice progresive, pe când elementele umorale sunt responsabile de inflamaţia acută.

Între manifestările extra-articulare ale PR se include şi osteoporoza, dar mecanismele patogenice sunt multiple, constelaţia citokinică proinflamatorie caracteristică bolii şi corticosteroizii cu impact asupra densităţii minerale osoase (DMO) şi *turnover*-ului osos fiind elemente definitorii (5,6,7). Cercetări recente au adus în atenţie evoluţia DMO raportat la durata şi tipul tratamentului, precum şi modalitatea evolutivă a bolii sub aspectul necesităţii instituirii cât mai precoce a unui tratament remineralizant (6,8,9,10), incluzând o dietă bogată în calciu, exerciţiile fizice, precum şi administrarea zilnică a 1000 mg calciu şi vitamina D 800 UI încă de la începutul corticoterapiei, iar la o lună după iniţierea acesteia, se dozează calciul urinar care, dacă depăşeşte 300 mg / 24 h, va necesita asocierea unei tiazide şi corectarea dozei de calciu şi vitamina D (11). În cazul în care DMO indică o scădere a acesteia, se instituie tratament corespunzător, în care un loc important îl ocupă bisfosfonaţii (12). Dintre bisfosfonaţii utilizaţi în practică, acidul alendronic, 70 mg /săptămână (Fosamax, Fosavance), acidul risendronic (Actonel 35 mg/săptămână), acidul ibandronic (Bonviva 150 mg /lună), au determinat o creştere a densităţii minerale osoase (13). Un rol important îl are şi terapia de substituţie hormonală (la femei, cu preparate de estrogen cu sau fără progesteron, iar la bărbaţi, cu testosteron) (14).

Datele acumulate au fost evaluate cu ajutorul programelor MS EXCEL şi SPSS 13.0, folosind intervalele de încredere la pragul de semnificaţie 95%, iar pentru testarea diferenţelor s-au utilizat testele χ^2 , t-Student, p.

Studiul si-a propus identificarea modificărilor masei şi *turnover*-ului osos la pacienţii cu PR trataţi cu corticosteroizi, în scopul elaborării unei atitudini terapeutice optime, având in atenţie: evaluarea modificărilor densităţii minerale osoase, identificarea particularităţilor etiologice, evaluarea modificării metabolismului mineral osos, identificarea mecanismelor patogenice implicate în degradarea masei şi *turnover*-ului osos, dinamica modificărilor masei şi *turnover*-ului osos induse de excesul de cortizon, investigarea modificărilor masei şi *turnover*-ului osos drept consecinţă a diferitelor intervenţii terapeutice.

MATERIAL ŞI METODE

În perioada 2010-2011, în Clinica de Reumatologie a Spitalului Clinic de Recuperare Iaşi, au fost studiaţi 55 pacienţi cu diagnosticul de PR (criteriile de diagnostic ACR, 1987), în ordinea prezentării în clinică. Aceştia au fost împărţiţi în două loturi, în vederea unui studiu comparativ: *lotul A,* alcătuit din 23 pacienţi (41.82 %) cărora, în cursul evoluţiei bolii, li s-a administrat tratament cu corticosteroizi (CS) şi *lotul B,* martor, alcătuit din 32 pacienţi (58.18%) care nu au luat niciodată tratament cu CS.

Datele asupra pacienţilor au fost obţinute pe baza unei fişe standard ce a urmărit evaluarea unor particularităţi demografice, clinico-biologice şi terapeutice legate de PR, precum şi determinarea densităţii minerale osoase prin absorbţiometrie duală cu raze X (*Dual X-Ray Absorbtiometry,* DXA)

folosind aparatul Hologique QDR cu raze X, care măsoară densitatea minerală osoasă, exprimând cantitatea de os pe unitatea de suprafață sau volum (fig. 1 A, B, C).

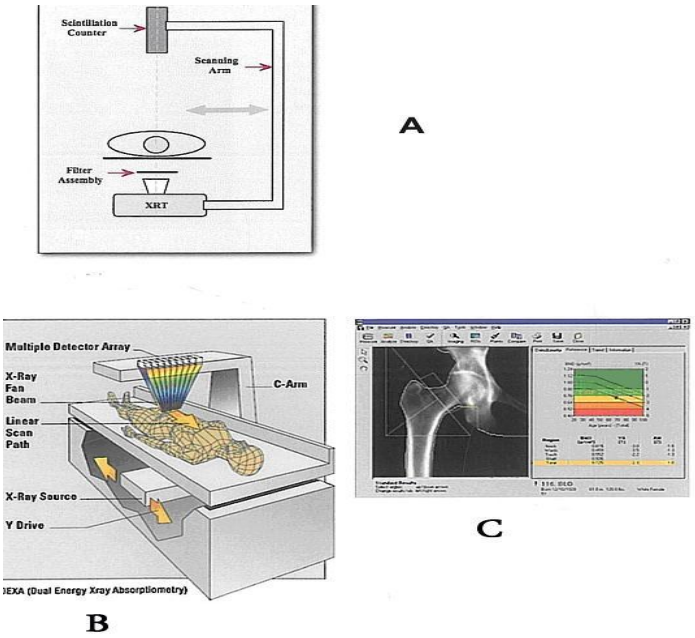

Fig. 1. A+ B - Diagrama aparatului de scanare DXA; C- Fişă de osteodensitometrie (15)

Criteriile Organizației Mondiale a Sănătății (OMS) din 1994 folosesc scorul T pentru delimitarea categoriilor diagnostice în osteoporoză. Astfel:

- scorul T > - 1 deviație standard (DS) definește populația cu masă osoasă normală;
- scorul T între -1 DS şi – 2.5 DS reprezintă criteriul pentru diagnosticul de osteopenie;
- scorul T ≤ - 2.5 DS este criteriul de diagnostic pentru osteoporoză;
- scorul T ≤ - 2.5 DS şi prezența unei fracturi de fragilitate reprezintă criteriul pentru diagnosticul de osteoporoză severă (manifestă).

Studiul s-a desfăşurat cu aprobarea comisiei de etică; toți pacienții au semnat un consimțământ informat înaintea includerii în studiu.

REZULTATE ŞI DISCUȚII

Aspecte epidemiologice. *Sexul.* Pacienții incluși în lotul A au fost 87% femei şi 13% bărbați, în timp ce în lotul B proporția a fost de 79% femei şi 21% bărbați. Statistic s-a demonstrat că structura pe sex a fost omogenă în cele două loturi (χ^2=0.23; GL=1; p=0.629), cu raportul cotelor de 1.87 (OR=1.87; IC95%: 0.36÷10.60).

Vârsta. Repartiția pe grupuri de vârstă a loturilor studiate a arătat o incidență crescută a PR începând cu decada a patra de viață, dar o frecvență mult mai mare fiind raportată în cea a cincea şi a şasea decadă. Distribuția pe grupuri de vârstă s-a dovedit a fi, de asemenea, omogenă (χ^2=3.45; GL=5; p=0.631). Ponderea pacienților cu

PR în vârstă de peste 40 de ani, a fost de 87% în lotul A şi 81% în B (tab. I).

TABELUL I

Repartiţia pe grupuri de vârstă a celor două loturi

Vârsta la momentul studiului (ani)	Nr.pacienţi/lot	
	A	B
<30	0	1
30-39	3	5
40-49	7	7
50-59	9	9
60-69	4	8
≥70	0	2

Aspecte clinice.

Redoarea matinală a fost mai intensă la pacienţii din grupul A, comparativ cu B (t=4.45, GL=53, p<0.001): 90± 20 minute, faţă de 60±30 minute (fig. 2).

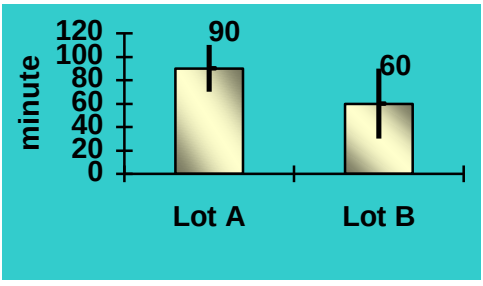

Fig. 2. Durata redorii matinale

În ceea ce priveşte *durata de evoluţie* a PR, s-a constatat la pacienţii din lotul A o medie de 9±6 ani, uşor mai crescută decât media evoluţiei bolii la cei din lotul martor (7± 3 ani), fără diferenţă semnificativ statistic (t=1.47, GL=53, p>0,05) (fig. 3).

Osteoporoza din PR impune o abordare diferenţiată, pe de o parte ca un fenomen atribuibil citokinelor proinflamatorii aferente patogeniei, a modificărilor metabolice (ale calciului) şi a hormonilor circulanţi, pe de altă parte, ca un efect bine cunoscut al corticoterapiei. Invariabil însă, osteoporoza reprezintă un element cvasiconstant întâlnit la pacienţii cu PR, dar un rol important în ceea ce priveşte evoluţia îl are riscul de fractură, în special cele vertebrale şi ale şoldului, indicând nivelul morbidităţii şi al mortalităţii, cu o scădere semnificativă a calităţii vieţii (8,9,10).

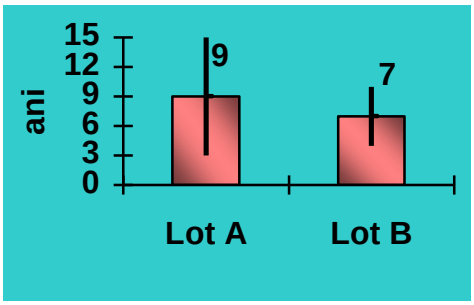

Fig. 3. Durata de evoluţie a poliartritei reumatoide

Două cazuri aparţinând lotului A, care au primit tratament CS, au prezentat fractură (8.7%), pe când în lotul B doar una (3.1%), distribuţia de frecvenţe nefiind semnificativ statistică (χ^2=0.09; GL=1; p=0.768).

Aspecte paraclinice. Examenele de laborator au vizat sindromul inflamator (*VSH*, proteina C reactivă-*CRP)* şi sindromul disimunitar (reacţia Latex şi Waaler-Rose pentru determinarea factorului reumatoid-*FR*).

Probele efectuate au arătat că sindromul inflamator este intens prezent la majoritatea pacienţilor studiaţi, atestând riscul de boală moderată sau severă, cu potenţial distructiv important şi predispoziţie la invaliditate prin apariţia deformărilor articulare(1,3)

VSH-ul, marker al gradului inflamaţiei reumatoide, a prezentat valori pentru lotul A: 20 - 40 mm/oră (3 cazuri), 40 - 60

mm/oră (5 cazuri), 60 - 80 mm/oră (10 cazuri), şi > 80 mm/oră (5 cazuri). Comparativ, în lotul B valorile *VSH*-ului au fost: 20-40 mm/oră (3 cazuri), 40-60 (12 cazuri), 60 - 80 mm/oră (9 cazuri) şi > 80 mm/oră (8 cazuri). În stadiul funcțional III şi IV valorile medii au fost dominante.

Inflamația şi caracterul eroziv al PR sunt traduse prin prezența *CRP*, care a fost înregistrată la 80% din cazuri în lotul A şi 60% în B, distribuția de frecvență fiind semnificativă statistic (χ^2=4.94; GL=1; p=0.026).

A fost determinat *FR* (explorare de rutină în PR), constituind unul din criteriile de diagnostic, iar titrurile serice înalte atrag atenția asupra unei evoluții severe, articulare şi extraarticulare. În 88% din cazurile care au beneficiat de corticoterapie, a fost evidențiată prezența FR şi doar în 60% din cele fără tratament, orientând decizia urmăririi pacienților tratați cu prednison (χ^2=18.94; GL=1; p=0.00001).

Aspecte terapeutice. În arsenalul terapeutic al PR evaluate s-a inclus: terapia patogenică remisivă în 76% cazuri; antiinflamatoarele non-steroidiene (AINS) în 96%; corticoterapia în 52% cazuri. În funcție de doza de CS indicată, două cazuri au primit doze între 5 şi 7.5 mg/zi, patru pacienți doze mai mari de 10 mg/z ; majoritatea (17 : 30,9 %) încadrându-se însă în doza zilnică de 7.5-10 mg.

Examenul densitometric. Osteodensitometria dual cu raze X este o metodă radiologică de măsurare a masei osoase, examinare unică, standardizată, acceptată de OMS pentru determinarea gradului de osteoporoză.(6). Diagnosticul de osteoporoză este stabilit la 15-20% din femeile aflate în postmenopauză prin aplicarea DXA la nivelul coloanei sau şoldului şi folosirea unui prag de diagnostic de -2.5DS. Incidența osteoporozei creşte exponențial după vârsta de 50 de ani, ca urmare a scăderii liniare cu vârsta a densității minerale osoase si distribuția gaussiană a valorilor DMO. Măsurarea DMO s-a realizat la toți pacienții studiați (tab. II).

TABELUL II

Structura loturilor în relație cu valorile scorului T

Scorul T	Diagnostic	Lotul A	Lotul B
> -1 DS	Normal	1	8
-1 DS - (-2.5 DS)	Osteopenie	2	5
≤-2.5 DS	Osteoporoză	17	10
≤ -2.5 DS + fractură	Osteoporoză severă	3	9

Statistic au fost evidențiate semnificativ mai multe cazuri de osteoporoză în lotul A (73.9%) comparativ cu B (31.3%), iar la lotul B s-a remarcat existența a 25% cazuri ameliorate, în timp ce la lotul A frecvența acestora a fost de numai 4.3% (χ^2=10.35; GL=1; p=0.016) (fig. 4, 5).

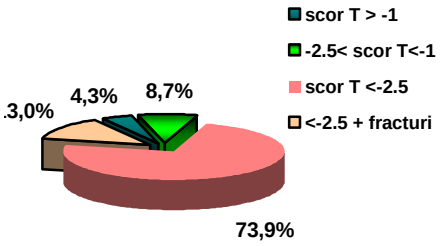

Fig. 4. Frecvența osteoporozei în lotul A

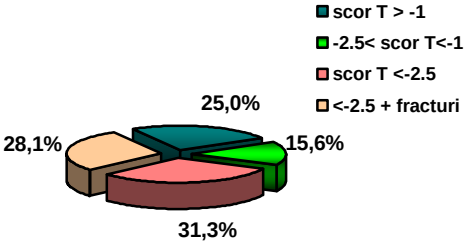

Fig. 5. Frecvența osteoporozei în lotul B

După iniţierea terapiei cu CS s-a constatat o fază rapidă de pierdere a masei osoase (primul an de tratament), urmată de o reducere mai lentă, dar continuă, a DMO, iar apariţia osteoporozei a fost corelată şi cu doza zilnică de prednison.

Cei doi pacienţi care au primit mai puţin de 7.5 mg pe zi nu au dezvoltat osteoporoză, în timp ce frecvenţa osteoporozei a fost depistată la 90% din pacienţii cu doze de 7.5-10 mg şi de 100 % la cei cu doză > 10 mg/zi (tab. III).

TABELUL III

Frecvenţa osteoporozei în relaţie cu doza de prednison

Doza de prednison	Total pacienţi	Pacienţi cu osteoporoză	%
5-7.5 mg/zi	2	0	0
7.5-10 mg/zi	17	15	90
> 10 mg/zi	14	14	100

Pentru valorile mai mici de 10, proporţiile sunt orientative S-a remarcat prezenţa osteoporozei la 31% dintre pacienţii ce nu au urmat tratament cu prednison, situaţie posibil a fi explicată de intervenţia şi a altor factori de risc (vârsta, menopauza, sedentarismul prelungit datorat bolii şi alimentaţia carenţială).

Repartiţia osteoporozei pe grupuri de vârstă la pacienţii în studiu a evidenţiat prevalenţa cea mai crescută pentru intervalul 40-69 ani (tab. IV).

Un alt factor de risc important în apariţia osteoporozei este menopauza, deoarece estrogenii modifică homeostazia calciului seric prin influenţa lor asupra glandei paratiroide sau a ţesuturilor ţintă pe care acţionează şi PTH. Repartiţia pacientelor cu osteoporoză în relaţie cu prezenţa menopauzei în cele două loturi studiate a evidenţiat că în lotul A, dintre cele 20 femei, 15 (75%) erau în etapa de postmenopauză, toate fiind diagnosticate cu osteoporoză; în lotulB, din 25,19 (76%) erau în post-menopauză dar doar 17 (68%) au fost diagnosticate cu osteoporoză.

TABELUL IV

Repartiţia bolnavilor cu osteoporoză pe grupuri de vârstă

Grupurile de vârsta (ani)	Lotul A		Lotul B	
	Număr pacienţi	Cazuri cu osteoporoză	Număr pacienţi	Cazuri cu osteoporoză
< 30	0	0	1	0
30-39	3	1	5	0
40-49	7	5	7	1
50-59	9	8	9	4
60-69	4	4	8	3
≥ 70	0	0	2	1

S-a dovedit că deficitul hormonal înregistrat la menopauză induce apariţia osteoporozei, aspect evidenţiat în ambele loturi de studiu (χ^2=0.32; GL=1; p=0.574).

Cercetările au dovedit prezenţa osteoporozei la 74% dintre pacienţii ce au beneficiat de tratament cu CS faţă de doar 31% la cei din lotul martor, dovedind efectele negative ale CS asupra metabolismului osos, accentuând resorbţia osoasă şi, în acelaşi timp, reducând procesul formării osului, având drept consecinţă reducerea masei osoase şi creşterea riscului de fractură.

CONCLUZII

1. Osteodensitometria este o metodă de diagnostic paraclinic ce trebuie obligatoriu aplicată pe parcursul evoluţiei bolii

pentru a surprinde modificările DMO şi pentru a interveni terapeutic corespunzător. Cu ajutorul osteodensitometriei a fost evidențiată prezența osteoporozei induse de CS la circa trei pătrimi dintre pacienții cu PR studiați.

2. Prin intermediul osteodensitometriei s-a constatat că în primele 6 luni de tratament cu prednison modificările la nivelul țesutului osos sunt de tip osteopenie, până la un an valorile indică osteopenie la limită cu osteoporoza, iar peste un an se instalează osteoporoza.

3. S-a dovedit că osteodensitometria are un rol major în evidențierea riscului de apariție a osteoporozei cortizonice, contribuind astfel la decizia instituirii unui tratament prevențional. Doza de prednison influențează apariția osteoporozei; dozele de 7.5 mg⁄zi administrate cronic pot determina osteoporoză în 90% din cazuri.

4. La începerea corticoterapiei s-a impus înlăturarea factorilor cu risc adiționali corectabili, cu obținerea cooperării informate a pacientului.

Suplimentarea cu calciu şi vitamina D a fost necesară la toți bolnavii incluşi în studiu care necesitau corticoterapie pe termen lung, din cauza balanței calcice negative induse de tratamentul cu CS.

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TRATAMENTUL CHIRURGICAL AL SINDROMULUI DE CANAL TARSIAN ÎN NEUROPATIA PERIFERICĂ DIABETICĂ

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SURGICAL TREATMENT OF TARSAL TUNNEL SYNDROME IN DIABETIC NEUROPATHY (Abstract): Posterior tibial nerve decompression surgery in tarsal tunnel syndrome in patients with diabetic neuropathy reduces pain, improves sensitivity and prevents foot ulcers and lower leg amputations. Aim: To observe and assess the recovery of plantar sensitivity recovery and the healing of ulcerative lessions of the foot, by clinical examination, exploration and analysis of quantitative neurosensory by surgical decompression of the tarsal tunnel. Material and methods: We evaluated a total of 10 patients and 12 symptomatic diabetic neuropathy feet in a prospective clinical study, surgically treated in the Clinic of Plastic Surgery and Reconstructive Microsurgery Iaşi, during January 2008 - June 2011, where we practiced tibial nerve decompression and neurolysis in tarsal tunnel syndrome. Results: Gender distribution of patients in the study group was predominantly male (60%), the ratio M/F = 1.5/1. Posterior tibial nerve decompression surgery resulted in recovery of plantar foot sensitivity in 90% patients in the study group. Testing Semmes-Weinstein 10 g monofilament was positive in 83.3% of the feet preoperatively whereas postoperatively only 25%, distribution of statistically significant ($\chi 2 = 6.04$, GL = 1, $p = 0.014$). Postoperative score to test a range of Riedel-Seiffer returned to normal in all patients: score 7 to 58.3% and score 8 to 41.7% of total standing tested. Conclusions: Tarsal tunnel decompression in diabetic patients with peripheric neuropathy improves plantar sensitivity, leads to healing of ulcerative plantar lesions and improves quality of life and should be performed in all patients with diabetic peripheral neuropathy in which conservative and / or medical treatment failed.
Key Words: DIABETIC NEUROPATHY, TIBIAL NERVE COMPRESSION, TARSIAN TUNNEL DECOMPRESSION

Neuropatia diabetică distală simetrică reprezintă forma cea mai comună, ocupând 75% dintre neuropatiile periferice. Pentru diagnosticul clinic al neuropatiei diabetice, 2 din cele 5 caractere sunt necesare: (i) semne, (ii) simptome, (iii) teste senzoriale cantitative, (iv) viteză de conducere nervoasă, şi (v) teste autonome (1, 2). Istoria naturală a neuropatiei diabetice este progresivă, cu pierderea sensibilității plantare care poate duce la apariția ulcerațiilor şi/sau amputațiilor (3). Studiile au arătat că decompresiunea chirurgicală a nervilor periferici de la nivelul extremităților inferioare la pacienții cu neuropatie diabetică poate reduce durerea, redobândi sensibilitatea, şi preveni ulcerațiile şi amputațiile (4, 5). În contextul acestor caracteristici ale

neuropatiei diabetice periferice, obiectivele acestui studiu au fost de a urmări şi evalua redobândirea sensibilității plantare şi vindecarea leziunilor trofice de la nivelul piciorului cu ajutorul examenului clinic, a explorărilor funcționale şi prin analiza cantitativă neurosenzorială la pacienții cu forma cea mai comună de neuropatie diabetică, şi anume polineuropatia senzorimotorie, după decompresiunea chirurgicală a canalului tarsian, într-un studiu prospectiv, la 3, 6 şi 12 luni după intervenția chirurgicală.

MATERIAL ŞI METODE

Am evaluat un număr de 10 pacienți şi 12 picioare diabetice cu neuropatie diabetică simptomatică într-un studiu clinic prospectiv, operați în Clinica de Chirurgie Plastică şi Microchirurgie Reconstructivă Iaşi pe perioada ianuarie 2008 – iunie 2011, cărora li s-a practicat decompresiunea canalului tarsian şi neuroliza nervului tibial posterior. Toți pacienții erau diagnosticați la momentul prezentării cu diabet zaharat tip 1 sau tip 2, aflați în evidența Clinicii de Diabet Nutriție şi Boli Metabolice Iaşi. Evaluarea redobândirii sensibilității plantare pre- şi postoperator a fost evaluată cu ajutorul examenului clinic în special prin urmărirea dispariției semnului Tinel, efectuarea testelor cantitative senzoriale care cuprinde testul sensibilității tegumentare cu monofilamentul Semmes-Weinstein (monofilamentul 10 g) şi evaluarea pragului de percepție a vibrațiilor cu diapazonul Riedel-Seiffer (Boehringer Mannheim GmbH).

Distribuția pe sexe a pacienților din lotul de studiu a fost preponderent masculină (60%), raportul M / F = 1,5/1. Distribuția pe grupe de vârstă relevă ponderea mare a cazurilor la grupa de vârstă 65-69 ani, cu precădere la sexul masculin. Vârsta medie a pacienților cărora li s-a practicat decompresiunea canalului tarsian şi neuroliza nervului tibial posterior, la intrarea în studiu, a fost de 61,1±6,52 ani, variind de la 51 la 69 ani.

1. **Evaluarea cantitativă senzorială**. *Testul senzorial cantitativ cu monofilamentul Semmes-Weinstein 5.07* care corespunde forței aplicate de 10 g (Baseline® tactileTM monofilament evaluator case, U.S.A.) - a fost efectuat înainte şi după decompresiunea canalului tarsian. Zonele de elecție unde se aplică monofilamentul sunt capul metatarsienelor I şi V, precum şi fața pulpară a halucelui. Monofilamentul se aplică perpendicular pe tegument şi o forță suficientă încât să se curbeze (6) (fig. 1). Sensibilitatea protectivă este prezentă dacă pacientul răspunde corect la cel puțin 2 din trei încercări. Senzația protectivă este absentă cu cel puțin 2 răspunsuri incorecte şi pacientul are un risc crescut de a dezvolta ulcerație plantară. Pierderea sensibilității protective a fost definită ca acel grad de pierdere a sensibilității care permite producerea unei leziuni tegumentare fără ca pacientul să o resimtă dureroasă (7). Sensibilitatea metodei este de 66-91%, iar specificitatea ei 34-86% (8).

2. **Evaluarea sensibilității vibratorii**. *Evaluarea pragului de percepție a vibrațiilor cu Diapazonul Riedel-Seiffer* (Boehringer Mannheim GmbH). Baza diapazonului se aplică pe proeminențe osoase, maleolele, articulația interfalangiană pe fața dorsală a halucelui, articulația metatarsofalangiană sau pe vârful halucelui (fig. 2) Valorile normale ale testului sunt cuprinse între 6/8 şi 8/8 pentru indivizii sub 60 de ani şi între 4/8 şi 8/8 pentru cei peste 60 de ani (9).

Fig. 1. Testarea sensibilității cu monofilamentul Semmes-Weinstein 5.07

Fig. 2. Aplicarea diapazonului pe suprafețe osoase

3. Descrierea tehnicii chirurgicale. Tratamentul chirurgical al decompresiunii canalului tarsian constă în practicarea unei incizii care începe la 4 cm proximal de maleola medială, urmăreşte îndeaproape partea posterioară a maleolei şi se extinde în jos către aspectul plantar al piciorului, fiind curbată anterior călcâiului. Fascia profundă este incizată, şi apoi se continuă distal cu incizia flexorului retinaculum (fig. 3).

Se secționează superficial fascia abductorului halucelui şi apoi septul dintre nervul plantar medial şi lateral (diviziunea nervului tibial în canalul tarsian) (10) (fig. 4).

Se poate efectua la nevoie pe lângă neuroliza externă şi neuroliza internă, precedată de epineurotomie longitudinală a nervului tibial posterior.

Fig. 3. Secționarea flexorului *retinaculum*

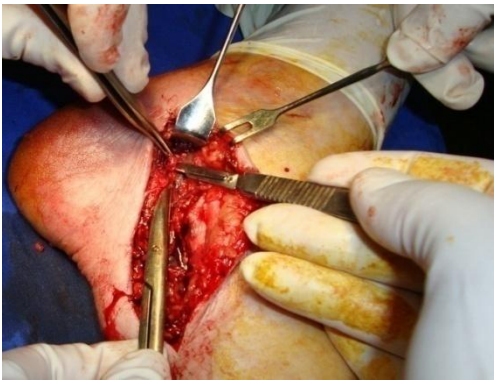

Fig. 4. Secționarea septului fibros între nervul plantar medial şi lateral

REZULTATE

1. Evaluarea preoperatorie. Pe cazuistica studiată, cel mai frecvent se remarcă hipoestezia plantară (50%), urmată de anestezia plantară (30%) (tab. I). Pe întreg lotul de studiu (100%) semnul TINEL a fost

pozitiv în spațiul retromaleolar intern.

În funcție de caracteristicile epidemiologice se remarcă frecvența ușor mai crescută a pierderii axonale severe la sexul masculin (p=0,673), însă distribuțiile nu au fost semnificative din punct de vedere statistic. La vârste sub 60 ani se remarcă semnificativ mai frecvent pierderea axonală severă (p=0,038) (tab. II).

Scorul preoperator cel mai frecvent întâlnit la testul cu diapazonul Riedel-Seiffer a fost 3 (41,7%), urmat de scorul 4 (25%), scoruri patologice indiferent de vârsta pacientului (fig. 5).

TABELUL I

Distribuția lotului în relație cu sensibilitatea plantară

Caracteristică epidemiologică	Hipoestezie plantară	Anestezie plantară	Hiperestezie plantară	Test de semnificație
Sex				
Feminin	10%	20%	10%	χ^2=0,14;GL=2 p=0,933
Masculin	20%	30%	10%	
Grupa de vârstă				
< 60 ani	0%	30%	20%	χ^2=5,20;GL=2 p=0,074
≥ 60 ani	30%	20%	0%	

TABELUL II

Distribuția lotului în relație cu viteza de conducere nervoasă

Caracteristică epidemiologică	Viteza de conducere nervoasă		Test de semnificație
	Pierdere axonală severă	Bloc de conducere nervoasă	
Sex			
Feminin	30%	10%	χ^2=0,18;GL=1 p=0,673
Masculin	40%	20%	
Grupa de vârstă			
< 60 ani	40%	30%	χ^2=4,29;GL=1 p=0,038
≥ 60 ani	30%	-	

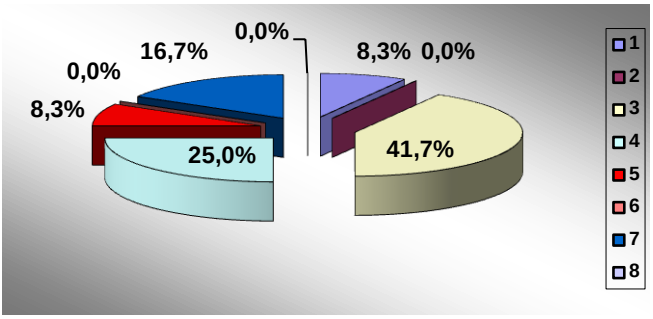

Fig. 5. Distribuția numărului de picioare în funcție de percepția vibratorie preoperatorie la testarea cu diapazonul Riedel-Seiffer

Testarea preoperatorie cu monofilamentul Semmes-Weinstein 10 g a fost pozitivă la 83,3% dintre picioare preoperator şi numai 25% postoperator, distribuție semnificativă din punct de vedere statistic (χ^2=6,04; GL=1; p=0,014).

2. Evaluarea postoperatorie. Pe cazuistica studiată, sensibilitatea plantară s-a îmbunătățit clinic, cu dispariția semnului TINEL la 9 pacienți (90%). În primele 3 luni semnul TINEL a devenit absent la peste 60% dintre pacienți, după care, la 6 luni a devenit absent la toți pacienții. Un pacient cu anestezie plantară nu a prezentat îmbunătățiri ale sensibilității plantare, dar clinic semnul TINEL a fost absent. Un singur pacient a prezentat la o lună postoperator dehiscența plăgii operatorii, refuzând reintervenția chirurgicală. La restul pacienților cu decompresiunea canalului tarsian, sensibilitatea plantară s-a îmbunătățit în proporție de 40% în primele 3 luni. După 6 luni, leziunile trofice plantare s-au vindecat complet fără alte complicații sau alte intervenții chirurgicale.

Scorul postoperator la testul cu diapazonul Riedel-Seiffer a revenit în limite normale la toți pacienții: scor 7 – 58,3% şi scor 8 – 41,7% din numărul total de picioare testate. Valorile postoperatorii ale scorului la testul diapazonului Riedel-Seiffer s-au încadrat în intervalul 7-8 indiferent de valorile inițiale, de aceea se remarcă o corelație moderat indirectă între aceste valori (r= -0,25) (fig. 6).

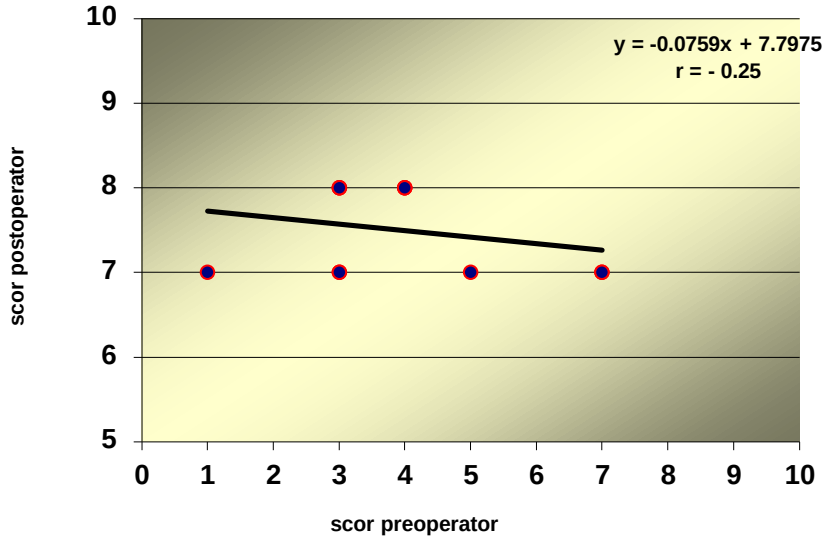

Fig. 6. Corelația dintre valorile individuale ale scorurilor pre şi post operatorii la scorul diapazonului Riedel-Seiffer

DISCUȚII

Neuropatia diabetică este complicația primară şi una din cele mai frecvente cauze de morbiditate legată de diabet. Este unul din factorii cei mai importanți în apariția ulcerației plantare la pacienții diabetici. Prevenirea acestei complicații este dificilă, în special din cauză că nu există o metodă fiabilă pentru a testa sensibilitatea plantei.

La pacienții diabetici nervii periferici se edematiază, tinzând să ducă la creşterea conținutului de apă prin creşterea conversi-

ei aldozreductazei din glucoză la sorbitol. Nervul tibial posterior este supus riscului cronic de dezvoltare a compresiunii ca urmare a edemului. Diametrul nervului tibial la pacienții diabetici cu polineuropatie este semnificativ mai mare decât la pacienții diabetici ce nu prezentau polineuropatie (24.0 vs. 12 mm^2) (1). Pierderea progresivă şi neuniformă a sensibilității pentru stimulii externi explică de ce se pot constata ulcerații la pacienții cu diabet la care o examinare neurologică grosieră a sensibilității superficiale poate duce la impresia greşită că aceasta este încă păstrată.

În acest studiu prospectiv am pus în evidență semnele clinice cele mai importante în sindromul de canal tarsian la pacienții diabetici, aspectele leziunilor trofice şi ulcerative plantare pe care polineuropatia diabetică periferică le induce alături de sindromul de canal tarsian, precum şi evaluarea pacienților din punct de vedere al testării cantitative şi semnicantitative senzoriale după decompresiunea chirurgicală a canalului tarsian. Totodată am evaluat şi pus în evidență redobândirea sensibilității plantare, dispariția durerii, şi vindecarea leziunilor plantare.

Decompresiunea chirurgicală a nervului tibial posterior a dus la redobândirea sensibilității plantare la 90 % dintre pacienți pe lotul studiat.

Vindecarea leziunilor plantare s-a produs la toți pacienții la diferite intervale de timp, cea mai lungă perioadă fiind de 1 an şi 6 luni, dar la acest pacient sensibilitatea plantară nu a revenit în limite normale după intervenția chirurgicală. In lotul de studiu, 1 singur pacient a prezentat dehiscența şi infectarea plăgii postoperatorii, dar a refuzat tratamentul.

Cuantificarea sensibilității plantare prin testarea cantitativă cu monofilamentul Semmes – Weinstein şi a percepției vibratorii cu diapazonul Riedel- Seiffer, a dus la concluzia că aceste teste pot fi folosite cu succes în evaluarea pacienților cu neuropatie diabetică pre- şi postoperator în chirurgia de decompresiune a canalului tarsian.

CONCLUZII

Cercetările au identificat că 40% dintre subiecții lotului de studiu au avut în antecedente amputații la diferite niveluri ale piciorului, toți prezentând leziuni trofice (ulcere plantare) la nivelul piciorului.

Dintre semnele clinice, cel mai frecvent s-a remarcat hipoestezia plantară (50%) iar la percuția în spațiul retromaleolar intern semnul TINEL a fost pozitiv la tot lotul de studiu.

Viteza de conducere nervoasă evidențiază cel mai frecvent pierderea axonală severă la nivelul nervului tibial posterior la nivelul gleznei (retromaleolar intern) (70%).

Sensibilitatea plantară s-a îmbunătățit clinic, cu dispariția semnului TINEL la 9 pacienți.

După 6 luni, leziunile trofice plantare s-au vindecat complet fără alte complicații sau alte intervenții chirurgicale.

Scorul postoperator la testul cu diapazonul Riedel-Seiffer a revenit în limite normale în proporție de 100%: scor 7 – 58,3% şi scor 8 – 41,7% din numărul total picioare testate.

Testarea preoperatorie cu monofilamentul Semmes-Weinstein 10 g a fost pozitivă la 83,3% dintre picioare preoperator şi numai 25% postoperator, distribuție semnificativă din punct de vedere statistic (χ^2=6,04; GL=1; p=0,014).

Decompresiunea canalului tarsian la pacienții diabetici cu polineuropatie periferică a determinat redobândirea sensibilității plantare, vindecarea leziunilor ulcerative plantare, prevenția altor ulcerații plantare și îmbunătățirea calității vieții și ar trebui luată în studiu la pacienții cu neuropatie periferică diabetică la care tratamentul conservator și/sau medical nu a dat rezultate satisfăcătoare.

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SINUS TARSI APPROACH FOR TREATMENT OF DISPLACED INTRA-ARTICULAR CALCANEAL FRACTURES

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SINUS TARSI APPROACH FOR TREATMENT OF DISPLACED INTRA-ARTICULAR CALCANEAL FRACTURES (Abstract): The optimal treatment for displaced calcaneal fractures involving the posterior facet is surgical. Several minimally invasive techniques have been developed in recent years. **Aim**: We evaluated the results of calcaneal posterior facet reduction by a limited sinus tarsi approach combined with percutaneous reduction and screw fixation. **Material and methods**: Twenty-three consecutive patients (mean age 41 years) with 27 intra-articular calcaneal fractures were treated. After reduction and fixation of the posterior calcaneal facet, the final fixation of the calcaneal fractures was performed percutaneously with two or three 6.5 mm, fully threaded cancellous screws. Patient follow-up was on average 1.4 years. **Results**: Superficial wound edge necrosis was observed in one case (0.27%) which has healed within one week with local wound care. The Bohler angle has improved from an average of -0.89^{o} preoperatively to 26.41^{o} postoperatively. Median AOFAS score was 89 points and median MES score was 87 points at the final follow-up. **Conclusions**: Minimally invasive open reduction and percutaneous screw fixation of selected displaced intra-articular calcaneal fractures can achieve good to excellent functional and radiological outcomes. Sinus tarsi approach appears to be an effective and reliable method for the treatment of these fractures being associated with fewer wound complications and a shorter waiting time before surgery. **Keywords**: DISPLACED INTRA-ARTICULAR CALCANEAL FRACTURES, SINUS TARSI APPROACH, CALCANEAL POSTERIOR FACET, MINIMALLY INVASIVE.

Calcaneal fractures account for 1-2% of all fractures and are the most common fractures of the tarsal bones, representing 65% of all tarsal fractures (1). Seventy percent of these fractures are intra-articular (2).

Nonsurgical treatment of displaced intra-articular calcaneal fractures is associated in general with poor results due to secondary subtalar arthrosis and modification of hindfoot morphology. For these reasons more and more surgeons advocate in favor of surgical over conservative treatment of displaced intra-articular calcaneal fractures (3-5). The optimal treatment for displaced calcaneus fractures involving the posterior facet is surgical and, for an extended period, the gold standard in the treatment of these fractures was represented by open reduction and internal fixation (ORIF) via an extended L-shaped lateral approach. However, this approach was frequently associated with major wound healing com-

plications, from 5.8% to 43%, such as skin edge necrosis, hematoma, and deep wound infection (6).

To minimize these risks, several minimally invasive techniques have been developed in recent years: closed reduction and percutaneous screw fixation (7, 8), open reduction of the posterior facet via sinus tarsi lateral approach (9, 10), limited posterior approach and arthroscopic-assisted fixation (11, 12).

In this prospective study we evaluated the results of calcaneal posterior facet reduction by a limited sinus tarsi lateral approach combined with percutaneous reduction and screw fixation of displaced intra-articular calcaneal fractures.

MATERIAL AND METHODS

Study design. Twenty-three consecutive patients, 18 (78.3%) males and 5 (21.7%) females, with 27 intra-articular calcaneal fractures were treated in the interval 2015-2016. The age of patients ranged from 24 to 78 years (mean age 41 years). The mechanism of injury was fall from height in most cases - 17 (73.9%) fractures, car accidents in 5 (21.7%) patients and direct trauma (a weight falling directly on the hindfoot) in one (4.4%) patient. Right foot was affected in 14 (60.8%) patients, left foot in 5 (21.7%) patients and four (17.4%) patients had bilateral calcaneal fractures. Two of the fractures were open: one type I and the other type IIIA according to Gustilo-Anderson classification.

Plain X-ray films were obtained for all the patients including lateral view, axial heel view (Harris view) and Broden's oblique views. Bohler's angle was measured in all patients pre- and postoperatively.

Computer Tomography (CT) scan was performed in all patients to assed the fracture patterns and for classification. Fractures were classified according to Sanders classification: 19 (70.3%) fractures Sanders type II and 8 (29.7%) fractures Sanders type III.

After the reduction and fixation of the posterior calcaneal facet, the final fixation of the calcaneal fractures was performed percutaneously with two or three 6.5 mm, fully threaded cancellous screws. The average time till surgery was 4 days (range 1-8 days) depending in general on the availability of surgical theater, and in older, active patients on comorbidity assessment (the oldest patient was 78 years old).

Seven (30.4%) patients were chronic smokers (on average more than 1.5 packs per day), 3 (13%) patients had diabetes and 1 (4.3%) patient had associated vascular disease.

Postoperative CT scan was performed in 17 (73.9%) patients to evaluate the reduction of calcaneal posterior facet.

All patients were assessed clinically and radiologically. Patients follow-up was on average 1.4 years (9 months to 1.8 years), two (8.7%) patients were excluded because were lost to after one-year follow-up. For clinical assessment we used The American Orthopedic Foot and Ankle Score (AOFAS) and Maryland Foot Score (MES).

Surgical technique. For exposure the patient is positioned in full lateral decubitus on the radiolucent operating table (fig. 1A). High tourniquet was used in all patients. The involved extremity is positioned on a cylindrical pillow and the other extremity is bent from the knee not to interfere during fluoroscopy.

Sinus tarsi approach consists in a small incision of about 3-4 cm under the tip of the lateral malleolus in the direction of the base of the fifth metatarsal bone (fig. 1B). This small incision is situated in the plane be-

tween the superficial peroneal nerve and the sural nerve and protects the lateral calcaneal artery which is responsible for most of the blood supply to the lateral calcaneal area. By sharp dissection care must be taken not to damage the peroneal tendons which are retracted laterally (fig. 1C). The incision is deepened by mobilizing the sinus tarsi fat pad dorsally. Sometimes the intermediate root of the inferior extensor retinaculum can be released to have better access to the fracture line. By now excellent visualization of the posterior facet is obtained (fig. 1D). In some cases, for better visualization of the medial articular fragment the interosseous talocalcaneal ligament could be transected, but this is not recommended because it creates subtalar instability.

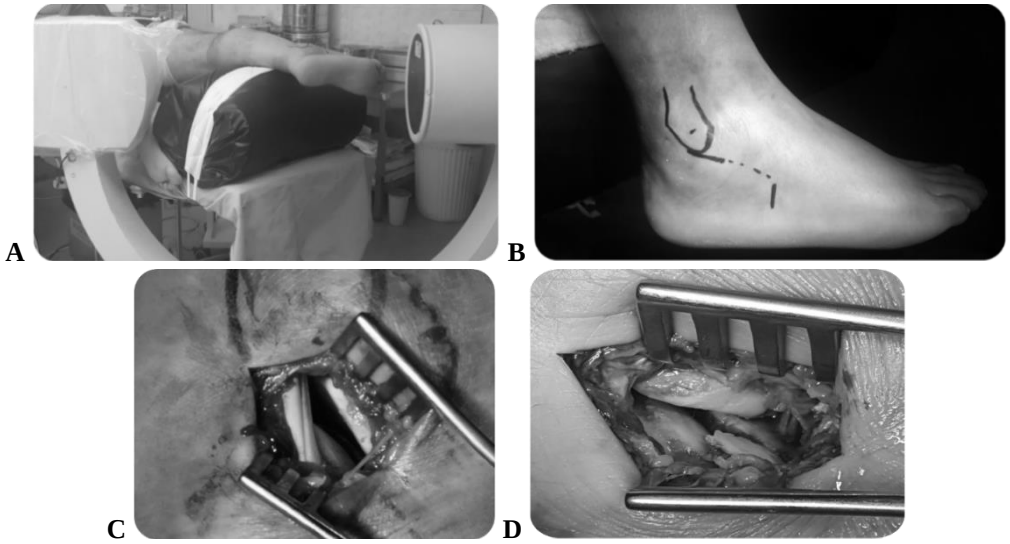

Fig. 1. A – position of the patient on the table, B – sinus tarsi approach, C – position of the peroneal tendons in the surgical field, D – visualization of the fractured calcaneal posterior facet.

A Shantz screw or a Steinman pin is used for traction and reduction of the tuberosity. After the fracture is mobilized and the interposed soft tissue is debrided, the posterior facet is reduced and fixed temporally with two, Ø 2mm, Kirschner (K) wires. The fractured fragments are reduced to the constant sustentacular fragment. Reduction of posterior facet fragments can be performed in an outside-in or inside-out-inside manner in case of a dislocated intermediate fragment.

Accurate articular surface reduction is assisted either by direct visualization or dry arthroscopy and fluoroscopically with lateral, axial heel and Broden's views. After confirming the anatomic reduction of the posterior facet, the two K-wires are replaced successively with two Ø 3.5 mm cancellous screws. The last step is the fixation of the calcaneal fracture.

Once the fixation is complete and final fluoroscopic or X-ray images are obtained, the wound is thoroughly irrigated, and the soft tissues are sutured in layers. A small closed suction drain is placed into the wound which is removed after 24-48 hours postoperatively.

All patients begun active and passive

range of motion exercises the second day after surgery.

RESULTS

No intraoperative complications occurred. The patient with open type IIIA calcaneal fracture needed plastic surgery reconstruction for skin closure. All the fractures had healed radiologically within three months (fig. 2).

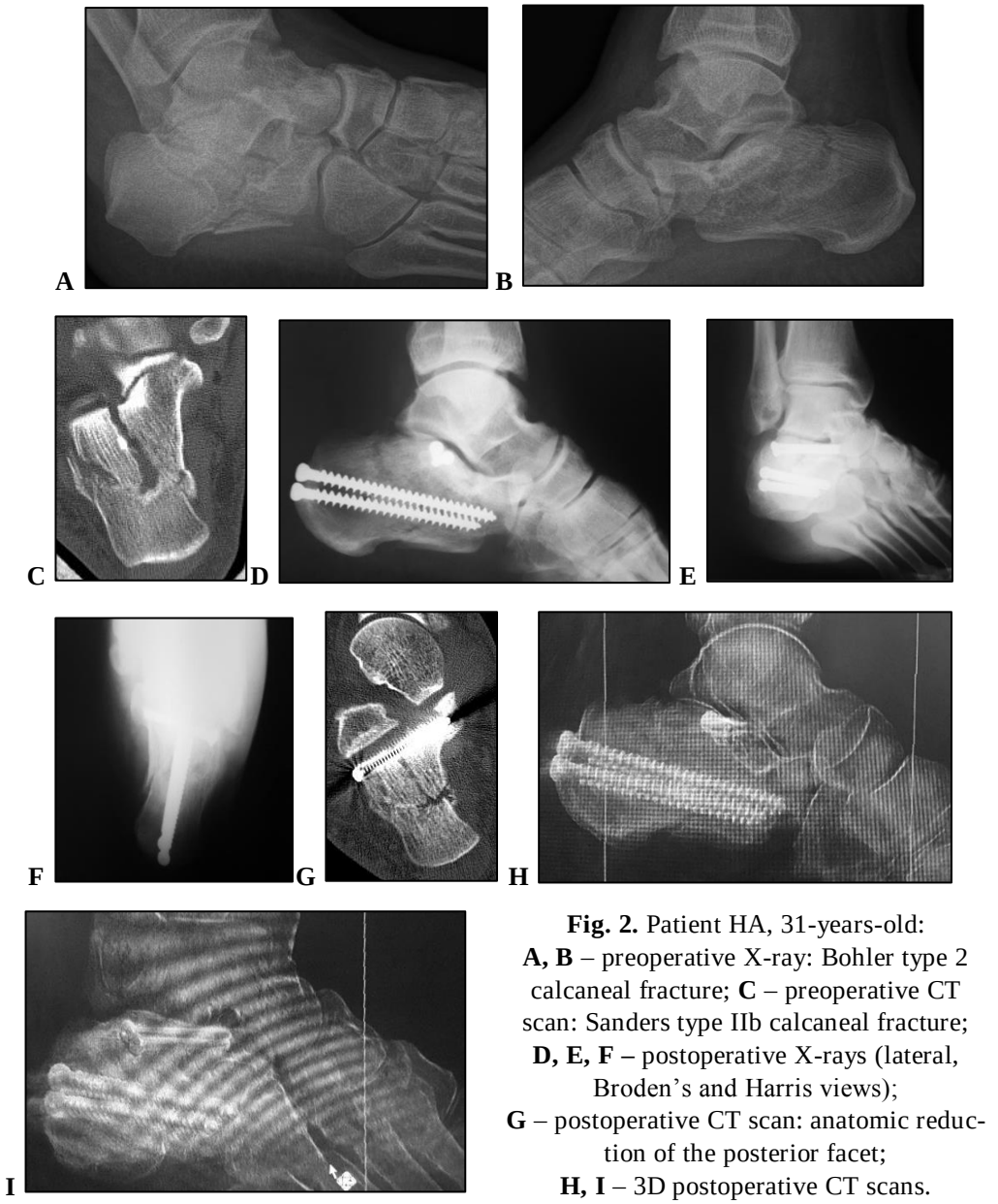

Fig. 2. Patient HA, 31-years-old:
A, B – preoperative X-ray: Bohler type 2 calcaneal fracture; **C** – preoperative CT scan: Sanders type IIb calcaneal fracture; **D, E, F –** postoperative X-rays (lateral, Broden's and Harris views); **G** – postoperative CT scan: anatomic reduction of the posterior facet; **H, I** – 3D postoperative CT scans.

Active and passive range of motions exercises were initiated from postoperative day 2. Non-weight bearing for thirty days was recommended followed by partial weight bearing (15-20 kg) for two weeks.

Superficial wound edge necrosis was observed in two cases (0.54%) which had healed within one week with local wound care. No superficial or deep infections developed in our series. In one patient we noticed, at three months follow-up, a lost in calcaneal varus reduction, without major clinical implication, probably due to intense anterior process comminution.

The Bohler angle has improved from an average of - 0.89^o preoperatively (range from -39.1^o to 11.39^o) to 26.41^o postoperatively (range from 15.23^o to 38.11^o). Postoperative CT scan showed a posterior facet step-off less than 1 mm in 12 patients, 1-2 mm in 4 patients and more than 2 mm in 1 patient.

Median AOFAS score was 89 points (range from 74 to 96) and median MES score was 87 points (range from 76 to 94) at the final follow-up.

DISCUSSION

Closed reduction and percutaneous K-wire or screw fixation have frequently been reported and can effectively reduce the risk of surgical wound complications because of the small incision (8). Open reduction of the posterior facet using a less invasive sinus tarsi approach and percutaneous screw fixation seems to be a better option (13).

In his study, Tornetta (14) was the first to report satisfactory results of percutaneous treatment of calcaneal fractures. He found a mean Maryland Foot Score of 87 in 22 patients with Sanders type IIc fractures at a mean follow-up of 2.9 years. In 2004, Rammelt *et al*. (15) found that percutaneous fixation of displaced calcaneal fractures had good to excellent results in patients with less severe fracture patterns, with a mean AOFAS ankle-hindfoot score of 94.1. In their study, Beltran and Collinge (16) including high-grade open calcaneal fractures treated with percutaneous screw fixation found good results in 17 patients, with an average AOFAS ankle-hindfoot score of 77, 1 patient developed a deep infection (6%) and 1 patient wound dehiscence (6%).

Van Hoeve *et al.* (17), in a 2016 review of the published data regarding minimally invasive treatment of intra-articular calcaneal fractures found that open and percutaneous reduction and screw osteosynthesis techniques have been used with increased frequency as an alternative to ORIF. Also, their review indicates that techniques using percutaneous reduction and screw osteosynthesis and minimally invasive open approaches result in significantly better outcomes in terms of the AOFAS ankle-hindfoot score and increased Bohler angle after treatment compared with external fixation and other techniques such as Kirschner wire fixation. No significant difference in the incidence of infection was found between these techniques. Only a few studies reported the congruency of the posterior facet of the subtalar joint as an outcome parameter.

Comparable results were found in our study, and it seems that excellent results are in close correlation with the restoration of Bohler angle and posterior facet step-out.

Open, less invasive, sinus tarsi approach with the application of the common calcaneal plate is limited to specially designed plates, which have not undergone biomechanical testing. Another option for osteosynthesis through sinus tarsi approach

are nails (18-20) which seem to offer better stability than the plates (21).

Another crucial element is that the lateral extensile incision requires delay in surgical treatment until swelling has improved, usually an average of two weeks. The current trend and interest is in small incision approaches which allow earlier operative fixation (22). The average time till surgery was 4 days in our series and this is due to prolonged preoperative period in some older patients (two patients were older than 70 years) who needed complex health assessment due to their frailty (23). In our country, older patients are sometimes self-neglected, and these conditions require a complex preoperative management in such cases (24).

CONCLUSIONS

We believe that minimally invasive open reduction and percutaneous screw fixation of selected displaced intra-articular calcaneal fractures can achieve good to excellent functional and radiological outcomes. Sinus tarsi approach appears to be an effective and reliable method for the treatment of these fractures being associated with fewer wound complications and a shorter waiting time for surgery.

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NEWS

GHRELIN PLAYS AN IMPORTANT ROLE IN METABOLIC DISEASES

Changes in ghrelin levels can directly affect immune responses and tissue homeostasis. Leukocytes, such as adipose tissue macrophages, express GHS-R and detect changes in energy status. Thus, ghrelin actions on ATMs may play a role in the maintenance of the tissue homeostasis, suggesting a link between the immune system and systemic metabolism in response to different physiological and pathological conditions such as obesity and insulin resistance. Immunoregulatory role of acyl-ghrelin and its beneficial effects to treat chronic inflammatory syndromes, especially acyl-ghrelin immunoprotected properties during endotoxic shock has been demonstrated. Ghrelin is not only a gastric peptide with CNS actions, but it is also an important hormone/cytokine with important pleiotropic functions. The wide distribution of GHS-R1a in different cell types, including immune cells, indicates that ghrelin acts as a potent immunomodulator with powerful anti-inflammatory roles. Ghrelin mainly acts on the innate and adaptive immune systems to suppress inflammation and induce an anti-inflammatory profile. (Jéssica Aparecida da Silva Pereira, Felipe Corrêa da Silva, Pedro Manoel Mendes de Moraes-Vieira, The Impact of Ghrelin in Metabolic Diseases: An Immune Perspective, *J Diabetes Res* Volume 2017:1-15)

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UNELE OBSERVAȚII PRIVIND INFECȚIA SIFILITICĂ LA GRAVIDE

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SYPHILIS IN PREGNANCY (Abstract): Inadequately treated, maternal syphilis often results in serious sequels for the fetus/newborn. Poor prenatal care is a major risk factor for the development of congenital syphilis. **Aim:** To determine the results of syphilis screening and follow-up programs on the incidence of syphilis in pregnant women and their offspring. **Material and methods:** Data on the incidence of syphilis, new syphilis cases, pregnant women diagnosed with syphilis and newborns confirmed with congenital syphilis in the Iasi district in the interval 2000-2009 have been collected. **Results:** In the interval 2000-2010 the incidence of syphilis in the Iasi district has decreased from 47.5/100,000 inhabitant in 2001 to 10.5% in 2009, and the new syphilis cases from 627 in 2001 to 87 in 2009. The highest number of pregnant women infected with syphilis was recorded in 2006 (74), and the lowest in 2010 (26). Most newborns confirmed with congenital syphilis were also recorded in 2006 (6), in the interval 2008-2010 no such case being recorded. **Conclusions:** All pregnant women have to be subjected to a serological screening test for syphilis at least twice during pregnancy: early during the first trimester and after week 28. Moreover, the women at risk for syphilis have to be examined serologically at delivery. All women who miscarry after 20 weeks of gestation have to be tested for syphilis. All newborns whose mothers' serological status for syphilis was not determined during pregnancy have to be tested before discharge. **Key words**: PREGNANCY, PRENATAL DIAGNOSIS, CONGENITAL SYPHILIS, NEWBORNS SEROLOGICAL STATUS

Sifilisul este o infecție cronică sistemică determinată de *Treponema pallidum*, transmisă de obicei sexual şi caracterizată prin episoade de boală activă întrerupte de perioade de latentă (1). După o incubație de aproximativ 3 săptămani, apare la poarta de intrare a treponemei o leziune primară, care este adesea asociată cu limfadenopatie regională; stadiul secundar este asociat cu leziuni mucocutanate generalizate, urmat de o perioadă latentă de infecție subclinică, cu o durată de mai mulți ani. În aproape o treime din cazurile netratate, boala evoluează spre stadiul terțiar caracterizat prin leziuni mucocutanate distructive, progresive, musculoscheletice sau parenchimatoase, aortită sau boală simptomatică a sistemului nervos central (2, 3, 4).

Agentul cauzal, un microorganism spiralat, subțire, mobil, de 5-15µm lungime şi 0,1-0,2µm grosime, cu 6-14 spire, poate fi pus în evidență prin microscopie în camp întunecat în stare vie în serozitatea din leziuni, prin metode de colorare şi impregnare, metode de imunofluorescență, microscopie în contrast de fază, microscopie electronică (5, 6). Aceasta din urmă relevă că microorganismul este constituit dintr-un

cilindru protoplasmic, un nucleu, un periplast, filamente axiale şi o membrană externă. Se reproduce prin diviziune transversală la 30 de ore.

Treponema pallidum este fragilă, supravieţuirea în afara organismului fiind redusă. Este distrusă prin căldură, uscare, spălare cu apă şi săpun, antiseptice uzuale. Congelată în ţesuturi la -78ºC, îşi păstrează viabilitatea şi virulenţa până la 9 ani. Nu poate fi cultivată pe medii artificiale, dar poate fi menţinută pe culturi de ţesuturi şi poate fi inoculată la animale (maimuţă, iepure (1, 2, 7, 8, 9).

Interesul pentru sifilis se menţine din următoarele motive:

- capacitatea acestei boli de a produce rapid epidemii, prin contagiozitate crescută, chiar şi în condiţiile unei morbidităţi scăzute;

- imposibilitatea eradicării ei şi menţinerea unei morbidităţi staţionare, dar cu posibilitatea apariţiei de noi epidemii la cea mai mică scădere a vigilenţei;

- repercursiunile infecţiei luetice asupra individului, la care produce, după mai mulţi ani de evoluţie, leziuni ireversibile ale sistemului nervos (paralizie generală progresivă) şi ale aparatului cardiovascular;

- posibilitatea transmiterii infecţiei şi pe cale nevenerianã (5), în special la descendenţi, cu consecinţe catastrofale asupra produsului de concepţie;

- uşurinţei cu care boala poate fi transmisă dintr-o ţară în alta, în epoca modernă (4).

MATERIAL ŞI METODE

Studiul a fost efectuat pe un lot de 426 gravide diagnosticate cu sifilis şi un lot de 31 nou-născuţi, confirmaţi cu sifilis congenital, din judeţul Iaşi, în perioada 2002-2010.

Culegerea datelor a fost realizată retroprospectiv, utilizându-se registrele de statistică ale Direcţiei de Sănătate Publică Iaşi.

Prelucrarea datelor s-a efectuat prin metode epidemiologice clasice şi computerizate, cu ajutorul programului MS EXCEL 2007.

REZULTATE

Distribuţia cazurilor pe ani calendaristici a arătat o incidenţă maximă în 2006 (74 cazuri – 17,4% din totalul lotului studiat, de 426) şi un minim în anul 2010 (26 cazuri – 6,1%). Se observă o tendinţă descrescătoare a incidenţei.

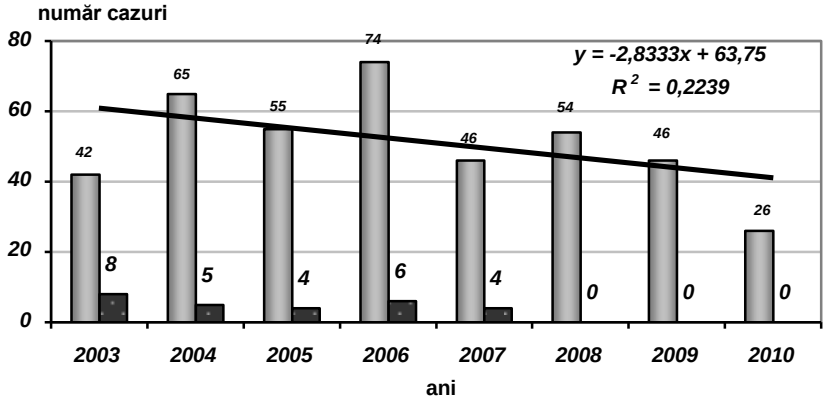

Fig. 1. Distribuţia anuală a cazurilor de sifilis la gravide şi şi nou-născuţi, în judeţul Iaşi, în perioada 2003-2010

Referitor la cazurile de nou-născuţi confirmate cu sifilis congenital, în perioada 2002-2010, acestea au prezentat o incidenţă maximă în anul 2003 (8 cazuri – 25,8% din totalul lotului de 31) şi zero cazuri raportate în anii 2008, 2009 şi în 2010 (tab. I, fig. 1).

Repartiţia pe medii de origine a femeilor luate în studiu a relevat o predominanţă a cazurilor din rural, cu valori de 19 (61,3%), comparativ cu cele provenite din urban – 12 femei (38,7%) (tab. I, fig. 2).

În perioada sarcinii, au fost luate în evidenţă au fost în număr de 12 gravide şi 19 nu au accesat niciodată un cabinet medical.

TABELUL I

Sistemul de supraveghere sifilis congenital la nou-născutul viu în Iaşi (10)

Anul	Mame infectate cu sifilis	Cazuri de nou născuţi confirmaţi cu sifilis congenital	Mediul de origine		Gravide luate în evidenţă		Test sifilis în sarcină	
			urban	*rural*	*da*	*nu*	*da*	*nu*
Sem.II 2002	18	4	2	2	0	4	2	2
2003	42	8	3	5	5	3	3	5
2004	65	5	4	1	1	4	0	5
2005	55	4	1	3	1	3	1	3
2006	74	6	1	5	2	4	3	3
2007	46	4	1	3	3	1	1	3
2008	54	0	0	0	0	0	0	0
2009	46	0	0	0	0	0	0	0
2010	26	0	0	0	0	0	0	0
Total	***426***	***31***	***12***	***19***	***12***	***19***	***10***	***21***

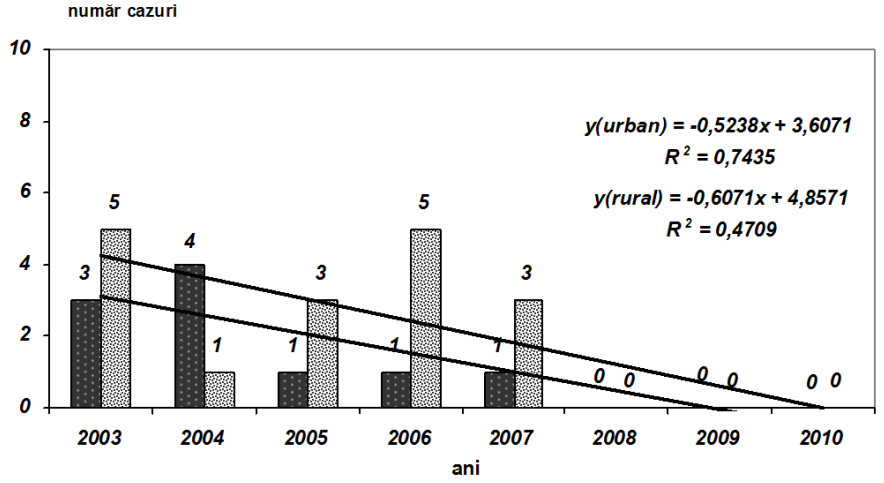

Fig. 2. Repartiţia anuală şi pe medii a cazurilor de sifilis la mamele care au născut copii cu sifilis congenital, în judeţul Iaşi, în perioada 2003-2010

Testarea pentru sifilis în timpul sarcinii a fost efectuată doar de 10 (32,3%) gravide din cele 31 care au născut copii cu sifilis congenital, iar restul de 21 (67,7%) nu s-au prezentat la aceasta, deşi testul este gratuit.

Rezultatele cercetărilor noastre sunt în acord cu cele ale altor studii publicate în ţară şi străinătate (4, 5, 7).

DISCUŢII

Intensitatea infecţiei luetice materne determină şi gravitatea manifestărilor fetale. Astfel, o septicemie treponemică a mamei are drept consecinţă o masivă infecţie spirochetică a fătului, conducând la avorturi spontane şi naşteri premature, moartea intrauterină a fătului, naşteri de feţi care prezintă imediat sau în cursul primilor 2 ani manifestări de sifilic congenital precoce.

Sifilisul congenital (prenatal) este împărţit în sifilis congenital precoce şi tardiv. Sifilisul congenital precoce reprezintă un coeficient de 1,5% din totalul cazurilor de sifilis congenital, sifilisul congenital tardiv cu manifestări clinice 72% din cazuri, iar sifilisul congenital pur seriologic 26,5% din cazuri.

Se consideră că agentul etiologic se transmite la făt transplacentar după 16-18 săptămâni de sarcină, datorita aparitiei discontinuităţii stratului celulelor Langhans (7). Riscul transmiterii la făt este direct proporţional cu durata infecţiei materne netratate.

Treponema pallidum nu traversează placenta în cursul primelor luni datorită grosimii trofoplastului vilozitar (8). Cu cât contaminarea maternă survine mai târziu în evoluţia sarcinii, cu atât este mai mare riscul ca nou-născutul să fie infectat. Studii recente au identificat *Treponema pallidum* în avorturile din primul trimestru la femeile cu infecţii sifilitice recente.

Evoluţia sifilisului latent nu este influenţată de prezenţa sarcinii. Dacă afecţiunea este dobândită în timpul sarcinii, perioada de incubaţie este mai scurtă (10-12 zile), şancrul sifilitic este mai mare, însoţit de edem, datorită vascularizaţiei mai abundente a organelor genitale. În absenţa tratamentului, evoluţia leziunilor se poate prelungi până la termen, împiedicând dilataţia colului atunci când sunt localizate la acest nivel (goma sifitică localizată pe colul uterin poate împiedica dilataţia şi naşterea pe cale naturală). În perioada terţiară, diagnosticul este serologic. Leziunile din perioada secundară sunt mai întinse şi uneori se complică cu ulceraţii vulvare.

Sifilisul florid al gravidelor este motiv legal de întrerupere a sarcinii în ţările în care avortul nu este legalizat.

Astăzi este admis că o mamă cu lues recent, netratat, transmite fătului boala în 70-100% din cazuri. Femeile cu sifilis latent tardiv pot naşte copii sănătoşi (dar care fac un sifilis congenital tardiv) şi rareori copii cu sifilis florid. În ultimile decenii, cazurile de sifilis congenital sunt rare, fiind totuşi prezente în majoritatea ţărilor. Încă nu se stie precis ce influenţă are boala mamei în primele 3 luni de dezvoltare a fătului. Specialistii sunt de acord că un copil născut dintr-o mamă sifilitică, care a fost corect tratată, nu poate fi prezenta sifilis congenital.

Potrivit unui studiu realizat de Institutul Naţional de Cercetare şi Dezvoltare în Sănătate (INCDS), începând cu anul 1989, incidenţa prin sifilis a început să crească de la an la an, ajungând în 2002 la o valoare de 58,3‰, faţă de 23,3 în 1990, iar în anul 2009 s-a inregistrat o incidenţă de 15,1‰. În România, incidenţa sifilisului ramane pe primul loc în ţările din UE (10).

CONCLUZII

Sifilisul rămâne o problemă de sănătate importantă pentru femei. Tratată incorect, boala maternală lasă deseori sechele serioa-

se nou-născutului. Îngrijirea prenatală precară reprezintă un factor de risc major în dezvoltarea sifilisului congenital. În mod evident, accesul mai uşor la îngrijirea prenatală este o componentă importantă a strategiei de prevenire a bolii.

Toate femeile gravide trebuie să efectueze un test de *screening* serologic pentru sifilis de cel puțin două ori pe parcursul sarcinii: în primul trimestru, cât mai precoce şi după a 28-a săptămână de sarcină. În plus, grupurile la risc pentru sifilis vor fi examinate serologic şi cu ocazia naşterii. Toate femeile care au pierdut o sarcină după a 20-a săptămână de gestație vor fi testate pentru sifilis.

Toți nou-născuții din mame al căror status serologic pentru sifilis nu a fost determinat pe parcursul sarcinii vor fi obligatoriu testați înainte de externarea din spital.

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OPTIMIZAREA STRATEGIEI DE DIAGNOSTIC GENETIC ÎN SINDROMUL VELO-CARDIO-FACIAL

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IMPROVEMENT OF GENETIC DIAGNOSTIC STRATEGY IN VELO-CARDIO-FACIAL SYNDROME (Abstract): Velo-Cardio-Facial Syndrome (VCFS) is characterized by congenital heart defects (CHD), palatal abnormalities, facial dysmorphism, neonatal hypocalcemia, immune deficit, speech and learning disabilities. SVCF is caused by microdeletion 22q11.2. Microdeletion is detected by fluorescence in situ hybridization (FISH). The highly variable phenotype makes diagnosis and selection for FISH more difficult. **Aim:** To retrospectively analyze and compare the phenotype of children with a clinical diagnosis of VCFS with/without 22q11 deletion; to verify the validity of literature guidelines and to describe combinations of clinical features that should lead to molecular analysis. **Material and methods:** The present study was performed in 21 patients with a clinical diagnosis of VCFS. Methaphase chromosome spreads were prepared from phytohaemagglutinin stimulated lymphocyte culture by standard methods before FISH. The patients were divided into two groups according to FISH test: positive and negative. **Results:** The features commonly noticed in FISH positive patients were: palatal abnormalities/ hypernasal speech, learning disabilities, facial dysmorphism, tapered fingers (6/6), CHD (5/6) and recurrent infections (2/6). In FISH negative patients the following were found: learning disabilities, CHD (12/15), facial dysmorphism (10/15), family history of CHD (7/15), short stature (6/15), hypocalcemia, tapered fingers (5/15), recurrent infections (3/15) and palatal cleft (2/15). In both groups, Tobias and McDonald-McGinn guidelines were positive. **Conclusions:** VCFS has a highly variable phenotype. Our study suggests that 22q11.2 deletion analysis by FISH should be performed in patients who have at least 2 (newborn)/ 3 (child, adult) specific criteria: CHD, hypocalcemia, palatal abnormalities, facial dysmorphism, learning disabilities, digital anomalies, and immune deficit. **Key words**: VELO-CARDIO-FACIAL SYNDROME, GENETIC DIAGNOSTIC.

Sindromul velo-cardio-facial (SVCF) reprezintă un spectru fenotipic larg şi variabil care cuprinde anomalii velo-palatine (cu diferite grade de severitate, de la despicături până la vorbire nazală), malformaţii cardiace (MCC) (în special conotruncale), dismorfie facială sugestivă (faţa lungă, posibil asimetrică în timpul plânsului, hipoplazie malară, nas bulbos/ tubular/ proeminent, cu hipoplazia aripilor nazale, urechi jos inserate/ protuberante cu anomalii ale helixului, filtru scurt şi microstomie), hipoplazia timusului

(deficite imune) şi a glandelor paratiroide (hipocalcemie), dificultăţi de învăţare şi tulburări psihice, la care se adaugă o multitudine de semne clinice. SVCF este produs prin microdeleţia 22q11.2 (1).

Numărul semnelor clinice identificate în SVCF depăşeşte 180 (2), fiind posibilă afectarea oricărui organ şi sistem, iar gravitatea spectrului variază extrem, de la forme severe cu potenţial letal în perioada neonatală (sindromul DiGeorge) până la forme uşoare cu dismorfie facială şi vorbire nazală sau hipoparatiroidism tranzitoriu. Variabilitatea clinică, raportată inclusiv intrafamilial, nu depinde de mărimea microdeleţiei (care poate varia de la 1,5 Mb la 3 Mb) (3).

Sindromul velo-cardio-facial este una dintre cele mai frecvente boli cromozomiale, prevalenţa sa variind între 1: 2.000 şi 1: 4.000 persoane (2, 4). Frecvent microdeleţia 22q11.2. apare *de novo*, dar poate fi moştenită în 6 - 28% din cazuri (5).

La nivelul regiunii implicate în deleţie au fost identificate mai mult de 50 de gene. Dintre acestea un rol major îl are gena *TBX1,* care codifică un factor de transcripţie implicat în reglarea proceselor de dezvoltare embrionară a arcurilor branhiale şi a câmpului de dezvoltare cardiac. Haploinsuficienţa acestei gene explică o mare parte din semnele ce alcătuiesc spectrul clinic al microdeleţiei 22q11.2: anomaliile de dezvoltare cardiace, timice, otice. Printre genele candidat pentru retardul mental, dificultăţile de învăţare şi susceptibilitatea la dezvoltarea de tulburări psihiatrice pot fi menţionate *PRODH, COMT* şi *RTN4R* (5, 6).

Diagnosticul de certitudine al sindromului velo-cardio-facial este pus prin tehnici de citogenetică moleculară: hibridizarea fluorescentă in situ (FISH), amplificarea multiplex a probei prin legare (MLPA) sau hibridizarea genomică comparată (CGH). Testele moleculare fiind costisitoare, este necesară o selecţie fenotipică a cazurilor folosind un algoritm bazat pe frecvenţa şi importanţa semnelor clinice. Diagnosticul precoce este util în stabilirea unui management adecvat şi acordarea unui sfat genetic corect (7-9).

MATERIAL ŞI METODĂ

Pacienţii

Studiul a fost realizat retrospectiv pe 21 de pacienţi, evaluaţi în Centrul de Genetică Medicală Iaşi, între anii 2003-2010, suspicionaţi clinic de SVCF, la care s-a efectuat testul FISH pentru confirmarea diagnosticului clinic.

Metode

Preparatele cromozomiale au fost obţinute prin metode standard, folosind culturi de limfocite stimulate cu fitohemaglutinină. Pentru tehnica FISH au fost utilizate sonda LSI TUPLE1 (HIRA) (Abbott Molecular, Vysis®), localizată în regiunea frecvent afectată de microdeleţie şi marcată cu fluorocromul Spectrum Orange şi sonda control LSI ARSA (Abbott Molecular, Vysis®), localizată în regiunea 22q13 şi marcată cu fluorocromul Spectrum Green. În funcţie de rezultatul acestei analize pacienţii au fost repartizaţi în 2 loturi: lotul A, FISH pozitiv - pacienţi la care diagnosticul de sindrom velo-cardio-facial a fost confirmat şi lotul B, cazuri infirmate, cu FISH negativ. Lotul A cuprinde 6 pacienţi, 2 de sex feminin şi 4 de sex masculin, cu vârste cuprinse între 1 lună şi 33 de ani. Lotul B cuprinde 15 pacienţi, 5 de sex feminin şi 10 de sex masculin, cu vârste cuprinse între 1 săptămână şi 16 ani. Semnele clinice prezente la pacienţii celor două loturi au fost comparate cu semnele clinice corespunzătoare algoritmului propus de Tobias *et al*. în 1999 şi scorului McDonald-McGinn *et*

al. din 2001 (3, 7).

REZULTATE

Analiza citogenetică a 21 de pacienţi cu diagnosticul clinic de SVCF a confirmat microdeleţia 22q11.2 în 6 cazuri (pacienţi ce formează lotul A) şi a infirmat-o în 15 cazuri (lot B).

În lotul A, cu diagnostic cert, patru semne clinice au fost prezente la toate cazurile: dismorfia facială, insuficienţa palatină/vocea nazală, dificultăţile de învăţare, şi prezenţa de degete lungi, conice. Alte două elemente de diagnostic au fost întâlnite: MCC (non-conotruncale) în 83% din cazuri, iar infecţiile repetate au fost prezente în 33% din cazuri. În lotul B (infirmat prin FISH), semnele clinice cele mai frecvente au fost MCC şi dificultăţile de învăţare, la 80% din cazuri, dismorfia facială în 66%, hipocalcemia în 33%, infecţiile repetate în 20%, iar anomaliile palatine în 13% din cazuri.

Scorul clinic al pacienţilor din lotul A a variat, pentru algoritmul Tobias, între următoarele asocieri de semne: (1 criteriu A + 4 criterii B + 1 criteriu C) şi (2 criterii B + 1 criteriu C) şi, pentru scorul Mc Donald-McGinn, între 4 - 5,5 puncte (tabelul I). În lotul B scorurile clinice au variat între (1A+1B+1C) şi (1B+2C) şi, respectiv, între 3 – 4 puncte (tab. I).

TABELUL I

Scoruri de diagnostic în cele 2 loturi

Semn clinic	Algoritm Tobias	Scor Mc Donald-McGinn	Lot A	Lot B
MCC conotruncale	Clasa A	1 punct	0/6	3/15
Un părinte cu SVCF	Clasa A	-	1/6	0/15
Dismorfie facială	Clasa B	¼ punct	6/6	10/15
MCC nonconotruncale	Clasa B	1p	5/6	9/15
Dificultăţi de învăţare/ retard psihomotor	Clasa B	1/2 punct / RM moderat	6/6	12/15
Insuficienţă velopalatină	Clasa B	1 punct	5/6	2/15
Hipocalcemie	Clasa B	1 punct	0/6	5/15
Imunodeficienţă	Clasa B	1 punct	2/6	3/15
Anomalii digitale	Clasa C	½ punct	6/6	5/15
Anomalii renale	Clasa C	½ punct	1/6	1/15
Hipotonie	Clasa C	-	0/6	1/15
Hipostatură	Clasa C	½ punct	0/6	6/15
Tulburări psihiatrice	Clasa C	½ punct	?	?
MCC familiale	Clasa C	-	2/6	7/15
Voce nazală	-	¼ punct	6/6	2/15
Indicaţie de FISH	1 criteriu A sau 2 criterii B sau 1B+minim 1C	Minim 3 puncte		

Pentru ambele loturi scorurile obţinute au fost superioare pragului la care era recomandată testarea pacienţilor în studiile respective.

DISCUŢII

Spectrul fenotipic al sindromului velo-cardio-facial este variabil, incluzând numeroase anomalii, în diverse combinaţii şi cu grade de severitate diferite. Această variabilitate este determinată de factori multipli: evoluţia fenotipului în funcţie de vârstă, variabilitatea genetică, prezenţa de gene modificatoare şi fenocopiile (10). Tabloul clinic clasic include anomalii palatine, MCC conotruncale şi dismorfie facială. În lotul A se remarcă asocierea între anomalii palatine (inclusiv voce nazală), dismorfie facială şi dificultăţile de învăţare. Anomaliile velopalatine, deşi cu un grad redus de severitate (insuficienţă velopalatină) sunt prezentate în lotul A la toate cazurile, spre deosebire de lotul B unde, deşi severitatea era crescută (despicături maxilopalatine), numărul de cazuri era mic (2/15). În acest context vocea nazală, care trădează deficitul funcţional al vălului palatin, a constituit un element valoros de diagnostic (6/6 în lotul A).

MCC au fost prezente în ambele loturi în proporţii similare (A - 83%, B - 80%) (corespunzând datelor de literatură - 80%) (4); prezenţa în lotul B a MCC conotruncale (3/15) sugerează că, deşi sunt tipul de MCC cel mai frecvent în SVCF (7-50%) (4, 9), este necesară asocierea acestora cu alte elemente sugestive de diagnostic pentru a necesita aplicarea tehnicii moleculare. Am observat că anamneza familială pozitivă pentru MCC a fost prezentă la un număr ridicat de cazuri în lotul B faţă de A, ceea ce pune la îndoială valoarea acestui criteriu, având în vedere frecvenţa mare în populaţia generală a cazurilor de MCC familiale.

Dismorfia facială s-a dovedit a fi un criteriu valoros, dar din păcate subiectiv în apreciere şi care devine evident doar odată cu înaintarea în vârstă. Dintre elementele dismorfiei faciale utile s-au dovedit: faţa lungă, posibil asimetrică în timpul plânsului, (5/6 faţă de 5/15), hipoplazia malară (5/6 faţă de 3/15), nasul bulbos/tubular/ proeminent (6/6 faţă de 6/15) şi hipoplazia aripilor nazale (5/6 faţă de 4/15). Alte elemente valoroase dar fără diferenţe semnificative între cele 2 loturi au fost urechi jos inserate/ protuberante (4/6 şi 13/15) şi anomaliile helixului (4/6 şi 10/15).

Dificultăţile de învăţare reprezintă un criteriu important pentru diagnosticul clinic de SVCF, dar din păcate corelat cu vârsta pacientului. Deşi scorul Mc Donald-McGinn cuprindea retardul mental (RM) moderat, mulţi dintre pacienţii noştri prezentau doar dificultăţi uşoare, iar în cazul studiului de faţă RM moderat a fost prezent doar în lotul B. Pe de altă parte, tulburările psihiatrice au debut tardiv, iar lotul nostru a cuprins preponderent pacienţi de vârstă pediatrică, astfel încât este necesară urmărirea ulterioară a cazurilor pentru a detecta astfel de modificări.

Deşi în literatura de specialitate, hipocalcemia este considerată un criteriu valoros pentru diagnosticul SVCF (simptom prezent la 17-60% din pacienţi) (8), aprecierea semnificaţiei acestei modificări biochimice la pacienţii noştri a fost dificilă deoarece ei au fost testaţi sporadic şi incomplet (doar dozarea calciului total, dozările calciului ionic şi parathormonului nefiind realizate), ceea ce nu ne permite concluzii pertinente. Aceleaşi rezerve există şi în privinţa imunodeficienţei care a fost apreciată doar declarativ (prin numărul de infecţii repetate).

Aspectul degetelor (lungi, conice) a fost un element valoros pentru diagnostic, fiind prezent în toate cazurile confirmate şi doar la 33% din pacienţii din lotul B. Anomaliile scheletice au fost întâlnite doar în câteva

cazuri: bloc vertebral (un caz lot A), *spina bifida* (un caz lot B) şi *pectus excavatum* (un caz lot B). Hipostatura nu s-a dovedit a fi un criteriu valabil de orientare, existând diferenţe semnificative între cele 2 loturi (0/6 faţă de 6/15).

Anomaliile renale, citate în literatură la 15 - 32% din cazuri (15), au fost întâlnite în 2 cazuri: agenezie renală unilaterală (un caz lot A) şi hidronefroză grad IV (un caz lot B). În 3 cazuri au fost prezente anomalii genitale: hipospadias (un caz lot A) şi criptorhidism (2 cazuri lot B).

Malformaţiile sistemului nervos central, hipotonia şi convulsiile nu au reprezentat criterii utile de orientare în acest studiu.

La pacienţii incluşi în studiu prezenţa semnelor clinice meţionate mai sus a fost punctată folosind o grilă elaborată de Centrul de Genetică Medicală Iaşi (tab. II). Astfel, efectuarea testului FISH este recomandată la nou-născut dacă scorul clinic este de minim 2 puncte, iar ulterior, la copil şi adolescent, de minimum 3 puncte.

TABELUL II

Grilă de evaluare a scorului clinic al pacienţilor cu SVCF în vederea testării FISH, elaborată în Centrul de Genetică Medicală Iaşi

Anomalii	Tipuri/observaţii	Punctaj
MCC	Conotruncale/ defecte septale	1 punct
Anomalii velopalatine	Despicături, insuficienţă velopalatină, inclusiv voce nazală	1 punct
Hipocalcemia	Ca ionic sau parathormon	1 punct
Retardul psihomotor/ dificultăţile de învăţare/ tulburări psihiatrice	Profil psihiatric caracteristic (deficit de atenţie, hiperactivitate, schizofrenie, tulburări bipolare)	1 punct
Dismorfia facială caracteristică	Faţa lungă, posibil asimetrică în timpul plânsului, hipoplazia malară, nasul bulbos/ tubular/ proeminent, hipoplazia aripilor nazale, urechi jos inserate/protuberante, anomalii ale helixului*	1 punct
Anomalii digitale	Degete conice	1 punct
Imunodeficienţă/ hipoplazie de timus	Scăderea numărului limfocitelor T, infecţii repetate, cu durata lungă (peste 14 zile)	1 punct
Anomalii renale	Hipo/aplazie renală	0,5 puncte

* necesită un evaluator experimentat

În condiţiile în care 6 - 28% din cazurile de SVCF sunt moştenite, iar variabilitatea intrafamilială poate fi importantă (adesea părinţii purtători ai microdeleţiei având un fenotip discret), screening-ul familial este un element indispensabil pentru acordarea unui sfat genetic corect.

Este evident că procesul de selecţie a pacienţilor rămâne elementul esenţial al oricărei strategii de diagnostic căci, chiar cu cel mai performant şi ieftin test de diagnostic, testarea globală a populaţiei nu

este posibilă.

CONCLUZII

Studiul retrospectiv prezentat în acest articol a permis diagnosticul a 6 pacienţi cu microdeleţie 22q11.2 într-o perioadă de 7 ani. În condiţiile în care incidenţa la naştere a SVCF este de 1/2.000 – 1/4.000 nounăscuţi, este evident că pacienţii diagnosticaţi reprezintă doar „vârful aisberg-ului".

Spectrul clinic al sindromului DiGeorge este extrem de variabil, atât intrafamilial, cât şi la nivelul populaţiei. Diagnosticul de certitudine se poate face numai prin tehnici de citogenetică moleculară, selectând cazurile cu elementele fenotipice cele mai evocatoare. Pentru a creşte eficienţa acestui proces de selecţie am elaborat un scor clinic, prezentat în tabelul II şi care considerăm că este util în practica medicală.

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ALGORITM DE DIAGNOSTIC ŞI TRATAMENT ÎN FARINGOLARINGITA DE REFLUX

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DIAGNOSTIC AND TREATMENT ALGORITHM FOR PHARINGOLARYNGEAL REFLUX (Abstract): Pharyngolaryngeal reflux (PLR) refers to backflow of stomach contents into the throat and larynx. Its diagnosis is difficult because of a variety of atypical symptoms, low sensitivity of traditional tests of gastrointestinal tract, and no consensus on the diagnostic algorithm. The aim of this study was to provide an algorithm for the diagnosis and treatment of PLR. There were no pathognomonic symptoms or findings, but the characteristic symptoms validate the Reflux Symptom Index and Reflux Findings Score as tools in the initial diagnosis. The first approach is empirical medical treatment for 3 month, based on endoscopic findings at laryngofiberscopy; then objective demonstration of reflux events using multichannel intraluminal impedance and pH monitoring. Proton pump inhibitors represent the mainstay of therapy for the patients with PLR, but they require a more aggressive and prolonged treatment than the patients with gastroesophageal symptoms. **Key words**: PHARYNGOLARYNGEAL REFLUX, PH MONITORING, PROTON PUMP INHIBITORS

Simptomatologia şi semnele clinice determinate de reflux au fost identificate la 4 până la 10% din toți pacienții consultați de otorinolaringologi (1). Datele din literatură arată că peste 50% din pacienții cu disfonie au afecțiuni induse de boala de reflux gastroesofagian (2). Simptomatologia şi semnele clinice obişnuite ale laringitei de reflux sunt răguşeala matinală, timpul de încălzire vocală mai mare de 20-30 minute, halitoza, hemaj, xerostomie, limba saburală, globus faringeus, disfagie, regurgitații ale conținutului gastric, tuse cronică, predominant nocturnă, tuse recurentă, ocazional pneumonie, laringospasm, decompensări ale astmului bronşic, infecții acute de căi respiratorii superioare la copii.

Uneori este dificil de diagnosticat faringolaringita de reflux, iar diagnosticarea incorectă conduce la costuri inutile pentru societate. Simptomatologia tipică de FLR este nespecifică şi poate fi cauzată de infecții (bacteriene sau virale), alergie, fumat, abuz de alcool (3), de aceea este important să existe un algoritm de diagnostic şi tratament pentru această afecțiune extra-

esofagiană indusă de reflux.

PATOFIZIOLOGIE

Incidența esofagitei de reflux este relativ scăzută, deoarece mucoasa esofagiană din porțiunea distală a esofagului are mecanisme proprii de protecție antiacidă. Aceste mecanisme includ peristaltismul (care, prin mișcările produse, indepărtează rapid acidul de pe suprafața mucoasei esofagiene), structura mucoasei (permite tolerarea unui contact intermitent cu acidul gastric), capacitatea salivei înghițită de a neutraliza acidul la nivel esofagian, producția de bicarbonat din esofag (4). Laringele şi faringele însă, nu au aceste mecanisme de protecție, astfel că expunerea la acid şi pepsina pot determina modificări substanțiale la acest nivel, în timp ce esofagul nu este deloc afectat. Datele preliminare raportate de Axford sugerează că mucoasa laringiană are un mecanism celular de aparare diferit de cel al mucoasei esofagiene şi că există diferențe specifice în expresia genei MUC şi a anhidrazei carbonice la nivelul laringelui şi faringelui (3,5).

Spre deosebire de BRGE, FLR nu se asociază frecvent cu pirozis şi regurgitații (doar 20% dintre pacienți, față de 83% în BRGE, prezintă această simptomatologie) (1). Nu există simptome sau semne clinice patognomonice pentru FLR, dar la nivelul mucoasei laringiene apar modificări care susțin implicarea refluxului în patogeneză: eritem, edem, ulcerații, granulații la nivelul aritenoizilor şi spațiului interaritenoidian (1,3) .

La nivelul esofagului există bariere fiziologice cu rol protector: sfincterul esofagian inferior, funcția motorie esofagiană cu clearance-ul acid, rezistența tisulară esofagiană şi sfincterul esofagian superior. Mai mult, esofagul prezintă o producție activă de bicarbonat în spațiul extracelular; la nivelul mucoasei laringiene nu sunt prezente toate aceste mecanisme de protecție. În timp ce BRGE apare ca urmare a disfuncției SEI, în FLR este implicată si disfuncția SES. Pacienții cu BRGE experimentează episoadele de reflux mai ales în decubit (noaptea), în timp ce la pacienții cu FLR pH-metria a aratat că cele mai frecvente episoade sunt ziua, în poziție verticală.

Modificările laringiene pot fi cauzate de afectarea directă a mucoasei sau prin mecanisme secundare. Astfel, afectarea directă se datorează contactului acidului şi pepsinei cu mucoasa laringiană, rezultând lezarea mucoasei. Alternativ, iritarea esofagului distal de către acidul gastric, poate cauza un reflex mediat de nervul vag, ceea ce conduce la tuse cronică şi hemaj, cu afectare secundară a mucoasei laringiene (2).

Refluxul biliar poate de asemenea cauza afectare laringiană (1, 3, 4). În plus, descoperiri recente ridică multe întrebări legate de patofiziologia laringitei de reflux. Ekley susține că scăderea factorului de creştere epidermal salivar poate fi asociat cu laringita de reflux. Altman descoperă prezența pompei de protoni la nivelul celulelor seroase laringiene şi a ductelor glandelor submucoase (4,5). Controversele sunt multiple în legatură cu patofiziologia FLR, şi s-a demonstrat că nu este doar o simplă acțiune mecanică a refluxului.

DIAGNOSTIC

Anamneza minuțioasă este importantă, pentru a putea realiza corect un diagnostic diferențial al simptomatologiei. Cel mai frecvent, laringita este o formă uşoară şi se vindecă spontan (5); dacă persistă trebuie definit factorul etiologic probabil: infecții virale sau bacteriene, alergie, abuz vocal, drenaj nazal posterior, leziuni benigne/maligne ale corzilor vocale, FLR. Faptul că

pacientul nu acuză pirozis şi regurgitaţii, nu exclude diagnosticul de FLR.

Asociatia Americana de Bronhoesofagologie (American Broncho-Esophagological Association – ABEA) a prezentat un studiu conform căruia cele mai frecvente simptome de FLR sunt hemajul (98%), tuse cronică persistentă (97%), globus faringeus (95%) şi disfonia (95%). (6)

Belafsky a dezvoltat un chestionar pentru pacienţi, indexul simptomatologiei de reflux (reflux symptom index - RSI) (7), în care pacienţii descriu gradul de severitate pentru disfonie, hemaj, dificultăţi la înghiţire, tuse, senzaţie de corp străin faringian, pirozis, regurgitaţie. Deoarece în studiul statistic efectuat pentru validarea acestui scor, limita de confidenţă la pacienţii din grupul de control, a fost de 13,6 în peste 95% din cazuri, un scor peste 13 este considerat patologic.

Laringoscopia are un rol important în evaluarea laringitei cronice. Modificările clasice pentru laringita de reflux sunt descrise în literatura ca "laringita posterioară", constând în edem şi eritem aritenoidian şi interaritenoidian. (3)

Autorul chestionarului pentru simptomatologia de reflux a dezvoltat şi un scor al modificărilor clinice induse de reflux (reflux findings score - RFS), care cuantifică semnele clinice care pot fi corelate cu prezenta FLR (7, 8). RFS depinde de obiectivarea următoarelor modificări: edem subglotic, obliterare ventriculară, edem al corzilor vocale, edem laringian difuz, hipertrofie a mucoasei laringiene din zona posterioară, granulom sau ţesut de granulaţie, mucoasă endolaringiană îngroşată. Scorul variaza între 0 şi 26, şi o valoare peste 7 are predictibilitate diagnostic de 95% pentru FLR.

Majoritatea pacienţilor (60-70%) cu simptomatologie laringiană de reflux au rezultate normale la endoscopia digestivă superioară, de aceea s-a recomandat utilizarea acestei metode de diagnostic numai la pacienţii care prezintă şi simptomatologie tipică de reflux - pirozis şi regurgitaţii, desi chiar şi la aceaştia, specificitatea metodei este de 83% (1,8).

Monitorizarea pH, deşi a fost considerată investigaţia de bază în multe studii clinice, nu este un instrument de diagnostic ideal; dacă în diagnosticul BRGE specificitatea este de peste 90%, în cazul FLR aceasta este de doar 54-67%, indiferent de localizarea cateterului-esofagian distal, proximal sau hipofaringian. (9-11). Mai mult, monitorizarea pH-ului la nivel esofagian proximal şi hipofaringian nu este o invesţigatie uzuală; în plus, nu există un consens în ceea ce priveşte cantitatea de reflux şi numărul episoadelor de reflux considerate patologice pentru mucoasa laringiană (1,7,9,11).

Impedansmetria esofagiană este superioară pHmetriei, datorită faptului că evidenţiază şi episoadele de reflux nonacid şi gazos; refluxul gazos este frecvent întalnit la nivelul laringelui datorită faptului că gazul are o difuzibilitate mai mare şi astfel poate ajunge la porţiunile superioare ale laringelui şi faringelui (11).

TRATAMENT

Modificările simple şi eficiente ale modului de viaţă sunt esenţiale în controlul simptomatologiei BRGE. Pacienţii trebuie informaţi în legatură cu necesitatea tratamentului de lungă durată, datorită recurenţei şi cronicizării BRGE şi FLR. Modificările stilului de viaţă sunt uşor de explicat şi implementat în tratament, iar costurile socioeconomice sunt scăzute.

Agenţii prokinetici sunt medicamente care cresc presiunea SEI şi accelerează clearance-ul esofagian şi golirea conţinutu-

lui gastric; îndeplinând aceste criterii, sunt teoretic medicamente ideale pentru a corecta mecanismele patogenetice ale BRGE, însă din pacate au numeroase reacţii adverse la nivelul sistemului nervos central şi al sistemului cardiovascular, chiar dacă sunt folosite pe perioade scurte de timp.

Antiacidele ameliorează doar simptomatologia BRGE, fără modificarea clearance-ului esofagian sau a activităţii sfincterelor esofagiene.

Agenţii acid-supresori sunt reprezentaţi de:

- antagoniştii de H2-receptori, care au un singur mecanism de acţiune: inhibarea secreţiei gastrice; nu au nici un efect asupra presiunii SEI sau asupra clearance-ului esofagian.

- inhibitori de pompă de protoni (IPP).

Inhibitorii de pompă de protoni (IPP) reprezintă cel mai eficient tratament nonchirurgical pentru BRGE. IPP (omeprazol, lansoprazol, rabeprazol, pantoprazol, esomeprazol) inhibă enzima H+/K+ ATP-aza care catalizează stadiul final al secreţiei acide în celulele parietale gastrice. IPP au un profil de siguranţă excelent, fără reacţii adverse (altele decât cele observate în studiile efectuate cu utilizare de placebo).

Chiar dacă IPP sunt medicamentele de elecţie în tratamentul BRGE, în FLR raspunsul la tratament este variabil, rata de răspuns variind în literatură între 60 şi 100% (12-15). Aceste studii subliniază variabilitatea răspunsului pacienţilor cu FLR la tratament, necesitatea tratamentului pe o perioadă mai lungă de timp pentru a avea rezultate (mai ales în cazul pacienţilor cu afectare severă), necesitatea unor doze mai mari de IPP, precum şi recurenţa rapidă a simptomatologiei în cazul în care tratamentul este întrerupt, sugerând că tratamentul de lungă durată este deseori necesar la pacienţii cu FLR.

Rata de eşec este mai mare la pacienţii care primesc IPP o singură dată pe zi (13). S-a demonstrat că o doză unică, dimineaţa, îşi menţine efectul antireflux pe o perioadă de doar 13,8 ore (mediana) (13,14). Problema creşterii secreţiei acide nocturne poate fi cauza limitării efectului dozei de seară a IPP (care ar trebui să acopere 10,2 ore) la 7 ½ ore (15). Unii pacienţi au prezentat un nivel normal al secreţiei acide numai după administrarea unor doze crescute de IPP. Nici unul din studiile efectuate nu a reusit să demonstreze că tratamentul cu IPP este o metodă incontestabilă de tratament eficient a FLR, astfel că specialiştii trebuie să fie conştienţi că tratamentul cu IPP nu are eficienţă în toate cazurile.

Deşi există aceste aspecte negative în utilizarea IPP, în lipsa altor metode de tratament rămâne tratamentul de bază pentru aceasta afecţiune. Mai mult, tratamentul cu IPP reprezintă o probă diagnostică în sine, comparabilă cu pH metria şi laringofibroscopia, tînând cont că în studiile clinice specificitatea probei terapeutice este 55-81% (16,17,18).

Tratamentul medicamentos este eficient în controlul refluxului acid în majoritatea cazurilor. Totusi, deşi controlează simptomatologia, tratamentul medicamentos nu corectează modificările mecanice care cauzează refluxul. Mai mult, pacienţii necesită tratament de lungă durată sau pe perioade nedefinite, iar întreruperea tratamentului duce la recurenţe. Indicaţiile tratamentului chirurgical includ simptomatologia persistentă, în pofida managementului medicamentos corect, precum şi intoleranţa pacienţilor la medicamente. Chirurgia este o alternativă şi pentru pacienţii care sunt preocupaţi de costul şi consecinţele unui tratament medicamentos de lungă durată (3, 7, 18).

Algoritmul de diagnostic şi tratament, prezentat în figura 1, reprezintă o modalitate adecvată de abordare a pacienților cu simptomatologie de FLR:

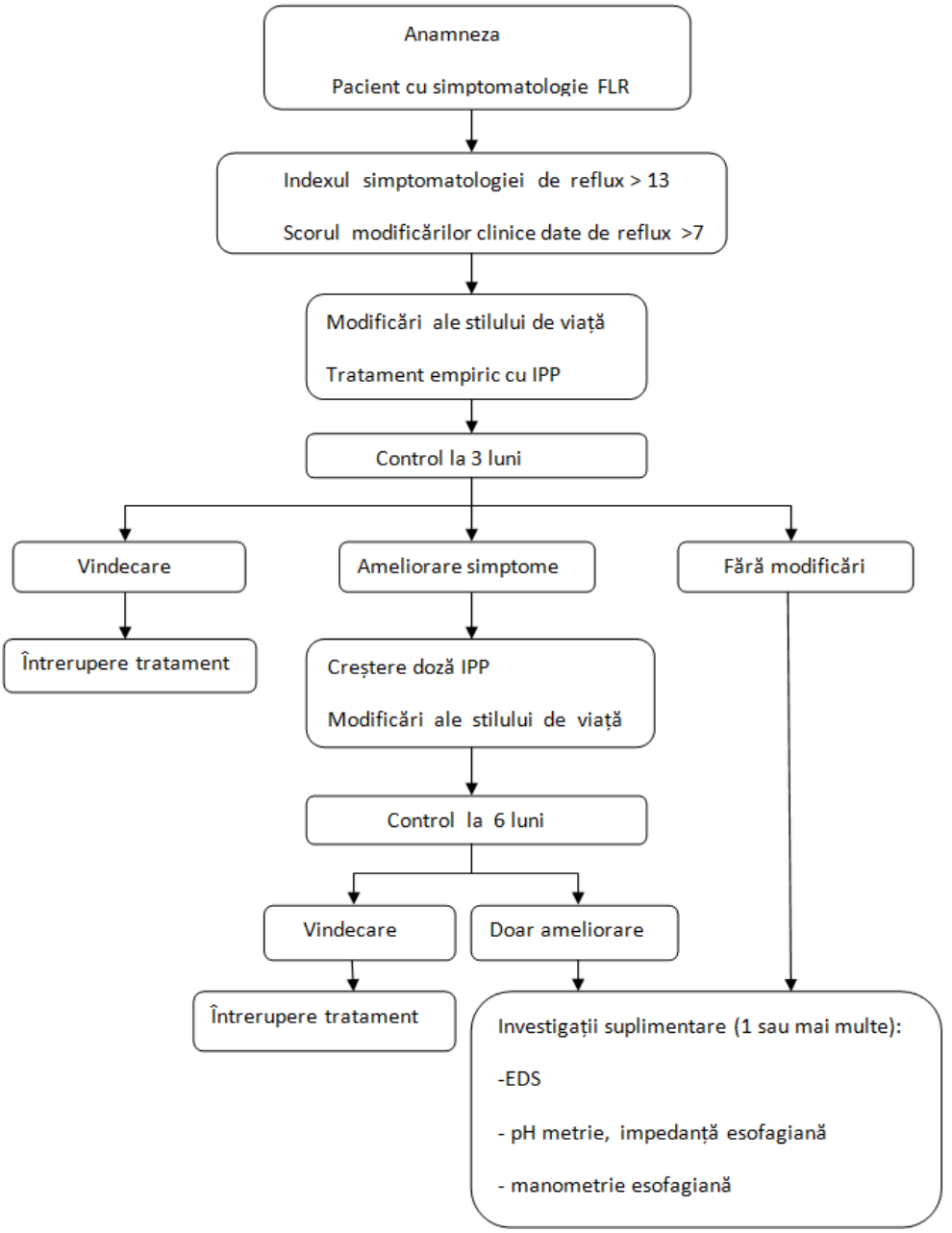

Fig. 1. Algoritm de diagnostic şi tratament pentru FLR (adaptată după Ahmed TF el al,M.Remacle et al) (19,20)

CONCLUZII

La pacienţii cu simptomatologie de FLR, este foarte importantă efectuarea atentă a anamnezei, examenului fizic general si laringoscopiei. Dacă disfagia este prezentă, se va efectua o evaluare endoscopică a deglutiţiei (functional endoscopic evaluation of swallowing – FEES).

Dilema în acest punct constă în efectuarea iniţială a testelor de diagnostic, în principal ph metria, sau în instituirea unei probe terapeutice empirice. Deşi pH metria pare ideală, există totuşi şi limitări: nu este întotdeauna disponibilă, sensibilitatea şi specificitatea nu sunt de 100%, pacienţii nu prezintă episoade de reflux cu aceeaşi frecvenţă în fiecare zi, există variaţii în expunerea la acid a esofagului proximal şi a celui distal, cu riscul de a obţine un studiu ph fals negativ.

Dacă anamneza şi investigaţiile iniţiale (laringoscopia) ridică suspiciunea de BRGE sau FLR, dacă există pirozis sau regurgitaţii (pentru BRGE) sau disfonie, hemaj (pentru FLR), atunci tratamentul antireflux este o alegere adecvată.

Dacă pacienţii nu răspund la acest tratament (IPP x2/zi, 12 săptămâni), se va efectua pH metrie şi se va continua tratamentul antireflux. Absenţa oricărui episod de expunere la acid a mucoasei esofagiene şi prezenţa unei supresii acide gastrice adecvate (pH > 4.5) sugerează că tratamentul medicamentos a fost eficient sau că trebuie cautat alt diagnnostic, dacă simptomatologia nu a disparut, deşi semnele clinice sunt absente. În cazurile în care, după tratament medicamentos, simptomatologia nu se îmbunătăţeşte, se impune efectuarea unor investigaţii suplimentare: impedansmetrie esofagiană, endoscopie digestivă superioară (pentru depistarea unor afecţiuni ca hernia hiatală, esofag Barret, leziuni ale mucoasei esofagiene), transit baritat, manometrie (pentru depistarea unui substrat de dismotilitate esofagiana).

Pacienţii la care simptomatologia de FLR nu se ameliorează semnificativ sau nu se vindecă, dupa tratament medical sau chirurgical, trebuie supravegheaţi pe perioade lungi de timp, datorită posibilităţii apariţiei afecţiunilor maligne.

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SFLT-1/PIGF NEW BIOMARKERS FOR MULTIPLE ADVERSE PREGNANCY OUTCOMES

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SFLT-1/PIGF NEW BIOMARKERS FOR MULTIPLE ADVERSE PREGNANCY OUTCOMES (Abstract): Placenta insufficiency and some related pregnancy outcomes are the result of the imbalance between angiogenic and antiangiogenic factors expressed at placenta level. From the angiogenesis factors, only a few have found practical applications, such as the serum soluble fms-like tyrosine kinase-1 (SFlt-1) / PIGF (Placenta growth factor) ratio, named also the angiogenic fraction, which is increased in pregnant women before the clinical onset of a Preeclampsia (PE), intrauterine growth restriction (IUGR) or uterine apoplexy. PROGNOSIS study demonstrated that sFlt-1 / PIGF ratio<38 predicts the absence of PE, eclampsia and HELLP (Hemolysis, Elevated Liver enzymes , Low Platelet count) syndrome within four weeks with a PPV(Positive Predictive value), (95%CI range) of 99.3% (97.9-99.9).Other approaches used different cutoffs for early and late preeclampsia (onset before or after 34 weeks), to enhance the diagnostic accuracy, or included the sFlt-1 / PIGF ratio in a statistical model, along with maternal risk factors, mean arterial pressure (MAP) measurement and uterine artery pulsatility index (UtA-PI), to improve first trimester prediction of PE. Considering the therapeutic applications of angiogenic factors, the use of statins (pravastatin) on animal models to treat PE seems to be promising, as well as the administration of (Vascular endothelial growth factor) VEGF 121 or extracorporeal removal of sVEGF-1.
Keywords: PREECLAMPSIA, ANGIOGENIC PLACENTAL FACTORS, ANTIANGIOGENIC PLACENTAL FACTORS, SFLT-1 / PIGF RATIO.

Placental insufficiency is responsive for many pregnancy pathological conditions as: preeclampsia (PE) (a pregnancy related disease associated with hypertension and proteinuria); uterine apoplexy (UA) or placental abruption (representing early separation of the placenta from the uterus before delivery). These can lead to maternal complications such as: convulsions, disseminated intravascular coagulopathy, hepatic and kidney failure and fetal reserved outcomes such as: fetal growth restriction (FGR), preterm delivery and stillbirth (1).

Placental insufficiency is due to alterations that start during placental development, when vessel formation occurs initially by vasculogenesis followed by branching and nonbranching angiogenesis. Vasculo-

genesis involves de novo formation of blood vessels from mesodermally derived precursor cells, while angiogenesis means new vessels creation from pre-existing vessels (2) and is classified in branching and nonbranching stages.

Branching angiogenesis occurs mainly in the first and early second trimesters and leads to the formation of an immature villous tree. During this phase, a dramatic decrease of the vascular resistance and increase in the blood flow through the placenta is produced by the progressive loss of the musculo-elastic media in the walls of the maternal spiral arterioles. The villous vascular branching adds this process on the fetal side, allowing the maternal and fetal circulations to convert to low resistance high -capacitance vascular bed circulations. Nonbranching angiogenesis stage starts at about 26 weeks of gestation and continues until term. During this phase, a new villous type arises, the mature intermediate villus that is specialized in gas exchange. In this type of villous tree, the tips of the villi are long and slender, and the capillary network creates several loops, the final capillary loop exceeding 4,000 m in length (3). Placentation process requires the involvement of angiogenic growth factors, but also of other molecules such as cytokines, extracellular matrix metalloproteinases, hormones and transcription factors (4). Immunohistochemistry studies shows that human placenta expresses angiogenic growth factors and their receptors, as well as antiangiogenic factors (5).

The angiogenic factors

The expression of angiogenic factors is, itself, regulated by oxygen pressure and mechanical stimuli (5). Placenta insufficiency and some abnormal pregnancy outcomes which derive from this insufficiency, are the result of the imbalance between angiogenic and antiangiogenic factors at the placenta level together with local inflammation.

Different studies described the expression of angiogenic factors at placenta level (2, 5, 6):

- fibroblast growth factor (FGF),
- hepatocyte growth factor, (HGF) and its receptor, c-met,
-vascular endothelial growth factor, (VEGF) and its receptors: VEGFR-1(Flt-1) and VEGFR-2 (flk-1/KDR),
- placenta growth factor (PIGF).

The anti-angiogenic factors

- placenta antiangiogenic factors are represented mainly by serum soluble fms-like tyrosine kinase-1 (s-Flt-1) and soluble endoglin (7).

Angiogenic and antiangiogenic placenta factors: Structure, Expression and Receptor-Binding Properties

Cysteine-knot proteins are a group of structurally related dimeric proteins comprised of eight characteristically spaced cysteine residues with intra- and interchain disulfide bonds between them. PlGF, VEGF(VEGF-A), VEGF-B, VEGF-C, VEGF-D, VEGF-E, and Fos-induced growth factor (FIGF), are the members of said group of growth factors and have in common several biochemical and functional features (i.e. PlGF and VEGF have the ability of forming heterodimeric molecules in the cells where both genes are expressed. VEGF was the first member of the above-mentioned family of growth factors to be identified and isolated. The VEGF-A homodimer exerts its biological activities

through activation of two distinct tyrosine kinase receptors: *fms*-like tyrosine kinase receptor-1 (Flt-1; also known as VEGFR-1) and the kinase domain-containing receptor/fetal liver kinase receptor (KDR/Flk-1; also known as VEGFR-2). Endothelial cells are the main target of this factor, because of their high expression of VEGF receptor-1 (VEGFR-1 or Flt-1) and VEGF receptor-2 (VEGFR-2 or Flk-1/KDR); the effect of VEGF over endothelial cells is to stimulate their proliferation (2, 6).

PlGF is a member like VEGF, more recently identified and described, and has, together with VEGF an important role in the stimulation of endothelial cell growth and, thus, angiogenesis. Placenta growth factor (PlGF), a member of the vascular endothelial growth factor (VEGF) family, is one of the key regulators of pathological angiogenesis, as demonstrated by gene inactivation studies. The PlGF primary transcript gives birth to four forms of the mature PlGF proteins (8) of which PlGF-1 and PlGF-2 (also denominated as PlGF-131 and PlGF-152, respectively), are the predominant forms, which differ in terms of structure by the insertion of a highly basic 21-aminoacid stretch at the carboxyl end of the protein, which consecutively gives the ability of binding to heparin to PlGF-2.

Antiangiogenic factors activated in preeclampsia are: soluble endoglin and sFlt-1(7). Endoglin is a protein that is abundantly expressed in the cell membrane of the syncytiotrophoblastic and vascular endothelium and acts as a co-receptor of transforming growth factor β1 and β3 (TGF-β1 and TGF-β3) (7). In consequence, soluble endoglin acts as an inhibitor to TGF-β1 signaling in the vascular endothelium, resulting in an antiangiogenic effect (18,19). In preeclampsia, soluble endoglin is released into maternal circulation, due to an up-regulation of placental endoglin (8). Circulating soluble endoglin level was shown to increase 2-3 months before onset of PE. Soluble fms-like tyrosine kinase 1 (sFlt 1) is a splice variant of the above-mentioned VEGF receptor, that is lacking cytoplasmic and transmembrane domains, thus acting like an important VEGF and PlGF antagonist (8).

According to one study found in literature, on a rodent model, modest hypertension without significant proteinuria and increased vascular permeability were obtained because of overexpression of soluble endoglin, by means of adenoviral vectors (8). In addition, overexpression of sFlt 1 and endoglin, caused, in a similar fashion, fetal growth restriction and severe vascular damage, nephrotic-range proteinuria, severe hypertension, thus a syndrome like HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets).

In conclusion, endoglin and sFlt 1 are two proteins with similar antiangiogenic effects, obtained through different mechanisms, which may combine producing endothelial dysfunction and severe preeclampsia.

Correlations of angiogenesis with fetal hypoxia

There are three different types of hypoxia that may occur in the fetoplacental unit and may influence fetoplacental angiogenesis: *pre-placental hypoxia, uteroplacental hypoxia and post-placental hypoxia* (5).

1. Pre-placental hypoxia. The mother, the placenta and the fetus are hypoxic, as in pregnancy at high altitude, maternal anemia and cyanotic maternal cardiac diseases. In this condition the peripheral placental villi show increased branching angiogenesis with formation of richly branched but

shorter terminal capillary loops.

2. Uteroplacental hypoxia. In this situation, maternal oxygenation is normal, but the placenta and the fetus are hypoxic, due to an impaired utero-placental circulation. This condition characterizes the preeclampsia with preserved end diastolic flow at umbilical artery.

In this case, the peripheral placental villi show formation of richly branching nests, as in the previous situation, and fetal blood flow is normal or slightly reduced.

Semi-quantitative Western blotting analysis shows in placentae of this type compared to normal placentae, an increased expression of VEGF and reduced expression of PlGF (5), suggesting that placental hypoxia, upregulates VEGF *in vivo* and causes changes in angiogenesis.

3.Post-placental hypoxia.

In this situation, the fetus is hypoxic, whereas the mother is normoxic and the placenta may show higher PO_2 levels than normal, a situation described as 'placental hyperoxia'.

The histopathological analyses show terminal capillaries that are poorly developed and capillary branching that are virtually absent. At ultrasound, the end diastolic flow at umbilical artery is zero or negative.

In this circumstance, the perinatal mortality is around 40% and survivors of neonatal intensive care are at risk of neurodevelopmental handicap. In these cases, PIGF is increased, while VEGF is decreased, suggesting that early onset of placental hyperoxia by enhancing PIGF, induces decrease of branching angiogenesis and failure of terminal villi formation.

Clinical applications of placental angiogenic/antiangiogenic factors

Preeclampsia is an imbalance between pro-angiogenic and anti-angiogenic factors. The ratio sFlt-1/PIGF is elevated in pregnant women before the clinical onset of preeclampsia, and many studies have been conducted, to establish the best sFlt-1: PlGF ratio cutoff to predict the clinical onset of PE in women with risk factors (9).

PROGNOSIS (Prediction of Short-Term Outcome in Pregnant Women with Suspected Preeclampsia *Study*), was a multicenter, prospective, double-blind, non-interventional trial, evaluating the short-term prediction of preeclampsia, eclampsia (maternal convulsions due to complicated PE) and HELLP (hemolysis, elevated liver enzymes, low platelet count) syndrome (PE complicated with hepatic insufficiency and coagulation disorders), in pregnant women with suspected preeclampsia. In this study, more than 1,270 pregnant women were enrolled at 30 sites in 14 countries, between December 2010 and January 2014. Results of the PROGNOSIS study were published by Zeisler *et al.* (10).

This study demonstrated that a ratio of the proteins sFlt-1/PlGF of 38 and below predicts an absence of preeclampsia, eclampsia and HELLP syndrome for one week , with a negative predictive value (i.e., no preeclampsia in the subsequent week) of 99.3% (95% confidence interval [CI], 97.9 to 99.9), whilst an sFlt-1/PlGF ratio above 38 predicts onset of preeclampsia, eclampsia and HELLP syndrome within four weeks, with a positive predictive value of 36.7% (95% CI, 28.4 to 45.7), with 66.2% sensitivity (95% CI, 54.0 to 77.0) and 83.1% specificity (95% CI, 79.4 to 86.3).

In 2013, the same team separated early preeclampsia (onset between 20 and 34 weeks of pregnancy) from late preeclampsia (onset between 34 and 37 weeks of pregnan-

cy). For each of the 2 gestational periods, 2 independent cutoffs framing an equivocal zone were determined: the first cutoff with focus on high sensitivity, and the second focusing on high specificity (11).

Between 20 and 34 weeks, the cutoffs at ≤33 and ≥85 resulted in a sensitivity/specificity of 95%/94% and 88%/99.5%, respectively. After 34 weeks, the cutoffs at ≤33 and ≥110 yielded a sensitivity/specificity of 89.6%/73.1% and 58.2%/95.5%, respectively. The approach to use multiple cutoffs for the early and late preeclampsia phases, enhances the diagnostic accuracy of the sFlt-1/PlGF ratio as a diagnostic tool for hypertensive complication during pregnancy (11).

Some teams, specialized in analyzing different statistical models in the screening of preeclampsia, included biomarkers of angiogenesis (Sflt/PIGF) in a combination of other markers and examined the performance of screening for PE by maternal factors alone and by maternal factors with the addition of each biomarker individually and combinations of biomarkers (12).

Using maternal risk factors, mean arterial pressure (MAP), uterine artery pulsatility index (UtA-PI) and serum placental growth factor (PlGF), this combination could identify, at a 5% false-positive rate (FPR), 98% of cases of early PE, but only 70% and 33% of intermediate and term PE, respectively. Screening at around 32 weeks by a combination of maternal factors with MAP, UtA-PI, PlGF and serum soluble fms-like tyrosine kinase-1 (sFlt-1) could identify, at a 5% FPR, 98% of cases of intermediate PE, but only 54% of term PE (12). PlGF and sFlt-1 levels are simultaneously affected in PE (especially in early pre-eclampsia); thus, the sFlt-1/PlGF ratio represents now a predictor element superior to the individual analysis of these markers.

Another approach in assessing these markers might consist in the sequential determination of serum levels, an approach that has provided better predictive results than the punctual assessment in of the pregnancy a certain moment. The sensitivity of sequential determinations of the sFlt-1/PlGF ratio is of 100% and its specificity is of 98-99% (13).

In the original article, sFlt-1: PlGF ratio, named also "preeclampsia fraction", was performed on Elecsys® Roche Platform (14). The sFlt-1/PlGF ratio is well described and it is a marker for prediction of the PE. Burke *et al*. (14) demonstrated that measurements of PlGF are reliable if we use different techniques and different platforms (R&D Systems, AlereTriage, Roche Elecsys or Abbott Architect).

Future therapeutic uses of angiogenic-antiangiogenic factors

We are waiting for the moment when angiogenic factors will be used as therapy for the prevention of adverse pregnancy outcomes due to placental insufficiency: preeclampsia, uterine apoplexy, fetal growth restriction, in utero fetal death and some cases of preterm delivery. For the moment, there are some promising studies on animals. Sulistyowati *et al*. (15) demonstrated that injection of recombinant VEGF121 has the potential to prevent fetal growth restriction in a newly proposed preeclampsia mouse model (*Mus musculus*).

There are studies on samples of placenta and blood vessels, as well as animal experiments suggesting that statins can reduce the level of abnormalities in angiogenic/anti-angiogenic balance, and perhaps reduce or eliminate the effects and risks of pre-eclampsia. This has been previously

demonstrated in stillbirths of unknown etiology. Statins (HMG CoA reductase inhibitors), seem to positively influence the inflammatory, anti-angiogenic milieu of pregnancies with underlying placental ischemia by their pleiotropic effects. This might prevent, ameliorate and delay preeclampsia (16). Esteve-Valverde *et al*. (17), in a systematic search to summarize the role of statins for preventing and treating severe preeclampsia, found 13 studies about statin's specific use during pregnancy. The review shows a potential beneficial role of statins in preventing and treating severe preeclampsia, meanwhile the rate of major congenital abnormalities in the newborn exposed to statins during pregnancy is no higher than the overall risk population.

Other proposed therapeutic interventions to reverse an anti-angiogenic status during pregnancy include the administration of VEGF 121 or extracorporeal removal of sVEGFR-1(18).

CONCLUSIONS

Our study wishes to be an update concerning the factors of placental angiogenesis and their role in the diagnosis and treatment of preeclampsia and other associated pathologies.

The structure of angiogenesis factors has been known since the '70s, but their clinical applications in placental insufficiency screening and the main obstetric syndromes induced by it, have only emerged in the last few years.

The ratio of variable pro-angiogenic and anti-angiogenic factors characterizes different situations of fetal hypoxia, which could be used in the clinical practice.

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NEWS

DIAGNOSIS IN MENINGITIS: AT THE BORDER BETWEEN MEDICINE AND COMPUTER SCIENCE

We all live in a world marked by the stamp of technology and information. In these circumstances, the biggest challenge for the information era is to establish some diagnoses using only computer science, without the help of human mind. In other words, the final goal is to develop performing programs which can diagnose a disease using just a set of parameters. For the first time, the researchers developed an original method that can differentiate between bacterial and viral meningitis. This is based on the DRSA methodology and it is constituted by a set of decision rules, like "if…then...". The method had results with a coverage factor between 2 and 95% and this can maintain the interest on the topic. Although the establishment of the diagnosis by computer seems to be utopian at this moment, this study opens the gate of a new universe of research with big chances of feasibility and practicability in future medicine (Gowin E, Januszkiewicz-Lewandowska D, Słowinski R, Błaszczynski J, Michalak M, Wysocki J. With a little help from a computer: discriminating between bacterial and viral meningitis based on dominance-based rough set approach analysis. *Medicine* 2017; 96: 32(e7635).

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TUMORILE STROMALE DE JEJUN ŞI ILEON

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STROMAL TUMORS OF JEJUNUM AND ILEUM (Abstract): Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors of the digestive tract and 25% are in the small intestine. **Material and method**: A retrospective study was performed on 73 patients diagnosed with intestinal cancer in three hospitals in Iasi and Galati, in a period of 12 years (1998-2009). **Results:** Stromal tumors represents 11.5% of malignant tumors of jejunum and ileum, and the average age of diagnosis was 55 years; male to female incidence was 1:2 ($\chi^2 = 0.66$, $p > 0.05$). 66.66% of patients came from urban areas ($\chi^2 = 0.66$, $p > 0.05$). The most frequent signs and symptoms were abdominal meteorism and abdominal pain (83%). There were no correlations between admission and discharge diagnosis. CD117 was present in all patients; CD34 was present in 66.66% of them. Survival at 2 years was 66.67% and at 5 years was 33.33%. **Conclusions:** Preoperative diagnosis is often difficult to make, many patients come to doctors in advanced-stage and the five-year survival rate is slow. The most frequent tumor markers were CD117 and CD34. **Key words:** GASTROINTESTINAL STROMAL TUMORS, SMALL BOWEL, IMMUNOHISTOCHEMISTRY

Tumorile stromale gastro-intestinale (GIST) sunt cele mai frecvente tumori de origine mezenchimală de la nivelul tubului digestiv. Intestinul subţire reprezintă al doilea sediu al tumorilor stromale, după stomac, 25% dintre ele apărând la acest nivel. Tumorile stromale intestinale reprezintă doar 14% din totalul tumorilor intestinului subţire (1). Vârsta medie de diagnostic este de 58 de ani (1,2,3).

Diagnosticul de certitudine se bazează pe examenul anatomopatologic şi pe testele de imunohistochimie (1).

Ca şi în cazul altor tumori ale intestinului subţire, datorită simptomatologiei fruste şi nespecifice, diagnosticul de tumoră stromală de intestin subţire se pune tardiv, în stadii avansate ale bolii (5) .

Supravieţuirea la 5 ani este de 30-60% în funcţie de stadiul tumorii la momentul diagnosticului (6,7,8,9).

MATERIAL ŞI METODĂ

A fost efectuat un studiu retrospectiv pe un număr de 73 de pacienţi diagnosticaţi cu tumori intestinale într-o perioadă de 12 ani (1998-2009) la Spitalul Clinic „Sf. Spiridon" Iaşi, Clinica III Chirurgie, Spitalul Judeţean Galaţi şi Spitalul General Căi Ferate Galaţi. 71,23% dintre pacienţi au fost diagnosticaţi cu tumori maligne, iar 28,76%, cu tumori benigne.

Tumorile stromale de jejun şi ileon au fost studiate din punct de vedere epidemiologic, imunohistochimic şi a manifestărilor clinice.

Pentru prelucrarea statistică a datelor s-a folosit *„Statistical Package for Social Sciences"* (*SPSS*) şi s-au folosit metode sta-

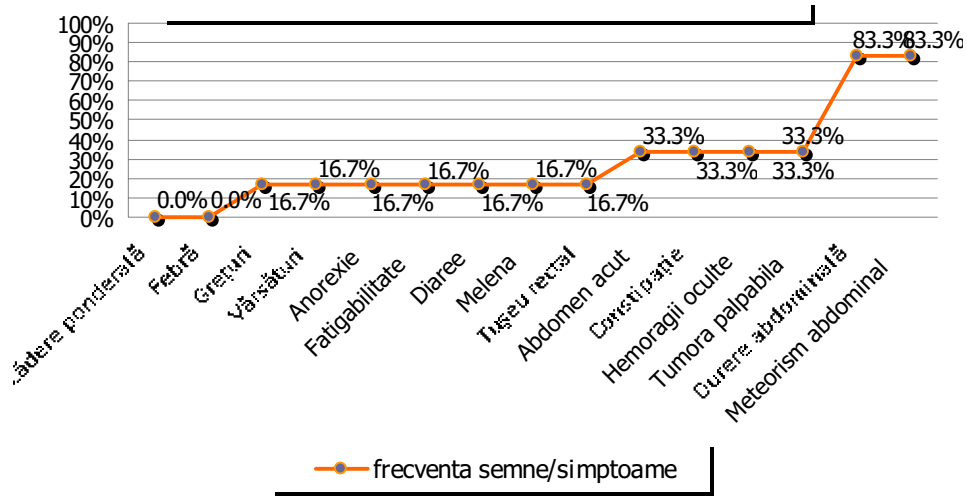

Fig. 1. Semne şi simptome ale tumorilor stromale de jejun şi ileon

tistice descriptive, cât şi metode care se încadrează în statistica inferenţială.

REZULTATE

Tumorile stromale au reprezentat 11,5% din totalitatea tumorilor maligne, iar vârsta medie de diagnostic în lotul studiat, a fost de 55 de ani. Raportul între subiecţii de sex masculin şi cei de sex feminin a fost de 1:2, (χ^2 = 0,66, p > 0,05). Sexul pacienţilor nu a influenţat frecvenţa tumorilor stromale, valoarea lui χ^2 (mai mică decât valoarea 3,84 din tabelul lui Fisher) determinându-ne să acceptăm ipoteza de nul.

Din punct de vedere a mediului de provenienţă, 33,33% din pacienţii cu tumori stromale de jejun sau ileon provin din mediul rural, iar 66,66% din mediul urban (χ^2 = 0,66, p > 0,05). Valoarea lui χ^2 de 0,66 cu un p > 0,05 semnifică că nici variabila mediul de provenienţă al pacienţilor nu a influenţat frecvenţa tumorilor stromale.

În acest studiu, toate tumorile stromale au fost localizate la nivelul ileonului. Localizarea tumorilor stromale pe ileon sau jejun nu a fost influenţată de sexul pacienţilor. Valoarea lui χ^2 (cu un prag de semnificaţie mai mare de 5%) pentru localizarea pe ileon, semnificând faptul că nu există o diferenţă semnificativă din punct de vedere statistic.

Cele mai frecvente semne şi simptome întâlnite la internare au fost: meteorismul abdominal şi durerea abdominală care se regăsesc în 83,3% din cazuri, iar hemoragiile oculte, tumora palpabilă, constipaţia şi abdomenul acut au avut fiecare o frecvenţă de 33,3%. Nu s-a înregistrat nici un caz cu febră sau scădere ponderală (fig. 1).

Examenele paraclinice efectuate au fost: ecografia abdominală de care au beneficiat toţi pacienţii şi care a identificat o formaţiune tumorală abdominală în 66,66% din cazuri; radiografia abdominală pe gol, s-a efectuat la 33,33% dintre subiecţi şi a constatat prezenţa nivelurilor hidro-aerice în 50% dintre aceste cazuri, iar computerul tomograf a fost efectuat în 16,66% din cazuri şi a fost pozitiv în toate situaţiile.

Cele mai frecvente diagnostice puse la internarea pacienţilor cu tumori stromale au fost de abdomen acut chirurgical şi sindrom dureros abdominal.

Concordanţa între diagnosticul de internare şi cel de externare a fost 0. Diagnosticele de internare au fost: abdomen acut chirurgical 33,33%, sindrom dureros abdominal, apendicită acută, ocluzie intestinală

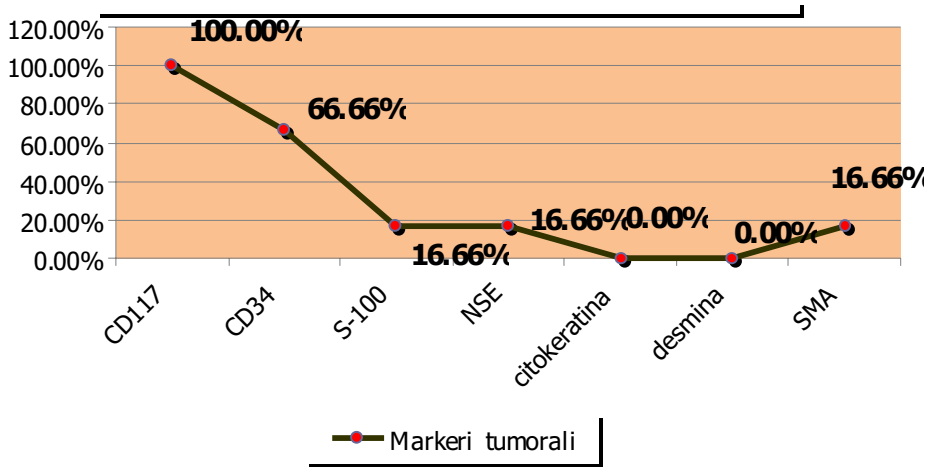

Fig. 2. Frecvenţa marcherilor tumorali pentru pacienţii cu tumori stromale de jejun şi ileon.

şi tumoare ovariană, fiecare reprezentând 16,66%.

Din punct de vedere imunohistochimic, CD 117 a fost prezent la toţi pacienţii luaţi în studiu şi care au fost diagnosticaţi cu tumoră stromală, CD34 în 66,66% din cazuri, S-100, NSE (neuron specific enolase) şi SMA (actina specifică muşchiului neted), în 16,66% din situaţii. Citokeratina şi desmina deşi au fost determinate, nu au fost evidenţiate în nici un caz (fig.2).

33,33% dintre pacienţi au prezentat metastaze ganglionare la momentul diagnosticului, iar 16,66% au prezentat metastaze la distanţă. Cei cu metastaze la distanţă nu au supravieţuit la 5 ani, spre deosebire de cei cu metastaze ganglionare, care au supravieţuit la 2 ani, dar nu şi la 5 ani.

Supravieţuirea la 2 ani a fost de 66%, iar la 5 ani de 33%. În ambele situaţii raportul între pacienţii de sex feminin şi cei de sex masculin, cât şi între mediul urban şi cel rural, a fost de 1 : 1.

Pentru subiecţii care au supravieţuit la 2 ani, toţi au avut CD117 prezent, iar CD34 a fost prezent în 60% din cazuri. Ceilalţi marcheri tumorali au fost absenţi. În cazul pacienţilor care au supravieţuit la 5 ani, doar CD117 a fost prezent.

DISCUŢII

Pentru tumorile stromale, intestinul subţire reprezintă al doilea sediu ca frecvenţă, după stomac.

În acest studiu, ele au reprezentat 8,3% din totalul tumorilor intestinale, procent care este în concordanţă cu studiile anterioare (1).

Vârsta medie de diagnostic a acestor tumori este, conform literaturii de specialitate, de 58 de ani (1,10,11). Studiul actual a obţinut o vârstă medie de diagnostic de 55 de ani şi un raport de 1 : 2 între subiecţii de sex masculin şi cei de sex feminin, spre deosebire de alte loturi studiate la care vârsta medie de diagnostic a fost de 60 de ani, iar numărul pacienţilor de sex feminin a fost egal cu a celor de sex masculin (12).

66% dintre pacienţii diagnosticaţi cu tumoră stromală, au provenit din mediul urban. Acest fapt poate fi explicat prin modul de alimentaţie, care în mediul urban este mai bogat în aditivi alimentari, dintre care unii s-a dovedit că sunt implicaţi în apariţia cancerului la nivelul intestinului subţire (13, 14,15).

S-a constatat că cele mai frecvente semne şi simptome apărute la prezentarea la medic au fost meteorismul abdominal şi durerea abdominală, iar cel mai frecvent diagnostic

de internare a fost abdomenul acut chirurgical, rezultate care sunt în concordanţă şi cu alte studii (12).

Examinările paraclinice cele mai relevante în cazul tumorilor stromale de jejun şi ileon sunt computer tomografia (CT) şi rezonanţa magnetică nucleară (RMN) (16, 17), CT-ul dând un diagnostic de certitudine şi în cazurile studiate de noi.

CD117, a fost prezent la toţi pacienţii, iar CD34 la 66% dintre aceştia. CD 117 este expresia lui c-kit în cazul tumorilor stromale gastrointestinale (GIST), fiind considerat ca principalul marcher pentru GIST, iar CD34, este un marcher nespecific, dar destul de frecvent întâlnit în tumorile stromale, între 47% până la 70% din cazuri (18).

Alţi marcheri, cum ar fi SMA, NSE şi S-100 sunt întâlniţi mult mai rar.

Prezenţa CD117 şi CD34 la pacienţii care au supravieţuit la 2 ani, poate fi explicată prin frecvenţa crescută a acestor markeri în momentul diagnosticului de tumoră stromală.

Supravieţuirea la 2 ani a fost de 66% iar la 5 ani de 33%. Supravieţuitorii la 5 ani nu au prezentat metastaze şi au beneficiat de rezecţie completă a tumorii.

Compararea ratelor de supravieţuire din diferitele studii publicate este dificil de realizat deoarece nu există un sistem standardizat de stadializarea a GIST iar multe studii sunt alcătuite dintr-un număr mic de subiecţi heterogeni. Cu toate acestea, s-a constatat o supravieţuire la 5 ani de 32-93%, în cazul rezecţiilor tumorale complete şi doar de 10% pentru situaţia în care tratamentul a fost paleativ (19).

CONCLUZII

Diagnosticul de tumori stromale de jejun şi ileon este dificil de pus preoperator, cei mai mulţi pacienţi prezentându-se la medic în stadii avansate. Vârsta medie de diagnosticare a fost de 55 de ani. Cei mai frecvenţi marcheri tumorali au fost CD117 şi CD34. Supravieţuirea la 2 ani a fost de 66%, iar la 5 ani de 33%.

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DETERMINATION OF GRADE OF GASTROINTESTINAL INJURY AND ITS PROGNOSTIC UTILITY IN PATIENTS WITH ACUTE NECROTIZING PANCREATITIS

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DETERMINATION OF GRADE OF GASTROINTESTINAL INJURY AND ITS PROGNOSTIC UTILITY IN PATIENTS WITH ACUTE NECROTIZING PANCREATITIS (Abstract): Acute necrotizing pancreatitis (ANP) is potentially lethal inflammatory process. Recent researches established that presence of multiorgan failure together with pancreatic infection are major determinants of its mortality. But significance of acute gastrointestinal injury (AGI) in course of ANP is still remaining unclear. **Aim:** To determine frequency and significance of AGI on severity and mortality of ANP. **Material and methods:** We performed a prospective observational cohort study of 151 patients with ANP which were admitted to single intensive care department. AGI was established according to European Society of Intensive Care Medicine (ESICM) recommendations. Clinical and laboratorial variables as well as plasma citrulline concentrations were studied. **Results:** Persistent organ failure was diagnosed in 33% and transient in 67% observations. Respiratory failure has been dominated in mortality structure, cardiovascular, renal and intestinal failures were met with equal frequency. Serum citrulline concentration reflected the level of AGI. **Conclusions:** Intestinal failure is strongly associated with unfavorable events in patients with acute necrotizing pancreatitis as well as pulmonary, cardiovascular and renal dysfunctions. Plasma citrulline concentration is simple and reliable marker for diagnosing and monitoring AGI. **Keywords:** ACUTE NECROTIZING PANCREATITIS, GASTROINTESTINAL DYSFUNCTION, CITRULLINE.

Acute necrotizing pancreatitis (ANP) is a surgical disease caused by primary aseptic necrosis of the pancreatic tissues, accompanied by the development of systemic inflammatory response syndrome in both aseptic and infected necrosis. In recent years there has been a significant increase in the number of patients with infected forms of ANP and the development of infected complications whose mortality reaches 17-70% (1). Assessment of the severity of the attack and predicting the course of the disease are important when determining the allocation of care for these patients or the need for transfer to a higher-level care early in the disease process. Recent researches established that presence of multiorgan failure together with pancreatic infection are major determinants of mortality during ANP (2-4). The mortality rates in the absence of organ failure, in the presence of one organ failure, and in pa-

tients with multi-organ failure are 0%, 3%, and 47%, respectively (5-6). The gastrointestinal system is considered critical to the development of organ failures, with bacterial translocation in intensive care unit patients supporting the concept of the gut having a role in multiorgan failure (7). In critically ill patients, the intestine is a vulnerable organ, and gastrointestinal dysfunction is common. It has been reported that almost 50% of patients in intensive care units (ICUs) have enterocyte damage at admission (8). It is therefore important to monitor the status of the GI tract in critically ill patients. In 2012, the Working Group on Abdominal Problems of the European Society of Intensive Care Medicine (ESICM) defined acute gastrointestinal injury (AGI) as the malfunctioning of the gastrointestinal tract in critically ill patients due to their acute illness and recommended a four-grade classification for AGI severity (9). However, this definition mainly depends on the symptoms and signs of AGI, which are usually not sufficient to objectively determine its severity and impact on patients' prognosis.

MATERIAL AND METHODS

This prospective observational cohort study was performed at Regional Emergency Hospital, Chernivtsi, Ukraine, between March 2010 and September 2017. The study was conducted in accordance with the principles of the Declaration of Helsinki. The ethics review board of each participating hospital approved the study. Patients or their legal representatives gave written informed consent. During this period 708 patients with acute pancreatitis were admitted to clinic. Patients were included in the current prospective study if they fulfilled the inclusion criteria of signs of pancreatic necrosis on contrast-enhanced computed tomography. Necrosis was diagnosed by lack of pancreas enhancement on contrast computed tomography, which was a sign of impaired or absent tissue perfusion. There were 192 cases of ANP. Patients were not enrolled to the study if any of the following criteria were present: a) age < 18 and > 80 years; b) recent surgical interventions; c) psychoses; d) pregnancy; e) previously history of chronic pancreatitis. After exclusion of abovementioned cases we enrolled to current study 151 patients with ANP. All of them were admitted to single intensive care department.

Severity of ANP was determined according to the recently revised Atlanta Classification (10). Organ failure was defined for respiratory, cardiovascular, and renal system by modified Marshall scoring system 10. Besides above organs dysfunction of neurological system, liver and intestine were estimated. Neurological failure was established by Glasgow coma score. Hepatic dysfunction was evaluated according to Child-Pugh classification (11), grade B or C were criteria for diagnosis of liver failure. AGI was estimated by European Society of Intensive Care Medicine recommended four-grade classification (grades I, II, III, and IV). Only 3rd and 4th grade of AGI was recognized as intestinal failure. Intra-abdominal pressure was measured via the bladder, with patients in the supine position, using the closed loop system repeated measurements technique (12). Additionally, venous blood citrulline concentration was determined as indicator of intestinal epitheliocytes functional activity (13). After admission, patients received fluid resuscitation, and full laboratory investigations were performed on the first 3 days and in the 24 hours before any inter-

vention. Nasojejunal enteral feeding was initiated if an oral diet was not tolerated after 48-72 hours after admission. If there were problems with nasojejunal intubation nasogastric tube feeding was started. Parenteral nutrition was only initiated when oral route was not tolerated or sufficient, but at least small amount of enteral feeding was present in all patients. In line with international guidelines, intervention was generally only performed in case of suspected or confirmed infection of pancreatic necrosis or peripancreatic necrosis alone. The decision to intervene was mostly based on clinical grounds (i.e. deterioration). Whenever possible, intervention was postponed until approximately four weeks after the onset of disease. In case of proven or suspected infection of pancreatic necrosis within four weeks after onset of disease, antibiotics were administered, and intervention was postponed as long as possible.

The following parameters were collected for each episode of acute necrotizing pancreatitis: length of hospital stay, in-hospital mortality, presence of organ failure, and local complications such as acute peritoneal fluid collection, pseudocyst, acute necrotic collection and walled-off pancreatic necrosis. Acute Physiology and Chronic Health Evaluation (APACHE)-II, Marshall scores, presence of organ failures and systemic inflammatory response syndrome (SIRS) were calculated daily during period of ICU treatment.

Statistics data were collected in a Microsoft Excel database. After completion of data collection, the database was imported into SPSS for Windows (SPSS 16.0., Chicago, IL, USA). Continuous variables were expressed as mean with standard deviation and compared using Student's t- test or Mann–Whitney test. Categorical variables were expressed as absolute numbers and proportions. Pearson's $\chi 2$ test or Fisher's exact test was used for comparison of categorical variables. Logistic regression models were created to analyze the independent effects of organ failures on mortality. To define predisposing factors, the odds ratio and its 95% confidence interval were used as a binary parameter. Receiver Operator Curve (ROC) was generated to determine cutoff value of citrulline for estimation of intestinal failure. A p value of < 0.05 was considered statistically significant.

RESULTS

Different levels of AGI were diagnosed in 141 (93.37%) of patients with ANP.

The 1st grade of AGI (risk of intestinal dysfunction) was detected in 37 (24.5%) cases. The general condition of patients was stable, with no organ failure, assessed according to the APACHE II scale from 6 to 9 points. Intestinal paresis, nausea, reflux, or vomiting were of a temporary nature and quickly passed by themselves, without affecting the general condition of the patients. IAP was within the range of 5-12 mmHg, citrulline in blood plasma – 28-38 μmol/L.

The 2nd grade of AGI (gastrointestinal dysfunction) was found in 54 (35.8%) persons. The general condition of patients was of moderate severity, assessed according to the APACHE II scale from 10 to 19 points. Patients demonstrated clinical signs and laboratory parameters of transient organ failure, which was corrected within 24-48 hours after hospitalization. Intestinal dysfunction was manifested by the acute development of intestinal paresis, large (more than 500 ml) residual volume of the stomach, reflux, intra-abdominal hypertension of the 1st stage (IAP was within the range

of 12-14 mmHg). Citrulline in blood plasma was lower than the index in the control group and equaled to 16.57±0.48 μmol/L ($p<0.01$).

The 3rd grade of AGI (intestinal failure) lasting more than 3-5 days was detected in 38 (25.2%) patients, whose condition severity was assessed according to the APACHE II scale from 19 to 30 points. There has been detected prolonged pronounced paresis of the intestine (caecum dilatation up to 9 cm, colon dilatation – up to 10 cm), high residual volume of the stomach (from 500 to 1,000 ml), progression of intra-abdominal hypertension (15 to 20 mmHg), accompanied by a low level of the abdominal cavity organs perfusion (perfusion pressure was less than 60 mmHg), in particular of the intestine and pancreas, leading to the development of persistent organ failure. Acute respiratory failure, renal failure and circulatory disorders were the most common. In accordance with the patients' condition severity and the intestinal dysfunction stage, the citrulline concentration in blood plasma decreased to 11.03±0.19 μmol/L.

The 4th grade of AGI with severe impact on the distal organs (critical intestinal failure) developed in 12 (8.0%) patients with severe ANP, whose general condition was assessed according to the APACHE II scale from 25-30 to more points. Their citrulline level dropped till 10.54±0.86 μmol/L. Later on, increase in the intra-abdominal pressure to 25-30 mmHg was accompanied by the development or deepening of existing permanent organ failure and is characterized as abdominal compartment syndrome. The abdomen is bloated, the abdominal wall is tense and painful when palpated, intestinal peristalsis is often not auscultated, there occurs reflux of gastric content or vomiting, residual volume of the stomach is more than 1,000 mL, dilatation of the caecum and colon is more than 12 cm. First of all, respiration is impaired, and patients need respiratory support already at the initial stage of ACS. The direct effect of high IAP on the lower hollow vein leads to a decrease in blood flow to the heart and, accordingly, cardiac output reduces (tab. I).

TABLE I

Grade of acute gastrointestinal injury in patients with acute necrotizing pancreatitis

AGI grade	Number of patients (%)	APACHE II score	IAP (mm Hg)	Citrulline (μmol/L)	Mortality, n (%)
0	10 (6.60)	6.30±0.11	7.50±0.11	32.1±0.29	- (0)
1st	37 (24.5)	8.10±0.13	9.80±0.13	24.5±0.25	- (0)
2nd	54 (35.8)	14.4±0.23	13.1±0.17	16.6±0.48	5 (9.30)
3rd	38 (25.2)	22.3±0.32	16.7±0.28	11.7±0.19	15 (39.5)
4th	12 (8.00)	27.5±0.16	23.5±0.31	10.5±0.86	11 (85.7)

The citrulline level below 12.5 μmol/L allowed to diagnose intestinal failure with the sensitivity of 90.4% and specificity of 84.6% (AUC ROC 0.905±0.0179, $p=0.001$) (fig. 1). A reliable correlation dependence between the grade of AGI and citrulline concentration in the blood ($r=0.743$ $p<0.05$) has been identified. Overall mortality rate was 20.5% and observed only among persons with severe form of ANP.

A number of organs with dysfunction were significantly higher in deceased pa-

tients compared with survivors (3.48±0.81 and 1.33±0.44, respectively, $p<0.05$) as well as APACHE II score, age, hematocrit and creatinine level.

Respiratory failure had been dominated in mortality structure, cardio-vascular, renal and intestinal failures were met with equal frequency.

Failures of these organs were independent factors of mortality in multivariate logistic regression model (Wald's criteria 8.441, 5.464, 5.660 and 3.847, accordingly, $p<0.05$). Neurological and liver failures did not influence on patient's survival (tab. II).

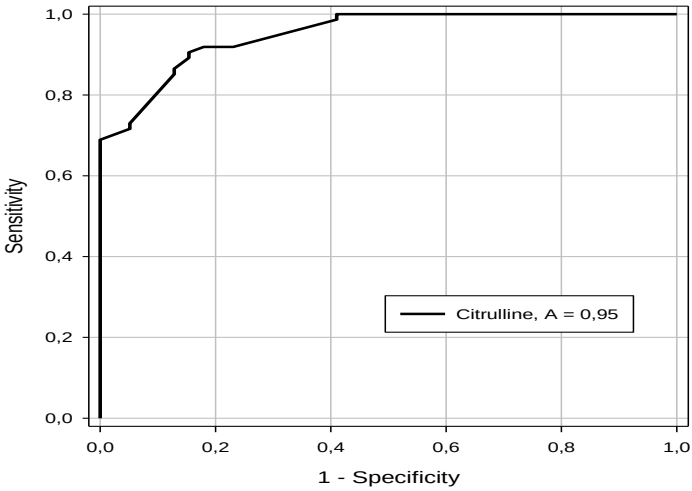

Fig 1. ROC curve of intestinal failure diagnosis by citrulline

TABLE II

Influence of organs failure on survival of patients with acute necrotizing pancreatitis

Factors	Deceased (n=31)	Survived (n=120)	p value
Respiratory failure, n (%)	28 (90.4%)	31 (25.8%)	0.001
Cardiovascular failure, n (%)	25 (80.6%)	11 (13.3%)	0.001
Neurological failure, n (%)	11 (35.5%)	26 (21.7%)	0.111
Renal failure, n (%)	25 (80.6%)	28 (25.0%)	0.001
Liver failure, n (%)	8 (25.8%)	22 (18.3%)	0.353
Intestinal failure, n (%)	24 (77.4%)	26 (21.7%)	0.001
Multiorgan failure, n (%)	28 (90.4%)	30 (25.0)	0.001

DISCUSSION

In our study respiratory failure occurred most frequently in patients with severe ANP and was followed by cardiovascular, renal and intestinal failures, which have similar impact on mortality

rate. Recommended by 2012 Atlanta classification committee modified Marshall score as well as other widely accepted organ failure scores like SOFA, MODS, APACHE II are dealing with respiratory, cardio-vascular and renal failures but missing intestinal dysfunction. Evaluating the intestine is difficult for two reasons: it is a deep organ, far from the mouth, anus, and abdominal wall, and critically ill patients are frequently not able to inform clinicians about a digestive complaint. This explains why its dysfunction may sometimes be occult or misdiagnosed, and the fact that it is not clearly integrated into the overall approach used to treat intensive care unit patients. However, even occult small bowel dysfunction may have clinical consequences on patient prognosis. The gut hypothesis of multiple organ failure was first put forward more than 20 years ago (7). According to this hypothesis, the multisystemic disorders defining this syndrome could be the late consequences of a previous small bowel injury. If the intestinal failure could be evaluated and protected on admission, gut-related sepsis will be reduced, and the mortality rate will fall down. Reintam Blaser *et al*. (9) found that 59% of critically ill patients have at least one gastrointestinal symptom during their stay in the intensive care unit. In 2012, The Working Group on Abdominal Problems of the ESICM has developed the definitions for gastrointestinal dysfunction in intensive care and proposed AGI score which was based on presence of feeding intolerance and intraabdominal hypertension. However, despite a welcome contribution to assessing AGI, it was noted that feeding intolerance was a subjective measurement, and that intraabdominal hypertension was not specific to gastrointestinal dysfunction (19). Although the AGI score proposed by ESICM appeared to be a new prognostic tool to evaluate critically ill patients, it was not based on a pathophysiological model of gastrointestinal dysfunction. We chose to examine changes of citrulline concentration as a marker of enterocyte mass in patients with acute necrotizing pancreatitis. This amino acid is synthesized exclusively by small intestinal mucosal epitheliocytes thus it may serve as a measure of intestinal mucosal functional activity (14). Recently it was established that plasma citrulline concentrations were able to reflect enterocyte mass in various small intestinal diseases including coeliac disease (15), short bowel syndrome (16), radiation enteritis (17) and Crohn disease (18).

In our research citrulline level decreased 1.5 times in patients with moderately severe pancreatitis and 3 times ($p<0.05$) in patients with severe form, which was highly inverse correlated ($r = -0.643$, $p<0.05$) with clinical AGI score. In deceased persons its concentration dropped less than 10.31±0.42 μmol/L, indicating critical level of intestinal failure due to deep injury of mucosal villi, desquamation of intestinal epithelia and high rate of bacterial and endotoxin translocation.

CONCLUSIONS

Acute gastrointestinal injury occurs in 93.4% of patients with ANP and appears as intestinal dysfunction in 35.8% and failure in 33.2%.

Intestinal failure as well as respiratory, cardiovascular and renal are independent factors of mortality at ANP.

Serum citrulline concentration below 12.5 μmol/L is simple and reliable marker for diagnosing of intestinal failure.

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THE THREE-DIMENSIONAL ULTRASONOGRAPHY OF THE FETAL FACE – HISTORY AND PROGRESSES

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THE THREE-DIMENSIONAL ULTRASONOGRAPHY OF THE FETAL FACE – HISTORY AND PROGRESSES (Abstract): Fetal face was the main concern of researchers who developed three-dimensional ultrasound. 3-D ultrasound beginnings date back to 1973. Parallel concerns of researchers from different parts of the world have led to a constantly changing the equipment and software. 3-D images of the fetal face were obtained since 1986, but those that are available on the ultrasound devices sold today dates from 1992, and the 4D since 1996. Techniques such as volum rendering and multiplanar volume rendering, led to the development of 3D ultrasound. Late 2000 revealed study fetal face by multiplane three-dimensional ultrasound, through the acquisition of volume and its processing. Study of fetal profile by this method provides new data on the jaw, naso-maxillo-mandibular angle, even a diagram of the fetal profile or of dimensions of the fetal face or nasal bone length. Fetal profile analyzed by 16 parameters measured by 3D ultrasound, with the creation of a craniofacial index variable is useful in assessing normal and abnormal fetal face. Today, the bones of fetal face - nose, jaw, bone palace, those of the orbit, can be studied in dynamics, from 12-14 gestational weeks. The best ultrasound images can be obtained after 21 gestational weeks, when fetal size and its ratio with amniotic fluid are optimal for the acquisition of fetal volume. **Key words**: 3D ULTRASOUND, FETAL FACE, HISTORY

A 3D fetus visualization has always been a concern for many investigators, including Tom Brown in Glasgow at the beginning of 1970 who made a"multiplan" scanner, in 1973, working for Sonicaid Ltd. (1). Starting with achievements in ultrasonography and computerised technology, the possibility of three-dimensional visualization appeared, at the beginning of 1980.

Kazunori Baba from the Institute of medical electronics, The University of Tokio, Japan, was the first who had reported a 3D ultrasonography system in 1984 and managed to get 3D fetal images, processing the 2D images on a mini-computer in 1986 (2,3). In 1992 Baba published, in Japanese, the first book of ultrasonography in obstetrics and gynaecology, a book which also contained chapters about the 3D ultrasonography (1,4,5).

In 1990, another group from the University Columbia led by Donald King, described other approaches and algorithms for the 3D spatial registrations and the visualization of ultrasonographic position and orientation of the images forreall (1,6).

In 1992, the team from the National University Hospital Cheng Kung of Taiwan, Chinese Republic, reported a 3D visualization of the fetal face, cerebellum and the spine, using a Combison 330. In 1994, Olaf von Ramm, Stephen Smith and his team, made an improved scanner which ensured a good resolution, up to 20 cm. The team has developed a *"state of the art"* of ultrasonographic medical images, being able to process signals from multiple sections, forreal (1). On the market it has been available since 1997.

Fig. 1. Fetal face obtained through surface rendering technique, with further acquisition of the volume processed in the following images (21 weeks' pregnancy)

In 1993, Wilfried Feichtinger from the University of Vienna, presented images with 10 weeks embryoes using the 3D transvaginal transducer. Alfred Kratochwil continued to support the development of 3D technology, and after retiring he continued to teach 3D ultrasonography. The group Urlike Hamper presented images with different congenital malformations using a 3D prototype scanner (1). În 1996, The group Nelson and the one from the medical imagistics of the University Hospital of London published independent researches on fetal echocardiography in 4D (3D in movement) (7,8,9).

In 1995, Eberhart Merz from the centre of ultrasonic diagnosis and prenatal care of the Mainz University, Germany, proved the utility of multiplanar orthogonal images and also the surface visualization and the transparent ones, in the diagnosis and confirmation of the fetal surface and the skeletal abnormalities, for example the cleft lip or multiple complex malformations (1,4). He and his contributors reported a great

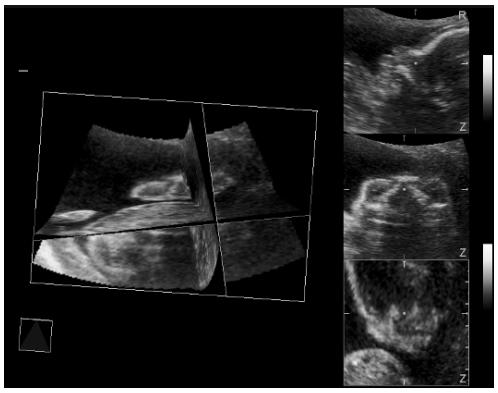

Fig. 2. The obtained and processed volume with the 3 slice technique. There can be seen the 3 orthogonal planes of the fetal face section, in the left side of the image

number of cases, more than 600, with fetal diagnosis using 3D ultrasonography. In 1993 his team made a diagnosis for facial abnormalities, using a 3D transvaginal scanning. The perception in 3D is done by the combination between deep shadows, a map of colours, the image texture and the combined volume of the rays track. The introduction of **multiplanar** reformatting has enabled the possibility of doing any section arbitrary chosen within the obtained data. In obstetrics this is important for measurements and the reconstruction of critical images (for example the visualization of the four rooms) or the orthogonal scanning of the face and soft palate. All these are dependent on the software' algorithms and the computer's power of processing, the most important of all the devices (1,4).

The volume rendering in medical Imagistics has its roots in the engineering computerized graphics, it has developed as a separate branch of the art, especially in literature computerized graphic before and independent of its application in medicine. One of the first pioneers in this field is Marc Levoy from the University of North Caroline (then at Stanford) (4).

The first English guide of 3D ultrasonography in obstetrics and gynaecology appea-

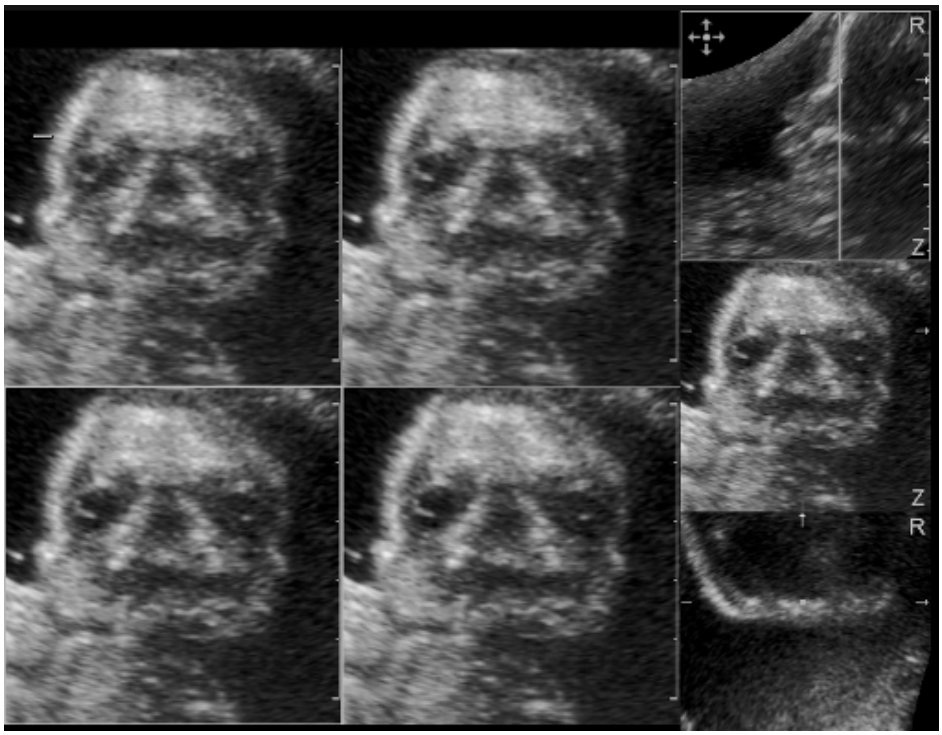

Fig. 3. In the fetus from the previous image, through multiplanar rendering technique, the coronal plane of the face making evident the bone structure of the face. Serial sections at 0,2 mm, having as starting point the base of the nasal bone. The fetal crystalline is seen in both eyeballs

red in 1996, edited by Kazunori Baba and Davor Jurkovic at the Royal University hospital in London. Eberhard Merz hosted the first World Congress of 3D ultrasonography in obstetrics and gynaecology in Mainz, 1997. Many topics of interest about 3D ultrasonography were discused. (1,7).

The important advantage of 3D ultrasonography over the conventional 2D, at the end of the millennium, is the ability to increase the mathernal-fetal relation, it improves the parents understanding of some fetal abnormalities, the recognition and clear confirmation of some abnormalities, for example the cleft lip, polydactyly, micrognatism, malformed ears, crooked legs, malformed spine and other "exterior" visible fetal abnormalities, as a consequence of the benefit brought by the interpretation made by rendering volumes and surfaces (1,9,10).

In an article, Asim Kurjak and his team concluded „Three-dimensional ultrasonography in the prenatal diagnosis: a luxury or a necessity?"

„The main advantage of three-dimensional ultrasonography in perinatal medicine and the prenatal diagnosis includes the scanning in coronal plane, it has improved the assessment of the complex anatomical structures, a surface analyse of the minor deffects, the volumetric measurement of the organs, the visualization of the fetal skeleton in a plastic way, the spatial presentation of the vasculary system and finally the volumes and scanned images storage. It is our definite opinion that the three-dimensional ultrasonography gained a valuable place in prenatal diagnosis, becoming a necessity for the modern centre of perinatal medicine....." (1,4,9,11)

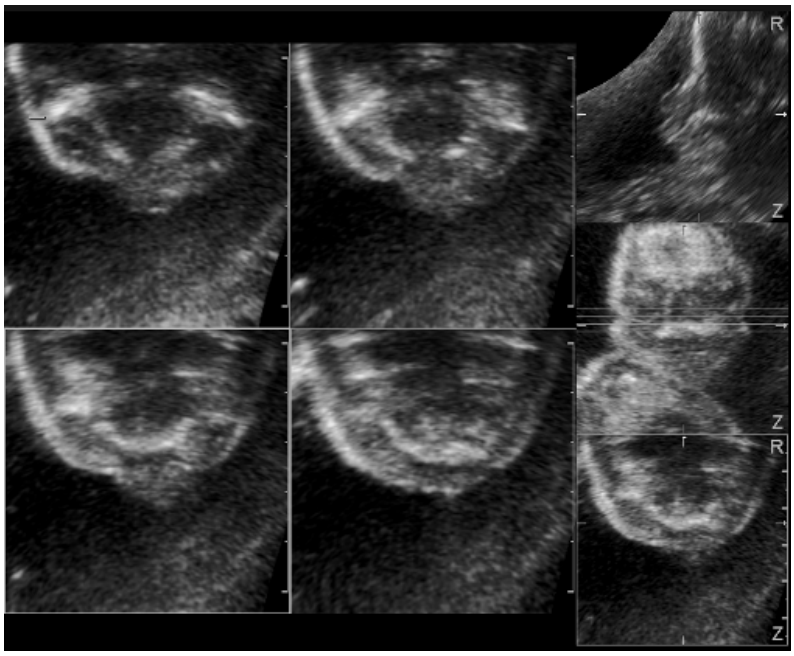

Fig. 4. Transversal sections from1 in 1 mm, through basic coronal plane, chosen in the prevoius diagram, this time with starting point from the palatine bone. It is noticed the rough palate, the orbits base and the malar bone

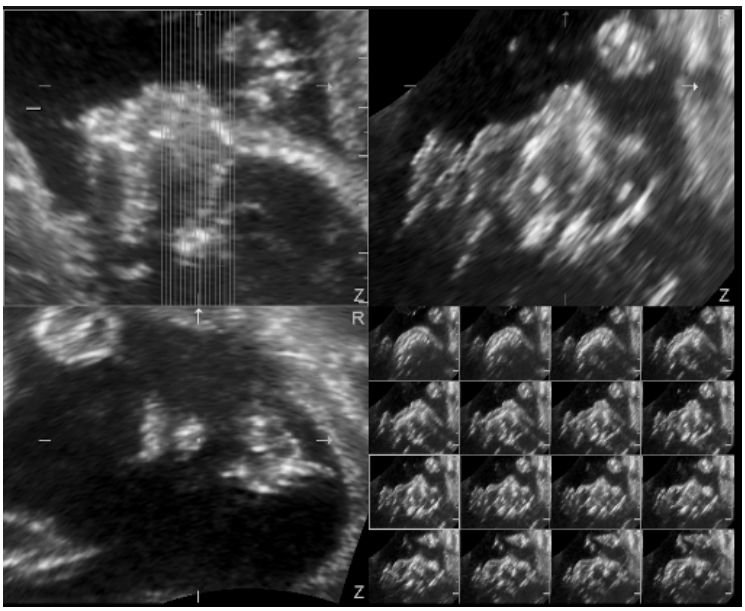

Fig. 5. Transversal sections through sagittal plane of the fetal face, at 1 mm distance. In the upper left part a nasal bone is seen, correspondent in the right corner with both bones in the transversal section. Bottom left to start the green sections, is on the nose, under which - to the left, it highlights the filter and the upper lip

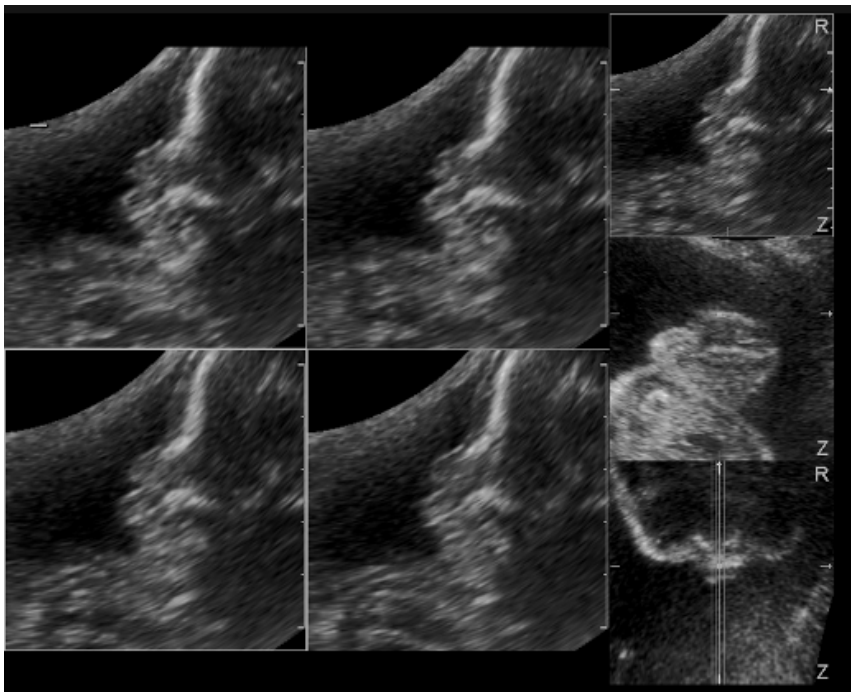

Fig. 6. Parasagitale serial sections, highlighting the nasal bone, the bone palate. On the right, the image from the middle represents the fetal chin, above which are distinguished the lips and the nasal cartilage with nostrils

Another possible benefit of 3D ultrasonography is represented by the possibility of documenting, data storage and communication and also the possibility of new research directions of the developing structures of the human embryo "in utero". Through a digital saving of the data, these can be transfered immediately for another interpretation or a second opinion.

Concerns for 2D ultrasonic study of the fetal face bones, with practical implications dates from the beginning of 2000 - Cicero and his contributors, when it has been noticed the relations between the absence of nasal bone and the trisomy 21 (12). Rotten and the contributors describe the main sections for the definition of normal face through 2D and 3D ultrasonography (13).

The end of 2000 has brought the study of the fetal face through multiplanar three--dimensional ultrasonography, through volume acquisition and its processing. The study of the fetal profile through this method brings new data about maxillary, mandible, naso-maxillo-mandibular angle (14,15). It has also been made a nomogram of the fetal profile sizes, ultrasonographically assessed according to the gestational age (16).

The fetal profile analyzed through 16 parameters measured by a 3D ultrasonography, with the composition of a variable craniofacial index, in *Roelfsema and* his contributors' opinion, it would be useful to estimate the normal and abnormal fetal face (17). Recently, Persico and his contributors have made a nomogram of the length of the nasal bone in fetuses with normal caryotype, at 16-24 gestational weeks, through three-dimensional ultrasonography (18).

The fetal face bones - nasal, maxillary, mandible, the bone palate, those of the orbits, can be studied in dynamic, starting with 12-14

gestational weeks. The best ultrasonographic images can be obtained after 21 gestational weeks, when the fetal size and the connection with the amniotic fluid are good for a volume acquisition, as for the 2D ultrasonography (fig. 1, 2, 3, 4, 5, 6).

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INCIDENCE OF ENDOMETRIOSIS - A MATTER OF PUBLIC CONCERN

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INCIDENCE OF ENDOMETRIOSIS-A MATTER OF PUBLIC CONCERN (Abstract): There is an increasing trend in the incidence of endometriomas, the disease affecting about 5-10% of women of reproductive age. **Aim:** To determine the incidence of endometriosis in patients undergoing surgery for gynecological diseases at the "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital from Iasi. **Material and methods:** Retrospective study of 12,178 patients followed up for 5 years (January 2010 to December 2015) assessing the detection rate of endometriosis and possible diagnostic methods, as well as differential diagnosis with other pelvic pathologies. **Results:** Distribution of cases by the six years of study identified an increase in the incidence of endometriosis in our clinic. A possible explication can be the improved diagnostic techniques and, also women's concern with infertility. Of the 12178 operated patients, only 1,100 were diagnosed with endometriosis. The highest incidence was recorded in 2015, 21.10 %. **Conclusions:** The incidence of endometriosis was of 9.03% and showed a rise in the past 6 years, due to the improved diagnostic techniques and women increasing preoccupation with the topic of infertility. **Keywords:** ENDOMETRIOSIS, INFERTILITY, INCIDENCE.

Endometriosis is one of the most frequent diseases in gynecological practice. It affects women of fertile age, being the second cause for surgical interventions after uterine fibromas during premenopause. There is an increasing trend in the incidence of endometriomas, the disease affecting about 5-10% of women of reproductive age (1, 2). It is one of the most important public health concerns, because of the continuous increase in the number of cases. Also, the risk of malignant transformation motivates the numerous researches focused on explaining de development mechanisms and the progression of this disease, 10% of endometrioid carcinomas of the ovary are associated with endometrioid carcinomas of the uterus (3, 4). Symptoms represent another problem, because they can vary from total absence to progressive course with multiple complications (5). Also, it is important to evaluate the implications of the disease regarding infertility. Both endometriosis and the surgical treatment for this disease have fertility consequences, with alterations of the AMH values (6).

The incidence of this disease grew ex-

ponentially, accounting for 2% of the general population, being the third cause for gynecological examination for infertility, chronic pelvic pain, dyspareunia and dysmenorrhea. The prevalence of endometriosis has also an increasing trend, up to 25-50% of infertile women being diagnosed with endometriosis, and about 30-50% of women with endometriosis being infertile. Endometriosis affects 7-15% of women of reproductive age, and 25-30% of all women with infertility, having a high social impact. The incidence of this disease rose exponentially following the introduction of new diagnostic methods such as endovaginal ultrasound and exploratory laparoscopy (7). Endometriosis affects 1% of asymptomatic women and up to 60% of women with chronic pelvic pain (8, 9). Endometriosis has a major social impact, and it was reported that quality of life was significantly reduced in women with endometriosis (9, 10). It is diagnosed in women aged 12-80 years old, with a peak incidence around the age of 28 years old (11, 12).

Studies show that endometriosis has a higher incidence in Caucasian, compared with Afro-Americans or Asian women (13, 14). Also, patients with endometriosis are taller, thinner, and have a significantly lower body mass index (BMI) (14).

The main objectives of this research are to evaluate the incidence of endometriosis in patients undergoing surgery for gynecological diseases at the Iasi "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital, and the impact of endometriosis on quality of life.

MATERIAL AND METHODS

Given that the global estimates of the incidence of endometriosis vary considerably, the aim of our study was to identify the incidence and prevalence of this disease among the gynecological patients admitted to the "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital, Iasi.

This is a retrospective study of 12,178 patients followed up from January 2010 until December 2015 with regard to the detection rate of endometriosis and possible diagnostic methods, as well as differential diagnostics with other pelvic diseases. This study included all patients admitted to the gynecology department. Patients admitted to the obstetrics department were excluded. Surgical interventions were performed either laparoscopically or by open laparotomy. A number of 1,100 of patients met the inclusion criteria, the ones diagnosed with endometriosis either pre or post operatory, histologically confirmed.

Data processing was carried out using *SPSS 18.0.*

RESULTS AND DISCUSSION

In the total of 1,100 surgeries performed during the interval 2010-2015 at the "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital from Iasi the highest incidence was recorded in 2015 - 21.1%. The trend for the following period was upward (fig. 1). The prevalence of surgical interventions in the Gynecological Department ranged from 18.5% in 2010 to 13.8% in 2015, while the trend during the study interval was relatively constant (y = 19.31-0.75x), approximately 14%.

Surgeries for infertility peaked in 2013-19.3% and 2014-19.2%, while the trend for the following period of time was relatively constant (y = 17.41-0.21x), with an estimation for the near future of 16%. During the study interval, the most frequent surgeries involved the ovaries (62.5%), and the peritoneum (31.8%) (fig. 2).

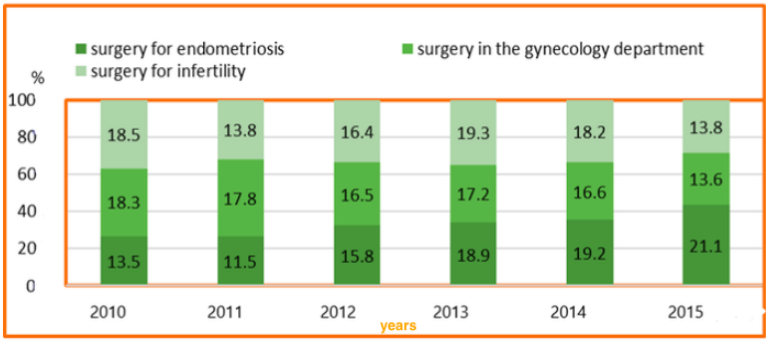

Fig. 1. Distribution of surgeries by study years

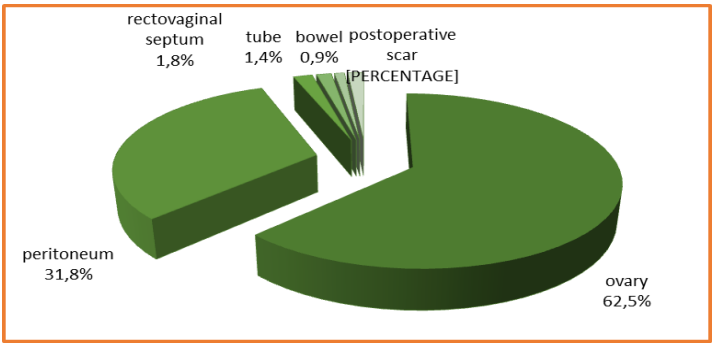

Fig. 2. Frequency of surgical interventions performed between 2010 and 2015 in patients with endometriosis, according to their location

Distribution of cases which underwent ovarian surgery showed a slight increase beginning with 2012 and ranged from 114 cases in 2012 to 139 in 2015.

The number of surgeries for peritoneal endometriosis was relatively constant between 2013 and 2015 (72-75 cases, while the number of surgeries for C-section scar, intestinal, rectovaginal s and fallopian tube endometriosis was low (fig. 3).

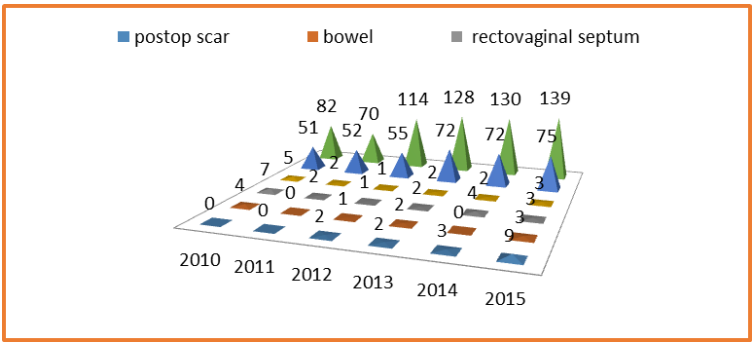

Fig. 3. Distribution of operated endometriosis cases by study years

During the study interval, surgeries for infertility accounted for 10-11% of the total surgical interventions, while surgeries for endometriosis ranged from 9.9% to 14% (fig. 4).

By analyzing the medical records of patients admitted for endometriosis treatment, we found that the main symptom described was chronic pelvic pain, followed by other complaints such as: infertility, dysmenorrhea, dyspareunia, menstrual spotting, ovarian tumor detected during a routine check-up, abnormal menstrual cycles, bowel malfunction and urinary complaints.

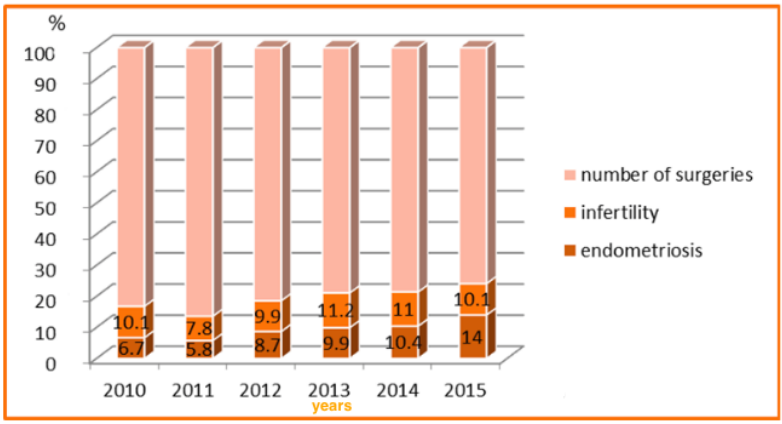

Fig. 4. Prevalence of cases with endometriosis and infertility from the total surgical intervention in the gynecology department

Infertility was described as the impossibility of conceiving after regular, unprotected sexual contacts for 12 months, after excluding any documented cause of male infertility. Also evaluated was the social status of these patients and we noticed that the patients with a higher education level and those with a good socio-economic status had better use of healthcare services, the last ones representing 94.5% of the total number of endometriosis patients. According to civil status, 847 of our patients were in a stable relationship. Of these 72.54% were married, 15.34% were not married, and only 12.12% were cohabitating. As to the place of residence, 953 of the 1,100 patients lived in an urban area and only 147 in rural areas.

Endometriosis is a major public health concern mainly because of the continuously increasing number of cases and the risk of ovarian malignancy. Ten percent of endometrioid carcinomas of the ovary were associated with endometrioid carcinomas of the uterus, fact that motivates the numerous researches focused on explaining de development mechanisms and the progression of this disease (6). The incidence of this disease rose exponentially following the introduction of new diagnostic methods such as intravaginal ultrasound and laparoscopy, an incidence of 2% in the general population being reported. It represents the third cause of gynecological assessment for infertility, chronic pelvic pain, dyspareunia and dysmenorrhea.

The prevalence of endometriosis rose dramatically going up to 25-50% in women with infertility, 30-50% of women with endometriosis suffering also from infertili-

ty. Endometriosis affects 7-15% of women of fertile age, representing 25-30% au sterility cases, with an important social impact (7). The main form of endometriosis that can be found prior to surgical intervention is ovarian endometrioma. Knowing the importance of early diagnosis, pelvic pain, dysmenorrhea, dyspareunia, or infertility can suggest it. Of the total number of patients, 1,100 had an intraoperative diagnosis of pelvic endometriosis, histologically confirmed, accounting for 9.03%. This finding agrees with the data in the literature reporting an incidence of up to 10% (6,15).

Distribution of cases by the six years of study identified an increase in the incidence of endometriosis in our clinic. A possible explication can be the improved diagnostic techniques, and also women's concern with infertility. Ovarian endometriomas are a common manifestation of endometriosis. These "chocolate" cysts are surrounded by a pseudo capsule adherent to the normal ovarian tissue. The ovary can be attached to the peritoneum, bowel and pouch of Douglas, frequently with modified pelvic anatomy.

The incidence of cesarean section delivery grew in the past years so the number of cases with endometriosis at the level of the cesarean scar was also rising. Because endometriosis is frequently underdiagnosed because of the absence of symptoms, its prevalence cannot be precisely estimated. According to studies endometriosis is present in 1% of asymptomatic patients, and in 60% of women with chronic pelvic pain (8, 9).

In 2012, a German study concluded that the incidence of endometriosis is 0.8% (8, 9), and a 2013 study showed that 62% of patients presenting chronic pelvic pain or dysmenorrhea had endometriosis (9, 16). But there are also studies reporting an incidence of endometriosis of 2% in the general population (17) associated with chronic pelvic pain in 70% of cases and with infertility in 25-30% (18, 19). Vercellini *et al.*, in a 2016 study state that 5% of women of reproductive age suffer from this disease (20, 21, 22).

Of the 12,178 operated patients in "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital from Iasi, only 1,100 were diagnosed with endometriosis. From January 2010 till December 2015, there was an increase for the incidence of endometriosis in women in the Moldavian area. Therefore, endometriosis is a disease with constantly increasing incidence, whose etiology is not fully elucidated and, moreover, with a major impact on physical, mental and social health.

From a demographic point of view, the patient with pelvic endometriosis is a woman residing in an urban area, married, with higher education and a high socioeconomic status, presenting to the physician with chronic pelvic pain, dysmenorrhea, dyspareunia and/or infertility. Although all these symptoms have a high sensitivity for endometriosis, their specificity is at the opposite pole, being low. Therefore, both the unique symptoms and the symptomatology should be noted by the clinician in a timely manner, with the exclusion or confirmation as soon as possible of the diagnosis of endometriosis. In our study, we brought up to date the literature data regarding the incidence of endometriosis, and also contributed with the demographic characteristics of our study group.

With the help of the data from literature, we conducted an empirical research of an actual theme, endometriosis being one of the great unknowns of medicine, a fluid

theme that continues to surprise elements of novelty that emerge to the surface, frequently succeeding to induce in error even a close researcher. So, as to endometriosis incidence, we provided not only a much needed up-to-date of known statistical data but also a precise analysis at regional level. The incidence of endometriosis and its impact on women life were studied in a large sample group.

That is why, one of the perspectives opened by this article is the need for a multicenter study, that can sum up data from all regions of Romania in order to obtain a clearer image of these parameters, nationwide, and to compare the Romanian regions and why not, international studies regarding this matter. Also, a multicenter study presents the advantage of identifying new risk factors for the development of endometriosis.

CONCLUSIONS

In our study, the incidence of endometriosis was of 9.03%, with an increase in the past 6 years due to the improved diagnostic techniques and infertility preoccupation among women. Demographically speaking, women with pelvic endometriosis represents the modern person who presents to the hospital with chronic pelvic pain, dysmenorrhea, dyspareunia and infertility. Patients with endometriosis confront themselves with a series of clinical symptoms described above, and also with emotional instability, high stress levels, all of these having a negative impact on the quality of their lives.

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NEWS

NEOADJUVANT CHEMOTHERAPY VERSUS UPFRONT DEBULKING SURGERY IN ADVANCED TUBO-OVARIAN CANCER

In a comparison of neoadjuvant chemotherapy and upfront debulking surgery, they noted no difference in overall survival among 1220 women with International Federation of Gynecology and Obstetrics (FIGO) stage III–IV tubo-ovarian cancer. Median overall survival, which was the primary endpoint of the analysis, was 26·9 months (IQR 12·7–50·1) in patients randomly allocated to receive upfront debulking surgery, and 27·6 months (14·1–51·3) in women randomly assigned to neoadjuvant chemotherapy (hazard ratio [HR] 0·97, 95% CI 0·86–1·09; p=0·586). In subgroup analyses, a gain of 3 months in median overall survival was noted for neoadjuvant chemotherapy as compared with upfront debulking surgery in 230 patients with stage IV disease, whereas overall survival was better with upfront debulking surgery than with neoadjuvant chemotherapy in 266 women with stage IIIC disease and extrapelvic metastases smaller than 5 cm (Anna Fagotti, Giovanni Scambia. Neoadjuvant chemotherapy versus upfront debulking surgery in advanced tubo-ovarian cancer.*The Lancet Oncology* DOI: https://doi.org/10.1016/S1470-2045(18)30625-9).

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ASPECTE CLINICO-EPIDEMIOLOGICE ALE EXCESULUI PONDERAL ŞI PARTICULARITĂŢI NUTRIŢIONALE LA COPILUL ŞI ADOLESCENTUL DE VÂRSTĂ ŞCOLARĂ

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CLINIC AND EPIDEMIOLOGIC ASPECTS OF OVERWEIGHT AND NUTRITIONAL HABITS OF SCHOOLCHILDREN AND TEENAGEERS (Abstract): Obesity is a complex, pluricausal disease that develops through the interaction of social, behavioural, cultural, physiological, metabolic and genetical causes. **Material and methods**: The study was conducted on 1764 school age children from Iaşi city. Data was collected by measuring their weight, height and arterial pressure. 283 children were diagnosed with weight excess and have been questioned about their nutritional habits and life quality. **Results**: It has been concluded that 50.17% of the children were breastfed in the first months of life. The weight excess was frequently correlated with: irregular meal schedule (57.59%), roast meal cooking (69.61%), eating white bread (77.38%), predilection for fat meat (66.78%), processed cheese and butter (69.25%), french fries (68.19%), everyday dessert (62.89%), highly concentrated sweets (cookies, chocolate) (63.95%), biscuits, snacks, chips (73.85%) and sweet juices (66.42%). Inactivity was also an important factor, often associated with weight excess, considering that 60.42% of the children spend their spare time watching TV or playing computer games and only 32.86% of the children practice sport during their spare time. **Conclusions**: Obesity and overweight are pathologies with increasing incidence in our country and they are caused by a combination between genetical factors and, more important, nutritional habits and the amount of time dedicated to physical exercise. **Key words**: OBESITY, ALIMENTARY INVESTIGATION, BODY MASS INDEX, DIAGNOSIS OF OBESITY.

Obezitatea este o boală complexă, multifactorială, care se dezvoltă din interacţiunea dintre factorii genetici şi cei de mediu. Mecanismele ei patogenice sunt încă incomplet cunoscute, dar implică integrarea factorilor sociali, comportamentali, culturali, fiziologici, metabolici şi genetici (1).

Folosind diverse tehnici de măsurare clinicienii pot identifica rapid copiii supraponderali sau obezi. Cu toate acestea diagnosticul este doar primul pas şi cel mai uşor. Cu adevărat dificilă este ajutarea familiior să înţeleagă obezitatea ca o problemă, să conducă măsurile de prevenţie şi să iniţieze tratamentul. Recunoaşterea obezităţii de către părinţi reprezintă un pas esenţial în dieta şi schimbarea stilului de viaţă al acestor copii (2). Atât factorii genetici, cât şi cei de mediu contribuie la etiologia obezităţii.

Obezitatea din cadrul sindroamelor genetice este rară, dar important de recunoscut deoarece poate fi asociată cu complicaţii medicale specifice, cu posibil răspuns la tratament. Multe din aceste sindroame sunt caracterizate prin deficit statural, întârziere în dezvoltare, trăsături dismorfice şi hipo-

gonadism. Ca regulă generală, se poate spune că în situaţiile în care copilul nu prezintă deficit statural pentru vârstă şi sex, nu are întârziere în dezvoltare sau trăsături dismorfice, este puţin probabil ca obezitatea să se datoreze unei tulburări endocrine sau unui sindrom genetic (3).

De-a lungul timpului s-au produs modificări în procentul de principii alimentare din dietă. Se apreciază că în ultimii două sute de ani consumul de proteine a crescut de la 10 la 13%, a scăzut consumul de glucide complexe de la 65 la 45%, iar consumul de lipide a crescut de la 25 la 40% (4). S-a remarcat, de asemenea, că încercarea de contrabalansare a consumului de grăsimi se face prin glucide simple şi nu prin glucide complexe (5). Alţi factori includ scăderea activităţii fizice, inclusiv la copii şi creşterea activităţilor sedentare. Activităţile sociale determină un aport alimentar independent de senzaţia de foame. Supraalimentarea la naştere poate duce la dereglări neuroendocrine care converg la formarea fenotipului obez (6).

MATERIAL ŞI METODĂ

Lotul de lucru a fost alcătuit din copii de vârstă şcolară din oraşul Iaşi, elevi ai şcolilor: „Petru Poni", „George Coşbuc", „Miron Costin", „Ion Simionescu", Grupul Şcolar Economic de Turism şi Şcoala numărul 39. Iniţial, aceşti copii au fost evaluaţi din punct de vedere al greutăţii, taliei şi tensiunii arteriale. Pe baza indicelui de masă corporală, raportat la valorile citate în tabele ca fiind normale pentru vârstă şi sex, grupul supus evaluării primare a fost împărţit în lot control şi lot de studiu (supraponderalitate = între percentila 85 şi 95, şi obezitate = peste percentila 95). Copiii cu exces ponderal (peste percentila 85), au fost chestionaţi ulterior în privinţa particularităţilor nutriţionale (ancheta alimentară) si a calităţii vieţii.

Tensiunea arterială a fost considerată normal înaltă dacă valoarea sistolică sau cea diastolică s-au aflat între percentilele 90 şi 95 pentru vârstă, sex şi înălţime şi am încadrat ca hipertensivi copiii având fie tensiunea sistolică, fie cea diastolică peste percentila 95. Valorile corespunzătoare pentru vârstă, sex şi înălţime au fost cele recomandate de Programul Naţional de Educaţie pentru hipertensiune arterială (7).

REZULTATE

1. Lotul a cuprins **1764** de copii de vârstă şcolară, dintre care 828 (**46,93%**) **băieţi** şi 936 (**53,06%**) **fete**.
2. Analiza IMC în funcţie de sex şi vârstă a permis clasificarea lor în: **obezi** – 54 de copii (**3,06%**), **supraponderali** – 229 copii (**12,98%**), **normoponderali şi subponderali** – 1481 copii (**83,95%**).
3. Determinarea TA la totalul de 1764 copii s-a concluzionat cu **83 de copii** (**4,70%**) având valori crescute ale tensiunii arteriale. Dintre cei 83 de hipertensivi, **14** sunt **obezi**, iar **21** sunt **supraponderali**.
4. Distribuţia copiilor cu exces ponderal după vârstă şi sex este prezentată în tabelul I:
   - Fete: **130 din 936** (**13,88%**), din care 25 obeze (2,67%), 105 (11,21%) supraponderale.
   - Băieţi: **153 din 828** (**18,47%**), din care 29 obezi (3,50%), 124 (14,97%) supraponderali.
5. Am constatat apartenenţa la un nivel socio-economic modest în 72 de cazuri (25,44%), mediu în 194 de cazuri (68,55%) şi superior în 17 cazuri (6%).

În urma anchetei alimentare realizate în cazul celor 283 de copii cu exces ponderal, atât obezi cât şi supraponderali, s-au obţinut următoarele rezultate:

- Din declaraţiile familiilor reiese că au primit o alimentaţie naturală în primele luni de viaţă un număr de 142 de copii (50,17%), mixtă 92 de copii (32,50%) şi artificială 49 de copii (17,31%).

TABELUL I
Repatiţia cazurilor pe grupe de vârstă şi sexe

Vârsta	7-10 ani	11-14 ani	15-18 ani
Obezi (nr.cazuri %)	9 (4%)	22 (3,13%)	23 (2,75%)
Supraponderali (nr.cazuri %)	36 (16%)	111 (15,76%)	82 (9,82%)
Normoponderali (nr.cazuri %)	180 (80%)	571 (81,11%)	720 (87,43%)

- 161 de copii (56,95%) declară un exces de aport alimentar impus, iar voit - 122 de copii (43,04%). În primul caz este vorba de copii la care familia, din diferite motive, insistă în favoarea unui aport alimentar crescut.
- 114 copii (40,28%) au afirmat că au primit în mod repetat suplimente de vitamine şi minerale.
- 86 de cazuri – 30,38% - au declarat că au urmat la un moment dat o dietă, în cele mai multe situaţii fiind vorba despre diete restrictive, care nu au cuprins un program regulat de mese şi de exerciţii fizice, soldate, în general, cu eşec.
- 163 de copii – 57,59% descriu un program neregulat de mese, în timp ce 120 de copii – 42,40% au afirmat că primesc trei mese principale şi două gustări, adecvate vârstei.
- 197 dintre copii – 69,61% - preferă alimentele prăjite, iar 86 dintre copii – 30,38% - pe cele preparate în alte moduri (fiert, la cuptor, grătar) .
- obişnuiesc să consume frecvent carne grasă, mezeluri 189 de copii – 66,78%, brânză topită, unt 196 copii – 69,25%, smântână 97 de copii – 34,27%, iar 148 de copii – 52,29% consumă mai mult de 3 ouă/săptămână.
- În ceea ce priveşte consumul de pâine : 218 copii preferă pâinea albă - 77,38% şi doar 65 primesc pâine neagră sau intermediară - 22,62%. De asemenea, 195 dintre copii consumă < 10 felii/zi - 68,90%, 68 consumă între 10-20 felii/zi – 24,02%, iar 20 de copii mănâncă > 20 felii/zi – 7,06%.
- Declară un consum zilnic de cartofi un număr de 43 de copii – 15,19%, de 4-5 ori/săptămână – 85 de copii – 30%, de 2-3 ori/săptămână – 129 de copii – 45,58%, şi mai rar de 2/săptămână - 26 de copii – 9,18%. Preferă cartofi prăjiţi 193 dintre copii – 68,19%, iar fierţi sau piure 90 dintre copii – 31,80%.
- Consumă lapte – zilnic – 128 de copii – 45,22%, de 4-5 ori/săptămână - 29 de copii – 10,24%, de 2-3 ori/săptămână – 67 de copii – 23,67%, deloc – 59 de copii – 20,84%. Preferă lapte normal 139 de copii – 49,11% şi degresat/parţial degresat 85 de copii – 30,03%.
- Primesc desert zilnic 178 de copii – 62,89%, de 4-5 ori/săptămână – 21 de copii – 7,42%, de 2-3 ori săptămână – 52 de copii – 18,37% şi rareori (< 2/ săptămână) 32 de copii – 11,30%.
- Consumă fructe zilnic un număr de 147 de copii – 51,94%.
- Primesc dulciuri foarte concentrate (prăjituri, ciocolată) 181 de copii – 63,95%.
- Declară un consum foarte frecvent de biscuiţi, snacksuri, chipsuri 209 copii - 73,85%.
- Primesc paste făinoase, pizza – zilnic - 10 copii – 3,53%, de 4-5 ori/săptămână - 19 copii – 6,71%, de 2-3 ori/săptămână - 80 de copii - 28,26% şi rar 174 copii – 61,48%.
- Orez – de 4-5 ori/săptămână - 6 copii – 2,12%, de 2-3 ori/săptămână - 57 copii – 20,14%, rar (< 2/săptămână), 220 copii – 77,73%.

- Consumă zahăr – zilnic 72 dintre copii – 25,44%, rar 102 copii – 36,04%, deloc 109 copii – 38,51%.
- Declară un aport crescut de sare un număr de 254 de copii – 89,75%.
- Apă < 1 l/zi – 131 de copii – 46,28%, 1-2 l/zi – 127 copii – 44,87%, > 2 l/zi – 25 de copii – 8,83%.
- Sucuri dulci – zilnic – 165 de copii – 58,30%, de 4-5 ori/săptămână - 23 de copii – 8,12%, de 2-3 ori/săptămână - 37 de copii – 13,07%, foarte rar – 58 de copii – 20,49%.
- Obişnuiesc să primească zilnic mic dejun un număr de 133 de copii – 47%.
- Cea mai consistentă masă a zilei este reprezentată de micul dejun pentru 11 copii – 3,88%, de prânz pentru 157 de copii – 55,47%, cină la 72 de copii – 25,44%, iar prânz şi cină în proporţii egale pentru 43 de copii – 15,19%.
- Primesc cina după ora 20 – 105 copii – 37,10%.
- Obişnuiesc să mănânce la TV – 150 de copii – 53%.
- Petrec timpul liber la calculator/TV - 171 de copii – 60,42%, iar în aer liber, cu activităţi fizice – 112 copii – 39,57%.
- Efectuează orele de educaţie fizică la şcoală – 184 de copii – 65,01%.
- Practică un sport în timpul liber doar 93 de copii – 32,86%.

DISCUŢII

1. Prevalenţa excesului ponderal în lotul de copii şcolari luat în studiu (16,04% dintre care 3,06% copii obezi şi 12,98% copii supraponderali) este mai mică decât cea descrisă în statisticile din ţările vest europene, dar mai ales din Statele Unite (15-16% doar copiii obezi), dar confirmă că problema excesului ponderal este reală şi în creştere şi la noi în ţară.

2. Repartiţia cazurilor pe grupe de vârstă arată o predilecţie mai mare a acestei patologii pentru vârstele mai mici, iar repartiţia pe sexe indică o uşoară predominenţă a sexului masculin (18,47% faţă de 13,88%), motivată probabil şi de preocuparea mai mare pentru aspectul exterior, pe care o manifestă fetele şi în special adolescentele, comparativ cu băieţii, la această vârstă.

3. Din totalul de 1764 de copii şcolari urmăriţi, 83 (4,70%) au prezentat valori crescute ale tensiunii arteriale. Dintre ei, un număr de 35 de copii au prezentat exces ponderal. Important este faptul că, deşi la vârsta copilăriei hipertensiunea arterială este în principal secundară, în cazurile întâlnite ea are caracteristicile unei hipertensiuni esenţiale, similară celei care apare la vârstă adultă.

4. Majoritatea copiilor luaţi în studiu au avut o alimentaţie naturală în primele luni de viaţă (50,17%). Cu toate acestea, s-a observat că procentul copiilor obezi alimentaţi artificial este aproape dublu faţă de cel al copiilor supraponderali alimentaţi artificial, sugerând că acest tip de nutriţie, iniţiată precoce, ar putea totuşi constitui un factor favorizant în dezvoltatea unui exces ponderal ulterior.

5. Au fost mai frecvent corelate cu excesul ponderal : programul neregulat al meselor (**57,59%** dintre copii), prepararea alimentelor prin prăjire (**69,61%**), consumul de pâine albă (**77,38%**), preferinţa pentru carne grasă, mezeluri (**66,78%**), brânză topită şi unt (**69,25%**), cartofi prăjiţi (**68,19%**), desert zilnic (**62,89%**), dulciuri foarte concentrate (prăjituri, ciocolată) (**63,95%**), biscuiţi, snacksuri, chipsuri (**73,85%**) şi sucuri dulci (**66,42%**).

6. Sedentarismul a fost, de asemenea, un factor mai frecvent asociat cu excesul ponderal, întrucât **60,42%** dintre copii au declarat că petrec timpul liber la calculator/ TV, şi doar **32,86%** practică un sport în timpul liber.

CONCLUZII

Prin urmare, obezitatea şi supraponderalitatea reprezintă o patologie a cărei incidenţă este în creştere şi în ţara noastră, în determinarea căreia se îmbină aspecte ce ţin de factorul genetic, dar mai ales de comportamentul alimentar şi de timpul alocat activităţilor fizice.

În apariţia excesului ponderal familia joacă un rol semnificativ, în primul rând prin adoptarea unui anumit stil de viaţă şi în al doilea rând prin solicitarea/impunerea unui aport alimentar crescut, lucru mai evident la copiii de vârstă mai mică.

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NOUTĂŢI

β4-TIMOZINA - UN CANDIDAT PENTRU TRATAMENTUL ACCIDENTELOR CEREBRALE ?

Următoarea frontieră în terapia accidentelor cerebrale ar putea purta numele generic de terapie neurorestaurativă. Exista ipoteza conform căreia imediat după accidentul cerebral apar anumite modificări celulare analoge diferitelor stadii de dezvoltare celulară iniţială. Creşterea expresiei proteinelor de maturare în zona limitrofă zonei infarctate sugerează un răpuns activ de reparare sau recondiţionare a leziunii ischemice. Terapia neurorestaurativă are ca ţintă celulele neuronale, oligodendrocitele, astrocitele dar şi celulele endoteliale, în vederea iniţierii neurogenezei endogene, angiogenezei, creşterei axonale cât şi a sinaptogenezei, cu rezultat final recuperarea funcţională. Tratamentul farmacologic include statine, inhibitori de 5-fosfodiesterază, eritropoetină şi donori de monoxid de azot. Această conduită s-a dovedit a îmbunătăţi manifestarile funcţionale după acidentul cerebral. β4-timozina (Tβ4) este o proteină de sechestrare a actinei, implicată în creştere, motilitate şi metastazare celulară ; acţionează atât în cazul celulei tinere cât şi în cazul celulei adulte. La nivel cerebral, precum şi la nivelul miocardului, determină stimularea proceselor de vasculogeneză, angiogeneză şi arteriogeneză. iniţiind devoltarea neuronală, migrarea şi diferenţierea celulară în aria ischemică (Morris DC, Chopp M, Zhang L, Zhang ZG. Thymosin beta4 : a candidate for treatment of stroke ? *Ann N Y Acad Sci* 2010 ; 1194 : 112-117).

M. Guţu

CLINICAL AND ECHOGRAPHIC EVALUATION OF THE EFFECTS OF EPINEPHRINE AND PLATELET-RICH PLASMA SOLUTION ON MODIFIED MCFARLANE SKIN FLAP IN RATS

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CLINICAL AND ECHOGRAPHIC EVALUATION OF THE EFFECTS OF EPINEPHRINE AND PLATELET-RICH PLASMA SOLUTION ON MODIFIED MCFARLANE SKIN FLAP IN RATS (Abstract): **Aim**: The aim of the study was to test the effects of the administration of the platelet-rich plasma solution in the same time with epinephrine in frequently used skin flap model in rats, and to notice the vascular response of the flap pedicles using ultrasound exploration. **Material and methods**: the experimental study took place at the Advanced Research Centre for Experimental Medicine (C.E.M.E.X), at the "Grigore T. Popa" University for Medicine and Pharmacy Iasi. We used 27 adults male Whistar rats, 6 of them were used as donor source for the PRP (platelet rich plasma) solution. The rest were split in 3 groups of study: on the first group we performed the modified McFarlane 8 x 3 cm, the second group received epinephrine 0.1 mg/kg intraperitoneally, and the third group received 1 mL solution of PRP subcutaneously, in the same points of the flap, alongside epinephrine. In the third postoperative day, a clinical assessment and photographic documentation was done. On the same day, the ultrasound exploration was done. **Results**: we calculated the percentage of skin necrosis, by calculating the areas of normal and necrotic skin, and comparing them. The highest rate of necrosis was observed in the second group (55.3 %) compared with the first group (48.5 %) and the group that received PRP (29.7 %). The second group also had the smallest pedicle's sizes, corelating with the area of skin necrosis. The biggest pedicles were found in the third group. The epinephrine only group had a very important variation of the pedicle's flow speed. **Discussion:** the ultrasonography is a machine and operator-dependent procedure, non-invasive and quick to perform, which brings valuable date regarding small perforators of the skin. In order to analyze such small perforator, like the pedicles of this modified skin flap, it is important to set the correct modes and to choose appropriate anatomical landmarks. Sometimes, it is difficult to measure the dimensions of the pedicles and their flow speed too. Clinical assessment of the flap was assessed in 5th postoperative day. Special digital software is helpful when we want to distinguish other colors apart from white and black, and to strictly delineate the normal skin from the necrotic one. **Conclusions**: our data do corelate with the literature, confirming the effect of the PRP on this type of rat skin flap, even though a small quantity of epinephrine was added. **Keywords**: MCFARLANE SKIN FLAP, PRP, EPINEPHRINE, ULTRASOUND, PERFORATOR PEDICLE.

Severe trauma or tumoral surgical interventions can lead to large soft tissue defects, which result in the exposure of vessels, nerves or osteo-articular structures. The defect has to be repaired taken into consideration the functional and esthetic requirements of the area involved. In time, the development of the vascular physiology of the human and animal skin flaps has led to a great variety of options regarding these reconstructions, no matter if it is a distant free flap or a local perforator free-style flap.

In order to better understand and to avoid the possible complications and failure of the skin flaps, which can lead to local or systemic infections, functional impairment or even amputations, several animal models were used to test a variety of substances that can produce vasodilation, or to study some vascular mechanisms, such as the delay phenomenon or ischemia-reperfusion injuries.

One of the most used animal skin flap models in experimental surgery is the modified McFarlane rat dorsal skin flap. The first description of the flap was in 1965, with a 10 x 4 cm dimension, being cranially based. Since then, there were many changes regarding whether the pedicle should be cranial or caudal, and the dimensions varied between 2 x 7 to 5 x 10 cm (1).

The rat dorsal skin has 3 main arterial sources, the lateral thoracic, the posterior intercostal and the deep circumflex iliac vessels. Usually, the flap is designed to measure 8 x 3 cm, raised at the level of the deep fascia, caudally based, with 2 pedicles coming from the deep iliac vessels.

This design has multiple advantages: it is a pedicled and random skin flap type, the so called rando-axial flap, it has a predictable skin necrosis area and the vascular pedicle is consistent (2, 3).

Using this McFarlane dorsal skin flap as an experimental model, many substances were tested in order to improve the necrosis rate of the random part of the flap, usually the cranial part, and to observe their angiogenic and vasodilatory effects. One of these substances is the Platelet-Rich Plasma solution (PRP), which has been injected in the subcutaneous layer of the flap, predominantly at a certain distance from the pedicles of the flap. The intradermal administration of an inactive PRP solution has led to the decrease of the flap necrosis (4).

Intraperitoneal injection of epinephrine decreased the skin survival of a modified McFarlane flap, which measured 7 x 2 cm, confirming the vasoconstrictive effects of the epinephrine on the cutaneous vessels (5).

Imagistic explorations are frequently used in planning a flap, especially for perforator-based flaps. Ultrasound based technologies are quite simple to practice, non-invasive and are much less expensive than computer tomography or magnetic resonance imaging (MRI). Taken into consideration the fact that the level of dissection when using a perforator flap is not very deep, the color Doppler ultrasonography was successfully used to locate the best and the nearest perforator pedicle adjacent to the defect (6). The use of the color Doppler ultrasound exploration was proved also in planning a free flap reconstruction, when using a free antero-lateral thigh flap for head and neck reconstruction (7).

The aim of the study was to test the effects of the administration of the platelet-rich plasma solution in the same time with epinephrine in frequently used skin flap model in rats, and to notice the vascular response of the flap pedicles using ultrasound exploration.

MATERIAL AND METHODS

The experimental study was performed at the Advanced Research Centre for Experimental Medicine (C.E.M.E.X), at the "Grigore T. Popa" University for Medicine

and Pharmacy Iasi. We used 28 adults male Whistar rats, which were hosted in special cages, with constant temperature and a day/night cycle from 7 a.m. to 7 p.m. The animals received water and food *ad libitum*. Their weight varied between 262-493 g.

The PRP solution was obtained from 6 donor rats, by cardiac puncture. The donor rats were sacrificed. From each rat a volume of 6 to 8 ml of blood was collected. The blood was transferred in special heparinized tubes. The tubes were then centrifugated at a speed of 3,100 rotations/minute, for 10 minutes. After this, the superior layer was separated from the sediment and the content was transferred in small tubes which were again centrifugated for 15 minutes, at a rate of 3,600 rotations/minute. The PRP solution was then transferred in 1 mL recipients.

There animals were divides in 3 groups, 7 animals each. Before any surgical procedure, the animals were shaved and the design of the caudally based dorsal flap (8 x 3 cm) was drawn after the induction of the anesthesia with Isofluran.

During the surgical procedure, the animals were under continuous inhalatory anesthesia, and for the group 1 (named BO1-BO7; animal BO4 died and was replaced by animal BO8*) the flap was raised at the level of the deep fascia, and sutured back to its place after the interposition of a sterile synthetic material, to prevent neovascularization from the subjacent tissue. Under the same sterile and anesthetic conditions, the group 2 (named BO8-BO14) received 0.1 mg/kg epinephrine by intraperitoneal injection, after the dissection and the suture of the flap. The third group (named BO20-BO27, BO22-donor) underwent the same protocol for anesthesia and dissection as the other two, but 1ml of PRP solution was injected subcutaneously at a distance of 5 cm from the pedicles of the flap, before the suture of the flap. After the suture was done, 0.1 mg/Kg of epinephrine was injected intraperitoneally.

The echography was done using a Vevo 2100 Imaging System machine, in the 5th postoperative day. The device is for animal study purposes, and we used the B-Mode for identifying the anatomical landmarks and for putting the transducer in the same place on the flap, the PW mode (pulsed wave) for measuring the direction and speed of the flow, and the color Doppler mode for a better identification of the vessels.

To perform the echography, the animals were under anesthesia with Isofluran and 0.3 U of Ketamine and Xylazine solution were injected (Ketamine and Xylazine solution was a mixture of 0.9 U Ketamine and 0.1 U Xylazine) throughout the procedure when necessary. The animal was placed in prone position. We set the General and Vascular mode for the exploration.

The transducer was placed at the base of the flap, on the cutaneous projection of the pedicles. We started by using the B mode, to observe the local anatomy and to set the landmarks. We switched to the Color mode to see the vascular flow and to identify the pedicles on each side.

To determine the size of the vessels we used the Freeze and Measure function alongside the option Linear, and the transversal diameter of the vessels was determined, above the place where they pierced the fascia and before the bifurcation into the cutaneous branches.

The speed of the flow was determined with the help of the PW function.

After the exploration, the animal was cleaned and taken back to its cage. If necessary, we supplemented the dose of Ketamine and Xylazine, depending on the status of the animal.

RESULTS

In the 5th postoperative day we clinically examined the flaps, and standard pictures were taken, using a professional device. We

calculated the rate of necrosis as a percentage from the total area of the skin flap.

The first group had a mean length of the flap of 7.17 cm and a mean width of 2.92 cm. The percentage of necrosis was 48.5 % from the total area of the flap.

At the second group, the one with injection of epinephrine only, the mean length was 6.98 cm, and the mean width was 2.54 cm. The percentage of necrosis was 55.3 %.

In the third group, the mean length of the flap was 6.67, and the mean width was 2.82 cm. The percentage of skin necrosis was 29.7 %.

The echographic examination found that there were no major differences between the animals from the first group regarding the dimensions of the right and left pedicles (0.45 mm and 0.46 mm). The mean of the flow speed of the two pedicles varied between 178 and 190 mm/s (tab. I).

TABLE I

Ecographic dimensions of the pedicles - First Group

First group	BO1	BO2	BO3	BO5	BO6	BO7	BO8*	MEAN
Right pedicle diameter (mm)	0.26	0.4	0.48	0.45	0.5	0.7	0.38	0.45
Left pedicle diameter (mm)	0.4	0.49	0.38	0.44	0.49	0.56	0.48	0.46
Right pedicle speed of flow (mm/sec)	120	110	220	-	300	200	120	178.33
Left pedicle speed of flow (mm/sec)	120	120	224	-	400	150	130	190.66

For the second group the diameter of the pedicles varied between 0.38-0.48 mm, with a speed of flow between 151-211 mm/s (tab. II).

TABLE II

Ecographic dimensions of the pedicles - Second group

Second group	BO8	BO9	BO10	BO11	BO12	BO13	BO14	MEAN
Right pedicle diameter (mm)	0.45	0.36	0.32	0.46	0.58	0.56	0.65	0.48
Left pedicle diameter (mm)	0.43	0.3	0.25	0.32	0.43	0.34	0.65	0.38
Speed of flow right pedicle (mm/sec)	90	150	-	180	280	280	290	211.66
Speed of flow left pedicle (mm/sec)	100	150	100	110	110	170	320	151.42

The diameters of the pedicles of the third group varied between 0.54-0.55 mm, and the speed of the flow varied between 172-198 mm/s (tab. III).

The dimensions of the pedicles did not vary too much in the first group, as well as their speed of flow. In the second group, the one with epinephrine injected intraperitoneally, the dimensions of the pedicles were smaller. This group had the greatest variation of the speed flow, as well as the highest and the lowest speed of flow.

TABLE III

Ecographic dimensions of the pedicles - Third group

Third group	BO20	BO21	BO23	BO24	BO25	BO26	BO27	MEAN
Right pedicle diameter (mm)	0.78	0.45	0.45	0.67	0.5	0.3	0.63	0.54
Left pedicle diameter (mm)	0.5	0.38	0.92	0.68	0.4	0.4	0.62	0.55
Speed of flow right pedicle (mm/sec)	300	210	180	210	100	190	200	198.57
Speed of flow left pedicle (mm/sec)	110	130	180	290	120	190	190	172.85

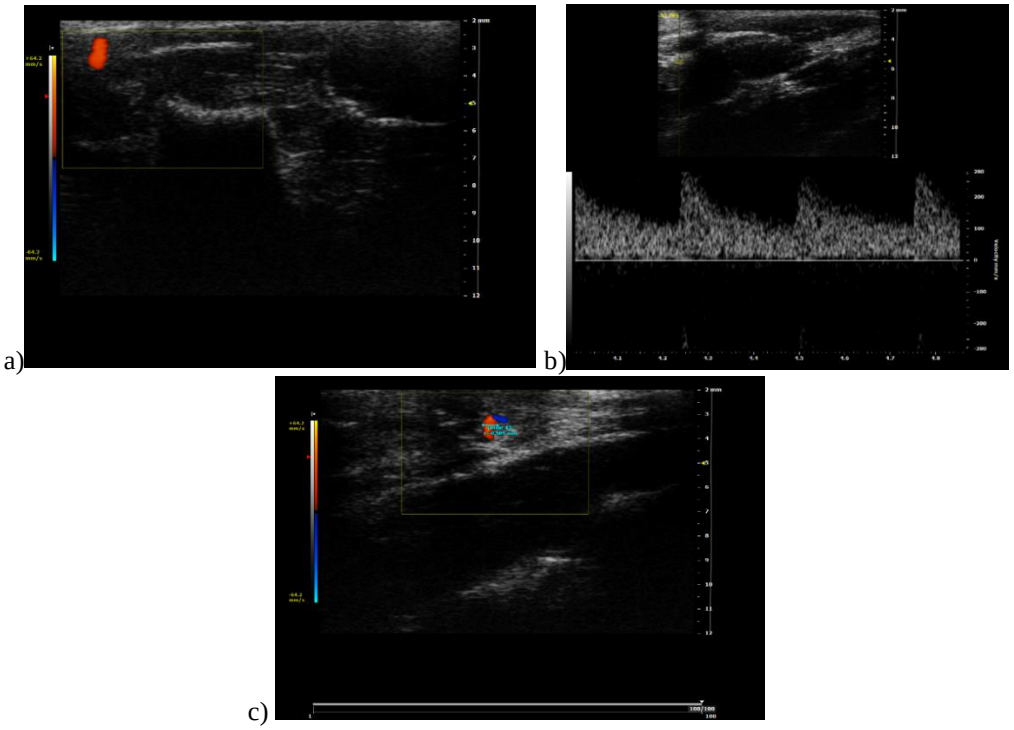

Fig. 1. Identification of the vascular pedicle (a), measuring the flow`s speed (b), measuring the pedicle`s diameter (c)

In the last group, the pedicles had the biggest diameters, a slightly higher variation of the speed flow compared to the first group and smaller compared to the second group.

One animal in the first group (BO4) died during the experiment (cardio-respiratory stop during anesthesia induction) and was replaced by another animal (BO8*).

At one animal (BO5) we did not manage to measure the speed of the flow of the pedicles.

Figure 1 shows the way we found the pedicle, and the measurement of the transversal diameter and the speed of the flow.

Tables IV-VII illustrate the *SPSS* descriptive statistics regarding the three groups with the diameter of the pedicles and the speed of the flows.

TABLE IV

Descriptive *SPSS* - Right pedicles diameters

Group	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	7	0.4514	0.13508	0.05106	0.3265	0.5764	0.26	0.70
Group 2	7	0.4829	0.12010	0.04539	0.3718	0.5939	0.32	0.65
Group 3	7	0.5400	0.16228	0.06133	0.3899	0.6901	0.30	0.78
Total	21	0.4914	0.13825	0.03017	0.4285	0.5544	0.26	0.78

TABLE V

Descriptive *SPSS* - Left pedicles diameters

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	7	0.4643	0.06079	0.02298	0.4081	0.5205	0.38	0.56
Group 2	7	0.3886	0.13284	0.05021	0.2657	0.5114	0.25	0.65
Group 3	7	0.5586	0.19887	0.07516	0.3747	0.7425	0.38	0.92
Total	21	0.4705	0.15279	0.03334	0.4009	0.5400	0.25	0.92

TABLE VI

Descriptive *SPSS* - Right pedicle speed of flow

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	6	178.33	75.476	30.813	99.13	257.54	110	300
Group 2	6	211.67	83.766	34.197	123.76	299.57	90	290
Group 3	7	198.57	58.716	22.193	144.27	252.88	100	300
Total	19	196.32	69.778	16.008	162.68	229.95	90	300

TABLE VII

Descriptive *SPSS* - Left pedicle speed of flow

Groups	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
Group 1	6	190.67	109.776	44.816	75.46	305.87	120	400
Group 2	7	151.43	79.042	29.875	78.33	224.53	100	320
Group 3	7	172.86	61.837	23.372	115.67	230.05	110	290
Total	20	170.70	81.340	18.188	132.63	208.77	100	400

We applied the one-way ANOVA method for testing the significance difference between the three means corresponding to group 1, second group and third group. The results are shown in the table VIII and find no statistical difference between pedicle diameters and the speed of the flows in the three groups.

TABLE VIII

ANOVA Test - Group 1-3

Parameters	p - significance
Right pedicles diameters	0.501
Left pedicles diameters	0.110
Right pedicle speed of flow	0.729
Left pedicle speed of flow	0.707

DISSCUSION

Doppler echography strictly depends on the quality and the settings of the device on which is performed. It is preferable to have only one person to do it. The choice of anatomical landmarks is crucial, as well as following the same sequence of choosing the modes. It is cheaper and takes a much shorter time than MRI exploration for example, but it may not give many information regarding the status of the microcirculation and the areas of perfusion. Even so, the Doppler exploration was used to identify capillary perforators smaller than 0.5 mm on the anterior border of the latissimus border

muscle, when planning a thoracodorsal artery perforator flap in humans.

Their identification guided the dissection which was safe and uneventful (8).

The pedicles of this model of rat dorsal skin flap are quite small, and sometimes very difficult to identify, and to measure their speed of flow. The movements of the transducer were very limited, as not to lose the vessel or to compress it with the transducer. Good anesthesia is of huge importance for this kind of exploration.

When analyzing a skin perforator, we have to determine the size of it and the perfusion pressure. These values are of importance when designing a perforator-based skin flap, which nowadays are more and more frequently used because of the minimal sacrifice of tissue and limited dissection. Recent studies confirmed a direct link between the internal diameter of a cutaneous perforator and its perfusion pressure. The perfusion pressure of a certain cutaneous perforator is decisive in establishing its skin territory that can be safely included in the flap (9).

In free flap models in rats, the perfusion pressure was better monitored by transit time ultrasound technologies, and for the venous congestion laser-Doppler flowmetry was used (10, 11).

The clinical aspect of the flaps does corelate with the dimensions of the pedicles. The smallest pedicles were found in the second group, the one with the highest necrosis rate.

There was a huge variation of the speed of the flow in the group were only epinephrine was administrated, with the lowest and the highest speed encountered, which illustrates the important rheologic impact that epinephrine may have, determining vasoconstriction and a possible compensatory vascular response. The speed of flow between the group with PRP and the first group were quite similar.

The fact that we found the biggest diameters of the pedicles in the group where PRP was administrated, confirms the vasodilatory effects of the platelet derived growth factors. They corelate with a smaller area of skin necrosis, and maybe with a bigger number of small skin anastomotic vessels to be opened. Even though the injections were done at a distance of 5 cm caudal to the pedicles, still there was a vascular response from them.

The injections with PRP were first recorded in sport related injuries, and nowadays it has a broad spectrum of indications: from articular degenerative disease to facial rejuvenation, or alopecia. When activated, platelets produce a variety of growth factors such as vascular endothelial growth factor, platelet derived growth factor, fibroblast growth factor, epidermal growth factor or interleukin 8. Its anti-inflammatory effects and the stimulation of different types of cell proliferation, along with being an autologous product, made its use safe and efficient (12).

It has been injected as well on the modified McFarlane dorsal skin flap. Apart from obviously improving the survival rate of the flap, it had proven proangiogenic stimulating effects (13).

There is a continuous search of vasodilator and proangiogenic substances, especially those who are safe to administrate and do not have impact on other organs or functions, that are autologous, such as the human placental extract, which seems to improve the survival rate of a random portion of the dorsal skin flap in rats, due to its antioxidant and antiapoptotic effects (14).

The form of administration is still debated, and can vary from various gels topically applied to subcutaneous injections or to subdermal gas administration, such as the nitrous oxide, that was found to improve the microcirculation of the dorsal skin flap in

rats (15).

CONCLUSIONS

Our study confirms the effects of epinephrine on the modified McFarlane rat dorsal skin flap. When injecting PRP in the flaps of the rats that received epinephrine intraperitoneally, the survival rate of the flap increases, proving the vasodilatory effects of the PRP. Clinical evaluation of these flaps should not be done before 3 days postoperatively and should have standard professional photographic assessment. It is preferable to have a dedicated soft for interpreting the images obtained, in order to better differentiate the colors that may appear on the surface of the flap.

Ultrasound examination gives very important information such as the diameter of the vessels, as well as the flow in each pedicle of this dorsal skin flap. In order to get this data, it is advisable to have one member of the working team to perform it and to set its position along with the anatomical landmarks. Using the correct sequence of exploration, like starting in mode B and switching to Collor function after identifying the pedicles ensures an optimal result.

There are some imagistic explorations that may be performed in order to analyze the skin perforators or microcirculation, such as MRI, thermography or contrast enhanced ultrasound, each one depending on the financial resources and the training of the personnel involved.

To sum, the ultrasound exploration provides valuable data even for this very small cutaneous perforator, but good anesthesia, skills and patience being mandatory for successful recording.

The PRP solution is an autologous product, already tested in chronic wounds and skin flaps, reminding about the intense studies in various fields of medicine regarding the use of the stem cells. Its effects on the endothelial layer and its actions in ischemic conditions are still a subject to be researched.

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NEWS

LUTEOLIN, A FLAVONOID AS AN ANTICANCER AGENT

Diet and consumption of bioactive compounds from natural sources (fruits and vegetables)have been recognized for their preventive role against a variety of human diseases including cancer. The inhibitory effects of phytochemicals found in plants against major diseases are well documented in literature. In this context, luteolin, a flavonoid found in different fruits and vegetables has been known as an anticancer agent through inducing apoptosis and cell cycle arrest and trough inhibiting metastasis and angiogenesis in multiple cancer cell lines such as breast, colon, pancreatic among other. Luteolin can be an important complementary medicine for the prevention and treatment of different types of cancers, owing to its natural origin safety and low-cost relative to synthetic cancer drugs. However, since most of the findings are based on in vitro and vivo studies, which do not necessarily represent the effect on humans, more work on different pharmacokinetic parameters and may involve human subjects is needed in the future before this substance becomes a prescribed drug. Luteolin (3,4,5,7 tetrahydroxy flavone) is an natural flavonoid, extensively present in many plant species. It is particularly present in fruits and vegetables such as celery, chrysanthemum flowers, sweet bell pepper, carrots, onion leaves, broccoli and parsley (Muhammad Imran, Abdur Rauf, Tareq Abu-Izneid, Muhammad Nadeem, Mohammad Ali Shariati, Imtiaz Ali Khan, Ali Imran, Ilkay Erdogan Orhan, Muhammad Rizwan, Muhammad Atif, Tanweer Aslam Gondal, Mohammad S. Mubarak, *Biomedicine & Pharmacotherapy*, April 2019, Volume 112).

PRO-HYPERTENSIVE ENDOTHELIAL DYSFUNCTION DUE TO LOWER EXTRACELLULAR MAGNESIUM INVOLVES IK1 CHANNELS

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PRO-HYPERTENSIVE ENDOTHELIAL DYSFUNCTION DUE TO LOWER EXTRACELLULAR MAGNESIUM INVOLVES IK1 CHANNELS (Abstract): Endothelium-dependent relaxation (EDR) in resistance arteries is largely independent of nitric oxide (NO) synthase and cyclooxygenase. It is mediated by endothelium-dependent hyperpolarization (EDH), based on endothelial Ca^{2+}-activated K^+ channels: IK1 with intermediate conductance (iK) and SK3 with small conductance (sK). Mg^{2+} modulates vascular reactivity directly and via NO-mediated EDR. **Aim:** We examined effects of diminished extracellular Mg^{2+} (Mg_e) concentration upon EDH-mediated EDR. **Material and methods:** Using wire myography, we tested isolated small mesenteric arteries from male rat. EDH-mediated EDR was induced by acetylcholine 10^{-8}-10^{-4} M, in vascular rings contracted by phenylephrine 10^{-5} M, in presence of l-NAME 10^{-4} M and indomethacin 10^{-5} M, with [Mg_e] kept at 1.2 mM (regular) or 0.8 mM (lower). **Results:** EDH-mediated EDR was inhibited by lower [Mg_e] (n=6; $p<0.001$; multiple *t-test*). Maximal EDH-mediated EDR was reduced from 75.79±2.6 % in regular [Mg_e] to 64.64±2.53 % in lower [Mg_e]. iK blocker TRAM-34 inhibited EDH-mediated EDR similarly in regular and lower [Mg_e], but sK blocker UCL 1684 inhibited EDH-mediated EDR more in lower [Mg_e] than in regular [Mg_e] (n=6; $p<0.001$; multiple *t-test*). Thus, lower [Mg_e] inhibits EDH-mediated EDR further when sK are blocked, but not when iK are blocked. **Conclusions:** Lower [Mg_e] inhibits EDH-mediated EDR, due to negative interference with iK. This novel pro-hypertensive mechanism is relevant for endothelial dysfunction in arterial hypertension associated with hypomagnesemia and for Mg therapy in general.
Keywords: MAGNESIUM, ENDOTHELIUM-DEPENDENT RELAXATION, RESISTANCE ARTERIES, EDH, CALCIUM-ACTIVATED POTASSIUM CHANNELS.

Systemic small arteries and arterioles ensure controlled blood flow distribution plus their dominant and regulated contribution to peripheral resistance. The latter determines arterial blood pressure (ABP) by hemodynamic laws. Vascular reactivity, including endothelium-dependent relaxation (EDR) of vascular smooth muscle (VSM), is key factor for local blood flow and ABP. Endothelial dysfunction has multiple facets and definitions. Here the term denominates all endothelial malfunction favoring vasoconstriction *vs* vasodilation.

Various studies examined Mg implica-

tions in the wide context marked above (1, 2). It has long been known that extracellular Mg^{2+} (Mg_e) induces vasodilation when [Mg_e] raises enough above normal. Mg_e-induced vasodilation involves direct VSM relaxation and EDR mediated by nitric oxide (NO) (3). In turn, NO-mediated EDR requires Mg_e (4).

EDR in resistance arteries is mostly independent of NO-synthase (NOS) and cyclooxygenase (COX), being mediated by endothelium-dependent hyperpolarization (EDH) (5). More precisely, EDH-mediated EDR depends on endothelial Ca^{2+}-activated K^+ channels (K_{Ca}) of two types (6, 7, 8, 9): (a) charybdotoxin-sensitive of intermediate conductance (iK) (alternative names: iK_{Ca}, SK4, $K_{Ca}3.1$, IK1, KCNN4); (b) apamin-sensitive of small conductance (sK) (alternative names: sK_{Ca}, SK3, $K_{Ca}2.3$; gene KCNN3). EDH-mediated EDR is an important functional opponent of sympathetic vasoconstriction (10) and it is clearly involved in flow autoregulation, myogenic response, and vasomotion (11). EDH-mediated EDR is compensatory in the endothelial dysfunction resulted from lower NO availability/effectiveness but is also involved in pathogenesis of endothelial dysfunction and hypertension (8, 9, 12, 13, 14).

Mg_e effects upon EDH-mediated EDR, or upon iK and sK, have not been previously investigated. Only one study seems available, but that is weakly related, because effects of Mg_e itself on EDH-mediated EDR were not examined (15). They found only that high $[Mg]_e$ attenuates the inhibition upon EDH-mediated EDR when such inhibition is exerted by the high [K^+] in cardioplegic solutions (15). Two other studies may come near, but Mg effects on EDH-mediated EDR were not tested therein either (16, 17). They studied the effect of chronic Mg upon l-NAME-induced hypertension and also tested EDR of isolated resistance arteries (16, 17), but in one study the effect of $[Mg]_e$ in the medium was not examined (16), while in the other study only the effect of $[Mg]_e$=4.8 mM (i.e. higher than normal) was tested (17). We started this study due to: (a) lack of previous investigations of Mg_e influence upon EDH-mediated EDR or upon the involved iK and sK; (b) vivid research on EDH-mediated EDR (5, 8, 9, 10, 11, 12, 13, 14, 18); (c) our group's interest in vascular research and hypertension (18).

MATERIAL AND METHODS

Male adult Wistar rats (~200 g) were euthanized. Jejunal loops were removed and placed in physiological saline solution (PSS), containing (mM): NaCl 119; KCl 4,7; $CaCl_2$ 2,5; $MgSO_4$ 1,2; $NaHCO_3$ 25; KH_2PO_4 1,18; glucose 5,5. In PSS at ~5°C, 1 mm rings from first order branches of superior mesenteric artery were dissected and mounted in wire myographs. Rings were then oxygenated (95% O_2 + 5% CO_2) and kept at 37°C (pH 7.2-7.4). After 30' equilibration under 1 g passive tension, rings were checked twice for complete EDR by 10^{-5} M acetylcholine upon contraction by 10^{-5} M phenylephrine. After drug washout for 15', the medium was either kept with regular [Mg_e] or switched to lower [Mg_e] and a 15' re-equilibration period was allowed for all rings. Lower [Mg_e] modified PSS contained 0.8 mM [$MgSO_4$] and was obtained by the required isoosmotic replacement of $MgSO_4$ with NaCl.

In presence of 10^{-4} M N(γ)-nitro-L-arginine methyl ester (l-NAME) and 10^{-5} M indomethacin, rings were contracted by phenylephrine 10^{-5} M and EDH-mediated EDR was induced by cumulative acetylcho-

line, 10^{-8} M to 10^{-4} M (5' of stable plateau before next dose). This was tested as control (without other drugs) or under selective full block (8, 19, 20) either of iK by TRAM-34 (TRAM; 10^{-5} M) or of sK by UCL 1684 (UCL; 10^{-7} M). Each ring was randomly subjected to only one of the six protocols described; one of the three tests above, performed either in regular or lower [Mg_e]. Inhibition of active tension at each acetylcholine concentration was expressed as % of complete relaxation from contraction by PHE 10^{-5} M. In the end each ring was subjected to 30' drug washout in regular [Mg_e], and again to relaxation by 10^{-5} M acetylcholine upon contraction by 10^{-5} M phenylephrine. All drugs were from *Sigma*. Statistics (using *GraphPad*): data expressed as mean ± SD; n=6 in each group; two-way *ANOVA*, multiple *t-test*, extra sum-of-squares F test for EC_{50} comparison; $p<0.05$ significance limit in all procedures.

This study was approved by Research Ethics Commission of the host institution. All procedures comply with guidelines from: Act on Animal Experimentation and Animal Health and Welfare from Romania; Universities Federation for Animal Welfare; Directive 2010/63/EU of the European Parliament and of the Council of 22nd of September 2010.

RESULTS

We observed that EDH-mediated EDR tends to decrease in lower [Mg_e], which could be explained by Mg^{2+} actions involving iK/sK, major players in EDH-mediated EDR. Our initial observation lead to an entirely novel hypothesis: a relatively small [Mg_e] lowering could induce endothelial dysfunction. The initial observation and subsequent hypothesis were confirmed (fig. 1, 2), but after some key preliminary tests, as described below.

First, we choosed the tested [Mg_e] to be 0.8 mM, a moderately diminished [Mg_e], that we expected to reduce EDH-mediated EDR without inducing VSM tone changes by itself. In rings at rest, in presence of l-NAME 10^{-4} M and indomethacin 10^{-5} M, isometric tension was similar upon simple [Mg_e] switch from 1.2 mM to 0.8 mM (n=6; $p>0.05$; paired *t-test*). This proved also valid for rings precontracted by phenylephrine 10^{-5} M (n=6; $p>0.05$; paired *t-test*). These preliminary tests show that [Mg_e] effects on EDH-mediated EDR are not biased by [Mg_e] effects on resting/activated vascular tone. We used TRAM (10^{-5} M) and UCL (10^{-7} M), concentrations that efficiently and selectively block iK and sK respectively, in terms of EDH-mediated EDR in small mesenteric arteries of the rat (19, 20). We separately confirmed (n=6) in the current setup the well-known abolition of EDH-mediated EDR by the combination iK-blocker + sK-blocker.

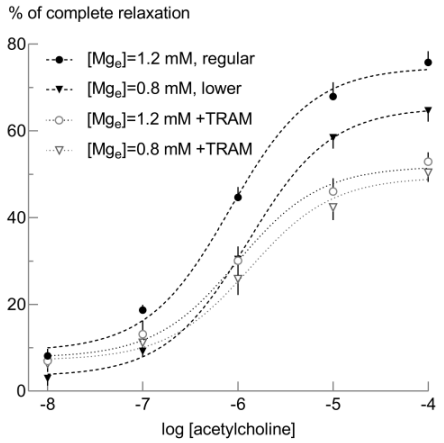

Fig. 1. EDH-mediated EDR is inhibited: (a) in lower [Mg_e], 0.8 mM *vs.* regular 1.2 mM; (b) by TRAM-34 (TRAM, 10^{-5} M), similarly in [Mg_e] 0.8 *vs.* 1.2 mM

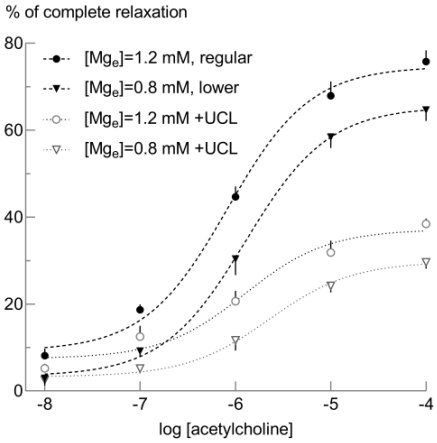

Fig. 2. EDH-mediated EDR is inhibited: (a) in lower [Mg_e], 0.8 mM *vs.* regular 1.2 mM; (b) by UCL 1684 (UCL, 10^{-7} M), more in lower [Mg_e] (0.8 *vs.* 1.2 mM).

DISCUSSION

In the context of massive research on EDR (5) and that of sustained interest in Mg relevance for vascular pathology (1, 2, 21), some studies tested the interplay between Mg and EDR of isolated arteries (3, 4, 15, 16, 17). Here we show that [Mg_e] influences EDH-mediated EDR, the major EDR pathway in resistance arteries. As detailed in the introduction, [Mg_e] effects on EDH-mediated EDR have not been tested before, not even in the three studies somewhat closer to the issue (15, 16, 17).

EDH-mediated EDR can be reduced through a yet unidentified path that allows lower [Mg_e] to hinder iK (but not sK) contribution within (figs. 1, 2). Whatever the mechanistic details, undiminished [Mg_e] could be required for normal function of the iK-based step in EDH-mediated EDR. This step is common, essential and rather late, somewhat beyond the preceding variable elements involved (5, 10, 11, 13, 14, 18). [Mg_e] effects (fig. 1, 2) could involve changed intracellular [Mg^{2+}] ([Mg_i]). As Mg_e effects on iK/sK and Mg_i effects on EDH-mediated EDR seem previously uninvestigated, we just note the only two studies on the effect of Mg_i on iK; patch-clamp showed that Mg_i inhibits iK (22, 23). There is no evidence of iK stimulation by Mg^{2+} and our results cannot be explained by Mg^{2+} inhibition of iK (either directly or indirectly by reducing Ca^{2+} influx), because such actions would lead to effects opposite to our data (figs. 1, 2). This includes iK in endothelial projections, which act in feedback with Ca_L in VSM (11, 24).

Mg_e inhibits L-type Ca^{2+} channels (Ca_L) in VSM (25, 26), the main Ca^{2+} influx path for VSM contraction. Mg_e stimulates K_{Ca} with large conductance (BK) in VSM (27). So, Mg_e promotes VSM hyperpolarization and relaxation, by Ca_L inhibition and BK stimulation. These Mg_e actions in VSM explain how EDH-mediated EDR is reduced in lower [Mg_e] (figs. 1, 2). The effect relies on iK (fig. 1) but not sK (fig. 2), so maybe the particular mechanism involving iK in endothelial projections (11, 24) is somehow affected by lower [Mg_e].

Endothelium-dependent contracting factors (EDCF) (5) may be involved in [Mg_e] effects (figs. 1, 2), except COX-dependent EDCF; COX was blocked by indomethacin. Given the exposure duration, the effects (figs. 1, 2) seem due to acute changes in cell signaling, not involving changes in gene expression, so we did not verify the opposite. However, all effects were rapidly and completely reversible, as shown in the end of each experiment: 30' washout in regular [Mg_e] and complete EDR by 10^{-5} M acetylcholine upon contraction by 10^{-5} M phenylephrine. Despite their high statistical significance, the differences that we revealed may look small (figs. 1, 2). However,

in vivo small changes in VSM active tension turn into small changes in diameter, which turn into corresponding 4th power changes in resistance to flow (Hagen-Poiseuille).

So, lower [Mg_e] reduces EDH-mediated EDR by negatively interfering with iK. This novel path is relevant in the hypertension with Mg deficit and also as reason for Mg supplementation (1, 2, 21). [Mg_e] relation with EDH-dependent EDR adds to known Mg benefits: direct and NO-mediated VSM relaxation, antioxidant, anti-inflammatory. Advances hereafter may impact the future of Mg therapy.

CONCLUSIONS

Low [Mg_e] inhibits EDH-mediated EDR, which is the major EDR mechanism in resistance arteries and relies on iK and sK. Low [Mg_e] further inhibits EDH-mediated EDR when sK are blocked, but not when iK are blocked. Regular EDH-mediated EDR presumably requires [Mg_e] not to be decreased. Otherwise, iK (but apparently not sK) would be hindered in their usual activation and/or involvement therein. Hypomagnesemia may well reduce EDH-mediated EDR in vivo, due to this ability of lower [Mg_e] to negatively interfere with iK. Most recent research shows that such a mechanism is relevant to endothelial dysfunction in hypertension. [Mg_e] effects on EDH-dependent EDR might become important in Mg pharmacotherapy.

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GHEORGHE MARINESCU (1863-1938) AND *LA CELLULE NERVEUSE*. 110 YEARS SINCE WRITTING OF THE MAJOR CHAPTER OF THE OLD TESTAMENT OF NEUROPATHOLOGY

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GHEORGHE MARINESCU (1863-1938) AND *LA CELLULE NERVEUSE*. 110 YEARS SINCE WRITTING OF THE MAJOR CHAPTER OF THE OLD TESTAMENT OF NEUROPATHOLOGY (Abstract): 110 years ago, in 1909 in Paris, Gheorghe Marinescu's *La Cellule Nerveuse* appeared. The work was practically a complete and extremely rich review of the literature of that time, in which Marinescu integrated the results of his own research. The book contained chapters on embryology, cytology and physiology of the nerve cell, but also on the pathology. *La Cellule Nerveuse* also recalls Marinescu's pioneering studies in the field of neuronophagy, as well as his studies in the field of chromatolysis. Nowadays, *La Cellule Nerveuse* and the whole activity of Gheorghe Marinescu in the field of neuropathology have been an important pillar not only for the development of the Romanian School of Neurology and Neuropathology, but also for the development of the European neurology that we should remember today. **Keywords:** GHEORGHE MARINESCU, NEUROPATHOLOGY, ROMANIAN SCHOOL OF NEUROLOGY AND NEURO-PATHOLOGY, NERVOUS CELLS.

HISTORICAL DATA

The founder of the Romanian School of Neurology and Neuropathology, Professor Gheorghe Marinescu (1863-1938) (fig. 1), was born on 23rd of February 1863 in Bucharest. In 1874 he was enrolled by his mother at the Central Orthodox Seminary, which he completed the second of his promotion. Refusing the mother's desire to continue his theological studies, young George is admitted to both the Faculty of Medicine and the School of Bridges and Roads.

After the first year, he will remain only at the medical school, where he will work as a student in the newly founded histology laboratories by Professor Petrini-Galatz, the first head of the Department of Histology of the Faculty of Medicine in Bucharest. Later, in 1886 he became assistant in this laboratory and intern of the Brancovenesc

Hospital from Bucharest (fig. 1) (1).

He was Professor Victor Babes' assistant, within the Institute of Bacteriology (2). He became his disciple and they collaborated together on the *Atlas of Pathological Histology of the Nervous System* (3). In 1889, the young George was elected president of the League of Medical Students and by a letter of recommendation signed by several teachers including Victor Babes, he was admitted to a scholarship for a period of nine years at the Salpêtrière Hospital in Paris (fig. 2), where he will study neurology (2).

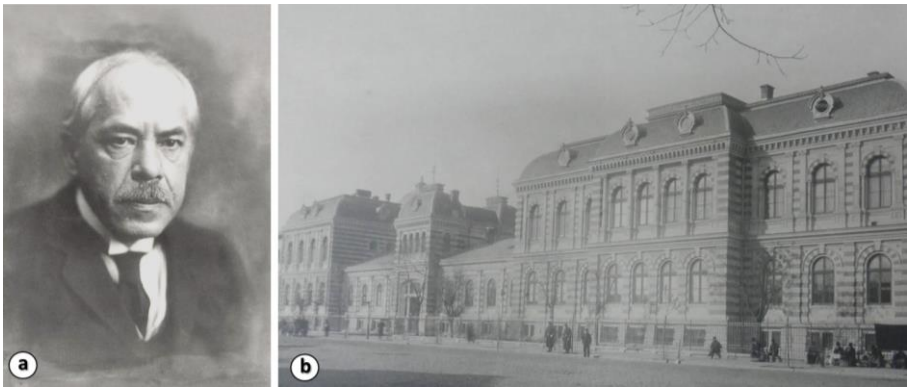

Fig. 1. Professor Gheorghe Marinescu (1863-1938) (a). Brancoveanu Hospital, Bucharest (b) (public domain)

Fig. 2. Jean Martin Charcot (1825-1893) (a). A Clinical lesson at the Salpêtrière (with Prof. Charcot and others) (1887), André Brouillet (1857-1914), Paris Descartes University, Paris (b) Marinescu is missing from the painting, because he arrived at the Salpêtrière in 1889, two years later (public domain).

At that time, the famous hospital was led by Jean-Martin Charcot (1825-1893) (fig 2), the founder of modern neurology. About Charcot, later in 1925 Marinescu noted: *I had the opportunity to meet personally not only the most renowned neurologists contemporaries, but also great scientists, such as R. Virchow, R. Koch, P. Ehrlich. Neither one made an impression as profound as Charcot, an impression that remains alive in my soul* (4).

Marinescu remained a volunteer assistant at Salpêtrière and collaborated with Pierre Marie (1853-1940), Gilles de la Tourette (1857-1904), Paul Oscar Blocq (1860-1896) and Joseph Babinski (1857-1932) (5). He also traveled to different universities in Europe, where he met and studied with Carl Weigert (1854-1904) in Frankfurt and Emil du Bois-Reymond (1818-1896) in Berlin (1, 3, 5, 6).

After defending his doctoral thesis in Paris, *The Succulent Hand in Syringomyelia,* Marinescu returns to Bucharest where he was appointed as Chief doctor at Pantelimon Hospital in May 1897 (6). For a period of more than 20 years he traveled daily around 12 km to the hospital, until the Neurological Clinic was transferred to the Colentina Hospital (4).

At Pantelimon Hospital, together with his first intern, Constantin I. Parhon (1874-1969), he reorganized the service starting with new laboratory researches in the field of neuropathology. One month later, in July 1897, at the age of 34, Marinescu becomes the first Head of the Department of Neurology of the Medical Faculty of Bucharest (6).

Marinescu's main area of interest was neuronal pathology, and his observations and hypotheses were published in 1909 in Paris, in his monograph *La Cellule Nerveuse (The Nervous Cell)* (3), in which he postulated that: *evolution and involution of neurons depend on active oxidation* (5). In this book he wrote about the cell biology of the neuron and its organelles, such as neurofibrils, pigments, Nissl bodies, the nucleus and nucleoli, both in the normal and the pathological status of the nerve cell. Although his work in this field of neuropathology has been extensively described and published in this book, he did not stop to work and continued until his death in 1938 (2).

THE MAIN NEUROPATOLOGICAL WORK: THE NERVOUS CELL

La Cellule Nerveuse (fig. 3) was practically a complete and extremely rich review of the literature of that time, in which Marinescu integrated the results of his own research (2), being the first book of its kind in the world, greeted with enthusiasm by scientists like Santiago Ramón y Cajal (1852-1934) and it has not been surpassed until today by any such work. This was due to the fact that Marinescu did not study the fixed and colored nerve cell only under the microscope, but also investigated it in the living state (4).

In volume I, Marinescu presents the general morphology of the neuron with all its components known at that time using optical microscopy and fixation and staining techniques (7). He reviewed in detail: nucleus and nucleoli, Nissl bodies, centrosome, neurofibrils, Golgi apparatus, bioblasts of Altmann (e.g. mitochondria) and the colored granules (black pigment for *locus niger* and *locus coeruleus*, as well as yellow pigment) (2, 7).

Regarding neurofibrils, Marinescu considered that they are inserted at the periphery of the cell and at the wall of the nucleus,

naming this structure *spongioplasma*. He was also the first to conclude that spongioplasma lesions can lead to degeneration of peripheral extensions of the nerve cell (8).

Regarding the role of the chromatophilic elements, Marinescu considers that they conducts the nervous impulses and furthermore, the large chromatophilic elements influence the intensity of the nerve impulse. He was also convinced that: *chromatophilic elements represent a substance with high chemical potentials, which is the place of a continuous phenomenon of integration and disintegration, and through these phenomena the nerve cell becomes an energetic device* (translation of the authors) (1). He also named these structures, generically, under the term *kinetoplasma* (9).

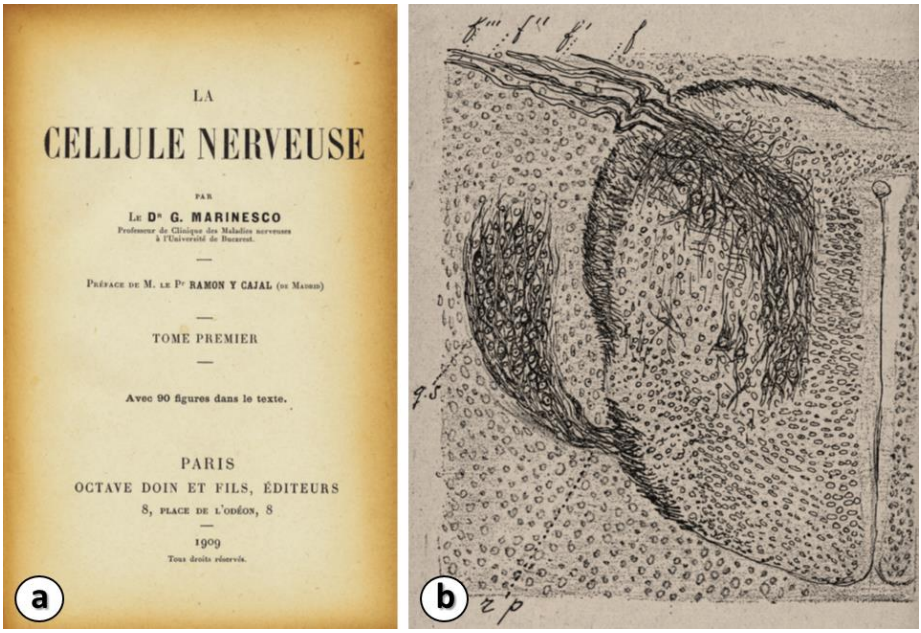

Fig. 3. *La Cellule Nerveuse*, published by G. Marinescu in 1909 (7) (a). Transversal section of a histological preparation showing the nervous fibrils. Chick embryo at six days of incubation. f – ventral roots, gs – spinal ganglion, rp - dorsal root (adapted after *Le Cellule Nerveuse* (G. Marinescu, 1909) (7) (b) (public domain).

Regarding the paranuclear corpuscles, small acidophilic inclusions present in the nuclei of pigmented neurons of the *substantia nigra* and locus *coeruleus*, Marinescu also described them in humans (10) (later named *Marinesco bodies*). He also found that they are more abundant in adults and elderly compared to young people, and also absent in children (7).

An entire chapter of the first volume was dedicated to neuroembryology (7), in which he reviews the knowledge of the time on this subject, to which he adds personal observations that do not depart from the vision of some of his mentors: Santiago Ramón y Cajal and Wilhelm His (1863-1934). One of the theories involved the existence of two types of cells in the neural tube or neuroepithelium: the germ cells that Marinescu considers neuronal precursors and the epithelial

cells that will generate the glial support. Marinescu's neuroembryology studies were largely based on chicken embryos, mice or even human embryos and fetuses (2). In addition, Marinescu also noted in his work that all neurons are not born at the same time, a phenomenon now known as a temporal gradient of differentiation (2).

The second volume of *La Cellule Nerveuse* contained Marinescu's contribution to the histopathology of the nervous system and in which he focused on two entities considered to be important for the neuropathology field: *chromatolysis* and *neuronophagia* (2, 8). He differentiated between the central chromatolysis that appeared in the cell body of the neuron after axon sectioning and the peripheral chromatolysis that could be observed in primary lesions of the nerve cell produced by traumatic or toxic causes (2).

In his experimental studies in rabbits, Marinescu also made observations on cell injuries secondary to nerve sectioning. In other experiments on small animals such as guinea pigs, cats or frogs, he also observed that trauma of the nerve centers leads to initial necrosis and that the neurons do not regenerate, while blood vessels and neuroglia proliferate (2).

Professor Marinescu was also a pioneer in the field of neuronophagia, concluding after multiple studies of chromatolysis and granular fibrillary degeneration, that oxidative stress is a major contributor to the process of neuronal aging (3). He introduced the term *neuronophagia* and demonstrated that phagocytes are not glial cells, but mesoderm-derived mobile cells located near the neuron or migrating from the blood vessels. Later, he assimilated them with microglia (11).

The Nervous Cells's preface was written by Santiago Ramón y Cajal (3), who wrote a laudatory preface: *honorable colleague and learned friend, Professor Marinesco is also an insightful observer and a strong and tenacious supporter of the experimental method* and *that his activity is undeniable, his fruitful research in the difficult field of the histology of the nervous system has earned him a reputation and authority more than enough to impose his own book on the attention and praise of the learned world* (translation of the authors) (7).

Professor Marinescu died in 1938 and on his tombstone is written: *Work ... Sincerity .... Silence ....* (1). He remained for the Romanian medicine as the founder of the School of Neurology, and his entire work together with *La Cellule Nerveuse* was an important pillar in the development of European neurology, which we should remember today.

At the turn of 156 years since his birth, 81 years after his death and 110 years since the publication of the *La Cellule Nerveuse* (1909), we wonder what is today's the inheritance of Gheorghe Marinescu. Of course, his name is closely related to the succulent hand in syringomyelia, to the Marinesco-Sjögren syndrome, to the first descriptions of lesions in the *niger locus* of Parkinson's disease (2), to the more than 1500 works in the field of neuroanatomy, neuropathology, neurophysiology, histochemistry (12), but especially because of his monumental work in the field of neurocytology and neuropathology of the nerve cell and his studies in the field of chromatolysis and neuronophagia - *La Cellule Nerveuse* (2).

In 1951, the anatomist Murray Barr said that *La Cellule Nerveuse, together with Cajal's better known Histologie du Système Nerveux de l'Homme et des Vertébrés, are*

major chapters in the neurocytologist's Old Testament (13).

Beyond these, Marinescu has left us a legacy of humanity. During World War I he offered himself as a volunteer at the Salpêtrière Hospital in Paris, where he cared of wounded soldiers with Pierre Marie. Later, for his humanitarian sacrifice, the French government granted him the title of *"Officer of the Legion d'honneur"* (5).

Three days before his death, Professor Marinescu wrote his own testament in which he mentioned*: No flower, no speech; those who loved me used the money for poor children and good words to encourage the suffering. Do not forget that those who are living in poverty and even in misery are too numerous, that those with money, those who are enriched by neon means live from the exploitation, in one way or another, of those who work. Leaving for the world from which no one has returned, I would not upset anyone, but the truth must be said: too much injustice is in the blessed country of Romania! It is due to the ease or haste with which the vital problems of the homeland were solved. The lack of true citizenship education and the feeling of duty, as well as immense pride or some passing ambitions have given rise to many social anomalies* (14).

CONCLUSIONS

Gheorghe Marinescu represents a European and international personality in the field of neurology and neuropathology. His works together with those of Ramon Y Cajal (1852-1934) represent the fundamental element in the development and understanding of the neurological sciences. In this domain, the keystone is his work published in France, 110 years ago.

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ADVERSE CHILDHOOD EXPERIENCES IN RELATIONSHIP WITH EATING DISORDERS

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ADVERSE CHILDHOOD EXPERIENCES IN RELATIONSHIP WITH EATING DISORDERS (Abstract): Studies indicate that patients with eating disorders report a history of childhood adverse experiences more frequently than the general population. Childhood abuse is reported by a significant proportion of people with eating disorders, with recent research highlighting other forms of child abuse, such as physical and emotional abuse, which may have consequences for later adult health. The association between childhood traumas and eating behaviors is very complex, leading to an increase in body mass index, obesity, metabolic and mental disorders. Bullying is a common childhood experience, with long-term psychosocial consequences and affecting eating behavior. Moreover, parental divorce and parental bonding have been implicated as important risks factors for eating disorders. In conclusion, it may be clinically useful to consider all abusive experiences in the evaluation of patients with eating disorders. Also, recognizing all these facts provides a clear opportunity for early intervention which may help prevent the development of eating disorders in traumatized people. **Keywords**: ADVERSE CHILDHOOD EXPERIENCES, TRAUMA, EATING DISORDERS, CHILDHOOD, POST-TRAUMATIC STRESS, EATING BEHAVIOR.

Adverse childhood experiences (ACE) are traumatic or stressful events exposed to a child that can continue to have long-term negative health effects throughout the course of that child's life (1). There are six categorizes of ACE: physical abuse by an adult in the household, sexual abuse by a family member, sexual abuse by a non-family member, a dysfunctional household caused by the use of drugs or alcohol in the family and witnessing physical abuse by a family member on another family member (2). Others include emotional abuse, failing to provide the necessities (food, medical care, good hygiene) and being the care of a member of the family having a physical or mental illness (3).

Various studies have found risk factors contributing to a child having ACE such as gender, socio-economic status, family type (single parent, adopted child) and the environment they grow up.

The consequences of such traumatic events are linked with self-induced harm to health, antisocial behaviors, a faster development of chronic diseases and premature death (4). ACE during childhood was related to chronic disease later in life and a strong relationship was identified between ACEs and risky behaviors like drinking alcohol,

consuming drugs or eating disorders.

Eating disorders represent a complex, multifactorial pathology, determined by biological, family, psychosocial and psychological factors (5). The lifetime prevalence of eating disorders in adults is about 0.6% for *anorexia nervosa* , 1% for bulimia nervosa and 3% for other eating disorders, disorders that are often chronic, relapsing and devastating. Numerous studies indicate that patients with eating disorders report a history of childhood trauma more frequently than the general population (6).

Freud said that events that occur in the first 6 years of life are particularly important for the rest of one's life, and from this point of view, adverse childhood experiences are associated with an increased risk for acute or chronic psychosocial disorders in adult life (7). Adverse childhood experiences are surprisingly common, generally unrecognized and may begin to show damage through somatization and illness even during childhood (8).

Moreover, several studies have documented an association between traumatic events in children and the development of obesity in life, finding that, out of a sample of adult patients seeking surgical treatment for obesity, about one-fifth were exposed to sexual and physical abuse and more than a third witnessed violence. In addition, other childhood events such as separation from mother or father or marital problems have been associated with an increase in obesity in adulthood (9).

Recognizing these facts provides a clear opportunity for early intervention, as it is demonstrated that what happens in childhood usually lasts a lifetime, like the traces of a child on soft cement, and in this case, time will not heal the wounds, but it will hide them (8).

CHILDHOOD TRAUMA AND EATING DISORDERS

Trauma is any event, usually an unusual one, that harms the body, self or spirit. It covers a wide range of painful experiences, including traumas that involve the physical, sexual, mental or emotional part of our being. The combination of these traumas is called adverse childhood experiences (ACE). If the trauma is accepted as real and the experience of the victim or survivor is validated and its expression sustained, its short-term effects, known as acute traumatic stress, can be expressed, processed, ameliorated or "metabolized" in a healthy way, so that in the end there are few or no harmful effects. In contrast, if the reality of the traumatic experience is denied or invalidated by the victim or other close members (such as family, friends, or help professionals), then the person may not be able to fully heal the adverse effects of the trauma. In order to heal from these traumas, the experimenter must be able to endure the pain associated with it and must remember the trauma well enough to name it accurately, the memory being a key to resolving the effects of the trauma (10).

Childhood traumas have been associated with a number of serious long-term psychiatric sequelae (including depression, alcohol and drug use, anxiety, post-traumatic stress disorder (PTSD), sexual disorders - especially in cases of sexual abuse, self-destructiveness, personality disorders), but in the past decade, clinicians and researchers have considered the possibility that childhood abuse and trauma, especially sexual abuse, may be a strong antecedent of eating disorders (11).

Childhood abuse is reported by a significant proportion of people with eating disorders, with recent research highlighting

other forms of child abuse, such as physical and emotional abuse, which may have consequences for later adult health (12). Also, child abuse promotes the development of mental disorders (mood disorders, post-traumatic stress disorder, substance use disorders, or clinical dimensions related to suicidal behavior, such as impulsivity or aggression), which in turn can lead to eating disorders (6).

Childhood trauma could lead to short-term post-traumatic symptoms, turning into obsessive-compulsive symptoms (such as obsessive fear of weight gain, extreme food concerns, body image and vomiting) in the longer term. Also, many researchers have suggested the need to elucidate the psychological mechanisms linking childhood trauma to subsequent eating disorders. Some investigators reported that depression is a significant mediator between childhood abuses and disordered eating in students, while others reported that anxiety and dissociation were important mediators, but that depression was not. In addition, obsession has been shown to be a predictor of bulimic symptomatology, and obsessive-compulsive traits could also be considered as an important mediator between childhood trauma and eating disorders.

A considerable amount of research has targeted childhood traumas as a risk factor predisposing to the development of eating disorders in adolescence or adulthood, but most research has focused mainly on forms of sexual abuse (5).

CHILD SEXUAL ABUSE AND EATING DISORDERS

Sexual abuse refers to sexual contact or behavior between a child and an older person, the explicit constraint being a frequent but nonessential feature of these experiences (5). There is no knowledge about the types of childhood trauma that are associated with the clinical characteristics and severity of eating disorders, some studies suggest a higher severity of eating disorders in cases of physical and/or sexual abuse, while others do not (6). For example, childhood sexual abuse may be associated with *bulimia nervosa*, whereas links with *anorexia nervosa* and other binge eating disorders are less clear (12).

Some studies consider that sexual trauma is a distal factor in the development of eating disorders - it does not directly cause the development of eating disorders but can precede the onset for a significant period. Based on the literature, it appears that there are two main etiological pathways between sexual assault and eating disorders: first is the survivor's negative perception of her body-including body dissatisfaction, shame and fear of future sexual trauma) and second is the need of a sexual trauma survivor to cope with psychological difficulties (such as failure of the average expected environment, need for control, coping with psychological diagnoses and regulation of emotions (13).

A literature review of the correlation between sexual abuse and eating disorders revealed that approximately 30% of patients with eating disorders had a history of sexual abuse (14). Results of another study indicate that the prevalence of childhood sexual abuse among hospitalized patients with *anorexia nervosa* is high, and these patients have reported higher mental disorders, including higher levels of depression and anxiety, lower self-esteem, greater interpersonal problems and more severe obsessive-compulsive symptoms compared to patients who had no history of childhood sexual abuse. Also, patients with a history

of sexual abuse have reported significantly more severe psychopathology of eating disorders (15).

The history of physical, emotional and sexual abuse in childhood is linked to all types of eating disorders, arguing that it can be both a reaction to abuse and a strategy to cope with the aversive states that result from childhood physical and sexual abuse. It has been found that a history of sexual trauma can increase body awareness and dissatisfaction, promoting eating behaviors and eating disorder symptoms. It has also been found that the incidence of bulimic syndrome in adolescence is up to five times higher among those who have experienced sexual abuse in childhood. Alternatively, childhood traumatic experiences can lead to disorders in the metabolism of norepinephrine, dopamine, serotonin, and endogenous opiate systems, all involved in the regulation of eating behaviors. However, the association between childhood traumas and eating behaviors is probably much more complex, leading to an increase in body mass index, obesity, metabolic and mental disorders (12).

A study of 10-15-year-old girls found that sexual abuse significantly predicts impulsivity and that impulsivity provides the strongest mediation between a history of childhood sexual abuse and purging and/or restricting eating behavior. Thus, sexual abuse has proven to be a predictor for impulse regulation and perfectionism. Another study found that sexually abused anorexic subjects had a higher degree of perfectionism than non-abused subjects, thus being more concerned with avoiding failure (5).

For men, sexual abuse is probably underreported because of the greater shame and stigma that accompanies men's abuse unlike women`s abuse, and because of underreporting, there seems to be a smaller number of men with a documented history of sexual abuse. Most perpetrators of sexual abuse are men; therefore, men who are victims of sexual abuse may develop issues related to sexual orientation and/or fear of experiencing sexuality. Through disordered eating, especially anorexia, men struggling with these problems can deny natural hormonal mandates as a solution to their crisis of sexual orientation. Studies showed that men are less likely than women to engage in bulimic "typical" compensatory behaviors, such as vomiting or laxative abuse; having at the same time more inclination to use excessive exercise as a compensatory method for controlling weight and body shape. The use of assessment tools, which emphasizes compensatory behaviors, binge eating habits, attitudes toward food and emotional triggers in men, would probably improve the accuracy of reporting by men, as well as lead to the development of appropriate interventions (14).

Thus, sexual trauma precedes eating disorders at the same rate as other psychiatric diagnoses and, therefore, may be just one of the many precursors of eating disorders. At the same time, sexual trauma can be a catalyst for mental distress and eating disorders can only be a manifestation of symptoms. It is also possible that there is a biological or neurological vulnerability that initiates an individual on the etiological pathway from a sexual trauma to a diagnosis of an eating disorder (13).

CHILD PHYSICAL ABUSE AND EATING DISORDERS

Physical abuse refers to bodily abuse of a child by an older person, predisposing a risk of or resulting injury (5). Studies have found an association between any form of

abuse and bulimia nervosa or binge eating disorder, but not *anorexia nervosa* - which seems to be related only to the childhood physical abuse. Other environmental factors, such as socio-cultural restrictive *anorexia nervosa*, genetics could be a major factor, possibly more important than environmental and family agents for children (16).

Physical abuse in children seems to be a more important factor in developing the distortion of the body image, its importance exceeding that of sexual abuse. Physical abuse, laxative abuse, and the binge-purge subtype *anorexia nervosa* are a considerable risk factor for the severity of the distorted body image, and their presence worsens the prognosis of the eating disorder. The differences between sexual abuse and physical abuse in terms of their role in the development of body image distortion must be due to the difference between the abuser's acceptances of the body during the abuse (17).

According to a study, when comparing groups of patients with eating disorders, 45% of those who had a history of physical abuse had more severe psychopathological symptoms, especially symptoms of anxiety and substance-related disorders, compared with patients without a history of abuse. Other studies found that if they were studied from the other hand, the appearance of self-destructive behavior was the factor that correlated highly with sexual and/or physical abuse in the history of eating disorders (5).

From previous surveys, it seems that about 5% of children between the ages of 6 and 12 have been physically or sexually assaulted and 10-15% of them have heard or seen such cases. Children who are sexually or physically assaulted feel intense helplessness, often being anxious or nervous. The assault can frequently lead to the so-called daily state of trance, when dissociative mechanisms such as detachment of the body are used to ease the trauma, and this can subsequently lead to rejection of the patient's own body, problems with representing the body's image distortion. During this process models of identification or rejection may appear, the victim of aggression may become an abusive parent herself and, depending on her personality, coping patterns, family conditions and interpersonal skills, she may behave more harshly (by showing herself self-destructive behavior and torture itself), for example by developing an eating disorder. Considering those mentioned, further studies are also needed to decide whether physical abuse plays a specific role in the development of eating disorders and which mediation factors are responsible for this effect (17).

CHILD EMOTIONAL ABUSE AND EATING DISORDERS

Emotional abuse refers to verbal aggression affecting the child's sense of well-being or any humiliating, derogatory or threatening behavior towards a child by an older person (5). Emotional abuse was the only type of abuse constantly linked to the symptoms of eating disorders; the emotional disturbance being positively associated with the symptoms of eating disorders. This highlights the importance of evaluating a history of emotional abuse, in addition to questions assessing the experiences of sexual or emotional abuse of children, which are often more common in clinical practice (16).

Emotional abuse may also increase the severity of eating disorders. It is assumed that specific genetic and/or epigenetic fac-

tors and induced emotional regulation may directly promote the occurrence of eating disorders, but it remains unclear what factors underlie the association between childhood trauma and eating disorders (6). Other studies have found an association between any form of abuse and bulimia nervosa or binge eating disorder, but not *anorexia nervosa* - which seems to be related only to the childhood physical abuse. Other environmental factors, such as socio-cultural influences, may play an essential role in the development of *anorexia nervosa* and especially in restrictive *anorexia nervosa*, genetics could be a major factor, possibly more important than environmental and family agents for children (16).

Emotional abuse has been shown to be a predictor of ineffectiveness, interceptive awareness, and impulse regulation, being the only form of childhood trauma that clearly predicts psycho-pathology in female students (5). Also, childhood emotional abuse was a predictor of bulimic symptoms and plays an important role in the development of psychological difficulties, including eating disorders and may be more traumatic than physical abuse (18). Kent and Waller (19) explained that childhood emotional abuse was a risk factor in the development of eating psychopathology, given the evidence of the links between childhood emotional abuse and self-esteem.

Another study also indicates that childhood emotional abuse is a predictor of "interoceptive awareness" (5). Traumatized individuals were deficient in "interoceptive awareness" for internal physiological and psychological conditions because they have often failed to develop the capacity to differentiate between complex internal states. Therefore, they often expressed their behavioral emotions rather than verbally, because they had difficulty expressing their feelings in words. In addition, the finding that emotional abuse is a predictor of impulse regulation was consistent with the idea that survivors of childhood abuse might turn into self-destructive and self-injurious behaviors, such as purging and vomiting, compulsive sexual behavior, compulsive risk, taking and using psychoactive drugs, to relieve stress and to regulate their internal emotional states, in the absence of the internal self-soothing capacity (20).

Because emotional abuse is likely to occur at a higher frequency than other types of abuse and may be perpetuated by multiple people simultaneously, the potential impact of emotional abuse on children should be viewed as seriously as other forms of children abuse (16). Also, a history of childhood abuse in people with eating disorders is associated with increased use of drug treatments, but with poor outcomes. Thus, both early detection of abuse and ongoing assessment for eating disorders are needed (11).

CHILDHOOD BULLYING AND EATING DISORDERS

Bullying is a common childhood experience with lasting psychosocial consequences because these victims are at an increased risk for physical health problems, behavioral and emotional problems, suicide, psychotic symptoms and weak school achievements; victims who attack others (so-called bully victims) having the worst results. Eating behavior can be affected by bullying for several reasons: bullying can increase one's perceptions of one's body, either directly by teasing about one's weight/appearance or indirectly, by its effects on one's overall self-esteem and emotional problems. The bullies, like vic-

tims and the aggressive victims, had increased results for eating disorders, even though they were perpetrators and probably saw the intimidation as a way of accessing resources. More surprisingly, observed associations were not attenuated in models that highlight the preexisting status, which suggests that experience of bullying itself may affect subsequent behaviors (21). Also, bullying, emotional symptoms, restricted eating and bodily dissatisfaction were all correlated, and emotional symptoms were found to significantly mediate the relationships between verbal intimidation and bodily dissatisfaction in girls, but not boys. Studies suggest that the experience of being verbally intimidated causes adolescent girls to be at a risk of developing emotional problems that can then lead to dissatisfaction in the body (22). In addition, involvement with bullying, as a victim, perpetrator or both, has lasting negative, educational, physical, social and emotional consequences for certain groups of young people (for example, those who are gay, lesbian, overweight or disabled), report higher rates of victimization than other young people (23).

There is growing evidence that victimization is related to either obsessive-compulsive behavior or bodily dysmorphic disorder. This pathology refers to a concern for an insignificant or unimaginable defect of the physical aspect that leads to the impairment of social or professional functioning. Although most individuals with body dysmorphic disorder are concerned with a part of the body (for example, nose, hair), some are concerned about the whole body, considering it is weak, is too small or is not muscular enough.

Nothing is known about the individual or social risk factors that lead to the development of this disorder, but the literature suggests that those who suffer from this disorder have suffered more abuse in childhood. The victimization rate of 21% of childhood bullying mentioned by body builders is comparable to the rates reported on community samples of children in primary schools, but higher than those of children in secondary school. It has been reported that women who were victims of rape as adolescents or adults were more often involved in compulsive dumbbells (24).

Few studies have highlighted the effect of bullying on adolescent women's psychopathology, namely body image difficulties and eating behavior problems. However, a study has revealed that this effect is mediated by shame of body image and self-criticism. Also, a number of studies have shown the relationship between being a target of negative interactions focused on the physical aspect (for example, weight and appearance-related teasing) and eating and body image issues; but peer victimization can have a deleterious effect on eating behavior even when not specifically focused on the physical aspect. Other studies have shown that these extremely negative experiences can have a persistent negative impact on body image and nutrition. In fact, patients with eating disorders often report experiences of being bullied by their childhood and adolescent peers (25).

There is considerable evidence that victims of bullying have a significantly higher rate of psychiatric problems and children who are bullied are often targeted because of perceived characteristics, such as being physically weak, differing in physical appearance, lacking assertiveness, being restless and having little or no friends. The autobiographical accounts of successful bodybuilders and the spontaneous reports

of bodybuilders in this study suggest that being a victim of bullying as a schoolboy and feeling weak and pushed around them can lead to bodybuilding (24).

PARENTAL BONDING AND EATING DISORDERS

The family plays an important role in the development of eating disorders, including *anorexia nervosa* and bulimia nervosa, studies focused on the overprotection of mothers and the indifference of fathers (26). The parental connection has been defined as the parental contribution to the parent-child relationships and is usually evaluated using the *Parental Bonding Instrument*. The maternal and paternal contribution to bonding is realized along two dimensions: care (varies from affection, emotional warmth, empathy and closeness, to emotional coldness, indifference and neglect) and overprotection (varies from control of parental ties and eating disorders, overprotection, intrusion, infantilization and prevention of independent behavior, to allow independence and autonomy (27).

The Parental Bounding Instrument score showed that patients with eating disorders and self-destructive behavior perceived that they received poor paternal care, more than half of these patients feeling that their father had "no affective control". Thus, it supported the hypothesis that the family environment of patients with eating disorders and self-destructive behavior is more complicated than that of patients with eating disorders without self-destructive behavior, in that patients with eating disorders without self-destructive behavior have conflicts with their mother, while patients with self-destructive behavior often have conflicts with their father (26).

A study conducted in a group of adolescent schoolgirls indicates that shame/defect and dependency/incompetence schemes act as perfect mediators in the relationship between parental care, maternal overprotection and symptoms of eating disorder. These results provide additional support for the link between family functioning, early adaptation schemes and symptoms of eating disorders, highlighting the importance of low parental care and maternal overprotection. Low levels of parental care can lead to the development of beliefs that one is flawed and not loved while high levels of maternal over-protection can lead to the growth of a child who believes he or she is unable to handle day-to-day responsibilities independently and in a competent manner. Theoretically, the combination of these early adaptation schemes and the presence of dysfunctional assumptions related to nutrition, weight and shape may contribute to the development of an eating disorder (28).

Parental divorce has been implicated as an important risk factor for eating disorders, with higher rates of divorce being observed in people with bulimia nervosa. Divorce provides an increased risk of an eating disorder (e.g., unspecified eating disorder, *bulimia nervosa* and *anorexia nervosa*) and the associations between divorce and disordered eating extend to community evidence, such as bodily dissatisfaction, control behaviors of weight (for example, diet, exercise) and binge eating. Body dissatisfaction is linked to depression and generally suggests that individuals who are depressed have an increased level of bodily dissatisfaction. This may help explain why the body's dissatisfaction, but not other forms of disordered eating, has shown unique gene-environment interaction effects. Depression is associated with

parental divorce and there is some evidence that childhood separation events increase the risk of depression only if the latent genetic risk is presently high). In addition, stress factors prior to divorce (marital discord, separation of parents) can affect the inheritance of disordered eating more than the divorce itself (29).

Scientific evidence suggests that eating disorders are multi-determined: individual vulnerabilities, family structure and dynamics can all interact in a social setting, which exposes the adolescent to a great weakness. Eating disorders in adolescence may be related to the failure or difficulties of separation in the individual during childhood, difficulties arising from disrupted parent-child relationships. Also, eating disorders usually develop among individuals in dysfunctional parent-child relationships: bulimics and, to a lesser extent, anorexic people, perceive their families as conflicting, hostile, disturbing, and denying the child's emotional needs (30).

CONCLUSIONS

The paper indicates that most respondents reported at least one adverse childhood experience and about one-third of adults experienced at least two adverse childhood experiences in childhood. This finding suggests that an ACE should warn the clinician to evaluate the patient for a history of exposure to other forms of domestic abuse or dysfunction (31). Numerous studies have shown the role of trauma in the development of psychopathology in adulthood. Psychiatric comorbidity rates are higher in patients with eating disorders with a history of childhood trauma (6). The lifetime prevalence of affective disorders in eating disorders is between 25% and 80%, most patients with eating disorders often feel lonely and desperate (32).

However, few studies have evaluated the links between psychiatric comorbidities and basic symptoms of eating disorders in patients who have experienced childhood trauma. It was found that maltreatment was not associated with most lifetime psychiatric diagnoses in patients with binge eating disorders, although specific associations were observed for post-traumatic stress disorders and alcohol use disorders. Another study found that depression fully mediated the associations between some forms of childhood trauma and eating psychopathology. Our results suggest that the links between ED severity and physical abuse as well as sexual abuse may be mediated by the presence of one or more psychiatric comorbidities (6).

Given the above, how can we prevent adverse childhood experiences from affecting so many people? The answer can be multiple. For example, professional health organizations may consider addressing the physical and mental health of their members, including recovering from the harmful effects of any adverse childhood experience. Healthcare companies should pay for adequate short and long-term treatment of the adverse effects of childhood experiences on trauma survivors, which would save them money in the long run. Also, each training program for health care professionals should include information about the effects of trauma, such as child abuse or other adverse childhood experiences, how to ask about them, and what to do with a patient who has suffered this type of trauma. Parents should stop treating their children as if they were their property, and instead give them compassion and love, not shame and fear. In addition, more monetary and political support should be given to organizations work-

ing to prevent child abuse (10).

It may be clinically useful to consider all abusive experiences in the evaluation of patients with eating disorders. Treatment options will be better understood and applied when the role of different forms of childhood trauma in eating disorders is more firmly established.

Finally, the findings that certain forms of childhood trauma are predictors of eating disorders and that depression is a significant mediator suggests that early intervention for childhood trauma and the resulting depressive symptoms may help prevent the development of eating disorders in traumatized people (5).

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THE IMAGING EVALUATION OF TYPICAL AND ATYPICAL CALCIFICATIONS IN DIFFERENT ANATOMICAL REGIONS STUDY IN BRASOV, ROMANIA

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THE IMAGING EVALUATION OF TYPICAL AND ATYPICAL CALCIFICATIONS IN DIFFERENT ANATOMICAL REGIONS STUDY IN BRASOV, ROMANIA (Abstract): Calcifications in different anatomical regions were identified by using ultrasound exam, radiography, mammography, and computer tomography, according to the anatomical region investigated. **The aim** of this research is to highlighting and evaluating typical and atypical calcifications in the organs of different anatomical regions, correlating them with age and pathology, finding the best imaging methods for detecting calcifications according to the anatomical region examined. **Material and methods** This was a prospective study was conducted for 2 months in 2018, in a private hospital, on 1338 patients of different ages, with or without symptomatology, who were randomly assigned to the Department of Radiology and Medical Imaging for the following types of examinations: radiography, computer tomography, ultrasound exam and mammography. **Results:** Calcifications found by imagistic methods were evaluated in the following anatomical regions: head, neck, thorax, abdomen, and pelvis. A database was generated where all data was collected and analyzed with SPSS 20.0 software. Various situations have been highlighted by anatomical regions. **Keywords:** AGE, CALCIFICATION, ANATOMICAL REGIONS, ULTRASOUND EXAM, RADIOLOGICAL EXAM, MAMMOGRAPHY, COMPUTED TOMOGRAPHY.

Calcifications may be physiological or pathological and may occur at different ages.

We will briefly describe a general assessment of these calcifications in some anatomical regions considered in the study to understand the importance of their identification, their benign and malignant characterization, and their association with the age and type of imaging examination. The most common intracranial calcifications were highlighted in the pineal gland (a calcification over 1 cm in diameter under the age of 9 years may be suggestive of a neoplasm), habenula, choroid plexus (frequently evident in the middle portion of the lateral ventricles), basal ganglia (calcifications are 0.3-1.5% and increase with age), falx, tentorium cerebella (affects 10% of the elderly population), sagittal sinus and petroclinoid ligaments (related to elderly degeneration) (1). The most common neoplastic calcification are: oligodendroglioma (70-90%), craniopharyngioma (50-80%), germ cell neoplasms and pineal neoplasms (60-80%), central neurocytoma and primitive neuroectodermal tumor (50-70%), ependymoma (50%), ganglioglioma (35-50%), dysembri-

yonic neuroectodermal tumor (20-25%), meningioma (20-25%), choroid plexus papilloma (25%), medulloblastoma and low grade astrocytoma (20%), and pilocystic astrocytoma (10%) (2).

In the chest region calcifications are highlighted in the pulmonary parenchyma, mediastinum, hilar and mediastinal lymph nodes, pleura chest wall, vascular, or any combination of these structures. Gross calcifications are evident on a conventional chest radiograph, but smaller calcific deposits are better evident on digital radiograph, computer tomography and high-resolutions CT (3). Pulmonary calcifications are the common asymptomatic finding discovered on chest X-ray and Chest CT (4).

In breast calcifications high-quality image and magnification on mammographic views are essential for the detection and evaluation of breast calcifications. It is important to differentiate the microcalcifications of benign origin (which increase their frequency with the age) from those that are suspected to be malignant, since 55% of non-palpable cancers are of ductal carcinoma form in situ. Distributions diffuse/scattered are a characteristic of benign calcifications. Regional distribution can signify a 26% probability of malignancy. Grouped/clustered calcifications more than 5 per 1 cm (this being the lower limit) indicate a suspicion of malignancy. Linear distribution calcifications may indicate a 60% and the segmental distribution a 62% malignancy probability (5). The positron emission mammography investigation is useful for assessing patients with suspected focal calcifications present on mammography. This is absolutely necessary for identifying invasive carcinomas and high-grade ductal carcinoma in situ. It becomes useful for avoiding false negative results of stereotactic biopsy (2).

For region of abdomen and pelvis calcifications can be visualized in normal and abnormal abdominal structure. They appear on radiographs and CT images as radiopaque or hyper-dense lesions (3).

On ultrasound, calcification appears as hyperechoic, posterior acoustic shadowing depending on the size and characteristics of the surface structure of calcification. The color Doppler twinkling artifact is a helpful sign for identifying small calcifications and thus can improve diagnostic (6). Computer tomography is regarded as gold standard for the identification of calcified lesions. Normal structures that calcify are costal cartilage, mesenteric lymph nodes, pelvic vein clots, and prostate gland. A high content of calcium in abnormal structures may indicate pathology of pancreas, renal parenchymal tissue, blood vessels and vascular aneurysms, gallbladder fibroids (leiomyoma). The same is true for biliary, renal, bladder calculi, appendicolith, and teratoma. Gynecological calcifications, fibroids, can become calcified and appear as rounded structures of varying sizes and locations in the pelvis (1). Frequently encountered abdominal and pelvic tumors often present a typical calcification pattern (7).

Miscellaneous Tumors appear as: calcifications with punctate pattern are unusual in the gastrointestinal stromal tumors (3-10%); solid pseudopapillary neoplasm is a peripheral curvilinear, rim (eggshell), punctate pattern of calcification. Calcification is seen as single or multiple form, it can be located peripheral, central or internal septa of tumor and can be punctate (round), amorphous (irregular), curvilinear or rim (eggshell) (8). Calcifications in the pancreas were found in 10-20% of cases

with a peripheral, curvilinear pattern or septal location, including punctate and coarse (amorphous) pattern. Intratumorally calcification can differentiate pancreatic neuroendocrine tumor (20% show calcifications) from adenocarcinoma (2%).

Calcifications in the liver are a rare form of the biliary neoplasm, such as solitary cyst adenocarcinoma, which is well encapsulated. These are rarely found but have been reported in 47% of the cases.

Calcifications in the appendix can be curvilinear, peripheral, and can occur in <50% of cases in mucinous adenoma, low-grade mucinous neoplasm, and mucinous adenocarcinoma. Mucinous neoplasm appears as a round or tubular cystic mass; wall thickening, and the irregular shape raise suspicions of malignancy (9).

Calcifications can be seen in all types of neurogenic tumors with various patterns. The tumor usually occurs in the retroperitoneum of the adrenal gland (10).

Castleman disease is an uncommon benign lymph proliferative disorder. Hyperplasia of lymphoid follicles is located in thorax in 70% of cases, 10-15% in the neck, 10-15% in the abdomen, retroperitoneum and pelvis. Calcifications patterns include punctate, peripheral, central, arborizing calcifications (8-11,12)

MATERIAL AND METHODS

This was a prospective study, conducted for a period of 2 months in the Department of Radiology and Medical Imaging of a private hospital in Brasov. The batch consisted of 1,338 patients of different ages who underwent various imaging investigations, namely: radiographic examination, computed tomography, ultrasound scan, and mammography. The devices on which the study was conducted are radiography device - Axiom Iconos R200 - *Siemens*, computed tomography - Somatom Emotion 16 - *Siemens*, ultrasound RS 80 with Prestige - *Samsung*, Logiq S7 - *General Electric*, mammography - Senographe Crystal - *General Electric*. Calcifications found imagistically were evaluated in the following anatomical regions: head, neck, thorax, abdomen, and pelvis.

The age-groups of the patients in the study are not homogeneous; thus, we have calculated percentages distributed according to each anatomical area from the total of persons in each age group.

The distribution of the patient batch by age group is unbalanced; therefore, the percentages distributed to each anatomical area were calculated from the total age group. Independent variables are age categories, while dependent variables are the percentage of people with calcifications in different age groups.

A database was generated where all data was collected and analyzed with *SPSS version 20.0* statistical software. The collected data was processed in Excel, the graphs being generated using Pivot tables. The X^2 homogeneity test was applied to determine whether there are statistically significant differences in the age groups of the patients participating in the study and the occurrence of calcifications in these five anatomical regions.

The purpose of this study was to highlight calcifications in different anatomical regions except limbs, in symptomatic or asymptomatic patients, and correlate them with the pathology and type of examination.

RESULTS

Analyzing the presence of the patients in the batch studied by age groups it was

found that the most representative incidence was in the 51-60 years old age group (28.7% of the patients), followed by the age group 41-50 years - 18.8% and then the group 61-70 years - 16.8%, these age-groups representing more than half of the studied total of patients. The low incidence was highlighted under the age of 30 and over 80 years, with an average of 4.5%. If we consider the distribution of patients depending on whether or not a symptomatology is present, symptoms in different regions were found in 67% of patients. Most of them accused frequent pains in the abdominal and cranial region (for both sexes). In the case of female patient's chest pain was noted, especially in the mammary gland. 33% of patients showed no symptoms; most of these patients came for a routine or regular control sent by an occupational physician.

In terms of gender of patients, a slightly higher (by 1%) incidence in the case of the females was noted (tab. I)

TABLE I

Distribution of patients with and without calcifications by age group

Age group	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	>80
With calcifications	3	6	43	85	185	286	191	105	74
Without calcifications	21	38	55	44	67	98	34	3	0
Percentage with calcifications	12.5	13.63	48.87	65.89	73.41	74.47	84.88	97.2	100

Analyzing the presence of calcifications according to the patients' age, it was found that their age-related linear increase was lower for those under 20, reaching 73% for the age group of 41-50, 97% for the group over 70 years, and 100% for the persons in the group over 80 years.

There is an increasing trend in the number of calcifications in total by age. Different trends have been observed for each anatomical region (fig. 1).

The sample we had was not equally distributed by age category and we took in consideration the percentage of patients presenting calcifications from each age group.

The regression was performed for each anatomical region to analyze age dependency of the anatomical area in the patients presenting calcifications. There was a 91% influence of calcifications on all anatomical regions studied in relation to age. Straights and regression equations have been drawn.

The determination coefficient, R square, for each anatomical region was obtained (fig. 2).

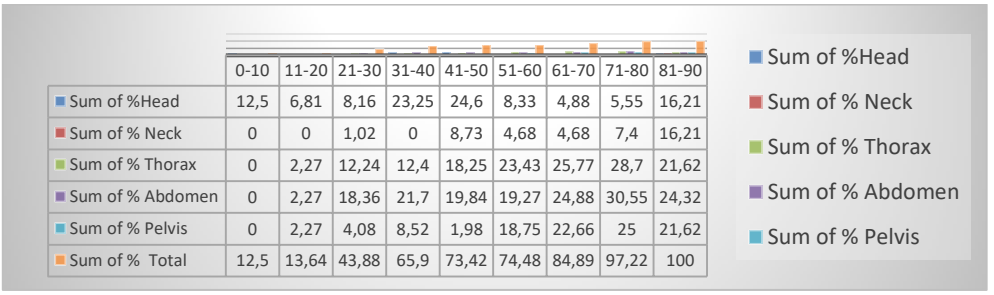

Fig. 1. Percentage distribution of patients according to age group and anatomical region

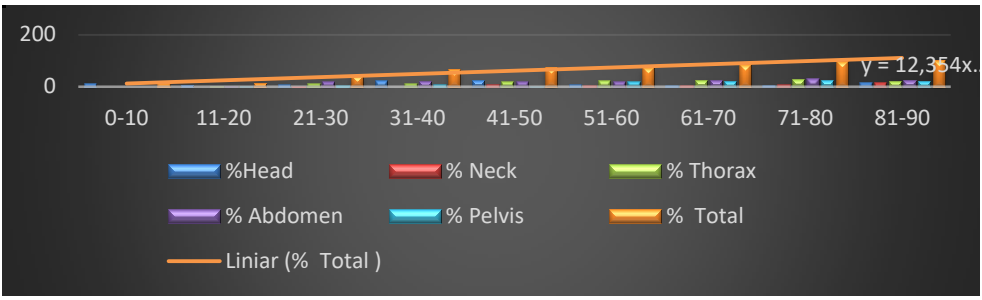

Fig. 2. Linear regression for each anatomical region

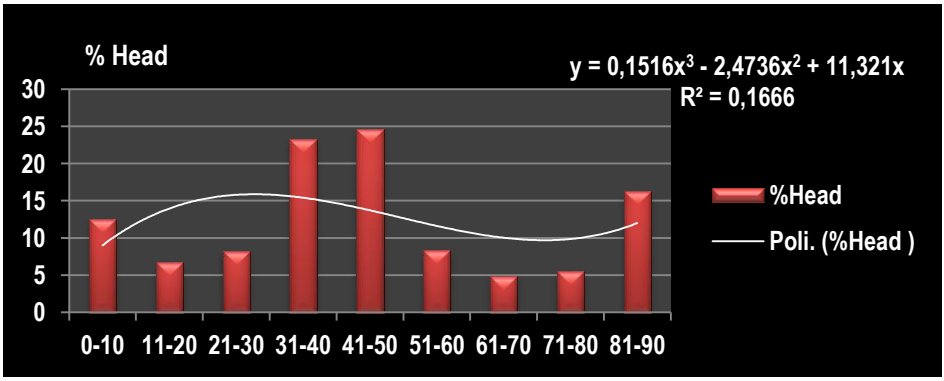

Fig. 3. Polynomial regression between head region and age

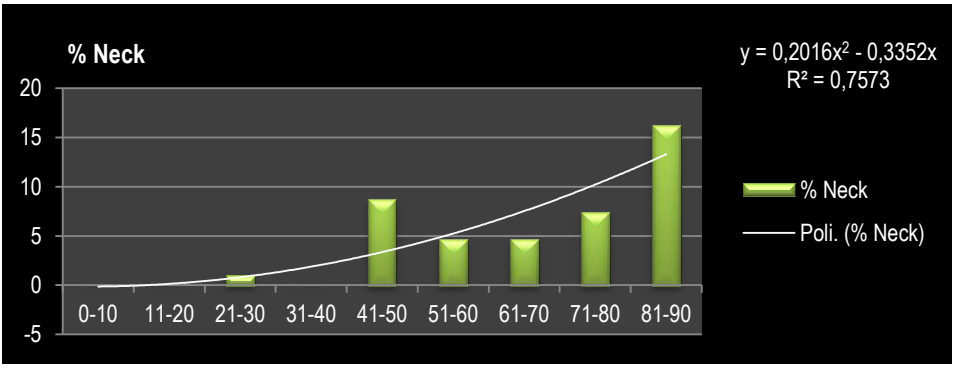

Fig. 4. Polynomial regression between neck region and age

R square shows how much age influences the presence of calcifications in various anatomical regions.

R square in the Head region has an influence de 16% in what calcifications are concerned, but it is inversely proportional with the increase of age. Between 30-50 years there is the highest peak (a mean of approximately 24%). The peak may be influenced by stress factors (fig. 3).

As far as the neck region is concerned, the number of people presenting calcifications increases with age, but a maximum appears around the age of 41-50 of 75% (fig. 4).

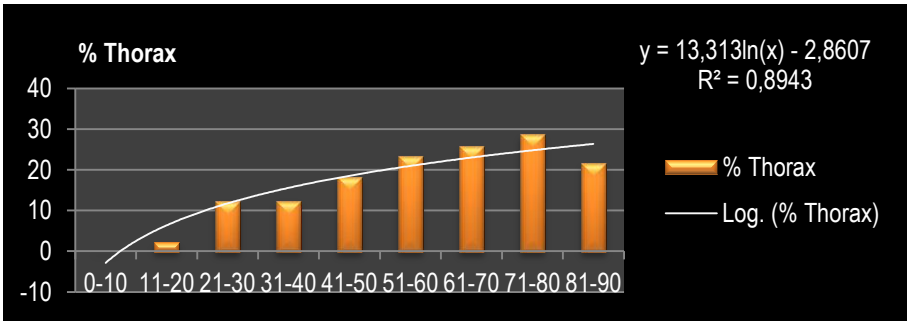

Fig. 5. Logarithmic regression between thorax region and age

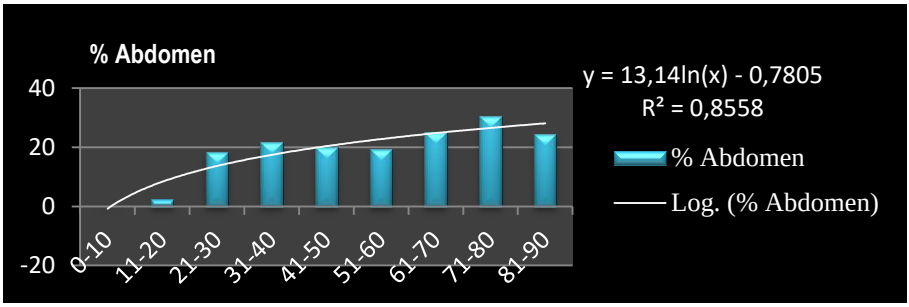

Fig. 6. Logarithmic regression between abdominal region and age

In the case of the thoracic region, we have the strongest correlation of 84% and a proportional increase with age of calcified individuals, but a reduction for the patients over 80 years due to the small number of persons in this age-group who came for an imaging evaluation in the period of the study (fig. 5).

In the abdominal region, we also have a close relationship of 85% and a rise of calcifications with age, but it falls in the case of the patients over 80 years. This may be due to the small number of patients who were present in relation to the other age groups, as was also the case for the results concerning the chest region (fig. 6).

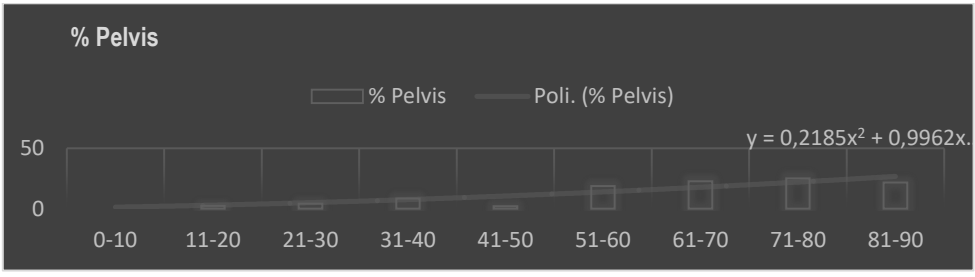

Fig. 7. Logarithmic regression between the pelvic region and age

In the pelvic region we have an 80% correlation, an increase proportional to the age of people with calcifications, similar to the chest and abdominal region (fig.7.)

TABLE II
Determination coefficient R square for each anatomical region

Anatomic region calcifications / Age	Head	Neck	Thorax	Abdomen	Pelvis
R Square	0.1666	0.7573	0.8943	0.8558	0.8094

By considering the R square determination coefficient for each anatomical region, an influence of age in the occurrence of calcifications in different anatomical regions was found. In the head region, there is a small influence (16%) of age on calcification. Age dependence in the occurrence of calcification in the neck is 75%. The risk of calcification at thorax level is higher, 89%. For the pelvis and the abdomen, the percentage is 85% and 80%, respectively (tab. II). Calcifications were less numerous in the cranial region due to its smaller volume and number of organs located in it than in, for example, the thoracic and abdominal region. Calcifications were more frequent in the elderly patients, but the number of patients who were part of the study, especially those over 80, was smaller. Using SPPSS we showed that there are statistically significant differences between the groups analyzed by X^2 = 25,674; p = 0.001, meaning that age does affect calcifications. Age influences cranial calcifications, as seen in the observed and expected counting table. It is statistically demonstrated that calcifications occur depending on the group of participants and on the age category. DATA-Weight Cases were weighted. By this statistical method, for each anatomical part we have determined the ages in which there is a tendency for the occurrence of more calcifications than is normally expected. In this study, the presence of typical and atypical calcifications in different organs, age groups, case correlations and pathology were analyzed.

DISCUSSION

In the head region, we identified the presence of calcifications in the young age up to 40 years in the pineal gland, habenular commissure, falx, tentorium cerebellum, and a case of congenital calcifications in the *Sturge Weber* syndrome. Between 40-50 years, calcifications occurred in the lenticular nucleus and in one case of cerebral tumor recurrence. After the age of 50, the presence of atheromatous calcium plaques was observed in the intracranial cerebral vessels, associated with the presence of the pathology. Calcifications in the neck region have been noted frequently in nodules in the thyroid gland (with a peak between 40-50 years), increasing also with age at the neck artery. Until the age of 40, calcifications in the thoracic region were revealed in the form of small calcifications at the level of mediastinal adenopathy, wall calcifications in the costal cartilage, while after 40 years at the level of some lung or solitary nodules, solitary or multiples. Calcifications in the abdominal region were surprisingly frequent in the 21-30 age group.

The presence of kidney and gall bladder stones and the environmental influences were more often observed among female patients in this group. After 40 years calcifications have been revealed in the spleen, adrenal gland, pancreas, calcifications associated with the history of pathology, in the male sex these being commonly encountered in the pancreas. At the age of 11-20 years, physiological calcifications in the prostate were present, the pathological calcifications being more frequent after the age of 50. At females, there have been calcifications in the genital

sphere, especially calcifications in the uterine fibromatous nodules. In both genders, calcifications of normal structures, namely pelvic vein clots -phlebolith, and at the level of some vessels were noted. Imaging investigations are useful tools for woman after 50, especially in the postmenopausal period.

CONCLUSIONS

In the revised literature, calcifications were highlighted in all anatomical regions, in most organs by different methods, without correlating them with the pathology of other organs, we tracked their incidence and correlated with age and certain pathologies existing only in the analyzed region. Highlighting intracranial calcifications by CT examination requires their description in terms of location of size and shape, which are necessary for a correct diagnosis and their correlation with age and neurological signs for true diagnosis and therapy. The method is original because the random group determined by collecting imaging investigations for two months, considering five anatomical regions, demonstrates the dependence of the number of patients with calcifications on their age, but also the particularities of each anatomical area. Using imaging investigation and correlating with patient's symptomatology or age is an accessible and noninvasive tool for screening neoplasms and benign tumors, with an impact on group ages people, especially on the elderly. In the future studies we intend to correlate the presence of calcifications in certain organs with their pathology in other anatomical regions.

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PROBLEME DE DIAGNOSTIC ÎN HISTIOCITOZA LANGERHANS LA COPIL

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CHILDREN LANGERHANS CELL HISTIOCYTOSIS DIAGNOSIS PROBLEMS (Abstract) : Langerhans cell histiocytosis is a group of idiopathic disorders characterized by the proliferation of cells of the mononuclear phagocyte system and the dendritic cell system. From the clinical point of view there are 3 forms of histiocytosis encountered in child pathology : acute, disseminated called Abt-Letterer-Siwe (it usually affects children under 2 years of age), eosinophilic granuloma and the intermediate clinical form called Hand-Schuller-Christian disease. We present the case of a premature born child that had, even from the day she was born, a rash disseminated on the entire surface of the body associated with enlarged lymph nodes, hepatosplenomegaly and thrombocytopenia. The diagnosis of Langerhans cell Histiocytosis is difficult to establish and this case is an example that confirms it. Therefore, we present the difficulties in diagnosing this case and its particular evolution. **Key words** : HISTIOCYTOSIS, CHILD, RASH

PERCEPTIONS OF COVID-19 VACCINATION AMONG HEALTHCARE PROFESSIONALS IN ROMANIA

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PERCEPTIONS OF COVID-19 VACCINATION AMONG HEALTHCARE PROFESSIONALS IN ROMANIA (Abstract): The first case of new coronavirus infection was reported first in the Wuhan city area from China in December 2019. The virus spread rapidly worldwide, produced an atypical cluster of pneumonia, was known all over the world as Coronavirus Disease 2019 (COVID-19) and, in Romania, first case was registered on the 26th of February. In Romania, the number of cases increased constantly, and a lot of healthcare professionals were infected with SARS-CoV-2. Aim: The aim of our study was to assess the opinion of healthcare professionals on the situation generated by the COVID-19 pandemic and to find what treatment against COVID-19 (a future vaccine, hydroxychloroquine) would be accepted by healthcare professionals. Material and methods: We used an online survey that was conducted from April 15th, 2020 to May 15th, 2020. The survey was developed using Google Forms and included 15 items. The respondents were recruited from health professionals (physicians, pharmacists and nurses), and future health professionals. Results: a number of 529 healthcare professionals were included in the study from different cities of the country. A total of 365 respondents (69%) agreed with the COVID-19 vaccine. Differences in accepting the vaccination against COVID-19 coverage by profession ($p=0.011<0.05$) were found. Conclusions: Based on the assessment of the current COVID-19 situation globally, the COVID-19 vaccines must be inoculated to the healthcare workers, but training on vaccination and compulsory measures may be needed in order to achieve better coverage. **Keywords**: COVID-19, VACCINATION, HEALTHCARE PROFESSIONALS.

In Wuhan city area from China was reported appearance since December 2019 of a new coronavirus which was named later Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2). This virus produced an atypical cluster of pneumonia, spread rapidly all over the world and known worldwide as Coronavirus Disease

2019 (COVID-19) (1). On 30 January 2020 to an international concern (PHEIC) the COVID-19 emergency was declared the sixth public health emergency by the World Health Organization (WHO) (2). On 11 March 2020 WHO declared COVID-19 officially pandemic (2). The symptoms associated with COVID-19 include cough, fever, diarrhea, shortness of breath, myalgia, sore throat, headache, and fatigue. Complications of the disease include pneumonia, acute severe respiratory distress syndrome, renal failure, or even death in certain cases (3, 4). In Romania, the first case of COVID-19 was registered on 26th of February, in the contact of an Italian infected person. In the next days, this case was followed by a few cases every day, until March 10, when the number of cases increased. A lot of Romanians (around 5 million) are working in Western Europe (around 1 million in Italy, but also In Spain, France, Germany, United Kingdom) (5). In all these countries and other western Europe countries, SARS-CoV-2 rapidly spread, the lockdown of most economical activities and tens of thousands of Romanians returned home. This emigration creating a huge potential for imported cases. Vaccines and treatments for COVID-19 are currently in development (6). Several studies reported that hydroxychloroquine has antiviral and prophylactic activity against SARS-CoV-2 (7-11).

In countries with weaker healthcare systems, prevention and supportive care are highly recommended (2, 12), as suggested by WHO director-general Tedros Adhanom Ghebreyesus (2). The COVID-19 pandemic causes mental health problems and panic for the public (13-15). Misinformation about this epidemic and myths, executive orders to quarantine travelers, and travel bans might affect the public's psychological health (14, 16, 17). Health literacy (HL) could help individuals and healthcare systems achieve a better lifestyle, quality of care, treatment decisions, disease management, and health outcomes (18, 19). Studies showed in previous pandemics that healthcare professionals were under strong stress from spreading the disease to their families, fear of becoming ill, and the heavy workload (20, 21). The threat of the disease can cause depression, anxiety, and stress (22-25).

This study aimed to assess the opinion of health professionals and future health professionals on the situation generated by the COVID-19 pandemic and to find what treatment against COVID-19 (a future vaccine, hydroxychloroquine) would be accepted by health professionals and future health professionals.

MATERIAL AND METHODS

Ethical Issues

This research was approved by the Academic and Scientific Ethics and Deontology Committee of the University of Medicine and Pharmacy in Craiova (Registration No. 26/2020) according to the European Union Guidelines (Declaration of Helsinki). All the respondents provided electronic informed consent starting with the first question of the survey.

Study design

The survey was developed using Google Forms and included 15 items. It was conducted from April 15th, 2020 to May 15th, 2020, and the respondents were recruited from health professionals and future health professionals. The online survey was anonymous and confidential. It was composed of three main sections: demographic characteristics, attitudes, and beliefs towards COVID-19 and treatment against COVID-19. The demographic section was com-

posed of seven items exploring age, gender, city, profession (physicians, pharmacists, nurses, medical students), the instruction level, the specialty, type of hospital (designated hospital vs. non-designated hospital). Three items about the treatment against COVID-19 composed the treatment section. Five items about beliefs were included in the third section.

Statistical Analysis

Continuous variables (age) were summarized as mean and standard deviation (SD). Kolmogorov-Smirnov Test was used to verify the normal distribution of continuous variables. Categorical variables were summarized as frequencies and percentages. Chi-Square tests or Fisher's exact tests were performed to compare categorical variables as appropriate. The analysis was performed with IBM *SPSS for Statistics version 25* (IBM SPSS, Armonk, NY). The significance level was 0.05.

RESULTS

A number of 529 health professionals and future health professionals were included in the study from different cities of the country, 362 of which were female (68.4%), with a mean age of 35.34±10.75 years (range: 21-66 years). The health professionals were the most of them from Craiova (309, 58%) and Bucharest (44, 8%). From the physicians, 115 (33%) were senior specialists, 114 (33%) specialists, and 117 (34%) residents. From the pharmacists, only 4 (5%) were senior specialists and 7 (9%) specialists.

A number of 154 (45%) physicians worked in a designated COVID-19 hospital and only 8 (26%) were nurses. From the pharmacist's respondents, 5 (6%) of them worked in a designated hospital, and the rest of them in a community pharmacy (tab. I).

TABLE I

Health professionals' characteristics

	Physicians (n=344)	**Pharmacists** (n=84)	**Nurses** (n=31)	**Medical students** (n=66)	**p-value***
Gender Female	207 (60%)	75 (89%)	26 (84%)	53 (80%)	p<0.001
Age (years old) <30 30-40 41-50 51-60 >60	 91 (27%) 137 (40%) 58 (17%) 40 (12%) 17 (5%)	 52 (62%) 24 (29%) 6 (7%) 2 (2%) 0	 5 (17%) 10 (33%) 11 (37%) 4 (13%) 0	 58 (88%) 8 (12%) 0 0 0	p<0.001
Working place Designated COVID-19	154 (45%)	5 (6%)	8 (26%)	-	p<0.001

*Chi-square test

In second table we define the attitudes about vaccination and COVID-19 treatment during the administration of the questionnaire.

A total of 365 respondents (69%) agreed with the COVID-19 vaccine. Differences in accepting the vaccination against COVID-19 coverage by profession ($p=0.011<0.05$) were found. In particular, physicians and medical students were more likely to accept COVID-19 vaccination than the other professions.

The medians of age (the distribution of age is not normal after One-Sample Kolmogorov testing) are the same across categories of accepting COVID-19 vaccination (p=0.138). Differences in accepting the vaccination against COVID-19 coverage were also found by depressed state (p<0.001): the depressive one was more likely to accept the vaccination.

The level of satisfaction with the protection measures taken did not influence the opinion on vaccination (p=0.094>0.05), nor knowing anyone infected with COVID-19 (p=0.316) or coming in contact with COVID-19 (p=0.063).

Fear of getting infected (p<0.001) and working in a designated hospital (p=0.023) made the difference in accepting COVID-19 vaccination.

Gender did not influence the opinion of acceptance of COVID-19 vaccination (p=0.664).

TABLE II

COVID-19 treatment opinion

N (%)	Physicians (n=344)	Pharmacists (n=84)	Nurses (n=31)	Medical students (n=66)	p-value*
If there was a vaccine against COVID-19, would you be vaccinated ?					p=0.011
Yes	242 (70%)	53 (53%)	19 (61%)	51 (77%)	
Do you know anyone infected with COVID-19?					p<0.001
Yes	161 (47%)	16 (19%)	8 (26%)	8 (12%)	
Do you think you have come in contact with COVID-19?					p<0.001
Yes	177 (51%)	24 (29%)	15 (48%)	5 (8%)	
Are you afraid of getting infected?					p<0.001
Yes	185 (54%)	48 (57%)	17 (55%)	49 (74%)	
What do you think about the protection measures in place ?					p<0.001
Yes, I am satisfied	212 (62%)	44 (52%)	22 (71%)	38 (58%)	
Do you feel sad / depressed during this period?					p=0.06
Yes	163 (47%)	32 (38%)	16 (52%)	41 (62%)	
Would you take hydroxychloroquine prophylactically?					p=0.03
Yes	87 (25%)	9 (11%)	9 (29%)	8 (12%)	
Would you recommend hydroxychloroquine?					p<0.001
Yes	186 (54%)	16 (19%)	11 (35%)	22 (33%)	

*Chi-square test

From all the respondents, 301 (57%) had a fear to get infected with SARS-CoV-2, from which the most were medical students (74%).

A total of 316 (60%) respondents considered satisfied with the available protection measures in hospitals/pharmacies. There is significant difference between the responses: the pharmacists were least satisfied with the protection measures taken, and the nurses the most satisfied.

255 of the respondents (48%) were sad/depressive during the COVID pandemic. Also, there are no differences in the depressive fillings between healthcare professionals from designated and non-designated hospitals (p=0.202). No differences were found in the depression level between feminine and masculine healthcare professionals (p=0.843). From healthcare

workers, nurses have the highest level of sadness/depression (52%).

Even if the treatment with hydroxychloroquine were controversial at the time of the survey, 113 (21%) from the respondents would administer prophylactically and 236 (45%) would recommend hydroxychloroquine in the treatment schedule.

DISCUSSION

This survey showed all factors that influence the acceptance of COVID-19 vaccination. More than 70% of the doctors and future doctors agreed with the vaccination, while 53% of pharmacists and 61% of nurses agreed with vaccination. Training on vaccination and compulsory measures may be needed in order to achieve better coverage. COVID-19 vaccination might be useful in the case of additional subsequent waves occur (26).

Regarding the sadness / depression during this period, all the healthcare professionals had a medium level, without differences between them or between the genders. The COVID-19 pandemic may be stressful for healthcare professionals because of the fear and worry about your health and the health of the loved ones, and the changing in live patterns. Furthermore, healthcare workers feel under pressure, particularly if they have not been involved in a similar situation. Other studies proved that rates of anxiety and depression were higher for female healthcare workers and nursing staff (27).

According to our results, a high percentage of the respondents are afraid of getting infected with COVID-19: 54% of physicians, 57 of pharmacists, 55% of nurses, and 74% of medical students. It is an extremely high percentage for medical students, despite the fact that only 8% of medical students thought that they have come in contact with COVID-19.

Community pharmacists were vital healthcare professionals during the outbreak, being on the frontline of public health with direct access for the patients (28-31). 52% of the pharmacists' respondents were satisfied with the protection measures provided. This small percentage is due to the fact that no Romanian organization or authority has dealt with ensuring the protection of pharmacists in the first month of the outbreak. The pharmacists themselves and their employers provided personal protective equipment. In the community pharmacies, the employers ensured that pharmacists are getting adequate breaks between shifts to ensure the disinfection of the space. Also, appropriate measures were taken to prevent the spread of infection and several modifications were made to the environment and work processes. Protection guidelines on COVID-19 were issued for the pharmacists by the *International Pharmaceutical Federation* (32) and other editorials (33). In Romania, the community pharmacies were encouraged to receive the prescriptions through email/electronic transfer. According to FIP, in order to expand capacity for mass immunization against COVID-19 when vaccines are available, the Romanian Government should consider enabling pharmacists to immunize adults (34).

Although the proved benefit of hydroxychloroquine is very limited against COVID-19 (34, 35), its demand was very high and there were problems in providing them to patients who were being treated with them for other chronic diseases, like rheumatoid arthritis, lupus, *porphyria cutanea tarda*. The percentages of recommend hydroxychloroquine (54% by doctors) or taking prophylactically (25% by doctors) are too high given that available clinical trials have not demonstrated that either of hydroxychloroquine or chloro-

quine are clearly effective. Furthermore, a study suggested that treatment with hydroxychloroquine could increase the risk of patients dying from COVID-19 (36), even if it has been retracted and more trials have recommenced.

The strength of this study is the big number of healthcare professionals, including healthcare workers and medical students. The main limitation was the large number of physicians among healthcare professionals that responded to our survey.

CONCLUSIONS

Based on the assessment of the current COVID-19 situation globally, the COVID-19 vaccines must be inoculated to the healthcare workers.

The moderate percentage of Romanian healthcare workers that agree with COVID-19 vaccination demonstrate the mandatory measures that may be needed in order to achieve better vaccination coverage, so they must be protected during the COVID-19 pandemic.

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RETINAL VASCULAR IMPACT IN SYSTEMIC HYPERTENSION. CASE REPORT

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RETINAL VASCULAR IMPACT IN SYSTEMIC HYPERTENSION. CASE REPORT (Abstract): We report a case of malignant systemic hypertension in the context of a multifactorial association (chronic kidney disease, hereditary thrombophilia, autoimmune thyroiditis, hyperaldosteronism, infectious optic neuritis) with ophthalmological onset symptoms only, highlighting the interrelationship between the retinal vascular impact and systemic hypertension, as well as between the systemic hypertension and the patient's underlying diseases. The paper presents the case of a 40-year-old female who complained of sudden loss of visual acuity in both eyes. The ophthalmological examination was suggestive for hypertensive neuroretinopathy, but the management of diagnosis and therapy was particularly difficult in the absence of systemic complaints and low patient compliance for a long time regarding the complementary interdisciplinary consultations that were recommended by the ophthalmologist. Confirmation of the diagnosis of hypertensive neuroretinopathy was possible only late in the evolution of the disease, with the appearance of systemic complications of hypertension such as: encephalopathy, cardiomyopathy, and nephropathy. In this context, a plurietiologic determinism was identified: chronic kidney disease, autoimmune thyroiditis, hyperaldosteronism, hereditary thrombophilia, and infectious optic neuritis. The ophthalmologic condition began to evolve favorably anatomically and functionally only after the establishment of pathogenic treatment. In conclusion, we are faced with a complex case that requires a multidisciplinary approach to assess a wide range of diseases, which analyzed separately, can have retinal vascular impact. **Keywords**: AUTOIMMUNE THYROIDITIS, CHRONIC KIDNEY DISEASE, HEREDITARY THROMBOPHILIA, HYPER-ALDOSTERONISM, HYPERTENSIVE NEURORETINOPATHY.

Systemic hypertension is a major cause of worldwide mortality (1). Any target organ can be affected by acute, severe, uncontrolled hypertension, the retina being no exception (2).

Vasoconstriction, arteriosclerosis and increased vascular permeability that occur in systemic hypertension cause retinal vascular damage which is clinically classified in: hypertensive angiopathy, retinopathy

and neuroretinopathy (2,3,4).

The pathophysiological changes in retinal vascular diseases include narrowing of the arteriolar lumen (vasoconstriction), damage of the vascular endothelium, degeneration of the smooth muscle fibers of the vascular wall, leakage of the plasma into the vascular walls that coagulates and produces mural thickening and luminal narrowing (fibrinous necrosis) (4,5,6), hypoperfusion and end-organ ischemia with subsequent target-organ dysfunction (6,7).

The blood-retinal barrier is a selective barrier that restricts the passage of molecules from capillaries to the retina, allowing optimal neural function. In hypertensive retinopathy, the internal barrier (retinal vascular endothelium) is disrupted, while the outer barrier (retinal pigment epithelium) is generally spared (8, 9). The increase in vascular permeability generates nerve fibers layers ischemia (cotton wool spots) and hemorrhages (flame shaped). Hard exudates resulting from lipid accumulations in the hemorrhagic areas, are characteristically arranged as macular star, due to the disposition of Henle fibers that are found only at this level, missing from the avascular foveal area (9, 10, 11).

Papillary edema is the important consequence of hypertension on the optic nerve, the ischemia causing swelling and blurred disk margins and the leakage causing peripapillary hemorrhage and disc edema (5, 9, 10, 11).

CASE REPORT

We report the case of a 40-year-old woman that presented in our clinic for painless decreased visual acuity (VA) in both eyes (OU) with sudden onset and no systemic complaint. The patient had a significant positive family history of type II diabetes mellitus (mother) and systemic hypertension (father). She was smoker and suffered from obesity (BMI = 35 kg/m^2), dyslipidemia and hypothyroidism.

VA of the right eye was 20/100 and 20/40 in the left eye. The intraocular pressure and anterior segment evaluation were normal for OU.

Fundus examination of OU revealed typical elevated and hyperemic papilla with blurred margins and with absent excavation, arteries with irregular caliber, turgid veins, arteriovenous crossings with vein deviation, the presence of cotton wool spots located parallel to the temporal arches and macular star.

Optical coherence tomography (OCT) assessed the macular structural impact and showed in OU an increase in retinal thickness with neurosensory retinal detachment, due to the underlying intraretinal fluid corresponding to the interpapilomacular area. The optic nerve OCT demonstrated an artefactual increase of the retinal nerve fiber layer (RNFL) thickness and the significant decrease of the tomographic parameters of the excavation on OU (fig. 1).

Corroborating the anamnesis, the ophthalmological examination, and the context of the patient's denial of the pre-existence of systemic hypertension, the first stage diagnosis was for OU: Papillophlebitis, Neuroretinopathy of unknown etiology, Papillary edema, Macular edema, and Foveal retinal neurosensory detachment. Consequently, we initiated treatment with nicergoline, acetylsalicylic acid, sulodexide and recommended paraclinical investigations and complementary interdisciplinary consultations: cardiological, hematological, endocrinological, nephrological, neurological and diabetes.

The patient returned to our clinic several times with incomplete, uncertain, or invalid results of the requested interdisciplinary examinations, noting on each ophthalmological evaluation the progression of structural and functional changes, despite the above-mentioned recommendations and the intravitreal treatment with anti-VEGF and anti-inflammatory steroid (figs. 2-5). During the last evaluation (fig. 6), the patient complained of occipital headache, marked decrease in VA and developed an exacerbation of retinal changes with severe alteration of retinal tomographic parameters.

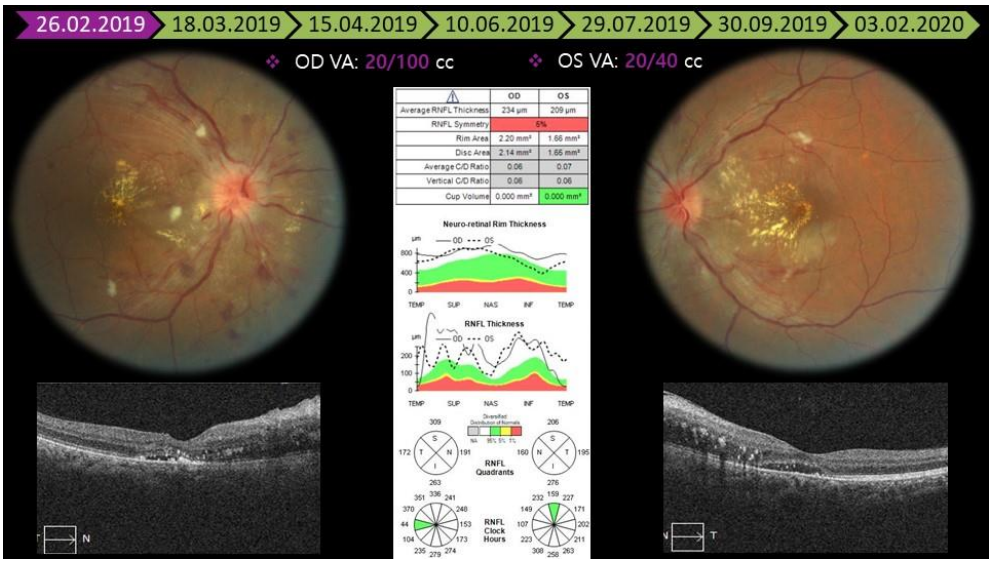

Fig. 1. Ophthalmological aspect at first presentation

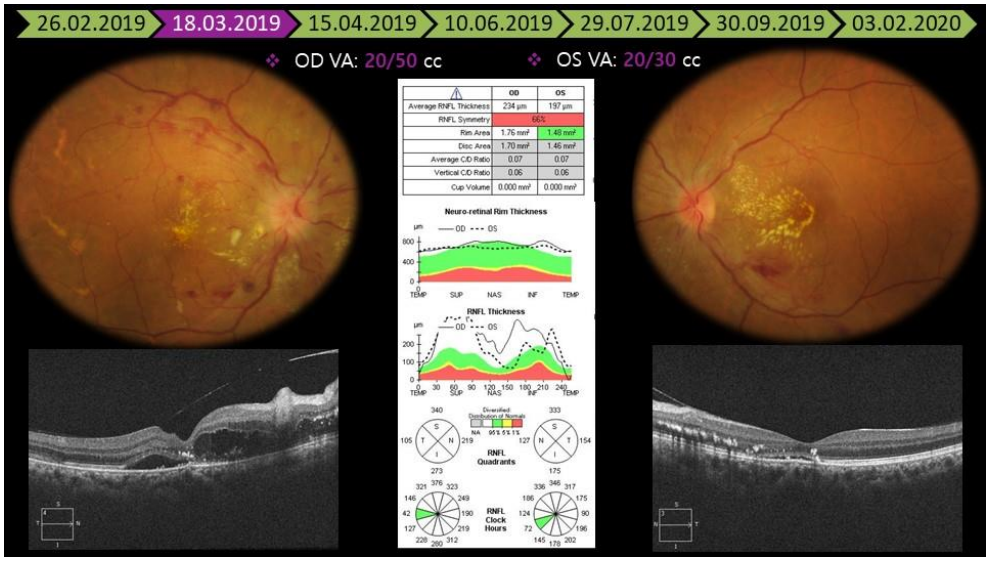

Fig. 2. Progression of structural and functional changes one month after presentation

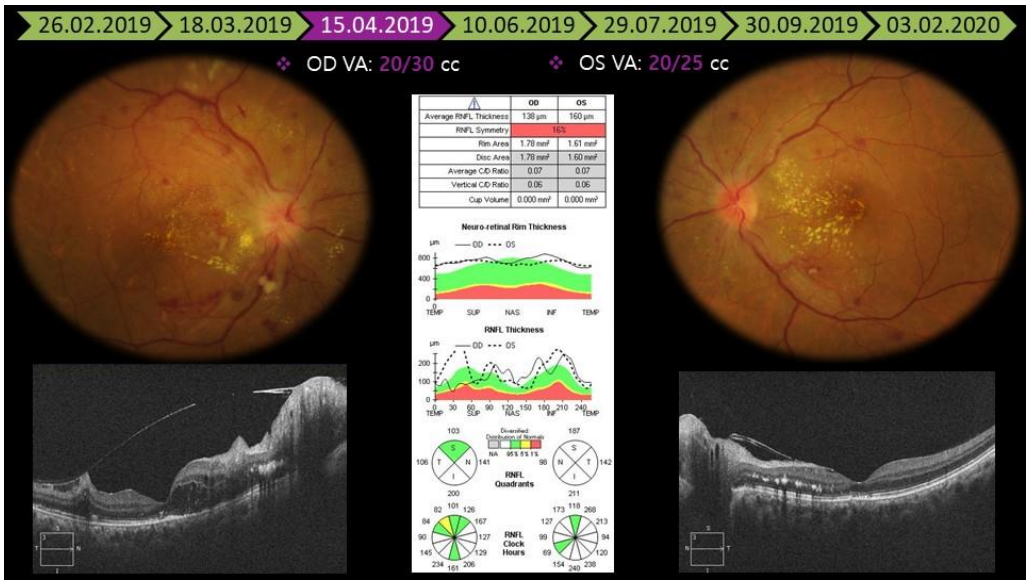

Fig. 3. Progression of structural and functional changes two months after presentation

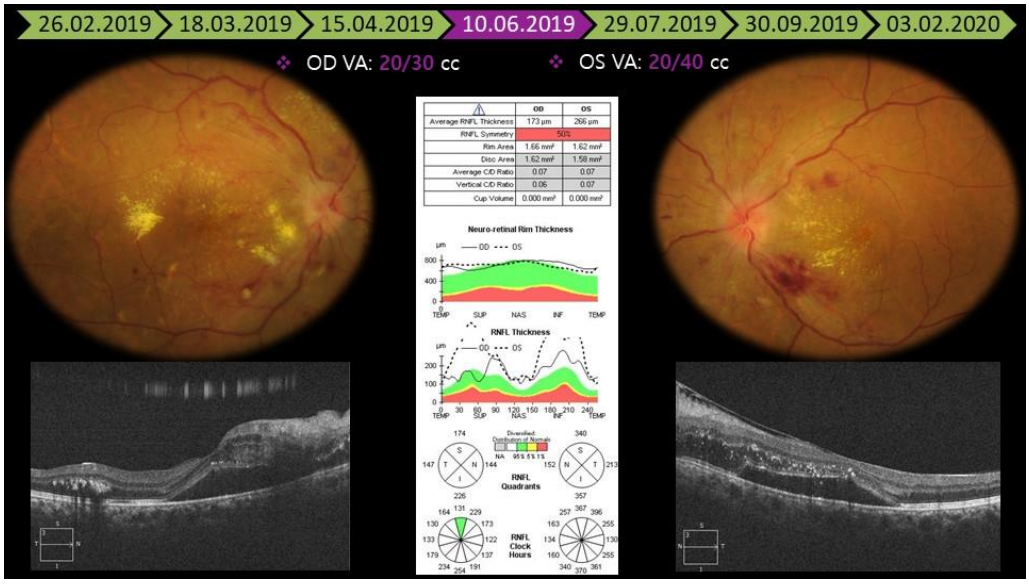

Fig. 4. Progression of structural and functional changes four months after presentation

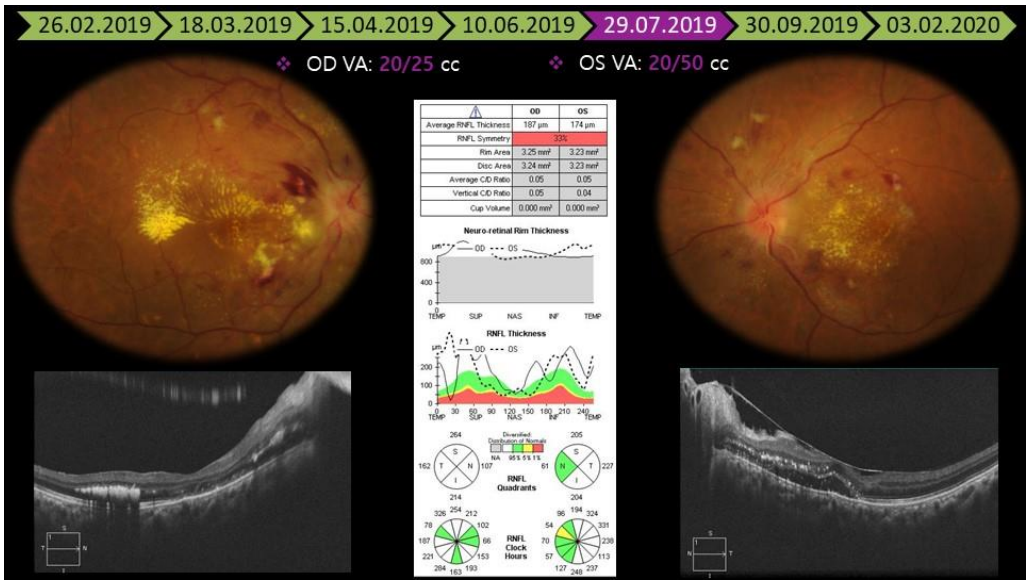

Fig. 5. Progression of structural and functional changes five months after presentation

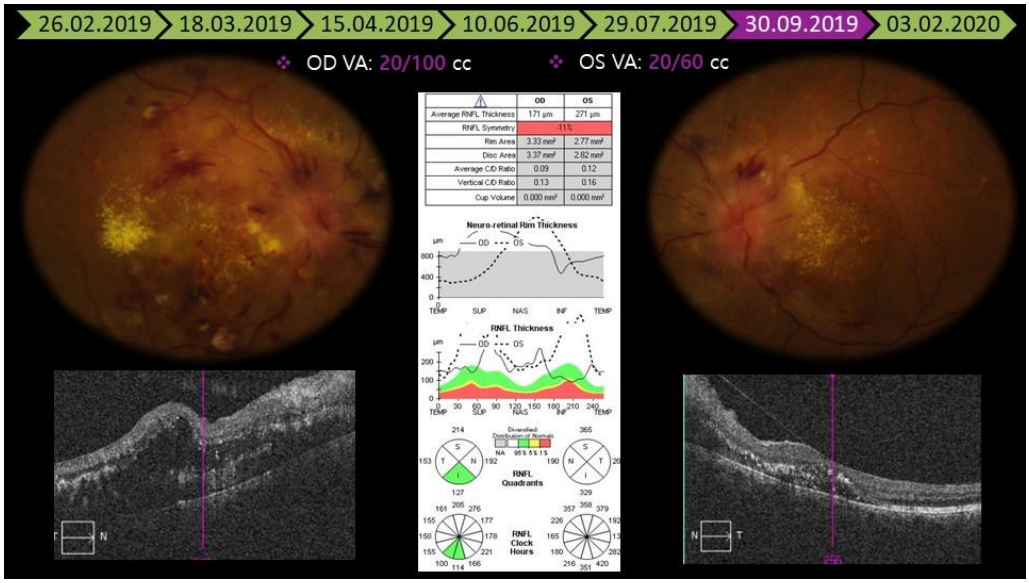

Fig. 6. Exacerbation of structural and functional changes seven months after presentation

Considering the given situation, the patient accepted the multiple interdisciplinary examinations, resulting several diagnoses as: malignant systemic hypertension (250/150 mmHg), hypertensive cardiomyopathy, hypertensive encephalopathy, chronic kidney disease stage G4, hypertensive nephropathy, hyperuricemia, secondary hypertension of endocrinological/renovascular cause, suspicion of prima-

ry or secondary hyperaldosteronism (aldosterone-renin ratio > 3), hypothyroidism and hereditary thrombophilia (MTHFR and PAI-1 genes mutations). The abdominopelvic MRI identified no tumors, urinary metanephrines and normetanephrines were in normal limits and the doppler ultrasound of the renal arteries showed no stenosis. As such, hygienic-dietary recommendations, and medical treatment (clonidine, amlodipine, spironolactone, furosemide, alopurinol and levothyroxine) were instituted, and the blood pressure reached the optimal parameters.

The VA improved in OU and we noticed the remission of the pathological elements: pink papilla with net edges and visible excavation, partial remission of macular exudates and disappearance of the cotton wool spots. The macular OCT revealed in OU the intraretinal fluid resorption, application of the neurosensorial retina and subsequently retinal thickness decrease, but with the appearance of macular atrophy due to ganglion cell complex - internal plexiform layer severe thickness decrease. The optic nerve OCT evaluation found normal optic disc tomographic parameters with normal RNFL thickness in OU (fig. 7).

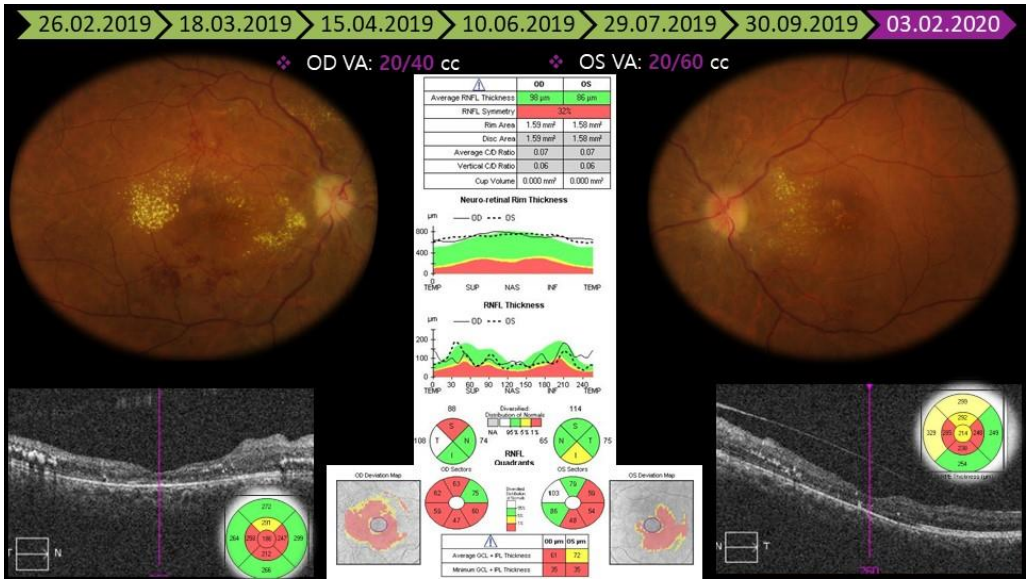

Fig. 7. Favorable ophthalmological evolution

The differential diagnosis of the ophthalmological aspect was done with retinal vascular diseases such as diabetic retinopathy which was excluded by normal laboratory examinations and with retinal vein occlusion (RVO) which could not excluded, it being frequently associated (8,12). It should also be noted that if the neurosensorial retinal application occurs after medical treatment, the diagnosis of hypertensive retinopathy is supported at the expense of RVO, as in the RVO, the outer blood-retinal barrier is affected while in hypertensive retinopathy is spared (9,13).

Regarding anemia and other blood dyscrasias (12), the presence of hereditary

thrombophilia can induce retinal changes.

We also considered other causes of papillary edema (14). The autoimmune or infectious cause were excluded by normal laboratory tests, except for the presence of antithyroperoxidase antibodies (TPOAb) which requires the differential diagnosis between the patient's hypothyroidism and autoimmune thyroiditis, respectively the presence of high titers of anti-Epstein Bar and anti-Varicella Zoster Viruses antibodies that raises the suspicion of a viral optic neuritis. Cerebral MRI was performed to assess intracranial hypertension of multiple causes, establishing the massive diffuse edema of the cerebral white matter which correlated with systemic hypertension and occipital headache led the neurologist to diagnose the hypertensive encephalopathy. Last but not least, we considered arteritis and non-arteritic anterior ischemic optic neuropathies (15, 16, 17) which were excluded by good VA and by the optic nerve aspect (3, 8, 11, 18,).

Secondary systemic hypertension can be caused by: pheochromocytoma, primary/secondary hyperaldosteronism, Cushing's syndrome, renal parenchymal/vascular disease, obstructive sleep apnea, hyperparathyroidism, hyperthyroidism, etc. (12). The patient is currently being evaluated by the endocrinologist and nephrologist.

Corroborating the data obtained so far, we established the positive diagnosis of Hypertensive neuroretinopathy in OU, along with the interdisciplinary diagnoses mentioned above.

The vital prognosis is correlated with multiple systemic complications that can occur over time. Visual prognosis depends on the evolution of the systemic diseases, severe retinal complications comprising retinal artery occlusion (19), RVO, retinal aneurysms, intravitreal bleeding, third and sixth oculomotor nerves palsy, hemianopathic deficiencies, etc. (3, 10, 11, 12, 18).

DISCUSSION

This case perfectly illustrates the retinal vascular impact of systemic hypertension. We would like to point out that many young patients with malignant systemic hypertension may actually present with bilateral vision loss due to serous macular detachment and bilateral optic disc edema (12, 13, 20). The relationship between young age and the retinal changes is consistent with the hypothesis that chronic hypertension, as it occurs in older patients, may have protective effects against the development of acute hypertensive damage (8, 21).

We also note the difficulty in distinguishing between essential and secondary hypertension in the context of renal impairment in this patient (chronic kidney disease stage G4, hypertensive nephropathy, aldosterone (523.5 pg/mL) - renin (107.44 pg/mL) ratio > 3, no renal arterial stenosis, no abdominopelvic tumors and normal urinary metanephrines and normetanephrines). Malignant hypertension is associated with activation of the renin-angiotensin-aldosterone system (RAAS), that plays a key role in the pathogenesis of hypertension. Severe increase in blood pressure would, in fact, lead to the activation of RAAS by microvascular damage and renovascular ischemia, and the increase in the production of angiotensin II would further increase blood pressure, leading to malignant hypertension. As such, we recommend following-up the renal function evolution and performing a renal biopsy if necessary (22, 23).

Hereditary thrombophilia (heterozygote

MTHFR and homozygote PAI-1 genes mutations), may play an important role in the etiopathogenesis of RVO. Smoking, obesity, dyslipidemia (hypertriglyceridemia - 157.6 mg/dL, cholesterolemia - 171.6 mg/dL, high-density lipoprotein cholesterolemia - 41.9 mg/dL and low-density lipoprotein cholesterolemia - 120.3 mg/dL) and the increased value of homocysteinemia (12.7 μmol/L) are also risk factors for systemic vascular thrombosis along of course with systemic hypertension (24, 25). Hyperhomocysteinemia is associated with both venous and arterial thrombosis in all vascular system, especially in the brain, therefore normalization of the elevated serum homocysteine may reduce the risk of a subsequent vascular event (26).

The thyroid gland has well-recognized effects on the cardiovascular system and blood pressure regulation. Blood pressure is altered over the entire spectrum of thyroid disease (27). The patient was already diagnosed with hypothyroidism and the presence of TPOAb was recently confirmed. First, we would like to emphasize that in the case of hypothyroidism and autoimmune thyroid disease there is a state of hypercoagulability given by increased fibrinogen level, factor VII level and activity, and plasminogen activator inhibitor-1 level, that may predispose to RVO (28). Another observation is the possibility of exacerbation of the autoimmune hypothyroidism in the primary hyperaldosteronism that the patient is suspected of (29).

Given the presence of antibodies in serum, both Epstein Bar and Varicella Zoster, viral etiology may be responsible for the appearance of neuroretinitis, as: anterior optic neuritis, also called papillitis (swollen optic disc), retrobulbar optic neuritis (normal optic disc), neuroretinitis (optic disc edema with macular star), anterior ischemic optic neuropathy, etc. (30). Another situation that could be considered would be vascular modification from vasculitis associated with papillary edema, which may pose problems of differential diagnosis. The pathogenesis of ischemia in retinal vasculitis is unclear, but it is suggested to be either thrombotic or obliterant secondary to the infiltration of inflammatory cells (31).

We identified several particularities in these case: the young age of malignant systemic hypertension onset; the ocular complaints were the first manifestations of a systemic disease; a multidisciplinary management was mandatory for the correct and complete diagnosis; the low compliance delayed the multidisciplinary evaluation; and the favorable evolution under pathogenic treatment with good functional and structural recovery.

CONCLUSIONS

We are faced with a complex case of hypertensive neuroretinopathy that requires a multidisciplinary approach to assess a wide range of diseases, which analyzed separately, can have retinal vascular impact.

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NEWS

ARTICULAR CARTILAGE REGENERATION: TISSUE-ENGINEERED CARTILAGE BRINGS NEW HOPE

Articular cartilage is non-lymphatic, non-vascular and non-neural structure and due to this fact is characterized by a limited capacity for self-repair. All traditional cartilage regeneration techniques have certain limitations. The technological leap in tissue engineering has brought hope in the regeneration of articular cartilage. Currently, three strategies are used to achieve "scaffold-cell" tissue regeneration, without scaffolding and without cells. The cell-scaffold construction, in which the seed cells are autologous or stem chondrocytes CaReS®, BioSeed®-C, together with NOVOCART® 3D are present on the medical market and are being the subject of several current clinical studies. Pluripotent stem cells and mesenchymal stem cells from different sources are mainly used for "scaffold cell construction". Cell-free strategies that indirectly use the repair and regeneration potential of stem cells, TruFit and MaioRegen are used in clinical practice. The scaffolding strategy represents a new direction of development, and the results of the short-term repair of the actor's product types are verry encouraging. (Shuangpeng J *et al*. Clinical Application Status of Articular Cartilage Regeneration Techniques: Tissue-Engineered Cartilage Brings New Hope. *Stem Cells Int* 2020; 2020: 5690252).

SURGERY **CASE REPORYS**

HIGH RISK PREGNANCY FOR ACHONDROPLASIA AND THE IMPORTANCE OF IgG AVIDITY TEST FOR DATING THE INFECTION WITH *TOXOPLASMA GONDII*. CASE REPORT

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HIGH RISK PREGNANCY FOR ACHONDROPLASIA AND THE IMPORTANCE OF IgG AVIDITY TEST FOR DATING THE INFECTION WITH *TOXOPLASMA GONDII*. CASE REPORT (Abstract): Achondroplasia is the most common disproportionate dwarfism caused by a mutation in fibroblast growth factor receptor 3 (*FGFR3*). The disease is inherited in an autosomal dominant manner, but 80% of cases are due to a *de novo* mutation. We present a couple consisting of a man with achondroplasia and a woman with normal stature. The man was diagnosed with achondroplasia at 5 years old, on clinical and radiographic basis. At age of 28 the molecular testing by PCR-RFLP confirmed the clinical diagnosis, being detected the G1138A heterozygote mutation in *FGFR3* gene. The couple asks for genetic counseling when woman was pregnant, and the couple was concerned about the possibility of transmission of mutation to children. The woman was tested by TORCH screen and was found positive testing for IgM and IgG antibodies against *Toxoplasma gondii*. The IgG avidity test for *Toxoplasma gondii* indicated the absence of recent infection with this parasite. Thus, the couple decided to continue the pregnancy, but demanded a prenatal diagnosis for achondroplasia. Molecular analyze was done on the DNA extracted from fetal cells obtained by early amniocentesis and the fetus wasn't a carrier of a mutation responsible for achondroplasia. A normal baby was born at term. In conclusion, we present the particularities of a high-risk pregnancy for achondroplasia and the utility of some tests for discrimination between recently acquired and an old infection with *Toxoplasma gondii*. **Keywords:** ACHONDROPLASIA, CONGENITAL TOXOPLASMOSIS, PRENATAL DIAGNOSIS, IGG AVIDITY TEST.

Achondroplasia (AC; OMIM 100800) is the most common form of disproportionate dwarfism. The main clinical signs are small stature, rhizomelic shortening of the limbs, macrocephaly, characteristic dysmorphic features with frontal bumps, flattened nose root, trident configuration of the hands, exaggerated lumbar lordosis (1). Birth prevalence of achondroplasia is 4/100,000 (2).

The disease is caused by mutations in the fibroblast growth factor 3 receptor gene

(*FGFR3*; MIM 134934). AC is transmitted in an autosomal dominant manner. In 80% of cases the mutation in the *FGFR3* gene is *de novo* (3, 4). *De novo* variants are exclusively produced during the paternal meiosis, being associated with advanced paternal age (5). The risk of an individual with AC to have children with AC when the partner is of medium height is 50%. If both parents are diagnosed with AC, the probabilities in the offspring are the following: 50% AC heterozygote form, 25% average waist, 25% AC homozygous form (lethal condition) (6).

Mutations in achondroplasia concern the *FGFR3* gene and all are located in the same region of this gene: the codon 380. The most frequent mutation is G1138A (98%) while in the rest of cases was discovered the G1138C variant. Both mutations generate the replacement of an arginine residue with glycine at position 380 of the protein. For this reason, the penetration of the disease is 100% (6).

The study based on the analysis of EUROCAT (European Surveillance of Congenital Anomalies) registries from 1991-2015 showed a stable, but heterogeneous prevalence in various regions and countries of Europe. In 20.2% of familial cases and 10% of sporadic cases there are associated congenital anomalies. The most important congenital anomalies associated with achondroplasia were anomalies of the nervous system (hydrocephalus 2.3%, Arnold Chiari malformation 0.5%), congenital heart defects (ventricular septal defect 1.6%, atrial septal defect 0.7%, patent ductus arteriosus 0.2%), urinary system (hydronephrosis 1.4%, double ureter 0.2%), omphalocele 0.2%, hypospadias 0.2%, and clubfoot 0.9% (3).

Complications associated with AC that can increase morbidity and mortality are: delayed motor and speech development in children, otitis media with hearing loss, respiratory dysfunction with obstructive sleep apnea, spinal stenosis and compression, dental malocclusions (7).

In AC, the change in quality of life refers to two areas: medical and psychosocial. The first category includes: pain, compromised physical function, overall diminution of functional health status (6,8-10). The second category is referred to: diminished income, less education and less successful employment, decreased self-esteem (8, 11).

Toxoplasmosis is caused by the parasite *Toxoplasma gondii*. It is one of the most common infections of humans worldwide (12). The birth prevalence for congenital toxoplasmosis was 33/100,000 according to European data (2).

Toxoplasmosis can be divided into 4 categories: 1) acquired in the immunocompetent patient; 2) acquired or reactivated in the immunodeficient patient; 3) ocular; 4) congenital (13).

T. gondii infection is asymptomatic in approximately 20% of cases of toxoplasmosis in immunocompetent individuals or shows minor signs (cervical lymphadenopathy or a flu-like illness) (13). Immunocompromised hosts may experience symptoms and severe damage: encephalitis, retinochoroiditis, fetal abortion, splenomegaly and pneumonitis (14).

The presence of the parasite during the pregnancy could generated different complications including vision loss, deafness, intellectual disability, severe developmental delay, neurological deficits. Prenatal treatment reduces the risk of developing these complications (15). Congenital toxoplasmosis can be subclinical at birth in

75% of cases, but over time the described complications could raise (13). In the case of symptomatic congenital toxoplasmosis, the classical triad includes chorioretinitis, intracranial calcifications and hydrocephalus, to which are added other elements of severity such as microcephaly, hepatosplenomegaly, jaundice, convulsions, chorioretinitis (often bilateral), cerebral calcifications, and abnormal cerebrospinal fluid (16, 17).

We present the case of a high-risk pregnancy for achondroplasia in which prenatal molecular diagnosis was made in the fetus and the IgG avidity test for *T. gondii* ruled out a gestational infection, invalidating a suspicion of congenital toxoplasmosis.

CASE REPORT

We present the case of a couple (28 years old, both members) who requested preconception genetic counseling because the man was diagnosed with achondroplasia. The diagnosis of achondroplasia was made on a clinical and radiological basis in childhood (at the age of 5 years), without a molecular test to reveal the gene mutation. The couple was unrelated. The family history was negative for achondroplasia. We discussed the risk of recurrence of the disease in offspring, the possibility of prenatal diagnosis and the need for molecular testing to highlight the mutation in proband.

Molecular testing for proband was requested. The PCR-RFLP technique analyzed the G1138A and G1138C mutations (*FGFR3* gene) associated with achondroplasia. The result of the analysis confirms the clinical diagnosis, the consultant being heterozygous for the FGFR3 G1138A mutation. In the same year, the couple returned for prenatal diagnosis. Molecular analyze was done on the DNA extracted from fetal cells obtained by early amniocentesis (12 weeks of gestation) and the fetus wasn't a carrier of his father's mutation in *FGFR3* or of the other mutation responsible for achondroplasia FGFR3 G1138C (fig. 1).

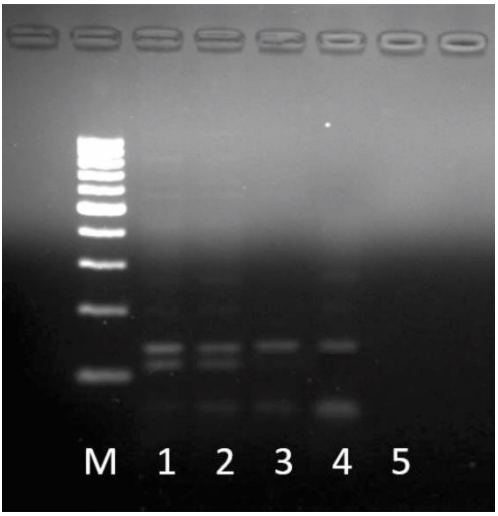

Fig. 1. PCR-RFLP analysis
M - molecular weight marker 100 bp;
1-positive control; 2-father;
3-fetus; 4-negative control; 5-water

The pregnant woman was tested by TORCH screen in the first trimester of pregnancy. The results showed that she was positive for IgM and IgG antibodies against *Toxoplasma gondii*: IgG antibodies 436.6 IU/mL (reference values: ≥ 30 IU/mL = positive); IgM antibodies = 0.96 IU/mL (reference values: ≥ 0.65 IU/mL = positive). Under these conditions, the question arose whether there was a risk of congenital toxoplasmosis in the fetus. To assess whether it was a preconception or acquired infection during pregnancy, it was recommended to perform the IgG antibody avidity test. Result of the IgG avidity test using ELFA (Enzyme-linked

fluorescent immunoassay) method was: *Toxoplasma gondii* IgG 82 IU/mL (< 4 negative; ≥ 4 and < 8 equivocal; ≥ 8 positive < 300 old immunity or onset of seroconversion; ≥ 300 recent or persistent seroconversion of an older high level). The avidity index was 0.488 (reference value: < 0.2 low avidity; 0.2-0.3 intermediate avidity; ≥ 0.3 high avidity). The increased avidity index ruled out recent infection (in the last 4 months) with *T. gondii* and thus ruled out the risk of congenital toxoplasmosis.

DISCUSSION

The molecular diagnosis strategy in AC includes as a first test, targeted analysis for the two common pathogenic variants. If the mutation is not highlighted, it can be done in the second stage of the multigene panel that includes *FGFR3* and other genes of interest involved in diseases with which a differential diagnosis can be made on a case-by-case basis (hypochondroplasia, thanatophoric dysplasia, SADDAN syndrome, cartilage-hair hypoplasia and other metaphyseal dysplasia, pseudoachodroplasia) (1, 18). In the case of our patient, the most frequent mutation was confirmed, so it was not necessary to proceed to the second stage of testing.

Pathogenic variant c.1138G>A (p. Gly380Arg) is identified in approximately 98% of patients with AC and variant c.1138G>C (p.Gly380Arg) in approximately 1% of cases (1). Thus, the molecular strategy and the genetic counselling in achondroplasia are easier than in the case of other monogenic diseases characterized by a high genetic heterogeneity (many mutations in the same gene / in different genes) (19, 20, 21).

Genetic counseling is a communication process, provided premarital or post marital, in the situation where there is genetic illness in the family (22, 23). The pregnancy of a couple in which one or both members of the couple are diagnosed with AC is a high-risk pregnancy. If the mutation was detected preconceptionally, the couple may benefit from prenatal or preimplantation diagnosis. Non-invasive prenatal testing could be performed on free fetal DNA cells in maternal blood, but this method is not yet wide used (24). In our case, molecular testing was performed by PCR-RFLP using fetal DNA obtained by early amniocentesis at 12 weeks of pregnancy.

Congenital toxoplasmosis is the consequence of infecting a woman with *T. gondii* in the first trimester of pregnancy or shortly before conception (25). The disease is preventable, treatable and reversible and therefore it is especially important to establish the diagnosis of acute or chronic infection during pregnancy. Treatment in utero during gestation significantly decreased mother-to-child transmission and improved clinical outcome (25-27).

In several European countries including Romania, the TORCH panel is recommended for pregnant women in the first trimester of pregnancy. If TORCH screening reveals IgM and IgG antibodies to *T. gondii*, it is important to distinguish between recent infection and past infection because the risks of congenital toxoplasmosis in the fetus are different and management is different also (fig. 2). *Toxoplasma gondii* - specific IgG avidity testing is an essential tool in establishing this in the immunocompetent host (28).

A high *T. gondii* specific IgG avidity result in 1st trimester is indicative for a past infection (older than 4 months). In our case it was a high avidity and, in this way, we

established that it was a primary preconception infection. When the result of the avidity test shows low or intermediate avidity this is not informative in dating the infection (28) (fig. 3). When the IgG avidity testing is made in the 2nd or 3rd trimester the results are more difficult to interpret because a high avidity is suggestive for past infection, but possibly during pregnancy (fig. 4).

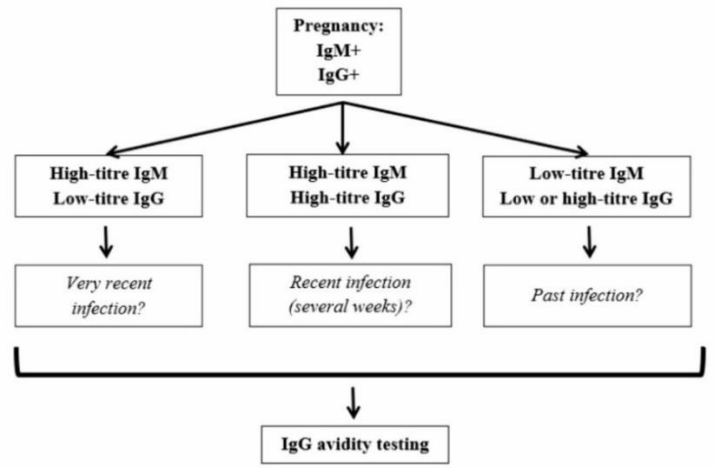

Fig. 2. Study of antibodies titers (Adapted from *Garnaud*, 2020)

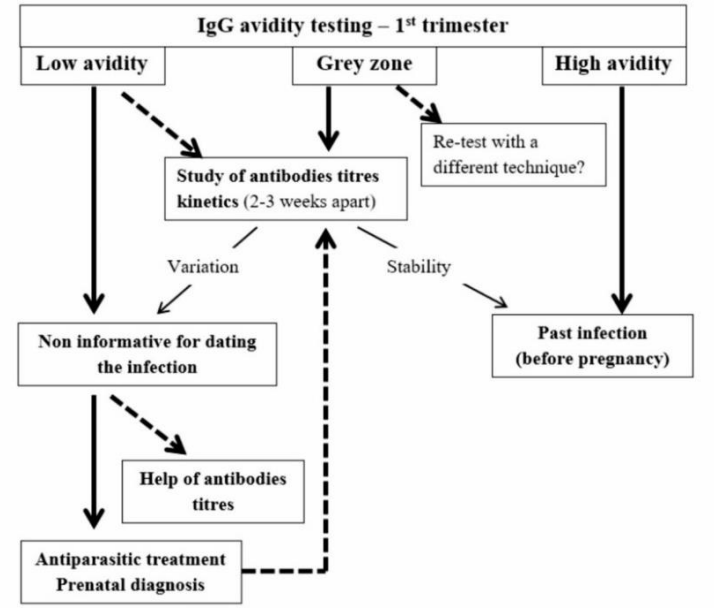

Fig. 3. Interpretation of IgG avidity testing and management in 1st trimester (Adapted from *Garnaud*, 2020)

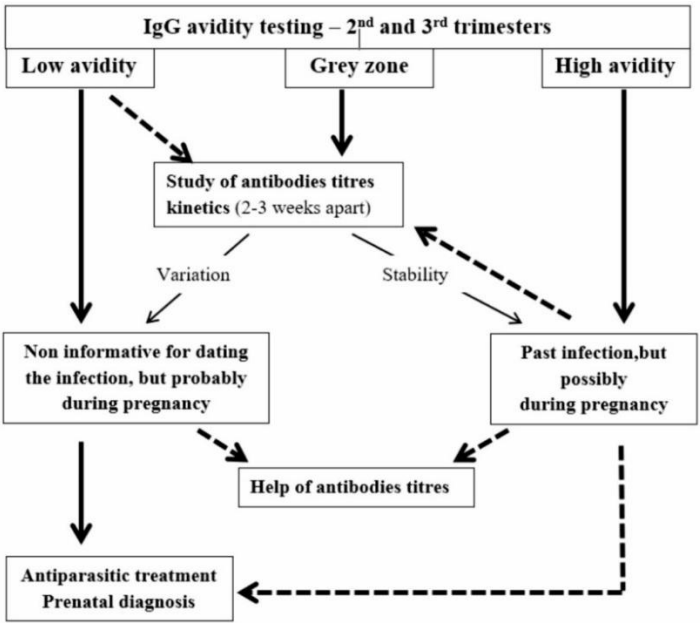

Fig. 4. Interpretation of IgG avidity testing and management in 2nd and 3rd trimesters (Adapted from *Garnaud*, 2020)

CONCLUSIONS

We present the particularities of prenatal diagnosis of a high-risk pregnancy for achondroplasia and the utility of IgG avidity test in 1st trimester of gestation for discrimination between a recently acquired infection and an old infection with *Toxoplasma gondii*.

CONFLICT OF INTERESTS AND FUNDING

The authors declare that there is no conflict of interest regarding this scientific research.

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SURGICAL COMORBIDITIES - A HIGH MEDICAL AND FINANCIAL BURDEN FOR HEMOPHILIA CARE

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SURGICAL COMORBIDITIES - A HIGH MEDICAL AND FINANCIAL BURDEN FOR HEMOPHILIA CARE (Abstract): Comorbidity in hemophilia, a pathology non-related to the primary disease, is a relatively new chapter, catching interest along with the revolutionary improvement of the outcomes of this coagulopathy. **Aim:** As prophylactic replacement therapy has been only recently introduced in our country, we aimed at assessing the frequency and the medical and financial impact of the surgical life-saving interventions of some challenging comorbidities of hemophilia. **Material and methods:** In the frame of a retrospective observational PRO (Patient Reported Outcomes) model survey conducted on 122 persons with severe congenital coagulopathies in the period of 2019-2020, we focused our attention on surgical comorbidities compared with surgical secondary morbidity. **Results:** There were performed 17 surgical interventions on 16 patients, 35.95% of them being for comorbidities. The comparative analysis of the two cohorts of patients, with comorbidities versus secondary morbidity, revealed some significant discrepancies: heterogeneity of the pathologic conditions claiming surgery and the modality of its performance, emergency or elective decision. There were registered also some similarities: age of patients and quality of outcomes. What concerns the financial impact, the medical direct costs were also similar. **Conclusions:** Surgical comorbidities are a high medical and economic burden for hemophilia care, claiming a comprehensive analysis of their approach in order to prospectively find out the best cost-effective and cost-efficient solution on country level for these unexpected, unpredictable diseases. **Keywords:** HEMOPHILIA, COMORBIDITY, SURGERY, COSTS OF INVASIVE INTERVENTIONS.

Comorbidities, as non-interrelated simultaneous diseases are a constantly increasing preoccupation in healthcare (1). It is estimated that >75% of healthcare expendi-

ture is spent on persons with multiple comorbidities, and that the average expenditure/person with comorbidities is roughly five times greater compared with individuals lacking a chronic disorder (2, 3). In the last decade along with the continuous improvement of treatment and of life expectancy of persons with hemophilia (PwH), they have spread also in the field of this rare, lifelong, hereditary coagulopathy (4, 5, 6). They are mainly age-dependent, as the aging population is most likely to catch a wide range of diseases, with decisive medical, economic and social consequences (7, 8).

As things are improving in the field of hemophilia care in our country, bringing along also demographic changes, it could be of interest to understand comorbidities and multiple morbidities in this disease also in our local conditions; it could be the way to plan the strategy for a predictive, personalized, preventive, participatory cost-effective, and cost-efficient solution of hemophilia care, closing the gap between its treatment between our country and that of other European countries (9, 10).

STUDY DESIGN

In the frame of a retrospective cross-sectional observational PRO (Patient Reported Outcomes) model survey conducted on 122 persons with hemophilia (PwH) and its related von Willebrand disease (vWD), diagnosed and treated in 11 neighboring counties from the Western part of Romania, we focused our attention on surgical comorbidities, justified by the fact that they represent the most challenging chapter for the medical care, involving persons with hereditary coagulopathy facing medical bleeding risky interventions. The study referred to the time interval of 1st January 2019 - 31st December 2020. Additionally, to the answers of PwH to a complex questionnaire, medical data have been obtained from the treating physicians and personal hospital records. All participants expressed their informed consent, and the Institutional Ethics Committee approved its performance.

In this comprehensive survey, we aimed at obtaining an insight into the group of comorbidities of PWH, in order to assess, comparatively with secondary morbidities, the frequency, clinical profile, and therapeutic solution of the surgical disease occurring concomitantly with hemophilia/von Willebrand disease, and to evaluate their medical outcomes and financial impact.

MATERIAL AND METHODS

In this survey, we focused our attention on surgical comorbidities, as they generate challenging situations of invasive interventions in the condition of a patient with risky hypo coagulopathy. Out of our 122 PwH and vWD we included in this study two cohorts of patients; 5 cases, presented in emergency, for surgical comorbidity and 11 with elective surgeries performed for the secondary morbidity aiming correction of disabilities or removal of uncontrolled pain in chronic secondary hemophilic hemarthropathies (tab. I).

For the evaluation of the invasiveness of interventions we considered the most used Sant Agostino classification: any elective or an emergent surgical procedure, requiring general anesthesia/respiratory assistance, in which a major body cavity is penetrated or exposed, a mesenchymal barrier is crossed, a fascial plane is opened or an organ is removed or normal anatomy is operatively altered are the main criteria for the definition of major intervention, the rest of invasive interventions being minor (11).

TABLE I

Classification of PwH with invasive interventions (2019-2020)

Type	Average age (years)	Intervention	Programmed	Emergency
Secondary morbidity	38.63±13.26	-total hip arthroplasty	3	-
		-knee arthroplasty	4	-
		-knee arthroscopy and bone biopsy	1	-
		-giant pseudotumor resection and amputation of the inferior third of the leg	1	-
		-cerebral hematoma evacuation	-	2
Comorbidity	36.52±24.17	-open tibial fracture with implantation of osteosynthesis material (case 1)	-	1
		-bilateral coxal-femoral fracture + arthroplasty + femoral neck cerclage (case 2)	-	2
		-testicular abscess evacuation (case 3)	-	1
		-cheilognatopalatoschisis plasty (case 4)	-	1
		-open heart surgery - aortic valve implantation + ascending aorta reduction plasty (case 5)	-	1
Total	37.97±16.55		9	8

SHA - severe hemophilia A, MHA - moderate hemophilia A,
SHB - severe hemophilia B, vWD - von Willebrand Disease

In accordance with the WFH guidelines, we assured a tailored, personalized replacement therapy, under the monitoring of the trough level of the coagulation factor concentrates (CFC) (12). In one child, who developed high titer inhibitors the treatment was assured with bypassing agents (BPAs - rFVIIa, aPCC). A special attention was given to the patient with aortic valve replacement, dilatation of ascending aorta, and cardiac insufficiency, whose intervention with cardio-pulmonary bypass and extracorporeal circulation needed a challenging mixture of contradictory medicines: CFC and unfractionated (UFH) followed by fractioned heparin (FH), antifibrinolytics (EAC), and antiaggregants (aspirin); he required a tough control of hemostasis with additionally thromboelastogram (TEG) and Thrombin Generation Assay (TGA). For the evaluation of the outcomes, we used the definitions proposed in the WFH Guidelines for Hemophilia Care (12). In the economic evaluation, we considered for direct medical costs: specific medicines (CFC and BPA), biological and imaging assessments for diagnosis and monitoring and hospital admission. All data regarding the real costs were sourced from the administrative departments of the treating clinics and from publicly available data, expressing them in national currency (RON) exchanged in EUR at the present rate; when necessary, in order to avoid some bias, we also calculated the costs/person/hospital day and body weight (BW) adjusted costs. Only the costs spent in the period of hospitalization have been considered, not calculating the consumption of CFC in the ambulatory treatment and during rehabilitation, physical therapy. We have not included the indirect costs for transport, sick leave, and loss of productivity.

Statistical analysis was performed using *Microsoft Office Excel* 2013 and *SPSS 14.0* for Windows Evaluation Version, calculating the average and standard deviation with significance at $p<0.05$ and a correlation coefficient significant when $r > 0.4$.

RESULTS

Our study group consisted of 122 patients, 114 (93.4%) with severe and 8 (6.56%) moderates with severe phenotype, 92 (75.4%) with hemophilia A (HA), 16 (13.1%) with hemophilia B (HB) and 14 (11.48%) with von Willebrand disease (vWD). We have assessed comorbidities in 79.51% *vs.* 25.64 % of cases with an age higher or lower than 23 years, respectively, with a Cumulative Illness Rating Scale (CIRS) of 373 *vs.* 64 in these groups of patients (13).

In the period of 2019-2020 there have been registered 17 surgical interventions (tab. II), 8.5/year, less than in previous years, considering that in 2020, dominated by the COVID-19 pandemic, only emergencies were accepted; 6 (35.29%) of them were dedicated to comorbidities; it is to mention that two major interventions were performed on the same patient with a post-traumatic orthopedic event. Their average age was 38.63 *vs.* 36.52 years in the secondary morbidity *vs.* comorbidity group (p=0.97), respectively, without age-dependence of both cohorts; surgical comorbidities (r=0.006) like secondary morbidities (r=0.06) were non-age related, in contrast with the global comorbidities (r=0.69), strongly dependent on the age. All were with severe HA, except one with severe type III vWD; one of the patients developed anti-FVIII inhibitors. Comorbidities had a rather heterogeneous profile of surgery: orthopedics-3, genitourinary-1, maxillofacial-1 and cardiovascular-1, in comparison with that of secondary morbidity dominated by orthopedic interventions (p=0.01). They all were performed as emergency or condition-assumed emergency (cardiac insufficiency, and cheilognatopalatoschisis) interventions (tabs. I, II), *vs.* PwH with secondary morbidity, all with elective programmed intervention (p=0.07). All interventions, in both cohorts of patients, were major procedures. Regarding the hemostatic efficiency in 14 cases the outcomes were excellent and in 3 cases were good, similarly distributed in the two cohorts.

Besides the important medical and social burden of these invasive interventions for the patients and caregivers, supplementary financial tasks for the society were also considered. The costs, dependent mostly (> 90%) on the replacement therapy, were very high in all the 3 scenarios:

- factor VIII/IX replacement in major surgery in PwH without inhibitors,
- switch to BPAs therapy in a patient developing a high titer anti-FVIII inhibitors and
- a challenging mixture of CFC with anticoagulants, antifibrinolytics, antiaggregant; considering the diverse BMI we calculated the real costs and also, the BW adjusted costs; beside the total costs during hospital admittance, we also expressed the costs/person/day.

The distribution of the annual direct costs/patient are presented in the third table. The daily direct medical costs in the cohort of patients with secondary morbidity without inhibitors, who underwent surgical interventions, were 1,873.8 ±725.15 Euros *vs.* cases with surgical comorbidity (1,687.76 ± 927.45 Euros), are not significantly different (p=0.35).

TABLE II

The etiopathogenic background of invasive interventions in PwH (2019-2020)

Type of intervention		Type of disease	Age (years)	Secondary morbidity	Comorbidity
Orthopedics	-total hip arthroplasty	SHA	42/52/29	3	-
	-knee arthroplasty	SHA vWD type3	46/39/37 50	3 1	-
	-knee joint arthroscopy and bone biopsy	SHB	44	1	-
	-giant pseudotumor resection and lower third leg amputation	MHA	36	1	-
	-post traumas open tibial fracture with implantation of osteosynthesis material	SHA	34	-	1
	-post-trauma coxofemoral fracture + arthroplasty neck cerclage	SHA	51	-	2
Genito-urinary surgery	-testicular abscess evacuation	vWD type 2	32	-	1
Oral and maxillofacial surgery	-cheilognatopalatoschisis plasty	SHA+ inhibitors	0.6	-	1
Cardiovascular surgery	-replacement of the aortic valve, ascending aorta reduction	SHA	65	-	1
Neurosurgery	-cerebral hematoma evacuation	MHA	4/46	2	-
Total				**11**	**6**

TABLE III

Direct costs (Euro) of surgical interventions in PwH, during the hospital stay

Case Number	1, 2, 3	4	5
Age (years)	42.5±12	0.6	65.00
Hospitalization costs	3,850.10 ±1,202.08	4,300.00	1,300.00
Costs of specific treatment (CFC / BPA)	55,051.54± 16,620.65	31,923.51 *266,561.40(BW adjusted)*	36,339.98
Percentage of total costs	92.67	86.90 *98.20 (BW adjusted)*	95.27
Costs for investigations	567.8 ±107.10	510.30	503.10
Total costs	59,404.64±15,418.57	36,733.80 *271,371.70 (BW adjusted)*	38,143.10
Hospital days	38.5±14.23	43.00	13.00
Costs / patient / day	1,687.76±927.45	854.30 *6,199.10(BW adjusted)*	2,934.00

DISCUSSION

The term "comorbidity" indicates any medical condition existing simultaneously with, but independent of a patient's primary disease, being mostly age dependent. In fact, the age-dependence of "secondary morbidity" varies with the quality of hemophilia treatment. In Sweden, before 1970, about 37% of PwH had the first joint replacement by the age of 30, reduced to 21% and 0% in the period of 1970-1979 and after 1980, respectively (14). The lack of such age-dependence in our survey is due to the fact that prophylactic replacement has been actually introduced in our country only recently (2016/2017), many of the adults and young adults suffering from important secondary morbidity; therefore, the average age in the two groups of PwH, with secondary- and comorbidity were similar, 38.63 *vs.* 36.52 years, respectively.

Due to the revolutionary advances of hemophilia treatment, the life expectancy and quality of life succeeded to get comparable with those of the non-hemophilic male population, concomitantly decreasing the secondary morbidity and increasing the frequency of comorbidity (14, 15). Middle-aged or elderly PwH had in 78% of cases at least one comorbidity and 15% of them at least 3 or more comorbid conditions (16, 17). In fact, they can be considered the downside of increased life-expectancy. This new landscape brings to the general attention the problem of aging hemophiliacs: firstly, for development of the comprehensive multidisciplinary medical assistance, and secondly, for an appropriate evaluation of the supplementary unexpected costs necessary to construct the best affordable and the most efficient strategy of their economic management (16, 17). Not to be neglected is the need for the establishment of appropriate treatment centers with a turn-around time activity of 24 hours/day and 7 days/week, with access to the proper medication, to the complex specific exploration and to a well-trained surgery team, facing a non-programmed major life-or limb-saving intervention.

Comorbidities in hemophilia can be very provocative. On one hand, the associated morbid condition itself or its treatment can interfere with hemostasis, generating an additional bleeding risk; on the other hand, hemophilia will impose supplementary attention in therapy or hemostasis monitoring. Our reported cases are illustrating these problems. The infant with cheilognatopalatoschisis, with recommended timely performance of surgical correction required a replacement with its missing CFC VIII; but, during its postoperative monitoring, there was an obvious unsatisfactory hemostatic response, with a low recovery of FVIII, justifying the laboratory reassessment, which revealed high titer FVIII inhibitors, compelling the switch to another life-saving therapy with BPAs. The other reported case of an adult with a life-threatening heart-disease, needing an emergent surgical solution created the most challenging situation : open-heart surgery with cardiopulmonary bypass, and extracorporeal circulation, having in his treatment protocol besides the CFC replacement, also anticoagulants, antifibrinolytics, and antiaggregants; daily coagulometric, TEG, and TGA assays were mandatory in order to balance between these contradictory agents for finding a safe hemosta-tic control.

Another aspect, which is worth to be mentioned is the non-evident, concealed,

sometimes non-investigated relationship between comorbidities and secondary morbidity in hemophilia. That issue justified our attention in the case of evident trauma-related double femoral neck fractures (tab. I). The highly osteoporotic image of the bones was striking, impacting also coxofemoral joints. The only therapeutical solution was the arthroplasty with neck cerclage. It raised the following questions: was it indeed a non-hemophilia-related fracture or was it a consequence of the hemophilia-related osteoporosis? To answer these questions, there are now many pre-and clinical studies, all agreeing that the prevalence of fracture is significantly higher in PwH than in the normal population. Besides low physical activity, low vitamin D, low body mass index, hypogonadism, tobacco, and alcohol use are discussed; additionally, the multifactorial substrate of this condition has been also proved, FVIII and vWF playing a role in osteoclastogenesis. Large studies revealed osteopenia in 43.5% and osteoporosis in 25.8% of PwH, increasing the fracture rate to 24.0%/1,000 PwH/year *vs.* 9.6%/1,000/ control group patient/year, with a yearly increase of 1-3%/year of its prevalence (18).

Concerning the costs of the surgical comorbidities, our short-term survey revealed its magnitude. Concordantly with the international data, the proportion of expenses dedicated to the specific CFC or BPA were 92.67-98.20%, being lower in the situation of long-term hospitalization (16, 17, 18, 19, 20). The occurrence of inhibitors is increasing the expenses by 3-5 times (20). In our infant patient, who developed high titer inhibitors, there was a significant rise in costs, but its dimension is much clearer if we calculate for comparison the BW-adjusted value of costs. The deleterious effect of the association of both types of morbidities has been once more revealed: secondary and comorbidity. Therefore, we have to accept that the avoidance of secondary morbidity, treatment-dependent, is the most efficient economic attitude, preserving at the same time a better quality of life. Hemophilia itself is an already costly disease. If we take into consideration also our affordability in the frame of our national hemophilia program, the average annual budget/PwH being about 30,000 Euros, the costs for such unexpected and unpredictable comorbidities are significantly increasing the financial burden of the incurred expenses.

As limits of our study, we have to mention the short-term horizon of our review, also including the period of the bad COVID-19 pandemic, impacting all the medical care in the country; additionally, the small number of the patients with this rare disorder and the low rate of surgical events, lead to difficult statistical evaluations. Despite not having included the important post-hospital direct and indirect costs, connected with this comorbidity, we could demonstrate the high economic burden of a such morbidity. It is also to highlight the risk of over-estimation of the costs of hemophilia care on the country level, as in our center is functioning a multi-institutional comprehensive hemophilia care with PwH addressed from all over the country.

CONCLUSIONS

This critical and provocative issue of surgical comorbidities in hemophilia requires a more extended approach in time

and space in our real life. It is suggesting the imperative lifesaving need to organize a network of comprehensive care centers, for finding economic arguments for immediate access to the right medical team, to the right medication and dosage, individualized for the right patient, according to complex hemostatic monitoring, basis for foreseeing a prospective predictive attitude for a cost-effective and cost-efficient solution to cover all their needs.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

ISOLATED MYOCARDIAL BRIDGING: A POTENTIAL CAUSE OF SUDDEN CARDIAC DEATH

Myocardial bridging (MB) is a congenital coronary anomaly in which a segment of coronary vessels is embedded into myocardium tissue. In sudden cardiac death (SCD) autopsy series, left anterior descending coronary (LAD), particularly the proximal segment, is the most common affected vessel in MB. However, several studies report involvement of multiple segments of MB in the same or different coronaries. As the majority of coronary blood flow occurs only during diastolic phase, delayed relaxation in MB during diastolic phase is responsible for coronary blood flow reduction. This pathophysiology plays an important role in the lethal consequences of this anomaly. The coronary obstruction of MB has been classified into either benign or pathologic according to their location, intra-myocardial length, and intramyocardial depth. Pathologic MB is considered if the bridging segment is 20–30 mm long and 2-3 mm deep. In some cases, pathologic MB may cause ischemia, infarction, malignant ventricular arrhythmias, and eventually SCD. However, there was a case report in which the evidence of ischemia and infarction were found despite benign MB affirming that the clinical consequences do not depend on the degree of coronary blood flow reduction. Although MB has been recognized for decades, the clinical significance of MB as a potential cause of SCD remains a subject of debate. According to previous studies, the probability of MB in Caucasian population as the only potential cause of SCD was quite low. The authors try to review the potential relation between MB and conventional risk factors and identify cardiovascular events for establishing the understanding of the clinical implications of MB **(**Vutthikraivit W, Rattanawong P, Prapaipan Putthapiban P, *et al.* Isolated Myocardial Bridging: A Potential Cause Of Sudden Cardiac Death. *Rom J Leg Med* 2020, 28: 133-136).

EFFICIENCY OF USING VISUALIZED TREATMENT OBJECTIVES IN ORTHODONTICS

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EFFICIENCY OF USING VISUALIZED TREATMENT OBJECTIVES IN ORTHODONTICS (Abstract): Lateral skull radiographs are used to perform cephalometric measurements and to evaluate the skeletal component of the orthodontic anomalies. It is difficult for the patients to imagine the expected outcome of their orthodontic treatments. so visual treatment objectives (VTO) can be a significant motivating factor. It can be used to illustrate the expected growth. the outcome of orthognathic surgery and the amount of teeth movements. This study was **aimed** to evaluate the accuracy of the visual treatment objectives through analysis of predicted and actual treatment outcomes. The second objective was the comparison of the initial cephalometric measurements with the final ones and the visualized treatment objectives prediction accuracy. **Material and methods:** Using the AudaxCeph cephalometric program and Roth-Jarabak analysis, lateral skull radiographs of 27 patients were analyzed. Visual treatment objectives were assessed by modification of the dental parameters as well as of the skeletal bases where indicated. **Results:** Lateral skull radiographs taken after the orthodontic treatment were used to evaluate the final cephalometric measurements. Statistical comparison of the dental and skeletal measurements of the final radiographs and the visual treatment objectives did not show statistically significant differences of the most important parameters. However, the following measurements were significantly different when compared with the visual treatment objectives Ar-Go-N angle (degrees), Go-Ar distance (mm) and S-Ar / Ar-Go ratio. **Conclusions**: Using the VTO, the expected orthodontic outcome can be well estimated, and it can be considered an effective tool for planning.
Keywords: ORTHODONTICS, CEPHALOMETRY, TREATMENT OUTCOME, RETROSPECTIVE STUDIES.

A visual plan can be very helpful in predicting patient growth and the expected effects of treatment, as well as determining the goals that need to be achieved for the patient. The use of Visual Treatment Objectives (VTO) also allows alternative treatment plans to be developed. Using VTO the advantageous aspects of growth should be exploited, interpreting the indicators from the cephalometric radiograph's

evaluation. If possible, the undesirable effects of growth should be also reduced during treatment. Progress can be continuously monitored during treatment, with the help of the dental VTO. In the 1980s, these visual goals were still manually drawn on tracing paper, but with the proliferation of digital X-rays and the advent of numerous cephalometric programs, this has now been completely pushed into the background. The time of preparation of the VTO depends entirely on the case and can be used as a supplement to traditional cephalometric analyzes (1, 2, 3).

Richard McLaughlin and John Bennett published a study in 1999 entitled "The Dental VTO: An Analysis of Orthodontic Movement" to provide simplified and organized information for diagnosis, design, and to resolve the issue of non-extraction / extraction, using the VTO. The study includes two case studies of patients with skeletal second-class anomalies (4).

Subsequent research has used VTO to design cases of orthognathic surgery and to accurately depict surgeries, with a strong emphasis on predicting soft tissue changes. For example, in the study of Pektas *et al.* (5), the accuracy of Dolphin Imaging System software prediction was examined at the soft tissue level, before surgery and then one year after surgery. In the study, the treatment approaches also included surgeries on both the maxilla and the mandible (5).

Robert J. Peterman *et al*. in a similar study. focused on soft tissue analysis. Pre- and post-treatment lateral skull radiographs were superimposed to determine the skeletal movements actually achieved during orthognathic surgery. This information was used to simulate soft tissue changes in patient profile in preoperative photographs. The accuracy of the VTO was further examined by comparing the projected images with photographs taken after the end of treatment (6).

This study was aimed to evaluate the accuracy of the visual treatment objectives through analysis of predicted and actual treatment outcomes. The second objective was the comparison of the measurements in order to determine the visualized treatment objectives prediction accuracy.

MATERIAL AND METHODS

The survey included the lateral skull radiographs of 27 patients (18 women and 9 men) aged between 9 and 30 years, who required orthodontic treatment. The number of growing patients was 15, while the number of young adults with completed ossification was 12. Patients visited the *Natural Smile Dental Clinic* for orthodontic treatment. Individualized treatment plans were established for each case considering clinical examination, study cast analysis, profile photos, age, anatomical boundaries and patient's intent. All patients signed an informed consent to participate in this study.

Photographic documentation was prepared for each patient. It included extraoral and intraoral photographs using a Canon 500 camera and 100 macro lenses. illuminated by a circular flash The intraoral images were taken from frontal, lateral, and occlusal views. The extraoral images were taken with a blue background, from the frontal and lateral view, both in rest and smile.

Panoramic and cephalometric radiographs (fig. 1) were taken for each patient before and after treatment. These digital X-rays were taken with a Pax Flex 3D +, Vatech X-ray machine with an exposure time of 12.9 seconds, 80 kVp and 9.0 mA. The EasyDent dental imaging software and

the AudaxCeph orthodontic cephalometry program were used in the study.

Fig. 1. Lateral cephalometric radiograph

During the survey, individual folders were created for all 27 patients, containing personal data of the patients, with lateral cephalometric radiographs and profile photos before and after treatment. Because not all types of analysis contain all the information needed to establish a VTO, in the present study X-rays were assessed by Roth-Jarabak analysis.

The given analysis type contains the following data:

- Geometry (reference points and planes) and measurements;
- Correlation of measurements with standard values;
- Predefined ‘superimposition’;
- Component sequences for sequential redrawing;
- Position and content of additional elements (measuring boxes, harmonic box, diagram and measuring table);
- Export template and print results.

The software automatically assigns the reference measurement points and planes (fig. 2).

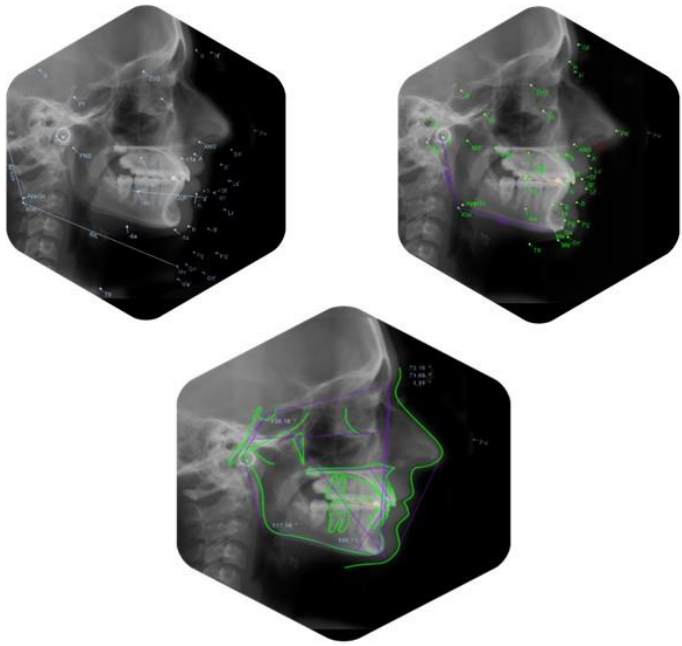
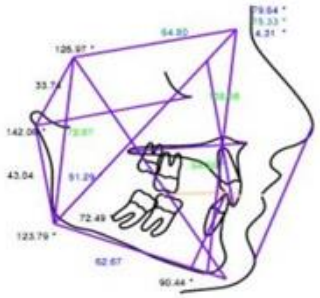


Fig. 2. Jarabak analysis steps

The precise positioning and adjustment of each cephalometric point to the corresponding anatomy formula is done manually. The X-ray image is calibrated by using the gradation visible on the cephalostat. During image calibration, the length and distance of objects are determined based on the known length of each element. The measurement process ends with the creation and editing of the outlines of the teeth, hard and soft tissues. Finally, by clicking the save option, the program provides the obtained values from the given patient (fig. 3).



MEASUREMENT		NORMAL VALUE	VALUE	DIFFERENCE	BIAS
Dental Analysis					
OcP/MeGo	°	14	24	10	I
Interincisal angle	°	135	149	14	I
+1/SN	°	102	88	-14	●●●I
+1/NL	°	N/A	95	N/A	●●●I
-1/MeGo (anatomic)	°	90	90	0	I
+1i/NPg	mm	5	2	-3	●I
-1i/NPg	mm	0	-1	-1	I
Ls/E-line	mm	-2	4	6	I●●
Li'/E-line	mm	1	5	4	I●●●
Skeletal Analysis					
N-S-Ar	°	123	127	4	I
S-Ar-Go	°	143	142	-1	I
Gonial angle (anatomic)	°	130	124	-6	I
Bjork (anatomic)	°	396	393	-3	I
S-N	mm	71	65	-6	●●I
S-Ar	mm	32	34	2	I
Ar-Go-N	°	54	51	-2	●I
ML/NGo	°	73	72	0	I
Go-Ar	mm	44	43	-1	I
S-Ar/Ar-Go	%	68	78	11	I●
Go-Me	mm	71	63	-8	●I
Go-Me/N-S	%	100	97	-3	I
Angle SNA	°	82	80	-2	●I
Angle SNB	°	80	75	-5	●●I
ANB	°	2	4	2	I●
SN/ML (anatomic)	°	36	33	-3	I
N-Go	mm	N/A	110	N/A	I
S-Me II FH	mm	N/A	52	N/A	I
Y/SN	°	N/A	70	N/A	I
S-Go	mm	N/A	73	N/A	I
Anterior Facial Heigth	mm	N/A	109	N/A	I
S-Go/N-Me	%	64	67	3	I●●
SNPg	°	N/A	77	N/A	I●●
NAPg	°	175	173	-2	I

Fig. 3. Lateral cephalometric radiograph imaging analysis summary (Print Out)

Based on the established treatment plans preparation of the visual treatment objectives (VTO) was done. The VTO permitted the following features:

- to move the items to the desired position;

- to calculate the expected growth;
- to create / present a view state at the request of the patient;
- to save the predicted changes.

With each interactive movement of the teeth, a delta box appears showing the relative distance and rotation. By opening this dialog box, can be also entered the X and Y distances manually. In patients with active growth after enrollment (5 children), the rate of growth was also assessed (approximately 1-2 mm / year) and then the physical advancement of the jawbones was plotted in the VTO.

The lateral view photograph was superimposed to the lateral skull radiograph. Based on the VTO, the soft tissue contour of the profile was automatically modified by the software, creating a new image showing the expected outcome of the treatment.

TABLE I

Initial dental and skeletal mean values

Dental measurements	Mean value	Standard deviation
Ocp/Mego°	23.11	5.87
Interincisal angle°	133.77	9.48
+1/Sn°	103.44	10.79
+1/NL°	111.92	9.52
-1/MeGo (anatomical)°	87.7	6.23
+1i/NPg mm	5.33	3.24
-1i/NPg mm	1.55	2.93
Ls/E-line mm	4.46	2.32
Li'/E-line mm	3.57	2.27
Skeletal measurements	**Mean value**	**Standard deviation**
N-S-Ar°	123.86	5.79
S-Ar-Go°	142.14	6.90
Go (anatomical)°	128.92	6.88
S-N mm	66.22	3.27
S-Ar mm	32.62	3.02
Ar-Go-N°	53.77	4.42
ML/NGo°	75.00	5.88
Go-Ar mm	41.59	4.82
S-Ar/Ar-Go%	79.18	7.38
Go-Me mm	66.62	5.22
SNA°	80.55	3.60
SNB°	77.92	3.71
ANB°	02.66	2.86
SN/ML(anatomical)°	35.07	7.31
Anterior facial height mm	109.74	6.82
S-Go/N-Me%	64.00	5.63
NAPg°	177.37	6.19

Following the dental and skeletal movements performed in this way, the software creates a table of estimated values for the changes made with the VTO, and the expected dental and skeletal changes provided by the treatment plan.

The Roth-Jarabak analysis was performed on the final lateral skull radiographs taken when the orthodontic treatment was completed.

The results of the obtained measurements of the initial and final analysis and the VTO estimation were entered into an *Excel* database manager. Statistical analysis was performed *t-Student paired test* was performed ($p \leq 0.05$) by using Graph Pad

.

RESULTS

Initial dental and skeletal mean values are shown in first table.

Data obtained from the VTO were compared with the final lateral skull radiographs dental and skeletal measurements.

The results are shown in tables II (dental parameters) and III (skeletal parameters).

TABLE II

Statistical data obtained from dental measurements Final and VTO estimation

Dental measurements	p-value
Ocp/Mego°	0.0708
Interincisal angle°	0.0696
+1/Sn°	0.1830
+1/NL°	0.1312
-1/MeGo (anatomical)°	0.0659
+1i/NPg mm	0.6976
-1i/NPg mm	0.1792
Ls/E-line mm	**0.0110**
Li'/E-line mm	0.0946

TABLE III

Statistical data obtained from skeletal measurements final and VTO estimation

Skeletal measurements	p-value
N-S-Ar°	0.3144
S-Ar-Go°	0.6198
Go (anatomical)°	0.219
S-N mm	1
S-Ar mm	0.3273
Ar-Go-N°	**0.0095**
ML/NGo°	0.2471
Go-Ar mm	**0.0033**
S-Ar/Ar-Go%	**0.0274**
Go-Me mm	0.4813
SNA°	1
SNB°	0.2327
ANB°	0.2110
SN/ML(anatomical)°	1
Anterior facial height mm	0.3526
S-Go/N-Me%	0.1478
NAPg°	1

DISCUSSION

The aim of the present retrospective study was to assess the effectiveness of the VTO. To demonstrate the effectiveness of the program, the estimated values of the visual treatment objectives should not differ significantly from the result and the projected changes should be as close as possible to the patient's final condition.

Statistical figures showed that there was a significant difference in the following three of the seventeen skeletal measurements:

- Ar-Go-N° (articular-gonion-nasion / degree)
- Go-Ar mm (gonion-articular / millimeter)
- S-Ar/ Ar- Go % (sella-articular/ articular-gonion / percent)

These measurements provide important information about the ascending ramus of the mandible. namely its length and degree of angulation. As demonstrated, the VTO prediction of the development and growth of the ramus of the mandible was less accurate than the other parameters.

Only one parameter was found to have a relevant difference, namely Ls / E-line mm (labralis superior to E-line in millimeter). The VTO estimation of the position of the upper lip in millimeters relative to the aesthetic line in the sagittal plane was significally different compared to the final result. This can be related to the protrusion of the upper incisors, as the key to achieving facial balance and harmony is not necessarily the placement of the bony tissues, but rather the position of the maxillary and mandibular teeth that determine the position of the upper and lower lips.

VTO-related studies in the literature have found that computer-generated predictions generally underestimated the nasal apex, under the nose, and A-point soft tissue projection, while they were overestimated for the upper lip (5). In general, predictive errors were smaller in the sagittal plane than in the vertical plane, similar to our results.

Peterman *et al.* found that for most measurement points within ± 2 mm, the software VTO estimate was accurate and the lower lip predictions were the most inaccurate (6).

Comparing the results of the present study with the data in the literature, very similar conclusions can be drawn. However, it should not be overlooked that the present study does not include any surgical cases and is not based on the prediction of soft tissue changes, but mostly on hard tissue measurements (angular values, size of bony anatomical formulas) and accuracy is not by analyzing the differences expressed in millimeters, using statistical procedures. The difference between the expected and the obtained treatment result reflects the effectiveness of the treatment. but in certain treatment procedures. without the patient's consent, the orthodontist is forced to submit the case to a compromise solution, making it impossible to achieve the ideal treatment plan. The goal of visual treatment as a motivational aid can be of great help in preventing these barriers, as it is a much more understandable way for patients to explain the most ideal treatment plans depicted with VTO. In addition to the simplicity, accuracy, and many other benefits of digital design, it should be borne in mind that the presentation of these predictions must be done very carefully and precisely to eliminate unrealistic expectations of patients.

CONCLUSIONS

The dental VTO is a great adjunct to the analysis of lateral cephalometric radiographs determining a diagnosis and treatment plan, but it alone cannot provide a complete picture of the situation.

The orthodontist performing the study can make excellent use of the VTO in communicating with patients in the form of an illustrative presentation as a motivational tool.

A projected graphical representation can provide adequate explanation to patients about the proposed treatment, facilitating its understanding and acceptance and an adequate estimate of the rate of growth for growing patients.

The ascending ramus of the mandible and the position of the upper lip were the least accurate in estimating the VTO, but there was no significant difference in the more important measurements, so its precision is not maximal, but its efficiency is satisfactory.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

Authors' contribution: SP - contribution to conception. design and interpretation of data; MK - analysis and interpretation of data, crafting of manuscript; KJ - acquisition of data; LC - critical revision; KH - analysis and interpretation of data and drafting of the manuscript.

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PROFESSOR DR. DUMITRU BAGDASAR (1893-1946) – 75 YEARS AFTER THE FOUNDER OF ROMANIAN NEUROSURGERY

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Professor Dr. Dumitru Bagdasar is considered to be the founder of Romanian school of neurosurgery. He was born in 1893 in Roșiești, Vaslui, România, in an Armenian countryside family, being the oldest brother. Passionate about knowledge and science, he got the attention of his professor from the beginning of the primary school in Idricii de Sus. He followed his high-school studies in Barlad, and in 1913 he applies for the Medical School in Bucharest. There were many times in his life when because of the lack of material resources, he faces difficulties in carrying on with his medical school. That's why at the end of his 3rd year he enrolled to the Medico-Military Institute trying to get some financial support (1). Quickly, he was sent to give aid to the military troops during the Second World War. Because of the harsh condition on the military front, he got sick of typhus and went through hardships.

After finishing his primary studies in highshool in Barlad, he studied medicine in Bucharest (1913-1919). His postgraduation thesis was published in 1922, entitled "Contributions to the study of postencephalitic parkinsonian syndrome".

Between 1922 and 1926 under the supervision of Dr. Gheorghe Marinescu and Dr. Ion Noica, he specializes in neurology. Under his guidance he published a series of manuscripts about Friedreich Maladie, mediastinal symphatic syndrome, Parkinson's Syndrome, etc. In 1927 Dr. Bagdasar was awarded with a Rockefeller grant and had the possibility to move to Boston, where under the guidance of Professor Dr. Harvey Cushing completed a volunteering-assistance program in neurosurgery.

His neurosurgery activity could be divided in 4 parts: in USA, at the Hospital in Jimbolia (27), Mental and Nervous Disease Hospital in Cernauti (149) and at the Central Hospital for Mental and Nervous Diseases (60) between 1930-1934.

His training in neurosurgery (1927-1929) is performed in Boston, USA, under the supervision of Harvey Cushing. (2). Together with Percival Bailey he published two papers "Treatment of cerebral goma" and "Intracranian cordoblastoma". In his first publication, Bagdasar highlighted that the incidence of intracranial gomas is low, and the surgical treatment is essential since 7 out of 8 had better results of medication treatment following the surgical procedures. His coming to USA was accompanied by his wife, owner of Fulbright grant, and who specialized in social medicine.

Fig. 1. The picture of young Dr. Dumitru Bagdasar

Starting with 1929, Bagdasar and his wife moved back to Romania and started practicing medicine in Jimbolia Hospital (Timis County). At this medical center there were performed the first 27 neurosurgical interventions in Romania, namely 15 ventricular punctures, 9 frontal-parietal approaches, two sub temporal decompressions, a cerebellar decompression, and other 18 procedures outside of the central nervous system.

In 1931, it was organized the first edition of the International Congress of Neurology in Bern, Switzerland whose chairman of the congress was the American neurologist, Bernard Sachs. At this time Cushing presented his memorable study of 2023 cerebral tumors and during the presentation Bagdasar was part of the audience. At the Gala Dinner event organized at Hotel Bellevue, Bagdasar was invited to stay at the left side of Cushing as a sign of dignity and friendship that was shared between them, near Thierry de Martel (France) and his second American mentor Percival Bailey with whom he published his articles in USA. His relationship with Cushing remained stable over time.

In 1932, Bagdasar and his wife Florica Bagdasar moved to Cernauti, where for one year he performed 28 surgical interventions namely 21 surgeries for intracranial lesions and 7 vertebral lesions. All of these surgeries were performed under local anesthesia with Novocain. Some of the cases were presented at the Neurological and Psychiatry Congress in Bucharest, September 1932 under the title "56 Surgical Interventions for Epilepsy. Critical Considerations". At Cernauti Hospital he also performs few neck dissections for peripheral nervous system, "but without the interest to delve seriously in comprehending the peripheral nervous system, since this such a surgery pertains in parts to the general surgery (…)".

In 1933, he performed 31 surgical interventions including laminectomies [2], peripheral nerve trauma surgery [1], cranial decompressions [4], spinal cord sections [6], osteoma (excision) [1], cerebral tumors (excision, exploratory look) [5], medullar tumors (excision) [2], cerebellar tumors (excision) [3], Dandy's operation (neurotomy of 5th nerve) [4], ventriculography [1] and abscess drainages [2].

Among the publication of that period is remarkable the one about the examination of ventricles and cysts gliomas. The authors pioneered a device called ventriculoscop (which is an endoscope that has 30 cm length and 3 mm diameter) and was brought to life by Wolf House in Berlin.

It is worth to be mentioned that besides

his neurosurgical interventions Dr. Bagdasar also performed surgical interventions at the Military Hospital which was supervised at that time by Professor Dr. Mihail Butoianu.

At the end of 1934, it is organized for the first time in Romania the first admission examination for a neurosurgeon place at the Central Hospital for Mental and Nervous Diseases. At that time on the examinations lists there were Dr. Bagdasar, Dr. Moruzi, Dr. Vasiliu. After 3 days of examinations Moruzi and Bagdasar were both on the first positions, however, taking in consideration the years of surgical experience the evaluation commission (which included: Prof. Butoianu, Prof. A. Jianu, and last but not least Prof. L. Balif) decided that Bagdasar should be Chief of Neurosurgery in Bucharest (3).

From 1929 to 1934, he performed over 200 surgical interventions on neurosurgical pathologies (4). Starting from 1935, dr. Dumitru Bagdasar started his activity in a single hospital, the Central Hospital for Mental and Nervous Diseases, in Bucharest as the attending primary neurosurgeon, having under his supervision 10 beds for hospitalization of neurosurgical patients (5). He succeeded in establishing the first independent Neurosurgical Department in Bucharest Central Hospital of Nervous Diseases - the second at a national scale, after Socola Psychiatry Hospital in Iași. Doctor Maria Niculescu, who operated with Bagdasar for a period of 7 years mentioned about the operative room conditions that "*sick patients were screaming, kept behind the bars, in this sinister atmosphere, the beautiful operations were performed on a wooden table, improvised, (...) instruments made according to Bagdasar's instructions, thus following Cushing's example, but without comparable material resources".*

In a filmed interview, Sofia Ionescu, the first female to be neurosurgeon, said that her entire future was marked by professor Bagdasar. She remembered that once Professor D. Bagdasar was unable to operate due to a wound on one of his fingers and he let her to perform her first surgery, saving the life of an 8-year-old comatose boy with an extradural intracranial hematoma. Professor Bagdasar remarkable praised her and required to stay with him during her training (6).

At the Emergency Hospital in Bucharest, ruled at that time by Prof. Iacobovici, Dr. Bagdasar worked day and night diligently with an impeccable technique and operative skill. Dr. Bagdasar worked with his own staff and always brought his own instruments with selflessness for science and for the sick.

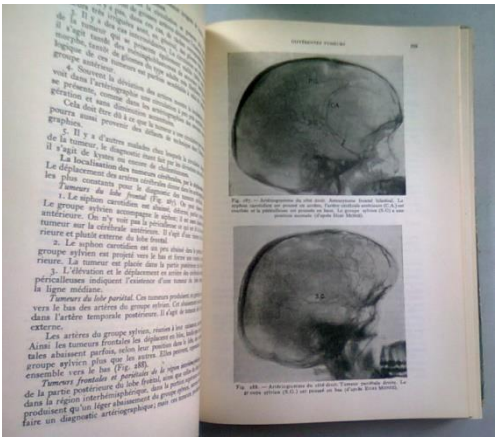

Fig. 1. First cover of *"Traité de Neurochirurgie",* D. Bagdasar & C. Arseni, Romanian Academy, 1948

During his activity Bagdasar participated to numerous medical congresses about history of medicine (International Medical Congress, in Bucharest, 1932), Neurological, Psychiatric and Endocrinological Congress (Chisinau, 1935) and National Surgi-

cal Congress (Bucharest, 1939). In one of his memories published in 1941, Bagdasar mentioned "besides our sick patients, I received cases from Hungary, Bulgaria and Palestine. Over the last 10 years, I practiced over 1800 surgical interventions on nervous system as follows: 473 ventriculographies in diagnostic scope, 988 big cranio-cerebral interventions, 259 laminectomies and 83 peripheral nerve surgeries". He was the author of over 50 specialty monographies. His most important work *"Traité de Neurochirurgie"* (fig. 2), was published postmortem in 1948, and represented the main study book for over 25 years for Romanian neurosurgery trainees (7).

Alexandra Bellow, the daughter of dr. Bagdasar mentioned in one of her late writings about the political involvement of his father. Dumitru Bagdasar enrolled in the Communist Political Party in 1943, participated in signing the memoir in 1944 according to they support Romania's exit from the Second World War. Dr. Bagdasar was assigned as the chief of Health Governmental Ministry in 1945 in the cabinet of Petru Groza. One of the stories from that time marked the moment when Bagdasar was assigned with a bodyguard and an official ministry car was given to him. He refused the governmental car, looking for riding his own car with the bodyguard. Once, coming back from the hospital he encountered a mass of drunken Russian soldiers who asked him and his staff to get out of the car. He remembered quite ironically that the first one who fled away was the bodyguard. In 1945 doctor Bagdasar was appointed professor.

He contributed to the "Red Aid" Romanian branch, an organization whose role was to support the communist hard workers and prisoners. He pleaded for the rights of the communist people in court as witness. All of this pro-socialist work should be understand looking through the eyes of the person who faced hard and difficult times, who felt poverty and agony at its thin limits. A person like Bagdasar who felt more for the Romanian people than for himself was determined to act for the common wellness of the population.

Dumitru Bagdasar died on 16th of July 1946, after several months of suffering. He was diagnosed with incurable pulmonary cancer at Wien, and he personally gave himself the second diagnostic of cerebral metastasis after the onset of apraxia. He gave up city life for a countryside retreat. On his deathbed, he called his collaborators: Dr. Constantin Arseni, Dr. Ionel Ionescu and Dr. Sofia Ogrezeanu Ionescu who then pronounced his last words: "Do your best to grow this specialty. Be united. Continue what you started at the same pace, with the same passion and, above all, with high morality". He wished Dr. Constantin Arseni to be his successor at the clinic. He also became post-mortem member of the Romanian Academy in 1948.

In conclusion, doctor Dumitru Bagdasar is one of the representative figures of a golden age of Romanian Medical School. Together with other representants of his period he pioneered in the domain of neurosurgery being coined as the father and founder of Romanian neurosurgery. His courage in acting in an unknown domain of medicine is remarkable despite any other obstacles that he encountered. His decision to dedicate his life to the neurosurgery made him an example for the Romanian school. His power for acting towards his goals resides in his deep moral character and values. In addition to his integrity and courage comes up his finest clinical sense of discernment. At the end of his path, he is

remarkable one of the personalities who fulfills his calling and completes his mission. Dumitru Bagdasar made history through his records he held, his innovations, the surgeons he trained and his dedication towards the goal of saving lives.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

CLINICAL MOLECULAR IMAGING FOR ATHEROSCLEROTIC PLAQUE

Atherosclerosis is a well-known disease leading to cardiovascular events, including myocardial infarction and ischemic stroke. These conditions lead to a high mortality rate, which explains the interest in their prevention, early detection, and treatment. Molecular imaging is able to shed light on the basic pathophysiological processes, such as inflammation, which cause the progression and instability of plaque. The most common radiotracers used in clinical practice can detect increased energy metabolism (FDG), macrophage number (somatostatin receptor imaging), the intensity of cell proliferation in the area (labeled choline), and microcalcifications (fluoride imaging). These radiopharmaceuticals, especially FDG and labeled sodium fluoride, can predict cardiovascular events. The limitations of molecular imaging in atherosclerosis include low uptake of highly specific tracers, possible overlap with other diseases of the vessel wall, and specific features of certain tracers' physiological distribution. Authors consider the need for a common protocol for patient preparation, data acquisition, and quantification in the area of atherosclerosis imaging research (Anton Kondakov and Vladimir Lelyuk. Review- Clinical Molecular Imaging for Atherosclerotic Plaque. *J. Imaging* 2021; 7(211): 1-15).

HISTO-ANATOMICAL CHARACTERISTICS OF THREE VARIETIES OF *PERILLA FRUTESCENS* LEAVES

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HISTO-ANATOMICAL CHARACTERISTICS OF THREE VARIETIES OF PERILLA FRUTESCENS LEAVES (Abstract): *Perilla frutescens* is a crop species, which belongs to the *Perilla* genus in the Lamiaceae family, and it is widely grown in Asia. *Perilla frutescens* species contain two varieties with a lot of forms, including *Perilla frutescens* var. *crispa* f. *purpurea*, *Perilla frutescens* var. *frutescens* f. *crispidiscolor* and *Perilla frutescens* var. *frutescens* f. *viridis*. Perilla has been studied by many researchers for its chemical composition and pharmacological effects. **Aim:** The description of the microscopic characteristics of the three varieties' leaves to emphasize certain differences that allow the identification of the taxons. **Material and methods:** The investigations consisted of the histoanatomical assessment using optical microscopy and scanning electronic microscopy, of each important structure found in leaves of the three genotypes of the *Perilla frutescens* species, harvested from Vegetable Research and Development Station Buzău in September 2020. **Results:** From the perspective of tissue anatomy, there is no significant difference in leaf structure between the three genotypes of the perilla. A difference is observed in *Perilla frutescens* var. *frutescens* f. *crispidiscolor*, which, in addition to all the structures of *Perilla frutescens* var. *crispa*, also contains a different number of peltate glandular trichomes. **Conclusions:** In the histoanatomical study of the three species of *Perilla frutescens* with the light microscope and the scanning electron microscope, we have obtained anatomical characters that show the similarities and differences between the three species. **Keywords:** *PERILLA FRUTESCENS*, OPTICAL MICROSCOPY, SCANNING ELECTRONIC MICROSCOPY, HISTO-ANATOMY.

Perilla is an annual herbaceous plant that belongs to the Lamiaceae family and is widely distributed especially in China, Japan, Korea, Vietnam, and other regions in Asia (1). In Europe, *Perilla* was brought late and used for its oils, but cultivated in small areas (2). *Perilla* contains four species: one that is cultivated

and most frequently named *Perilla frutescens* and three wild species named *Perilla hirtella*, *Perilla setoyensis*, and *Perilla citriodora* (3, 4). *Perilla frutescens* species was divided by Yu according to leaves color in green and purple (5).

In 2010, Hu classified *Perilla frutescens* species in five varieties: *acuta*, *auriculato-dentata*, *frutescens*, *crispa* and *arguta* (6). Nowadays, there are three varieties: *frutescens*, *crispa*, and *hirtella*, that are accepted by The Plant list (7, 8), while the Integrated Taxonomic Information System accepts only two (var. *crispa* and var. *frutescens*) (9, 10).

Perilla frutescens species includes multiple forms of the two varieties, among which: *Perilla frutescens* var. *crispa* f. *purpurea*, *Perilla frutescens* var. *frutescens* f. *crispidiscolor Perilla frutescens* var. *frutescens* f. *viridis*. These varieties are distinguished by the following morphological characters: *Perilla frutescens* var. *crispa* f. *purpurea* present purple leaves on both sides and ruffled surface, *Perilla frutescens* var. *frutescens* f. *crispidiscolor* presents green leaves on the adaxial side and purple on the abaxial side (10), and *Perilla frutescens* var. *frutescens* f. *viridis* present large green leaves on both sides with many wrinkles (11).

Perilla frutescens has been investigated by many researchers in terms of its chemical composition and pharmacological actions (12). A variety of compounds have been isolated and identified from this plant, including flavonoids, volatile oils, fatty acids, triterpenes, phenolic compounds, and so on. The isolated compounds showed pharmacological actions such as antioxidant, anti-inflammatory, anticancer, antidepressant, antiallergic, etc. (12).

Photonic and electron microscopy investigations by Zhou *et al.*, 2021, indicated the presence of three distinct types of secretory hairs, which the authors classified as peltate, capitate, and digitiform. According to the analyses performed, the authors conclude that the secretion of pelted hairs contains perilla ketone, isoegomaketone, and egomaketone, which are major constituents in the leaves of *Perilla frutescens* (13).

Although many species (*Lavandula sp.*, *Teucrium sp.*, *Ajuga sp.*) belonging to Lamiaceae family have been previously characterized by microscopic means, similar data referring to various taxons of *Perilla* is scarce (14, 15, 16). Therefore, our study aimed to describe the microscopic characteristics of leaves from the three genotypes and to identify if there are differences between them.

MATERIAL AND METHODS

The plant material used in this study for histo-anatomical examination under an optical microscope and scanning electron microscope was represented by the leaves harvested from the three genotypes of the species *Perilla frutescens*, respectively: *Perilla frutescens* var. *crispa* f. *purpurea*, *Perilla frutescens* var. *frutescens* f. *crispidiscolor*, and *Perilla frutescens* var. *frutescens* f. *viridis*.

The leaves were harvested in September 2020 from the crops made at Vegetable Research and Development Station Buzău and preserved in 70% ethyl alcohol. The vegetal material is properly

labeled and preserved in the collection of Plants anatomy and morphology laboratory "Academician Constantin Toma" from Biology Faculty within "Alexandru Ioan Cuza" University of Iaşi, having the value of voucher specimens.

The samples to be analyzed were fragments of the petiole, leaf tongue, and rectangular portions of the leaf tongue, including the median rib. Different sections were made for each sample using a botanical razor, botanical microtome, and two halves of extruded polystyrene (shock marrow replacement).

The obtained sections were exposed to the coloring process with iodine green and red ruthenium, but before, were immersed in sodium hypochlorite solution for 10 minutes, then washed with acetic water. After washing, the sections were stained with iodine green, then washed with 90% ethyl alcohol, then with distilled water, after which they were stained with red ruthenium. Finally, the sections were washed with distilled water and mounted on microscope slides in a drop of water. The sections were examined and photographed under a Celestron 44341 LCD built-in optical microscope. The color photos obtained were digitally processed using the Photos software.

As for the scanning electron microscopy investigations, they were performed using the TESCAN Vega II SBH microscope with a secondary electron surface (biological material is dried with carbon dioxide using the EMS 850 critical point dryer, metallization the samples are made of gold using the EMS 550x metallizer). For TESCAN Vega II SBH scanning electron microscopy investigations of plant material, plant fragments were deposited on aluminum foil coated with double-sided carbon tape. The material was then coated with gold by the metallization process on the EMS 550x Metallizer and placed under a microscope for examination.

The use of this modern method of investigation provided additional information on the conformation of the cuticle, epidermal formations (ordinary epidermal cells; surface trichomes (non-glandular) and secretory (glandular) hairs; stomata; cuticular striae), information that completed the data table on the of the surfaces of vegetative organs investigated.

RESULTS AND DISCUSSION

The leaf structure from a histo-anatomical point of view has no significant dissimilarity between the three genotypes of *Perilla frutescens*.

Petiole

Cross-section throw petiole revealed only one difference between the basis and the top of the leaf blade: the numbers of trichomes that are several on the adaxial side than abaxial one. On all three genotypes, the cross-section is semi-circular modified by two slightly protruding lateral-adaxial wings. The adaxial side of the epidermis has besides regular epidermic cells many trichomes, including uniseriate multicellular surface trichomes and capitate glandular trichomes, that are short.

There is one difference observed on *Perilla frutescens* var. *frutescens* f. *crispidiscolor* that contains besides all structures of *Perilla frutescens* var. *crispa*, also a various number of glandu-

lar peltate trichomes. Making this observation we can suppose that *Perilla frutescens* var. *frutescens* f. *crispidiscolor* have at least theoretically the most quantity of volatile oil (fig. 1).

On the other hand, on the abaxial side we can find the same trichomes, but in a very small number. What we can find on the abaxial side in a large number are the stomata located well above the external level of the epidermis. This condition appears in two situations if the vegetal material is excessively watered, especially if it comes from a greenhouse or represents an ecologic adjustment. Considering that two samples were harvested from the greenhouse (*Perilla frutescens* var. *frutescens* f. *viridis*, *Perilla frutescens* var. *frutescens* f. *crispidiscolor*), and one from the field (*Perilla frutescens* var. *crispa* f. *purpurea*) we can say that this position of stomata represents an adaptation to the Romanian climate and type of soil. Below both epidermic sides are located two-three layers of angular collenchyma, a mechanic tissue that has a purpose in support and guidance of the leaves after the light source.

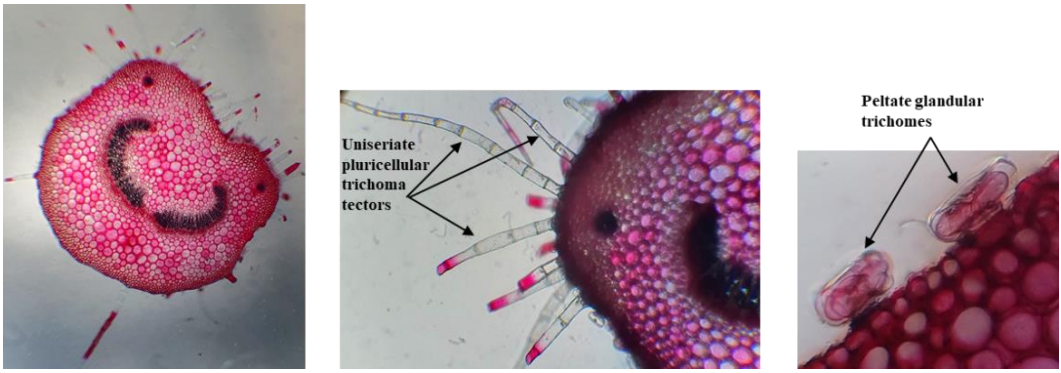

Fig. 1. Petiole of *Perilla frutescens* var. *frutescens* f. *crispidiscolor* - cross section view and detail (x20; x100; x400)

In fundamental parenchyma, there are conducting tissue that is organized in two arches that are oriented with phloem tissue through the abaxial side and with lignified tissue through the adaxial side. The two arches are separated by fundamental parenchyma cells. In patches, near phloem cells, there is a thin slightly sclerified and lignified sheath (fig. 2). On *Perilla frutescens* var. *crispa* forma *purpurea* we couldn't find that sheath (fig. 3). This finding may be a characteristic element of the separation between *Perilla frutescens* var. *crispa* f. *purpurea* and *Perilla frutescens* var. *frutescens* f. *viridis*. The two slightly protruding lateral-adaxial wings have the same structure as fundamental parenchyma as follows conducting tissue with the same orientation, many stomata placed above the outer level of epidermic cells, and from place to place near phloem cells we can find one layered sheath with elements of sclerenchyma.

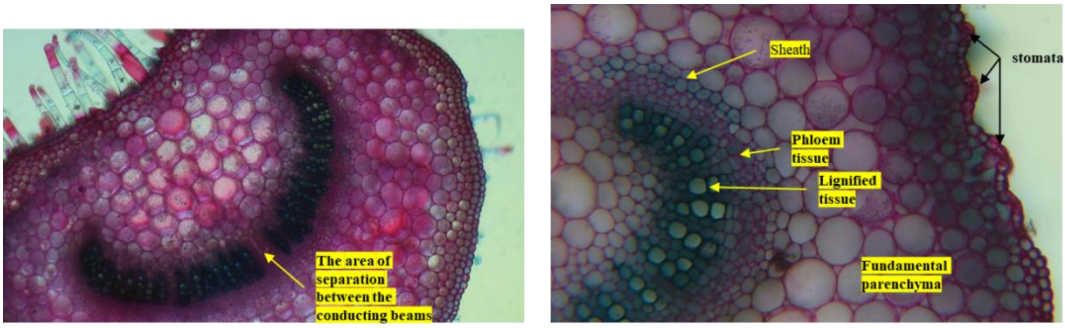

Fig. 2. Petiole of *Perilla frutescens* var. *frutescens* f. *viridis* - cross section view and detail (x100; x400)

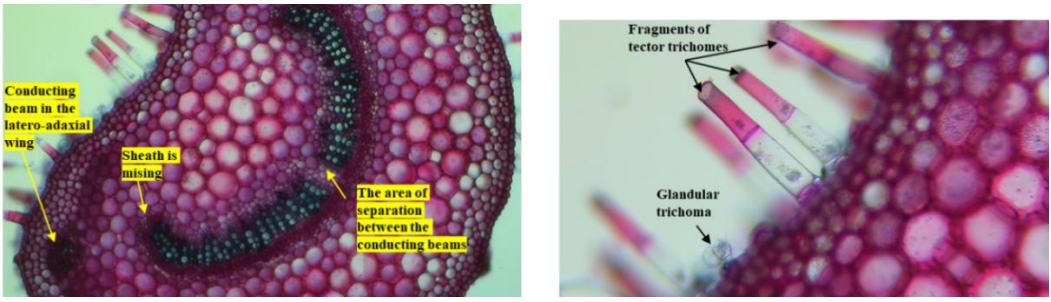

Fig. 3. Petiole of *Perilla frutescens* var. *crispa* f. *purpurea* - cross section view and detail (x100; x400)

Foliar Limb

In the cross-section of the foliar limb, the median rib protrudes strongly on the abaxial side to all three genotypes of *Perilla frutescens* (figs. 4, 5, 6). As in petiole at the level of the epidermis, there are lots of uniseriate pluricellular surface trichomes and less capitate glandular trichomes. Under epidermic layers, there are two-three sheets of angular collenchyma.

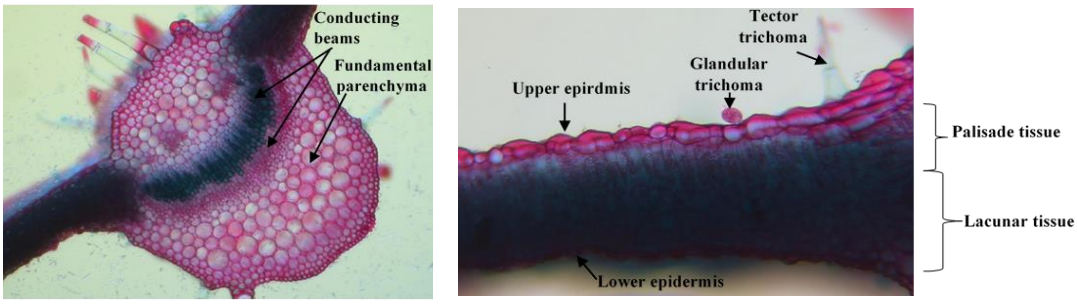

Fig. 4. Foliar limb of *Perilla frutescens* var. *frutescens* f. *viridis* - cross section view and detail (x20; x200)

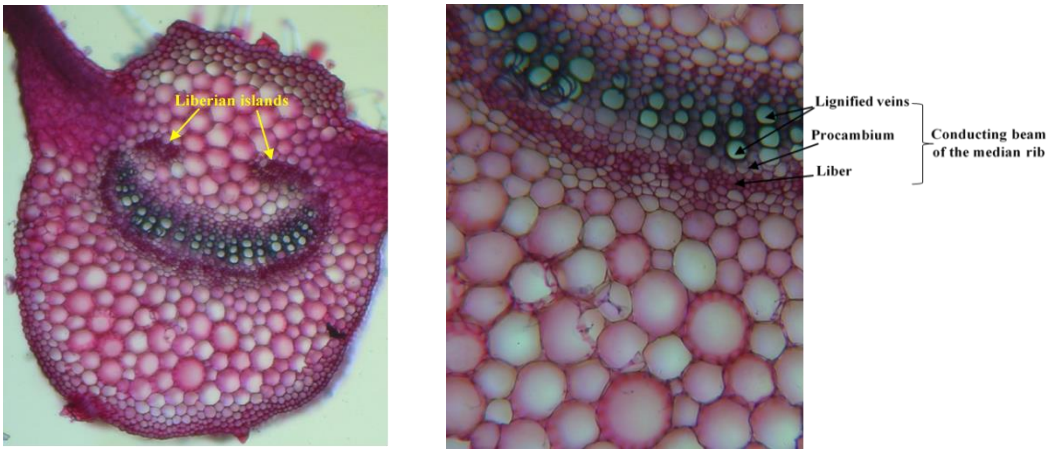

Fig. 5. Foliar limb of *Perilla frutescens* var. *crispa* f. *purpurea* - cross section view and detail (x100; x400)

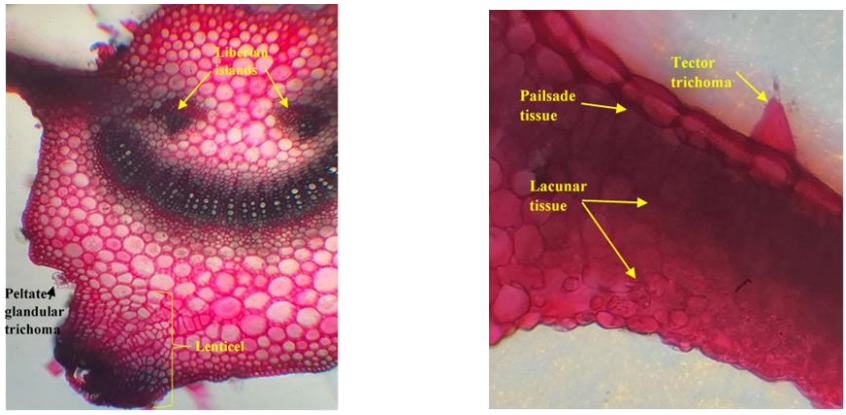

Fig. 6. Foliar limb of *Perilla frutescens* var. *frutescens* f. *crispidiscolor* - cross section view and detail (x100; x400)

Unlike in petiole, in foliar limb, there is only one conducting tissue orientated with phloem tissue to abaxial side and with lignified tissue to adaxial side. Near big principal conducting tissue there are two phloem mass orientated to the adaxial side of the median rib.

The last characteristic is not applied to *Perilla frutescens* var. *frutescens* f. *crispidiscolor*, which besides the two-phloem mass near the principal conducting tissue has two phloem-lignified small beams on both sides (fig. 7). This finding can represent an histo-anatomical character that identifies *Perilla frutescens* var. *frutescens* f. *crispidiscolor*.

In-between the veins, under the upper epidermis there is palisade tissue, the exterior layer being compost by higher cells. Under the lower epidermis, there is multi-layered lacunar tissue. Having these two characteristics we can conclude that the perilla foliar limb has a hetero-facial structure. The palisade tissue occupies

two-thirds of the thickness of the foliar limb, so the leaf realized a quality photosynthesis activity.

For a better and more detailed understanding of perilla leaves structure we used scanning electronic microscopy. The characteristics obtained from the three genotypes are summarized in table I.

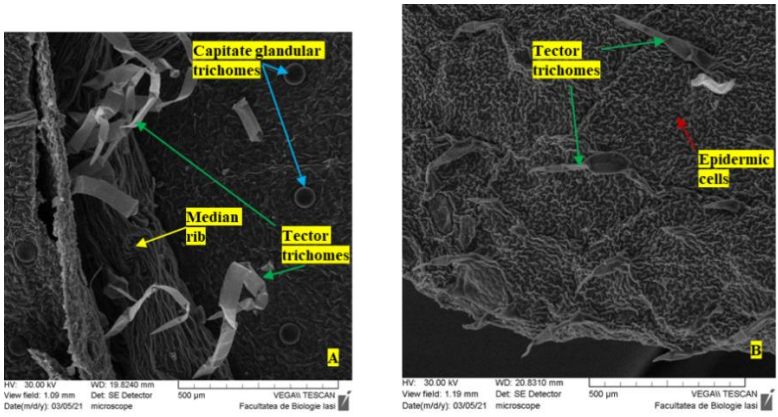

Fig. 7. SEM of *Perilla frutescens* var. *frutescens* f. *viridis* leaf: A - lower epidermis, B - upper epidermis.

TABLE I

Anatomical characteristics of genotypes of Perilla frutescens species on electronic microscope scanning

The samples	Lower epidermis	Upper epidermis
Perilla frutescens var. *frutescens* f. *viridis* (Fig. 7)	- capitate glandular trichomes, are located between the secondary veins, with the glandular part, covered by a smooth cuticle; - uniseriate pluricellular surface trichomes arranged along the ribs, very rarely between the secondary veins; - stomata are present (hypostomatic limb).	- uniseriate pluricellular surface trichomes are observed between the secondary veins; - glandular trichomes are missing between the secondary veins; - stomata are absent.
Perilla frutescens var. *crispa* f. *purpurea* (Fig. 8)	- capitate glandular trichomes are present between the secondary veins; - surface trichomes are missing between the secondary veins; - stomata are present (hypostomatic limb)	- rare mono/multicellular surface trichomes are placed between the secondary veins; - very rare capitate glandular trichomes are located between the secondary veins; - stomata are absent.
Perilla frutescens var. *frutescens* f. *crispidiscolor* (Fig. 9)	- peltate glandular trichomes are located between the secondary veins; - uniseriate pluricellular surface trichomes are arranged along the ribs; - stomata are present (hypostomatic limb).	- glandular trichomes are missing; - short unicellular surface trichomes are located between the secondary veins; - uniseriate long pluricellular surface trichomes are located between the secondary veins; - stomata are missing.

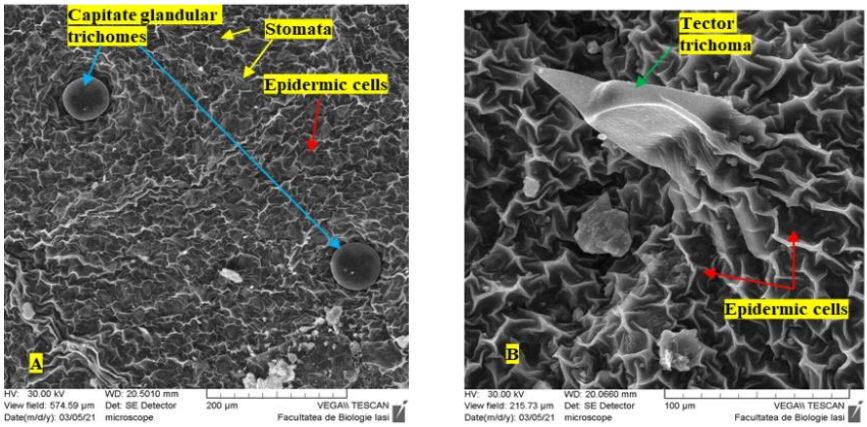

Fig. 8. SEM of *Perilla frutescens* var. *crispa* f. *purpurea* leaf:
A - lower epidermis, B - upper epidermis.

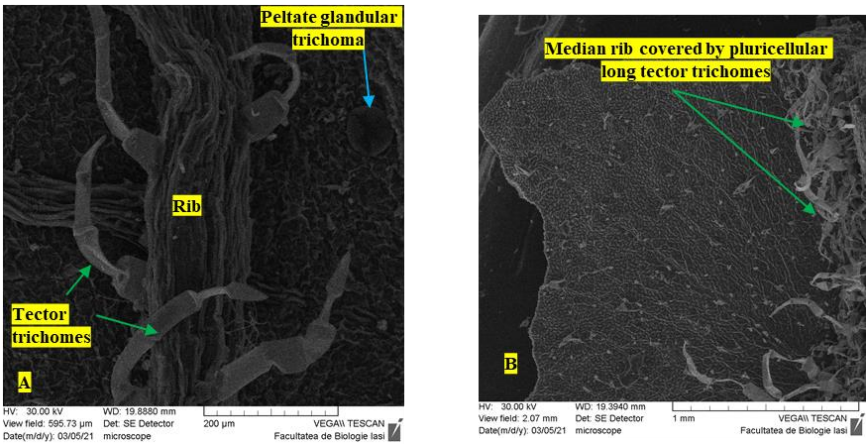

Fig. 9. SEM of *Perilla frutescens* var. *frutescens* f. *crispidiscolor*:
A - lower epidermis, B - upper epidermis.

CONCLUSIONS

Appling the histoanatomical investigation using an optical microscope and scanning electron microscope to the three types of *Perilla frutescens*, we obtained morphological characteristics that presented the similarities and differences for the three ones. As far as we know, there is no such study that involves perilla leaves. The described characters could have taxonomic value in the Lamiaceae family.

There are no significant differences between the three genotypes. Cross-section shape epidermis, stomata, angular collenchyma, and conducting tissue organization are mostly the same. An important finding that can help us correctly identify *Perilla frutescens* var. *crispa* f. *purpurea* is the absence of the sclerenchyma sheath at the level of the two large convex conducting tissue.

Also, we discover that *Perilla fru-*

tescens var. *frutescens* forma *crispidiscolor* has the most peltate glandular trichomes and so offers a bigger quantity of volatile oil. Other characteristics that helped us to recognized *Perilla frutescens* var. *frutescens* forma *crispidiscolor* were the two small phloem-lignified beams located on either side of the fascicular convex arch of the median rib.

These features, which are important for general level taxonomic classification, represent the novelty of our research. However, the chemical composition and pharmacological effects of the extract, which are closely related to the similarities and differences between the three genotypes of *Perilla frutescens*, still need to be further studied.

CONFLICT OF INTEREST AND FUNDING

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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NEWS

MODERATE HYPOFRACIONED VS. CONVENTIONALLY FRACTIONED RADIOTHERAPY IN BREAST CANCER

Recently, a group of oncology specialists undertook a critical review that included data from 149 articles on the use of moderately hypo fractionated post-operative radiation therapy for breast cancer in clinical practice. The main aim of this study is to provide an overview of the applicability of moderately hypo fractionated RT for breast cancer patients, focusing on factors influencing clinical decision-making. They conclude that moderately hypo fractionated RT to the breast, chest wall (with/without breast reconstruction), and regional lymph nodes is at least as safe and effective as conventionally fractionated regimens and could be considered as the treatment option for the vast majority of the patients. For those who are still concerned about its generalized application, we recommend participating in ongoing trials comparing moderately hypo fractionated RT to conventionally fractionated RT for breast cancer patients in some clinical circumstances. The conventional radiation doses ranged from 50 to 50.4 Gy, given in 25-28 fractions over a course of 5-6 weeks. This empirical schedule assumed that a total dose around 50 Gy, prescribed in 1.8-2.0 Gy fractions, might maximize tumor control while minimizing normal-tissue injury. The results of six randomized phase III trials that were published with formal comparisons between moderate hypofractionation and conventionally fractionated irradiation for breast cancer patients The conventionally-fractionated whole breast irradiation 50 Gy in 25 fractions or hypo fractionated whole breast irradiation 42.56 Gy in 16 fractions was used. The patients in the conventionally fractionated whole breast irradiation group received a boost of 10-14 Gy (in 5 or 7 fractions, respectively), and those in the hypo fractionated whole breast irradiation group received 10-12.5 Gy (in 4 or 5 fractions, respectively). There were no significant differences between the conventional fractionation group and the 41.6 Gy hypofractionation group. Likewise, other side effects (such as shoulder stiffness, arm oedema, and breast shrinkage) were similar between the hypofractionation and conventional fractionation groups. Normal-tissue effects, such as breast oedema, telangiectasia, and breast shrinkage, were significantly less common in the hypofractionation than in the conventional fractionation group; no significant differences existed in arm oedema, shoulder stiffness, or breast induration. This pragmatic trial design, employing a biologically estimated lower dose led to, not surprisingly, a lower rate of acute side effects as the total dose was reduced, but it also produced lower late normal tissue side effects, which are more dependent on dose per fraction. Importantly, local control was at least as good. Overall survival was unexpectedly better, raising the exciting hypothesis that a shorter overall treatment time could be beneficial for survival, which could not be concluded in view of the non-inferiority design of this trial for local control. The critical review concludes the fact that existing data confirms that moderately hypo fractionated RT for breast cancer is efficient, convenient and safe for all indications, target volumes and techniques, with most evidence available for 2D- and 3D techniques for treatment of the breast and the chest wall, with and without a boost. Based on a more limited set of data, combined with radiobiological considerations, they recommend extrapolating these results to other indications, target volumes and techniques including regional nodal irradiation and treatment after mastectomy with or without breast reconstruction (Nader Marta GN, Coles C, Kaidar-Person O, *et al.* The use of moderately hypo fractionated post-operative radiation therapy for breast cancer in clinical practice: A critical review. Published online 2020 Aug 26. doi: 10.1016/j.critrevonc. 2020. 103090).

BIOMIMETIC COMPLEX CREAM FORMULATION, PROPERTIES, TOLERANCE AND MOISTURIZING EFFECTIVENESS ON SKIN

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BIOMIMETIC COMPLEX CREAM FORMULATION, PROPERTIES, TOLERANCE AND MOISTURIZING EFFECTIVENESS ON SKIN (Abstract): This study consisted of developing and evaluating a new biomimetic cream with vitamins, hyaluronic acid and active phytocomplexes from different plants, such as *Centaurea cyanus, Aloe barbadensis, Achillea millefolium and Spilanthes acmella*. Efficiency of the new formulated products was demonstrated by testing the hydration degree of human subjects' skin. **Material and methods**: It were developed a lipophilic cream with a 64% hydrophilic phase and 36% lipophilic phase and a new type of lipophilic emulsifier. The physico-chemical properties (appearance, pH), technological parameters (consistency, spread ability and rheology) and *in vivo* moisturizing effect were evaluated. **Results**: The proposed moisturizer cream presented a pleasant texture and a pH of 7.1. From the rheological behavior, it can be seen that the product can be easily applied to the skin and in terms of effectiveness the results are excellent. After a single application on dry skin, the proposed formulation can improve by 80% the degree of hydration. **Conclusions:** The developed water/oil biomimetic formulation, which has a pH compatible with the skin, is easy-to-apply and have a very good tolerance on dry skin with immediate hydration. **Keywords**: BIOMOMETIC, COSMETIC CREAM, SKINCARE, MOISTURIZING EFFECT.

The process of aging in the skin causes certain changes: the quality of the skin's support matrix tends to change, the endogenous water retention capacity decreases, the dermal fibers tend to atrophy, and the skin becomes dehydrated and loses its elasticity (1). The main role of cosmetic product after application, is to help remedy these changes and to maintain healthy skin, through the supply of water that it provides from the outside. The use of water/oil (W/O) creams is generally indicated for all

skin types, especially for dry skin, where it is a necessity, they also replace the lack of natural secretion of fat from the skin. The creams, through their composition, correct dryness and restore elasticity, especially through the content of emollients that soften the skin (the stratum corneum), helping to maintain its suppleness by delaying the dehydration process. Skincare also aims to provide as pure fat as possible, to replace those that have been removed by numerous washes. Regardless of their mode of action, the creams have been broadly called emollients, but the term may include multiple categories of creams (2-4).

When formulating and preparing cosmetic creams (emulsifying systems) the aim is to ensure the following conditions: not be irritating (avoid irritating substances); to be formulated with the help of excipients and active substances capable of deep hydration; to be able to incorporate biologically active substances, favoring their penetration into the skin; be prepared with the best quality raw materials, brought in a state of fine division, to ensure penetration into the skin; not rancid easily, otherwise causing skin irritation and redness; be suitable for the skin on that it is applied; to make a protective layer on the skin, both in summer and in winter, having at the same time an emollient action; not to be overly fragrant (5-12).

The latest trend in cosmetics is biomimetic creams because are the key to effective and sustainable skincare. The term of biomimetic come from the prefix "bio" which refers to natural while "mimetic" is derived from the word "imitate". Biomimetic cosmetics include all kinds of products that are formulated in such a way as to adapt to the structure of the skin. Only natural cosmetics aren't always the most sustainable, and pharmacies can use biomimetics to formulate new alternatives that are more sustainable with less environmental impact. In addition to anti-aging skincare, biomimetics has also been crucial in creating better moisturizing products for people who suffer from chronic dry skin. Niacinamide improve overall skin texture by rebuilding a lipid layer, minimizing the appearance of pores, preventing UV damage and inflammation as an antioxidant (9*). Spilanthes acmella* flower extract works as key system because stimulates the production of normal moisturization factor in the skin and achieves moisturization rather than being a topical hydrator. This biomimetic ingredient is a concentrated firming and wrinkle which stimulates the filaggrin (13). Targets cell communication to reorganize and retighten the dermal architecture. Has a natural lifting and smoothing effect, visibly reducing deep wrinkles and firming the skin. The concept of biomimetics products goes beyond basic product formulation by delivering a synergistic combination of phospholipids, pre/probiotics, natural moisturizing factors, skin-identical lipids, peptides, vitamins, or amino acids.

The main role of creams is to moisturize and maintain healthy skin, by providing water from the outside, after application (14). The choice of a cosmetic cream by a consumer is the combination of good marketing and proven product quality to gain notoriety, consumer attention, last but not least, the satisfaction of commercial interest (15-17). The quality and attractiveness of a cosmetic product are decided by factors such as its texture, appearance, smell and performance. The objective evaluation of cosmetics has attracted increasing attention lately, on the one to hand, to improve

their performance and on the other hand to meet the requirements of legislation requiring continuous and scientifically substantiated proof of the claims used in marketing on quality of the products. This information can be obtained through the rheological study and the ability to spreadability on the skin. These characteristics can influence the quality of the cosmetic product and even its effectiveness.

The aim of this study consisted of developing and evaluating a biomimetic formulation, which constantly applied to the skin produce a moisturizing effect.

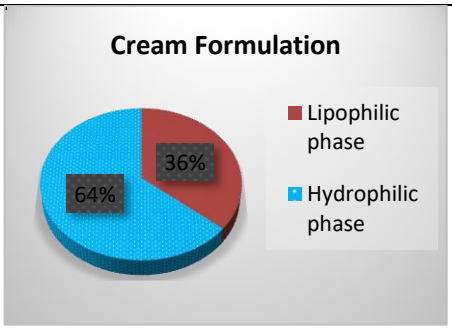
MATERIAL AND METHODS

A W/O cream with moisturizing properties was developed and analyzed. The cosmetic product contains 64% hydrophilic phase and 34% lipophilic phase, emulsify system in which the following ingredients have been incorporated: vitamins, hyaluronic acid, and active phytocomplexes from *Achillea millefolium* oil, *Cocos nucifera* oil, *Argania spinose* kernel oil, *Spilanthes acmella* flower extract, *Centaurea cyanus* flower water and *Aloe barbadensis* leaf juice. The physical-chemical properties (appearance, pH - determined with a digital pH-meter), technological parameters (consistency - determined with a semi-automatic Penetrometrer, spreadability - determined with an extensometer, and rheological parameters, determined using a Rheotest equipment) and the moisturizing effect (*in vivo* studies on human subjects using a Corneometer) were investigated.

TABLE I

Creams composition

INGREDIENTS	(%m/m)
Lipophilic phase	
Achillea millefolium oil	10
Cocos nucifera oil	7
Argania spinosa kernel oil	7
Methyl Glucose Sesquistearate	5
Stearic acid	3
Spilanthes acmella flower extract	1
Glyceryl Stearate SE	2
Tocopherol	1
Hydrophilic phase	
Centaurea cyanus flower water	40
Aloe barbadensis leaf juice	20
Niacinamide	2
Hyaluronic acid	2



Centaurea cyanus flower water (Logis-Tech Jolanta Czepulonis, Poland), *Aloe barbadensis* leaf juice (Fagron, Greece), *Achillea millefolium* oil (Fagron, Greece), *Cocos nucifera* oil (Fagron, Greece), *Argania spinos*a kernel oil (Fagron, Greece),

Stearic acid (Vitamar, Romania), Methyl glucose Sesquistearate (Evonik Industries, Germany), Glycerol (Vitamar, Bucharest, Romania), *Spilanthes acmella* flower extract (HerbPharm, India), Niacinamide (Fagron, Greece), Glyceryl Stearate SE (Evonik Industries, Essen, Germany), Tocopherol (Medchim, Romania), Hyaluronic acid (Fagron, Greece).

Cosmetic cream preparation

The emulsifier, Methyl Glucose Sesquistearate (MGS) was dispersed in the lipophilic phase, heated to 65°C and then the hydrophilic phase was incorporated at the same temperature.

pH measurement

Samples of 5.0 g cream were heated in 15 g of distilled water until the fat phase melted, stirred for one minute, cooled to 20 °C and filtered through filter paper. The pH of the aqueous filtrate was measured using Consort C831 multiparameter analyzer. The results shown for each cream represent the average of 6 measures.

Rheological analysis

The spreadability of the prepared formulation was determined using a extensometer. The sample with a uniform thickness was placed between the two glass slides and pressed with different weights were placed on the upper side and then drawing the appropriate curves (18). Consistency assessment was performed using a penetrometer (PNR 12 Petrolab, Germany) with a micro cone and a suitable container (19). All measurements were carried out in triplicate. The rheological characteristics evaluation of the prepared formulations was carried out using a rheometer Rheotest RV (RHEOTEST Medingen GmbH, Germany), provided with coaxial cylinders operating with twelve shear rates (20).

Moisturizing test

The instrument used to measure the hydration level was Corneometer® CM 825 (Courage + Khazaka). Due to its ease of use and short measurement time, this device is the most widely used system in the world to determine the level of hydration on the surface of the skin and especially in the stratum corneum. The device runs at an average frequency of 1Mhz, between 0.95 MHz (very hydrated medium) and 1.15 MHz (very dry medium). The device is equipped with a 0.64 cm^2 probe consisting of a 75 μm network of gold electrodes, coated with a vitrified material with a low dielectric constant. The probe exerts a constant pressure of about 1.5 N on the skin and covers an area of 49 mm^2. Measuring the degree of hydration with this device is based on measuring the capacity of a dielectric medium. Uses field capacity sensors to measure the dielectric constant of the skin. The dielectric condition of the skin will change with the water content. This allows you to measure any changes in skin hydration through the precision measuring condenser. These changes in the water content of the stratum corneum are transformed into arbitrary units of hydration. There is a piece of fine glass on the probe head to make sure that only the capacities are changed due to the water content. Even small changes in the water can be detected. The measurement time is short at only one second, minimizing the effects of occlusion. The depth of measurement was 10-20 μm in the stratum corneum, so the deeper layers of skin do not influence the measurement (21, 22).

The moisturizing effect was *in vivo* test on eight subjects. The evaluation of the hydration grade of the stratum corneum indicated that the products have a moisturizing effect four hours after application. The study was carried out under the responsibility of a dermatologist following the protocol, the principles of good clinical

practice, international recommendations ICH E6 (R1) of 10.06.1996, Directive of the European Parliament, and the Council 2001/20 / EC-OJ / EC of 01.05.2001), in ethical conditions towards the subjects, they preliminary filling in an informed consent form (23).

Statistical data analysis

For absolute variations compared to T_0 ($\Delta = T_i - T_0$), the following parameters were defined or calculated for the treated area and for each experimental time (T_i): number of subjects (n), mean, standard deviation, minimum and maximum values. The data were analyzed with *SPSS* software *Windows version*. For statistical analysis, the degree of significance of 5% was used (tests with paired data - statistical significance: p value <0.05), and for normality tests 1%.

RESULTS

In this study, a cosmetic cream with an emulsifying system W/O has been developed with a pleasant, homogeneous appearance and odor, without signs of phase separation or foaming.

pH measurement

The tested formula has a pH = 6.8. The value of pH is used in W/O creams so that it can be applied around the eyes without causing stings due to the pH.

Rheological analysis

The analysis of the rheological properties of the proposed cosmetic formulation showed a plastic behavior (fig. 1). The spreadability and consistency curves are presented in second figure.

Moisturizing effect

To perform the hydration test on human subjects using a corneometer, only those with dry skin are chosen from a panel of healthy subjects, those with a corneometric index value higher than 35-40.

From the experimental data obtained and presented in second table, S_2 and S_4 subjects can be excluded.

Comparative interpretation of research results: for each experimental time (T_1, T_2, T_3, T_4) the relative variations of the corneometric index (compared to T_0) were calculated according to the following formula:

$$\Delta\% = [(T_i - T_0) * 100] / T_0$$

The hydration efficiency, after applying the cosmetic products, is expressed by average corneometric index (I), as well as the variation% compared to T_0 are shown in fourth figure.

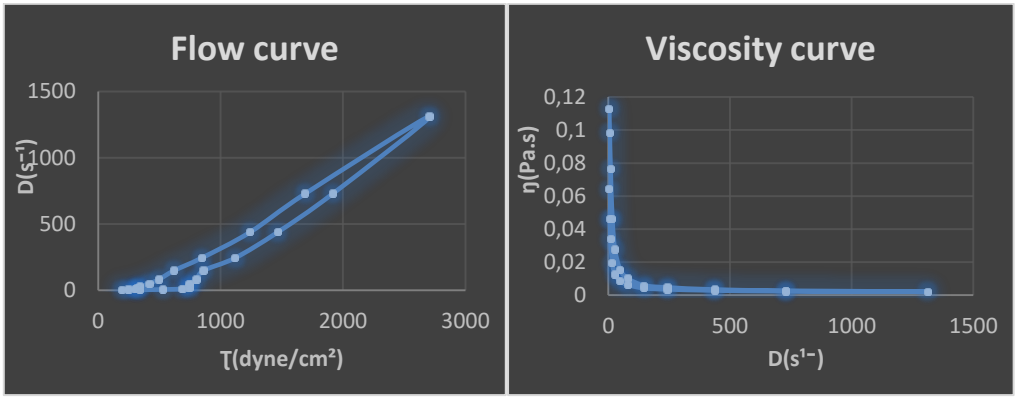


Fig. 1. Rheological behavior of the biomimetic cream

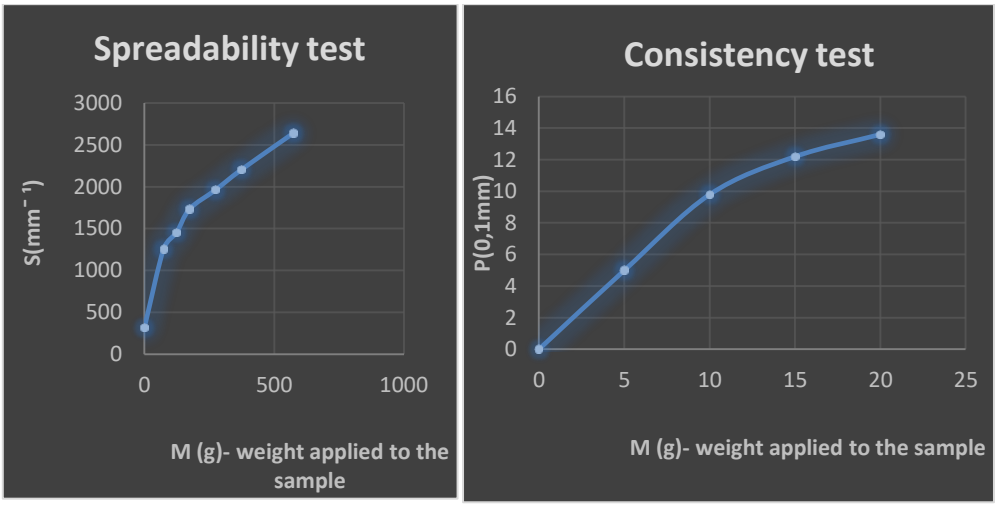


Fig. 2. Spreadability and consistency of the biomometic cream

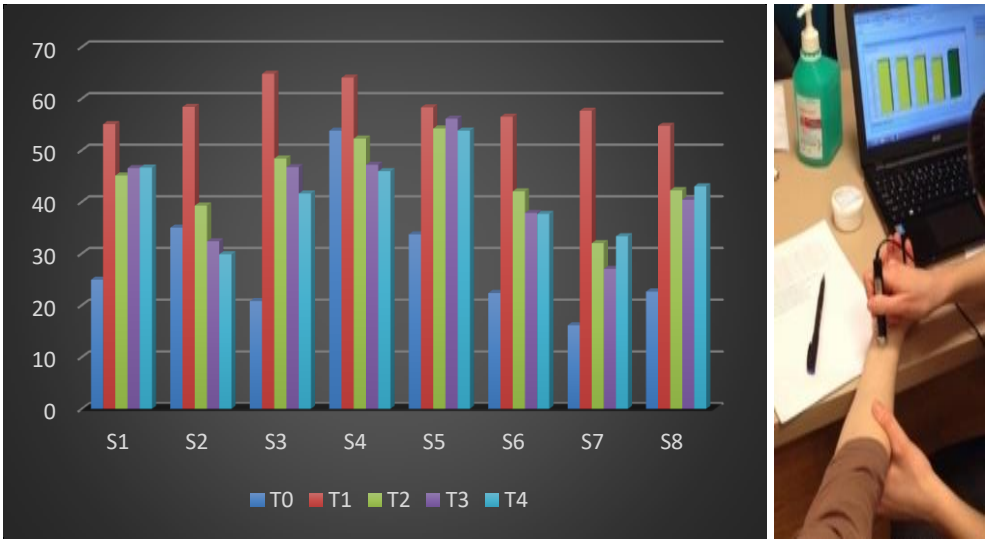


Fig. 3. Corneometer index measured at different time after cream application

*T_0 - before cream application; T_1 - immediately after cream application; T_2 - at 30 minutes after cream application; T_3 - at 2 hours after cream application; T_4 - at 4 hours after cream application

TABLE II.

The size of the variation of the hydration effect on the treated area

Human Subjects	Variation of corneometric index (%)			
	T_1 *vs.* T_0	T_2 *vs.* T_0	T_3 *vs.* T_0	T_4 *vs.* T_0
S_1	121.08	81.08	86.90	87.22
S_2	66.85	12.28	-7.57	-14.74
S_3	211.53	132.69	124.61	100.00
S_4	19.03	-2.86	-12.28	-14.66
S_5	72.96	60.80	66.46	59.61
S_6	152.73	88.09	69.38	68.62
S_7	257.28	98.32	67.32	106.50
S_8	142.06	86.77	78.68	90.22
N	8	8	8	8
Mean	130.44	69.63	59.19	60.34
Minimum	19.03	-2.86	-12.28	-14.74
Maximum	257.28	132.69	124.61	106.50

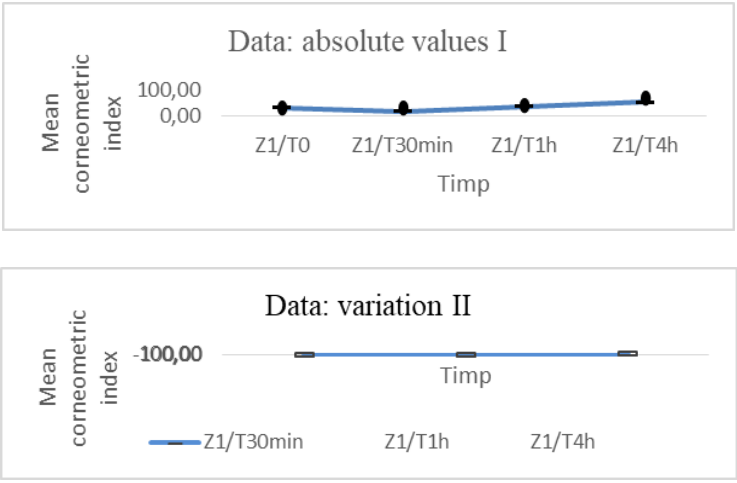


Fig. 4. Moisturizing efficiency after applying the cream

Following the correlations made with Dunnett t test, Z_1/T_0 -Z_1/T_1, Z_1/T_0 - Z_1/T_2, Z_1/T_0 - Z_1/T_3 and Z_1/T_0 - Z_1/T_4 there were no differences statistically significant.

TABLE III

Multiple comparisons after statistical interpretation data

(I) Time	(J) Time	N	Test	Average difference (I-J)	p value
Z1/T_1	Z1/T_0	8	Dunnett t	30,01	0,152
Z1/T_2	Z1/T_0	8	Dunnett t	15,77	0,069
Z1/T_3	Z1/T_0	8	Dunnett t	13,1	0,085
Z1/T_4	Z1/T_0	8	Dunnett t	12,8	0,176

DISCUSSION

Skincare with cosmetic products that contain mostly natural ingredients, without a high content of preservatives, is a desire of the first choice of users. The skin contains a large amount of water, respectively over 70%, of which 1/3 is found in the stratum corneum.

In our extensometry test the pressure is gradually exerted at the same time on the entire surface of the sample, unlike the penetrometer test in which the pressure is gradually applied to a single point on the surface of the sample. Practical, the extensometry curves show the ability to deformation throughout the bulk mass of the cream.

The cream shows a plastic behavior is observed for the prepared moisturizer, the increase of the tangential tension determines the decrease of the viscosity. When evaluating the restructuring behavior during shearing with successive decreasing speeds, a hysteresis loop was observed. The analyzed sample initially showed a high viscosity, but with the increase of the tensile stress, the viscosity decreases, with the destructuring of the system. Then, from the moment the tangential stress decreases, the viscosity begins to increase, with the restructuring of the system, but not with the same speed with which the destructuring took place.

From the experimental data obtained by the hydration test on human subjects only subjects with very dry skin were tested, because the capacity of a moisturizer cannot be determined if the skin is already hydrated or has excess sebum. After four hours to the application of the cream proposed in the study, the hydration level increased in S1 by 87%, in S3 by 108%, in S5 by 59%, in S6 by 68%, in S7 with 106%, and in S8 with 108%. Given that a moisturizer should theoretically improve hydration by 30%, it can be said that an average of over 80% is a very good percentage.

CONCLUSIONS

In this study a biomimetic cosmetic formulation, with 64% hydrophilic phase and 34% lipophilic phase, emulsive system in which the following ingredients have been incorporated like vitamins, hyaluronic acid, and active phytocomplexes from *Achillea millefolium* oil, *Cocos nucifera* oil, *Argania spinose* kernel oil, *Spilanthes acmella* flower extract, *Centaurea cyanus* flower water and *Aloe barbadensis* leaf juice, was developed, able to restore the optimal hydration level. The cream had a regenerating effect through its ingredients, the skin becoming more elastic and hydrated. Although, it is a W/O cream that does not leave the skin oily, the concentration of the lipophilic phase and the emulsifier used do not leave oily skin after application, the absorption of the components is maximum after application. The tested formula had a pH = 6.8 and it was observed a plastic flow.

In vivo tests confirmed the increase of the hydration level on average of 89% in comparison with T_0. Taking into consideration that a moisturizing cream has to improve the hydration by at least 30%, it can be concluded that over 80% hydration improvement is an optimal result.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

FINDINGS AND PERSPECTIVES USING ENCAPSULATION TECHNIQUES

The association between obesity and vitamin A has been studied. Some studies point to the anti-obesity activity related to this vitamin, carotenoids with provitamin A activity, and carotenoid conversion products. This performance has been evaluated in respect of adipogenesis, metabolic activity, oxidation processes, secretory function, and oxidative stress modulation, showing a new property attributed to vitamin A in preventing and treating obesity. However, vitamin A and its precursors are highly sensitive and easily degraded when subjected to heat, the presence of light, and oxygen, in addition to losses related to the processes of digestion and absorption. In this context, recognizing the instability of vitamin A and carotenoids with provitamin A activity in the face of environmental conditions, and thereby incorporating these bioactive compounds in encapsulating agents that bring stability and promote positive absorption effects, is a strategy of considerable interest. In this way, it is possible to expand this nutrient's use in food or pharmaceutical products, as well as potentiating the effects in the treatment of deficiencies related to vitamin A. Thus, it is believed that the encapsulation of vitamin A or its precursors brings essential benefits since it preserves and maintains functionality. It also favors these compounds' action in target tissues and/or organs, thus allowing these bioactive compounds to be better used in the treatment of obesity and associated comorbidities. (de Carvalho Gomes C, *et al.* Vitamin A Status Improvement in Obesity: Findings and Perspectives Using Encapsulation Techniques. *Nutrients* 2021; 13(6): 1921/ doi: 10.3390/nu13061921).

SIGNIFICANCE OF NON-HLA ANTIBODIES IN LOW-RISK KIDNEY TRANSPLANT RECIPENTS

This prospective study included total 94 recipients among 201 consecutive recipients who underwent an ABO-compatible living donor kidney transplant (LDKT). Angiotensin II type 1 receptor antibodies (anti-AT1R) and anti-endothelial cell antibodies (AECA) were measured before, during and after LDKT. The prevalence of acute rejection (AR) within 12 months post-transplant did not differ significantly based on anti-AT1R levels and AECA results. However, when a cut-off value of 11.5 U/mL was applied, it independently predicted a higher risk for AR. AECA (+) recipients had a higher risk for AR or microvascular inflammation (MVI) only during the follow-up period and also showed a rapid increase in creatinine and decrease in eGFR at approximately three months; after this point, they showed persistently higher creatinine and lower eGFR until 20 months. Pre-transplant anti-AT1R level was a significant risk factor for the development of AR, while AECA status was associated with impaired renal function regardless of AR. (Shinae Yu, *et al.* Pre-Transplant Angiotensin II Type 1 Receptor Antibodies and Anti-Endothelial Cell Antibodies Predict Graft Function and Allograft Rejection in a Low-Risk Kidney Transplantation Setting. *Ann Lab Med* 2020; 40(5): 398-408).

INFLUENCE OF CARBOPOL 971P NF ON FLOW RATE AND SWELLING PARAMETERS IN ANTIFUNGAL TABLETS

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INFLUENCE OF CARBOPOL 971P NF ON FLOW RATE AND SWELLING PARAMETERS IN ANTIFUNGAL TABLETS (Abstract): The purpose of this research laid in developing oromucosal bioadhesive tablets with miconazole nitrate based on hydrophilic bioadhesive polymers, Carbopol 971P NF, capable to form a good adhesion to prolong the duration of the active ingredient action for several hours. The aim is to observe the correlation between the disintegration time, the swelling index and the amount of polymer used in mucoadhesive tablets. **Material and methods**: The tablets were made using direct compression method, the mucoadhesive tablets differing in the amount of Carbopol 971P NF. The first proposed formula (FI) contains 40 mg polymer and the second one (FII) contains 10 mg polymer. After the formulation and development of mucoadhesive tablets using different grades of polymer, physicochemical parameters such as surface area, thickness, diameter, friability, hardness, disintegration time and swelling index were determined. **Results**: Both proposed formulas have an optimal disintegration time of 75 and 98 minutes. The swelling index for FI (18% polymer) is 0.4435 and for FII (4.5% polymer) is 1.0316. **Conclusions:** Considering that the mucoadhesive tablets imply a swelling index over 1 and a longer disintegration time, the FII formula, with 10 mg carbomer, can be considered optimal. **Keywords**: CARBOPOL 971P NF, MUCOADHESIVE TABLETS, ANTIFUNGAL TABLETS, MICONAZOLE NITRATE.

The proliferation of stressful conditions, as a result of the diversification of new activities in human society with the appearance of the corresponding noxious substances, as well as the diversification of microbial species, mainly due to the rapid

movement of large numbers of people around the globe, has led to the emergence and intensification of fungal infections.

Many fungi have low virulence and only cause superficial infections of the outer layers of the skin, where antibodies and immune cells cannot reach. Although these infections are not serious, they are very common. These infections are usually chronic or recurrent if the underlying cause has not been eliminated and can become very stressful for patients.

Increased susceptibility to infection has been reported in people whose immune systems are physiologically impaired or as a consequence of a disease or treatment, or whose natural defenses and normal microbial flora have been affected. The incidence of fungal infections in immunocompetent organisms with a severely compromised immune system has also been observed.

It is now noted that the most common fungal infections in humans are urinary, vaginal and oromucosal. In recent years, their etiology is changing. If a decade ago they were produced almost exclusively by *Candida albicans* (85-90%), today non-albicans strains of *Candida* are increasingly involved (1, 2).

Miconazole nitrate is a topical antifungal agent used for local treatment of vaginal, skin and nail infections due to yeasts and dermatophytes, being active against *Candida* spp., *Trichophyton* spp., *Epidermophyton* spp., *Microsporum* spp. However, oral administration of drugs has disadvantages, such as metabolism of the first hepatic passage and enzymatic degradation within the gastrointestinal tract. These disadvantages may limit or prevent the oral administration of certain drugs, especially peptides and proteins, when they are inserted into special colon administration systems (3). These limitations have led to the exploration of other mucous membranes as potential locations for drug absorption (4). The mucosal routes of drug administration (e.g., mucosa of the nasal, rectal, vaginal, ocular and oral cavities) offer various advantages over oral administration of drugs (5, 6). These benefits include possible alternative pathways for the first-pass effect and avoidance of presystemic elimination within the gastrointestinal tract (7, 8).

Strategies for oral administration of drugs are represented by the new oral dosage forms, which consist mainly of sustained release systems for oral administration, designed to deliver the drug over a period of time (9, 10).

Mucoadhesive tablets are widely used in the formulation and preparation of topical antifungal products for prophylactic or curative purposes in infections of the oral and pharyngeal mucosa, such as acute oral candidiasis. This oral candidiasis is a major problem for patients with long-term HIV infection, with corticosteroid, antibiotic, anti-inflammatory agents treatment (8).

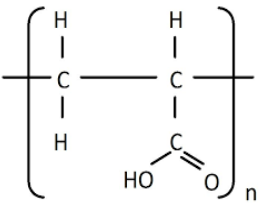
To improve the bioavailability of active substances in the oral cavity, various bioadhesive tablet systems have been developed in recent years with different active substances. Oral tablets can be applied in the oral cavity, for example in the palate, the mucous membrane of the cheeks, and the upper lip and the gums. They soften and adhere to the mucosal substrate is retained in the fixed position until complete dissolution and/or complete release. After a while, the patient no longer feels the tablet in his mouth. The location of the tablet in the mouth seems to have a big impact on tolerance and retention time. Depending on the location, either palatal or gingival, the retention time varies from 20-40 minutes to

7-12 hours.

It is important that excipients for the development of tablets do not stimulate salivation, as in this case much of the drug may reached the gastric level, instead of being available locally and absorbed. Thus, the main characteristic of bioadhesion is the force of the attachment of the pharmaceutical form to the biological tissue and this is obtained by using polymers that adhere to the mucin-epithelial surface. Oral mucoadhesive tablets are modern modified-release pharmaceutical forms used in current therapy, to achieve a controlled release of the active substance at the site of application as a result of maintaining the pharmaceutical preparation at this level. The principle of formulation of modified release bioadhesive tablets is to disperse the active substance in various hydrophilic polymeric matrices.

Many oral products on the pharmaceutical market use carbomers as polymers for bioadhesion due to the benefits of this kind of formulation (11). Carbopol-type polymers have different rheological properties, influenced by particle size and molecular weight. The rheological properties can be related/correlated with the molecular weight of the polymer. Carbopol 940, 941, 971 and 934 are types of polymers very well tolerated by the body without toxic effects. The resulting levels should be low those shown to have no risk to human health. For example, in a toxicology study of Carbopol® 974P NF polymer with dogs, the no observed adverse effect level was 50,000 ppm (12). Carbopol® 971P NF polymer (tab. I) is used in oral and mucosal applications such as extended/controlled release tablets, oral liquids, and suspension and bioadhesive formulations (13).

TABLE I.
Physico-chemical properties of Carbopol 971P NF

Appearance	white power	
Density	208 kg/m^3	
Specific weight	1.41	
Humidity	2.0% maximum	
pKa	6.0 ± 0.5	
pH value (1% solution at 25°C)	2.5 - 3.0	
pH value (0.5% solution at 25°C)	2.7 - 3.5	
Equivalent weight	76 ± 4	
Ash content	0.009 ppm (average)	
Glass-transition temperature	100-105° C	

The research was focused on the development of solid pharmaceutical forms, such as oral mucoadhesive tablets with miconazole nitrate, and the changes that occur regarding the disintegration time and swelling index using different excipients.

MATERIAL AND METHODS

The following substances were used to develop mucoadhesive tablets: miconazole nitrate 99% (Sigma Aldrich, Germany), Carbopol 971P NF (Lubrizol, USA), mannitol (East Chemical, China), aerosil (Degussa, Germany), and magnesium stearate (Sigma Aldrich, Germany). Miconazole nitrate was used as an antifungal active substance of mucoadhesive tablets, which were prepared according to table II in two formulations.

TABLE II.

Formulation of mucoadhesive tablets with miconazole nitrate

Ingredients (mg/tablet)	F I	F II
Miconazole nitrate	50	50
Carbopol 971P NF	40	10
Mannitol	122	152
Aerosil	6	6
Magnesium stearate	2	2
Total weight (mg) 220		

Determining the flow rate and compressibility parameters of powder mixtures

There were monitored the typical parameters: flow rate (t), friction coefficient (tg α), repose angle (α), Hausner ratio (HR) and Carr index (CI).

Flow rate (t) the time needed for 100 g of formulation to flow through a funnel with a 10 mm orifice;

Friction coefficient (tg α) – dynamic method, according to the equation:

$tg\ \alpha = h/r$

where: h = height and

r = radius of the cone

Repose angle (α) – dynamic method;

Haussner ratio (HR) – calculated by determining the density before and after settling, according to the equation:

$HR = pt/pi$

where: pt = density after settling and

pi = initial density

Carr index (Ci) – identical determinations and recordings with the ones presented in Haussner ratio, but calculated according to the equation:

$CI = (pt-pi/pt)x100$

Preparing the oromucosal bioadhesive tablets with miconazole nitrate

Mucoadhesive tablets were made by using the method of direct compression of the powder mixture with a Korsch tablet equipment double-sided press (size of upper punch φ = 9 mm) to a compression pressure of 5 – 6 kN, for 30 seconds.

Characterization of the oromucosal bioadhesive tablets with miconazole nitrate

Dimensional characteristics. The dimensions of the tablets: diameter (d^2) in mm and height (h) in mm were determined using the automatic device PHARMA-TEST, Germany, on a number of 10 tablets and the average was calculated. The surface area of the tablets was then calculated according to the formula:

$S=\pi*d^2/4$

Resistance to crushing. The resistance to crushing of mucoadhesive tablets was also automatically determined in PHARMA-TEST device.

Friability. Also called tablet wear, it is obtained after exposure to rolling the tablet. Due to the phenomenon of rolling some of

the particles of the tablet fall off. The phenomenon is also known as an abrasion and is expressed as a percentage. The friability is calculated according to the formula:

$$\text{Friability} = (\text{Initial weight} - \text{Final weight}) / \text{Initial weight} \times 100$$

Disintegration. According to USP 32 method A, the test was carried out on a number of 6 tablets of each formula. *The Pharmacopoeia* provides the determination on 6 tablets which are placed in a 500 mL conical vessel with 300 mL of water maintained at a temperature of 35-39°C. During the determination, the vessel is shaken gently twice a minute. After four hours, the contents of the vessel is filtered through a 0.8 mm mesh sieve, the vessel is washed with 300 mL of water at 37±20°C, which is brought over the remaining particles from the disintegration. It is allowed to remain on the sieve particles, which when lightly pressed with a flattened wand at one end pass through the sieve. If the result of the determination is negative, the test is repeated twice with 6 tablets each, establishing the duration of disaggregation for both determinations. The product is considered suitable if the average of these two determinations does not exceed the expected time by more than 20%. The disintegration test determines the time required to turn the tablets into fine particles or the time required to release the contents of the capsule wrapper when they are placed in a liquid medium under specific working conditions.

Swelling index

The swelling index (SI) of a mucoadhesive preparation is an important parameter for evaluating the prolonged release of the drug and the adhesive action. The swelling index of the mucoadhesive tablets has been determined by weighing only one of the two formulas and recording the weight before placing them separately in Petri dishes. The individual initial weight was marked with (W1). In each dish, we added 15 mL of phosphate buffer (pH=6.8) and the temperature was thermostatically maintained at 37°±0.5°C. At regular intervals (60 minutes, 120 minutes), the tablets were extracted, and excess surface water was removed, carefully and weighed again (W2).

The swelling index was calculated for each formulation, using the formula:

$SI = (W2-W1)/W1 \times 100$

where: W1 = the initial weight of the tablet and W2 = the weight of the tablet after 60,

respectively, 120 de minutes at 37ºC in phosphate buffer solution.

Statistical data analysis

Statistical analysis of the data was performed using IBM *SPPS 20* software.

RESULTS AND DISCUSION

Determining the flow rate and compressibility parameters of powder mixtures

The first parameter that was determined for the flow behavior of the powders according to the cohesive material was the value of the repose angle. If a particle is temporarily outside this limit angle, it can slide to the adjacent surface under the influence of gravitational force until the shape of gravity is balanced by the friction between the particles. The lower the repose angle value, the lower the powder flow rate. Thus, a value of the angle below 20 indicates an excellent flow and a value between 20-30 indicates a good flow and up to 40 a poor flow rate. FI formulation contains 40 mg polymer compared to 10 mg of polymer in FII, and this matrix-

forming polymer makes it difficult for the powder to flow even if it contains aerosol and magnesium stearate (tab. III).

TABLE III.
Flow rate and compressibility parameters for FI and FII formulations

Formulation	Parameter			
	α (º)	tg α	HR	CI (%)
FI	41.9	0.90	0.8800	13.99
FII	36.80	0.73	0.8300	18.65

The Haussner ratio is the ratio of the density of the final powder bed to compaction and is based on the friction between the particles which demonstrates a good or less good yield. Thus, in all formulations the result of the Haussner ratio is below 1.2 which indicates a slight flow. We can see a better flow in the FII formula with a smaller amount of polymer (tab. III).

The Carr index, also called the compressibility index, is closely related to the Haussner ratio, which is another parameter of the fluidity of the powder mixture that indicates the need to add a thinner or not. According to specialized monographs, CI with a value between 5-12 means an excellent flow of the powder and between 12-16 a good value for flow. Compressibility is the ability of a powder to decrease its volume instantly, under a pressure applied vertically (14-21).

Characterization of the oromucosal bioadhesive tablets

The macroscopic characteristics of the mucoadhesive tablets made have flat, uniform, flat-rimmed, flat-surface lenticular discs, which means that these pharmaceutical forms are suitable both in terms of Romanian Pharmacopoeia Xth edition (FRX) and optimal for administration to the oral mucosa. The diameter and thickness are suitable for mucoadhesive administration, the deviations varying within the limits of the size of the punch chosen for compression. The diameter obtained is ideal for this type of administration because the gum above the canine has a maximum size of 12-13 mm and a thickness greater than 4 mm would change compliance.

The tablets are white, uniform, without spots, without odor, slightly bitter taste diminished due to the mannitol added as a sweetener. The value of the friability test is according to the accepted parameters, a value of less than 1% for both proposed formulas (tab. IV).

All values of the mechanical strength parameter are within the corresponding limits, respectively between 30-90 N. Disintegration time is an extremely important parameter because the product must be released gradually in the oral mucosa (21). A rapid disintegration can lead to an ineffective antifungal. The longer the tablets stay in the mucous membranes, the longer the effect lasts and the longer the antifungal has time to act. The disintegration time of the proposed formulas is closely related to the amount of polymer used. In the case of the FI formula, which contains 40 mg carbopol, the disintegration time is 75 minutes and in the case of the FII formula, which contains 10 mg polymer, the disintegration time is 98 minutes. A smaller amount of polymer can soak with water forming a gel around the tablet (tab. IV). Thus, this gel slows down the disintegration of the tablet.

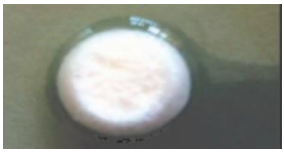
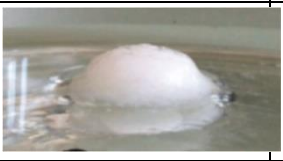
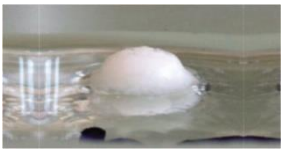
TABLE IV.
Physical properties of mucoadhesive tablets with miconazole nitrate

Formulation	Thickness (h) (mm)	Diameter (mm)	Surface area mm²	Hardness (N)	Friability %	Disintegration time (min)
FI	3.280	9.260	66.75	50.29	0.8965	75
FI-SD	±0.057	±0.174	±3.376	±7.601	±0.515	±4.393
FII	4.109	9.398	71.67	61.10	0.9100	98
FII-SD	±1.02	±0.981	±2.342	±10.245	±3.456	±5.601

Swelling index

The swelling index was calculated according to time (tab. V). When performing this test on sample for swelling, the weight changed with the swelling of the polymers in an aqueous solution. The swelling rate increased as the weight of these tablets increased in proportion to the rate of hydration up to two hours and then gradually decreased as a result of disintegration in the dissolution medium. It has been observed that total release is achieved when a lower concentration of polymer is added to the formula. The reason for this is the erosion of the gel layer in tablets containing smaller amounts of polymer. The higher the swelling index, the better the tablet will adhere to the oral mucosa, gradually releasing the amount of antifungal.

TABLE V.
Swelling index of mucoadhesive tablets

Formula	W1 initial (g)	W2 at 60 minutes (g)	W2 at 120 minutes (g)
FI			
	0.2390	0.3450	0.4700
	SI (%)	**0.4435**	0.9665
FII			
	0.2210	0.4490g	0.3200
	SI (%)	1.0316	0.4479

A swelling index at 60 min is 1.0316% for formula FII, which contains 10 mg polymer. The amount of polymer used in the FI formula swelled in the presence of water, greatly increasing its volume. The swelling index is 55% lower, 0.4435% in the formula

FI, which contains 40 mg polymer. For prolonged-release tablets, the swelling index needs to be greater than 1 and a higher concentration of the polymer leads to a longer soaking time with an aqueous solution. A film is needed that is made on the outside of the tablet when it is positioned at the level of the oral mucosa. The higher the swelling index, the more effective the mucoadhesive tablet can be. A large amount of polymer in formula FI, 40 mg, did not have enough surface contact with water to have higher SI results. The polymer, being a network-type structure, needs time and large contact surface with water to be able to soak water. The proposed mucoadhesive tablets have a small contact surface, 9 mm, and large amount of polymer does not allow interpenetration between the upper layers. A smaller amount of polymer, FII formula, evenly dispersed in the tablet may develop a higher SI.

In the case of SI at 120 minutes, the values differ a lot. For FI formula, the SI at 120 minutes is 0.9665%, about 50% more than the SI value at 60 minutes. In this case the large amount of carbopol had enough time to come into contact with water and to swell the polymer network. In the case of FII formula, the SI value at 120 minutes is 57% lower than the SI value at 60 minutes. If the polymer is in a lower concentration, it will be dispersed uniformly in 60 minutes, which conducts to a maximum water-soaked network structure. At 120 minutes the SI value of FII decreases because the gel formed around the tablet is gradually released.

CONCLUSIONS

Given that the antifungal active substance should be released gradually, FII formula with 4.5% carbopol may be proposed for future studies. The value of the swelling index and the disintegration time are important parameters for oral antifungal drugs in the form of mucoadhesive tablets. For FI formula, with 18% polymer, the swelling index is very small, being 132% lower than the FII formula with 4.5% polymer. Also, the disintegration time for FI formula is 30% shorter than the FII formula. The swelling index provides information about the ability of the new pharmaceutical form to stand on the oral mucosa. By disintegrating the tablet, the bioadhesive polymer in the aqueous medium forms a gel as it disintegrates. Thus, the longer this gel stays on the oral mucosa, the more active substance is gradually released from the pharmaceutical form and the contact time between the mucosa and the active substance will increase, which can provide a better action of the pharmaceutical product.

CONFLICT OF INTEREST AND FUNDING

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THE STUDY AND DEVELOPMENT OF PERSONALIZED ORTHOTIC INSOLES THROUGH 3D PRINTING TECHNIQUE

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THE STUDY AND DEVELOPMENT OF PERSONALIZED ORTHOTIC INSOLES THROUGH 3D PRINTING TECHNIQUE (Abstract): The human foot is the main connection of the body with the environment. It has a complex anatomical structure allowing different movements, such as gait, orthostatic position or running, on different surfaces, without any pain or injuries occurring. Upon it, there are many types of impact forces acting, therefore, the human foot has to absorb and dissipate these forces. The study of plantar pressures and their distribution across the foot aid in diagnosing and treating different pathologies. The plantar analysis helps in creating personalized tools for treating pathologies associated with the foot. **Objective:** This paper aimed to create a personalized insole, 3D printed, made out of flexible materials that would be fitted for this study's participant. **Material and methods:** The study consisted in collecting data from 12 subjects, with no known pathologies and performing the plantar pressure analysis, both static and dynamic analysis, then, modeling and printing the anatomical insole, fitted for the participant of the study. **Results:** The result is an anatomical insole, personalized, 3D printed, using flexible filament, with different thicknesses along the arches of the foot and different infill densities for unloading uneven plantar pressures across the foot. **Conclusions:** After all the stages of the study, a personalized insole was printed. The insole strictly follows the participant's foot anatomy, takes into account the distribution of plantar pressures across the foot and the deformities that were noticed as a result of the static and dynamic plantar analysis. **Keywords:** PLANTAR PRESSURE, INSOLE, 3D PRINTING, FLEXIBLE FILAMENT.

People spend the majority of their time in orthostatic position, walking or engaged in other types of physical activities. In this case, the feet and all the surfaces walked on are in direct contact. The plantar forces, forces that are acting upon the feet can sometimes have an uneven distribution. This uneven distribution can lead to a series of issues that can affect the general health of an individual (1).

Since the foot is the main surface of interaction during gait or every other physical activity, it is important to quickly identify any types of pathologies and deformities in their beginning phases so they could be either treated, prevented or corrected. One of the easiest, fastest and most reliable sources to do so could be considered the plantar pressure analysis (2).

Plantar pressure and its measurement offer information about foot and ankle function in orthostatic position and during

physical activities. The data obtained during the analysis of the important parameters involved in these movements are important in evaluating different pathologies and deformities that can affect the feet (1).

There is a great relationship between plantar pressure and the posture and balance of human bodies (3).

There are a lot of advantages in using this technique to assess different issues upon the foot, such as the usage of complex software able to perform and evaluate gait analysis, in both static and dynamic regimes, distribution of the plantar pressures across the feet, support areas, mass repartition, body posture. All the information can be easily saved and viewed in a database. The method in itself is easy to perform for both the specialist and the patient, fully objective and it allows modelling and creating personalized insoles fitted for each patient and their issues. Performing the analysis and taking the measurements are, as mentioned before, easy to obtain, the more difficult part is interpreting and analyzing the results obtained after the measurements (4).

Inadequate support of the feet can lead to numerous injuries in the short or long term. For better support, an anatomical insole can be one great solution. To create a personalized insole, the morphology should be perfectly adapted to the patient's feet. 3D printing of the insoles is the newest and best technique to create solutions for treating or preventing deformities, to ensure an even distribution of the pressure across the feet for better support and better comfort (5,6).

For designing shoes and the components of the shoes, such as the insoles, a detailed analysis of the feet is a key point, considering the different characteristics that every individual has. Given the fact that the feet of every individual are so different, it is important to understand and recognize certain patterns to obtain products that are both supportive and comfortable enough (7).

As stated before, the foot has an anatomical structure that is different for every person, therefore, when the plantar analysis is taken, a series of other parameters and measurements are important, such as: the length of the foot, width at different points, the height of the hallux and other toes, along with some information about the patient: age, sex, height, weight, shoe size (7,8).

MATERIAL AND METHODS

In this study, plantar analysis was performed on 12 participants. The plantar analysis was performed for both the static and dynamic regimes. The participants had no diagnosed pathologies but had different ages, heights, weights and shoe sizes.

The data was collected using the pressure plate Footwork (AMCUBE) and was visualized using the associated software.

The first step of the study was collecting data from the participants: age, sex, height, weight and shoe size. All this information is relevant because they change the way the plantar pressure is distributed across the foot (9, 10). The next step was performing the plantar analysis. For the static analysis, the subjects were asked to stand in a natural position for 10 seconds, their arms on the sides of the body, looking ahead, feet shoulder-width apart, both feet on the pressure plate. For the dynamic condition, subjects were asked to take two steps before striking the plate, right foot first, then in the same way for the left foot. Once the data was collected, it was visualized and organized for better understanding

in tables. The data that was looked at was mean and maximal pressure for both feet, in both conditions, the forces chart and mass distribution.

TABLE I.
General data collected from subjects

Subject	Sex	Age	Height (cm)	Weight (kg)	Shoe size (EU)
S1	F	48	165	69	40
S2	M	19	168	60	42
S3	F	20	160	48	38
S4	M	20	182	110	46.5
S5	M	23	181	85	40
S6	F	19	165	54	38
S7	M	20	177	68	41
S8	F	20	160	36	37
S9	F	19	158	48	38
S10	F	20	165	54	37
S11	M	21	180	86	44
S12	F	24	173	59	40

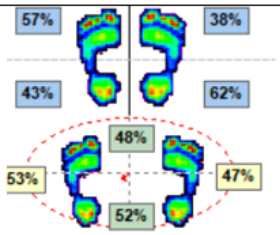
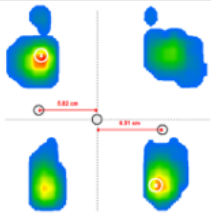
Fig. 1. Pictures taken during the scanning process.

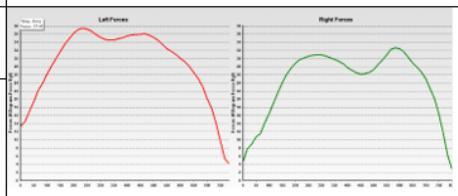
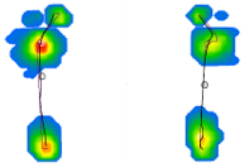
To create the insole, out of the 12 analyses, only one was chosen given the different deformities and the pressure distribution that were noticed on the subject. Table I displays the plantar analysis for the chosen participant.

Once the plantar analysis was chosen, the next step was to create the model of the insole for printing. Firstly, Gensole was used. Gensole made possible the creation of a template for the insole with all the dimensional parameters of the participant's foot, along with certain modifications on the thickness and heights across the insole, in accordance with the foot's deformities,

TABLE II.

Plantar pressure for static and dynamic analysis.

Static					
Mean Pressure (kPa)		**Maximal Pressure (kPa)**		**Mass Repartition**	**Plantar Pressure**
Left Foot	Right Foot	Left Foot	Right Foot		
56.53	51.66	151	125		

Dynamic					
Mean Pressure (kPa)		**Maximal Pressure (kPa)**		**Forces Chart**	**Plantar Pressure**
Left Foot	Right Foot	Left Foot	Right Foot		
186	198	306	301		

Autodesk Fusion 360 was used to modify the specific zones according to the plantar pressures. For this, the 3D model previously obtained and the plantar pressure for the subject were overlaid to create a support for the transverse arch, as well as to mark zones for decreasing plantar pressures in areas where high values were observed.

For the printing of the model, Prusa Slicer was used to modify the local densities and geometries inside the insole.

In the last phase, the obtained personalized insole was printed using PRUSA i3 MK3. The used material was Filaflex, a flexible filament from Recreus with great proprieties for this type of application.

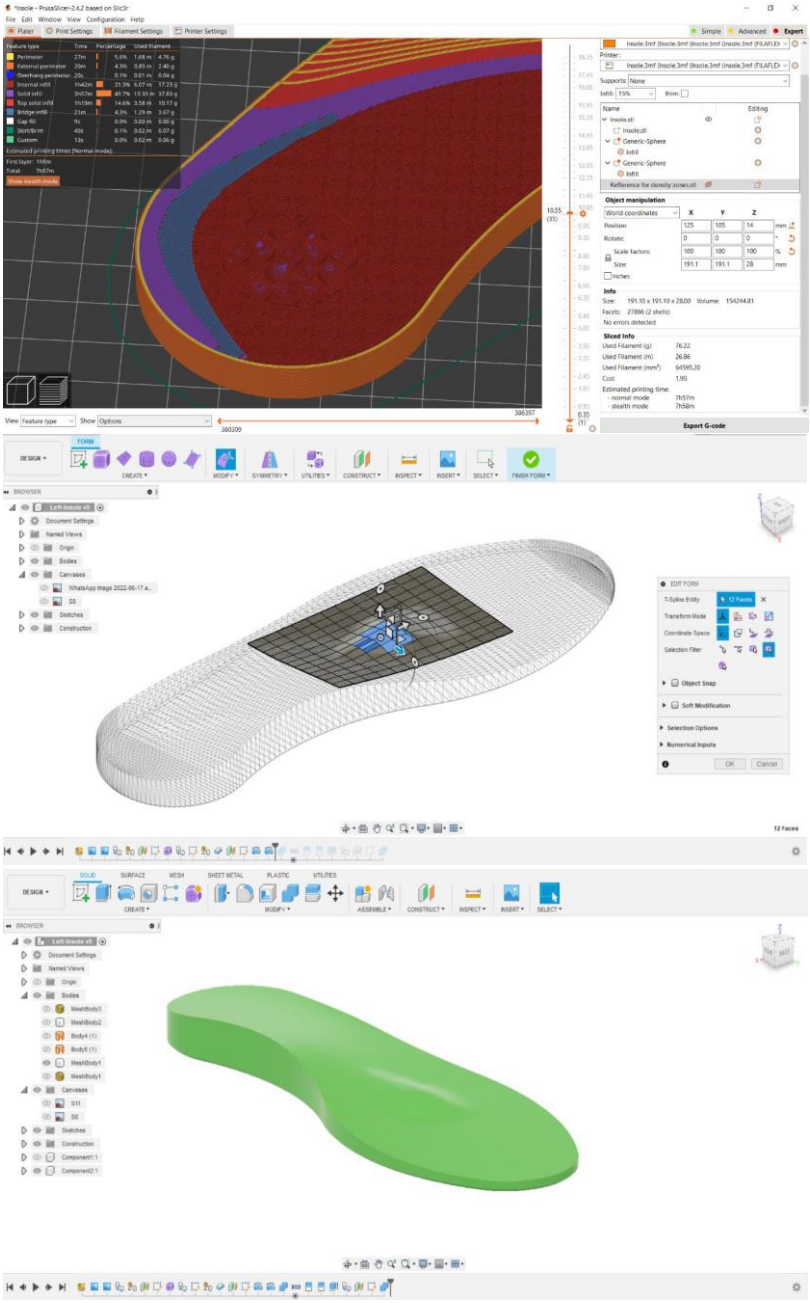


Fig. 2. Images during the modelling process.

RESULTS

After the 3D printing of the insole was performed, the result was a personalized insole, fitted for the study's participant. Different heights, thicknesses and heights were obtained across the plantar arches,

with the purpose of correction of pes cavus hindfoot valgus. Also, another advantage of the personalized insole is that it has zones of different infills that will serve as unloading areas of the high plantar pressures that were noticed in the first part of this study.

Fig. 3. Images of the personalized insole.

DISCUSSION

Over the last few years, the topic of personalized medicine, based on treating each patient individually and not the pathology itself has been presented as the future of the medical field. This type of approach has a series of advantages, such as the optimization of specific treatment schemes, better solutions fitted for every patient and lower final costs.

The study has presented such an application, a personalized solution for treating certain pathologies or for improving patients' comfort in their day-to-day life.

While many researchers have been working on creating customized insoles through various techniques and materials, the method presented in this paper has not been done before. Most of these papers were done regarding the study and comparison of thermoplastic elastomers. Mogan *et al.* (11) has conducted a series of studies regarding the proprieties that Filaflex has and the infill pattern impact on the mechanical proprieties of the final product and has concluded that this particular material has remarkable proprieties.

Zuniga *et al.*(12) developed a 3D printed insole, using two types of flexible filament, for comparison and evaluated plantar pressure distribution in walk trials. The development of the insole was, however, done using a 3D scanner along with the usage of Gensole and then printing the insoles. The authors have declared this method as being expensive. Also, their proposal was time-consuming compared to the method this paper presented.

Jandova *et al.* (8) have presented a study in which they developed a model of a 3D printed insole, which was based on specific parameters and characteristics of one of the three specific European feet, as they classified them, but their proposed method was not a personalized, custom made for patient application.

The method presented in this paper is a new method to design and create custom-made insoles to treat feet's pathologies or for better support and comfort. The combination of techniques that were used, such as the usage of just a pressure plate to identify the most affected areas across the feet, the usage of Gensole software to briefly design the template that was later used to shape and design different areas on the

insole in Autodesk Fusion 360 that would make the insole truly personalized and then the fabrication process, respectively 3D printing using a flexible material, Filaflex, a material with truly remarkable proprieties. All these methods combined have not been done before, making it possible to create a custom-made insole, perfectly personalized without needing a 3D foot scan, but only a pressure plate and the 3D printing machine.

CONCLUSIONS

This study's purpose was to create personalized insoles using flexible materials. Plantar analysis performed in both static and dynamic allowed for adequate data that was needed to use in the design and 3D printing of the insole. This technique has the potential of easing the technician's work through optimized and digitalized processes, with lower final costs and much quicker than using the standard methods, with better results for the patient as well.

CONFLICTS OF INTEREST AND FUNDING

Authors declare that there is no conflict of interests, and they received no funding for this study.

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INVESTIGATING LIFESTYLE, EATING BEHAVIORS, OBESITY, AND ORTHOREXIA NERVOSA AMONG MEDICAL STUDENTS

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OBESITY AND ORTHOREXIA AMONG MEDICAL STUDENTS (Abstract). Lifestyle and eating behaviors change during the academic year. The aim of the study was to assess the lifestyle, eating behaviors, the presence of obesity, and orthorexia nervosa among students enrolled in medical studies in Romanian faculties. Data collected from 231 students gathered sociodemographic, medical, as well as anthropometric and psychological information. The statistical analyses were performed using IBM *Statistical Package for Social Sciences (SPSS)* for Windows, version 24.0 (SPSS Inc., Chicago, IL, USA). To identify the presence of orthorexia nervosa, the *Düsseldorf Orthorexia Scale* was used. The research identified that almost 70% of students have a normal status. Less than half of the students (42%, N = 97) consumed food supplements with the aim of improving cognitive performance during exam sessions. Students registered M = 3.02 ± 1.36 hours of physical activity per week. More than half of the students (53.7%, N = 124) estimated that they spent between 2 and 5 hours a day in front of screens. The collected data identified the presence of orthorexia nervosa in 6.1% of students, and 16.5% were evaluated as being at risk. More correlations were identified. Healthy lifestyles are difficult be maintained during academic years, and specific attention should be paid to dietary patterns and physical activity. Psychoeducation should be an important aspect of medical students' personal and professional development. **Keywords**: MEDICAL STUDENTS, HEALTHY LIFESTYLE, EATING BEHAVIOURS, OBESITY, ORTHOREXIA NERVOSA.

The 21st century is facing a global obesity epidemic, and this obesity crisis has become one of the greatest challenges of our time with widespread recognition as an important global public health issue. The prevalence of obesity has skyrocketed in these early years of the 21st century, affecting populations in both developed and developing countries, and this increase has rapidly become a public health problem (1).

Obesity entails a significant economic burden on individuals and their families, but also on health systems and society in general, both through direct healthcare costs for treating obesity-related complications and indirect costs through reduced productivity and job performance. It is

important that strategies to address obesity involve individuals and communities as well as health care providers and policy-makers and include creating supportive environments for at-risk populations, promoting healthy lifestyle habits, obesity prevention strategies, and improving access to health care treatment (2).

Obesity profoundly impacts health and is associated with numerous health risks and chronic non-communicable diseases. Thus, it is a risk factor for developing insulin resistance and type 2 diabetes, as excess adipose tissue influences the body's ability to regulate blood glucose levels. Visceral fat causes the accumulation of atheromatous plaques, narrowing arteries and restricting blood flow, thus increasing the risk of cardiovascular disease, high blood pressure and stroke. Certain cancers are thought to be closely linked to obesity, including breast, colorectal, ovarian, liver, kidney, and pancreatic cancers. Obesity also leads to the development of respiratory problems such as sleep apnea, hypoventilation syndrome, and asthma. Because of the extra stress on the bone system and joints, people with obesity are at risk of developing osteoarthritis and back pain. Mental health is influenced by obesity, as people with obesity have higher rates of depression and anxiety due to social stigma and compromised body image.

According to the World Obesity Federation, it is estimated that nearly 30% of men and women will develop obesity by 2030 (1). Furthermore, the Eurostat report for 2019 concluded that in Europe, 53% of adults are overweight or obese, depending on BMI, and the percentage of obesity is directly proportional to age, except for people over 75 (3).

In terms of the proportion of young adults who are obese, this varies from country to country and according to gender; thus, the European average of obese people in the 18–24 age group was, according to Eurostat, 25% in 2019. The lowest value was recorded in Italy (18%), and the highest value, 31%, was recorded in Hungary. Romania recorded 25.4%, a percentage close to the European average (4).

There are several factors that can affect the rise of obesity among students. Among them is changing eating habits by adopting eating patterns that are mainly characterized by frequent consumption of high-calorie foods, fast food, and snacks instead of meals, as well as sweetened carbonated drinks, all of which contribute to weight gain and obesity. Restricting access to nutritious food or skipping breakfast (5) may also be contributing factors to obesity.

The goal of the present study is to investigate the presence of eating behaviors, obesity, and orthorexia nervosa among medical students in Romania, and the factors that influence them. There is a gap in the data concerning obesity among medical students in Romania, so the research focused on identifying the underweight, normal, and overweight students enrolled in medical studies, but also, considering the specialization - medical studies – the research team took into consideration that students enrolled in medical studies should be more focused on eating healthy and maintaining a good physical and mental life (6). That is why the investigation also took orthorexia nervosa as a variable. This is defined as an obsession with healthy eating accompanied by restrictive behaviors, sometimes being associated with malnourishment, loss of relationships, and poor quality of life (6).

MATERIALS AND METHODS

Participants and data collection

The study was conducted between April and May 2023 among students from the "Grigore T. Popa" University of Medicine and Pharmacy of Iasi. The participants were informed about the purpose of the study, about the confidentiality of the data, and that they could withdraw from the study whenever they wanted, without consequences. The inclusion criteria were being students of the medical field and filling out the questionnaire before the deadline. The exclusion criteria were questionnaires that were incomplete or submitted after the deadline. Finally, 231 questionnaires were included in the research.

The online questionnaire was created using the Google Forms application (Alphabet, Mountain View, CA, USA).

The first part of the questionnaire gathered socio-demographic information (like age, gender, living environment, year of study, the faculty where they study, and if they have employee status), medical information (if they or another family member suffers from chronic diseases and follows any treatment for their disease), and anthropometric data (height, weight, abdominal circumference, following a diet).

The second part of the questionnaire includes several questions grouped by various lifestyle components and factors that can influence lifestyle:

- substance consumption (tobacco, alcohol, psychoactive substances, or food supplements),
- the perceived influence of emotional factors on food (frequently consumed foods and changes in appetite during states of nervousness, stress, boredom, eating without feeling hungry, the level of consciousness felt while chewing food etc.),
- the influence of family income on eating behavior (the source where the money comes from, the percentage of income invested in buying food, proximity to restaurants, supermarkets, agri-food markets)
- the behavior related to the carried out physical activity,
- sleep-related behavior and time spent in front of screens,
- acquired food patterns and the frequency of food and beverages consumed.

The final part of the questionnaire includes one instrument, which refers to assessing and screening orthorexia nervosa: Düsseldorf Orthorexia Scale. This instrument was developed in 2015 by Barthels *et al.* and consists of 10 items with proven reliability, ranging from satisfactory to good, with an internal consistency of $\alpha = 0.84$. This scale is based on a four-level scoring system from "this does not apply to me (1)" to "this applies to me" (4), with scores ranging from 10 to 40 points (7, 8).

Statistical analyses

The statistical analyses were performed using IBM *Statistical Package for Social Sciences* (*SPSS*) for Windows, version 24.0 (SPSS Inc., Chicago, IL, USA). The results for descriptive statistics were expressed as means and standard deviations (SD). The normality of the data distribution was assessed by using the Kolmogorov-Smirnoff test. Given the fact that all data are not normally distributed, bivariate analysis will be performed and non-parametric tests will be applied. To assess comparative results considering gender, employee status, and chronic disease diagnosis, the Mann-Whitney test was performed.

Comparative results considering sex, living environment, and changes in appetite depending on the emotional state following

a diet, were assessed by using the Kruskal-Wallis H test to determine if there were statistically significant differences between more than two groups of an independent variable on a continuous or ordinal dependent variable.

The Spearman correlation was used to test the relationship between variables. A p-value < 0.05 was considered statistically significant.

Ethical approval

The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee of the University of Oradea, No. CEFMF/3, March 16th , 2023.

RESULTS

Socio-demographic, medical, and anthropometric data

Sociodemographic data were gathered, along with household-related information and medical information. The average age of participants was M = 23.61 ± 6.71. More than half of the students lived in a rented apartment (56.3%, N = 130), while the rest lived at home with their parents (16.5%, N = 38), or in a student dormitory (27.3%, N = 63).

Most of the participants (88.7%, N = 205) and their family members (70.1%, N = 162) did not suffer from chronic diseases, and a very small part took medication for chronic diseases (8.2%, N = 19). Additional information about anthropometric and medical data is presented in first table.

TABLE I.

Socio-demographic data[1]

Socio-demographic characteristics	M±S.D and %
Abdominal circumference	73.66 ± 15.15
Nutritional status	
Underweight	24 (10.4%)
Normal status	161 (69.7%)
Overweight	37 (16.0%)
Obesity	9 (3.9%)
Following a diet	
Yes, it was recommended to me by a health professional	9 (3.9%)
Yes, I chose it personally	31 (13.4%)
Yes, I tried but failed	24 (10.4%)
No, I didn't follow a diet	167 (72.3%)

[1]Means and standard deviations (M±D), frequency and percentages (%)

Lifestyle components and factors that can influence lifestyle

Substance consumption

The results of the study showed that more than half of the participants had never smoked (64.9%, N = 150), and a small part smoked daily (14.7%, N = 34) or occasionally (19.5%, N = 45). Moreover, more than half of the participants' family members had never smoked (59.7%, N = 138), while more than a third smoked daily (32.5%, N = 75).

Less than half of the students (42%, N = 97) consumed food supplements with the aim of improving cognitive perfor-

mance during exam sessions. Regarding the consumption of other substances, detailed results are presented in the table below.

TABLE II.

Substance consumption[1]

Substances	N, %
Alcohol	
never	52 (22.5%)
sometimes	173 (74.9%)
often	6 (2.6%)
daily	0
Psychoactive substances	
never	217 (93.9%)
sometimes	12 (5.2%)
often	1 (0.4%)
daily	1 (0.4%)

[1]Frequency and percentages (%)

The perceived influence of emotional factors on food

In terms of emotional eating, half of students (50.6%, N = 117) admitted that they ate more when they felt stressed, nervous, or bored, the most consumed foods like sweet puff pastry such as Fornetti products, doughnuts, strudel, etc. (20.3%, N = 47), cream cakes (18.6%, N = 43), salted foods - such as sticks, pretzels, breadsticks (18.6%, N = 43), or salted puff pastry (7.8%, N = 18), and very few (3.9%, N = 9) were those who consumed more fruits during these tense periods.

However, less than half of the participants admitted that their eating pattern had changed largely (32.5%, N = 75) or to a great extent (14.3%, N = 33) after admission to college. Additionally, less than half of the students (44.6%, N = 103) rarely ate without being hungry, and most of them (71.9%, N = 166) were moderately aware when chewing food.

In addition, more than a third of the participants (39.8%, N = 92) said that it was difficult for them to leave food on the plate, only when it came to their favorite food.

The influence of family income on eating behavior

More than half of the students had a source of income from their parents (69.3%, N = 160), with a few having a stable job (17.7%, N = 41). Regarding the income allocated to buy food, less than half of the students (42.4%, N = 98) said that they spent between 25 and 50% of their income on food. Similarly, less than half of the students (43.3%, N = 100) largely considered that the income they had was enough to ensure their daily lives.

Most students lived in areas close to supermarkets (73.2%, N = 169) or neighborhood stores (14.7%, N = 34), and fewer lived in the areas of agri-food markets (4.8%, N = 11) or fast-food stores (3.0%, N =7).

The behavior related to the physical activity carried out

Students performed an average of **M = 3.02 ± 1.36** hours of physical activity per week. However, 48.1% of them (N = 111) declared that they rarely conducted physical activities, and a third of them (33.3%, N = 77) conducted physical activities often. The most practiced physical activities were walking (57.1%, N = 132), fitness activities in the gym (22.1%, N = 51), jogging (4.8%, N = 11), or others (5.6%, N = 13).

Sleep-related behavior and time spent in front of screens

More than three-quarters of the students (75.8%, N = 175) declared that they slept between 6 and 8 hours a night. Most students (91.8%, N = 212) declared that they

never woke up at night to eat, with very few being those who did this sometimes (7.4%, N = 17). Regarding sleep during the day, more than half of the participants (57.6%, N = 133) declared that they rarely slept during the day.

Additionally, more than half of the students (53.7%, N = 124) estimated that they spent between 2 and 5 hours a day in front of screens, be it a TV, smartphone, tablet, video game, or laptop.

Acquired food patterns / frequency of food and beverages consumed

More than half of the students (63.2%, N = 146) cooked or ate cooked food daily. Regarding the main meals of the day, less than half of the participants (43.3%, N = 100) served breakfast daily, with lunch being the most consumed meal of the day (35.5%, N = 82).

Sometimes, the main meals were replaced with pretzel-type snacks, sweets, and puff pastry by the majority of students (75.3%, N = 174). Moreover, more than a third of the participants (38.1%, N = 88) declared that they rarely ate with family members.

Most students (67.5%, N = 156) ate fast food only 1-2 times a month. More than half of the participants (61.5%, N = 142) had never consumed sugar-free carbonated drinks. More than half of the students consumed fish only 1-2 times a month (55.0%, N = 127), fruits 2-4 times a week (56.3%, N = 130), and vegetables a few times a week (55.4%, N = 128). More than a third of the participants (39.0%, N = 90) had never consumed cereal products. Other detailed results are presented in third table.

Less than half of the students (41.6%, N = 96) often read the food label, with the most sought-after checked components being sugar (24.7%, N = 57), calories (24.2%, N = 56), or additives (13.0%, N = 30).

TABLE III.

Frequency of food consumed[1]

Food	N, %
Meat (chicken, beef, pork)	
daily	45 (19.5%)
5-6 times a week	40 (17.3%)
3-4 times a week	92 (39.8%)
1-2 times a week	46 (19.9%)
never	8 (3.5%)
Eggs	
daily	18 (7.8%)
5-6 times a week	12 (5.2%)
3-4 times a week	40 (17.3%)
1-2 times a week	152 (65.8%)
never	9 (3.9%)
Milk/dairy and cheese	
daily	58 (25.1%)
5-6 times a week	41 (17.7%)
3-4 times a week	56 (24.2%)
1-2 times a week	60 (26.0%)
never	8 (3.5%)
Fried food	
daily	11 (4.8%)
5-6 times a week	22 (9.5%)
3-4 times a week	67 (29.0%)
1-2 times a week	111 (48.1%)
never	20 (8.7%)

[1]Number of answers (N) and percentage (%)

Assessing and screening of orthorexia nervosa

Cronbach's alpha of 0.868 showed that the scale had a high level of internal consistency. The total score for the *Düsseldorf Orthorexia Scale* was on average M = 19.56 ± 6.08, the scores ranging from 10 (4.8%, N = 11) to 39 (0.4%, N = 1).

A small part of the students had a high

score on this scale, which would indicate the presence of orthorexia nervosa. No risk of developing ON was observed in 77.5% of the students in the present study, as shown in table IV.

TABLE IV.
Prevalence of ON among students[1]

DOS	N, %
Presence of ON (≥ 30)	14 (6.1%)
At risk of ON (25–29)	38 (16.5%)
No risk of ON (< 25)	179 (77.5%)

[1]Number of answers (N) and percentage (%)

Comparative and correlational analysis

Comparative analysis showed that students who had employee status (Mdn = 21.00) had a higher score on the Orthorexia Scale, being more fixated on health-conscious eating behavior ($p = 0.007$, $U = 2905.50$, $z = -2.720$) than students who were not employed (Mdn = 18.00). Similarly, the results of the Mann-Whitney test ($U = 1430.00$, $z = -2.055$, $p = 0.040$) showed that underweight students had a lower score on the Orthorexia Scale (Mdn = 17.00) than normal-weight students (Mdn = 18.00). No gender difference was identified.

The Kruskal-Wallis H test revealed that there were statistically significant differences in total Orthorexia Scale score among groups with different levels of physical activity: those who practiced physical activity daily ($n = 34$), those who practiced physical activity often ($n = 77$), those who practiced sports rarely ($n = 111$), or those who never did such activities ($n = 9$); $\chi^2(3) = 15.855$, $p = 0.001$. The Mann-Whitney U post hoc analysis ($p = 0.009$, $U = 65.50$, $z = -2.621$) showed that participants who had never done sports had a much lower score on the Orthorexia Scale (Mdn = 14.00), as opposed to those who did sports daily (Mdn = 22.00). Additionally, the Mann-Whitney analysis ($p = 0.021$, $U = 3428.00$, $z = -2.310$) showed that those who did sports often (Mdn = 18.00) had a higher score on the Orthorexia Scale, in contrast to those who rarely did sports (Mdn =17.00).

Comparative analysis ($p < 0.001$, $U = 1366.50$, $z = -4.230$) showed that students who smoked daily (Mdn = 14.50) had a lower score on the Orthorexia Scale than students who had never smoked (Mdn = 19.00). Similarly, students who never drank alcohol (Mdn = 21.00) had a higher score on the Orthorexia Scale than students who drank alcohol often (Mdn = 15.00) ($p < 0.007$, $U = 50.50$, $z = -2.701$).

Significant differences ($p = 0.011$, $U = 168.50$, $z = -2.534$) resulted between students who were not at all conscious when chewing food, being rather attentive to their laptop or phone (Mdn = 17.00) and those who were extremely conscious when eating, the latter having a higher score on the Orthorexia Scale (Mdn = 22.00).

Our results showed that there was a positive correlation between body mass index and abdominal circumference. Thus**,** we identified that the higher the abdominal circumference value of the students, the higher the BMI ($r = 0.61$, $p < 0.01$).

A strong negative correlation was identified between the consumption of tobacco and the frequency of consumption of carbonated drinks ($r = -0.27$, $p < 0.001$), in the sense that the more the students smoked, the more they tended to consume fewer soft drinks. Tobacco consumption was also negatively correlated with meat consumption, so that the more the students smoked, the more they tended to decrease the frequency of meat consumption ($r = -0.17$, $p = 0.09$).

Positive correlations were identified between the frequency of tobacco consumption and the frequency of alcohol consumption ($r = 0.36$, $p < 0.001$), respectively of

the consumption of psychoactive substances ($r = 0.28$, $p < 0.001$), in the sense that the more the students smoked, the more alcohol and psychoactive substances they consumed.

A negative correlation was identified between the level of self-consciousness that students had when chewing food and using tobacco. Thus, the more the students were aware of the way they ate, the less they tended to smoke ($r = -0.20$, $p = 0.002$). Significant results regarding various items and the score obtained on the Orthorexia Scale are presented in table V.

TABLE V.

Correlation analysis results

Variables	DOS
BMI	r = 0.187**, p = 0.004
Tobacco consumption	r = -0.254**, p = 0.000
Alcohol consumption	r = -0.270**, p = 0.000
How conscious are you when you chew your food?	r = -0.158*, p = 0.016
Performing physical activity	r = 0.255**, p = 0.000
Frequency of consumption of eggs	r = -0.189**, p = 0.004
Frequency of consumption of meat	r = 0.144**, p = 0.029
Frequency of consumption of fish	r = -0.138*, p =0.036
Frequency of consumption of cereal products	r = -0.194**, p = 0.003

* $p < 0.05$; ** $p < 0.001$.

DISCUSSION

Maintaining a healthy lifestyle throughout adulthood is crucial for ensuring a healthy life in old age. Academic years are very challenging because, due to the chaotic schedule, the increased fast-foot intake and less time to practice sports could lead to a lower quality of physical and mental life.

The present study identified that medical students were at some risk: underweight and overweight was registered in 30% of students, and the presence of orthorexia nervosa or the risk of developing it was identified in 22.5% of respondents. Orthorexia nervosa was identified in students, and results from the scientific literature revealed that it was linked to the cognitive domains affecting attention, verbal long-term memory, visuospatial functioning, and executive functioning (9, 10). For example, orthorexia nervosa was measured among undergraduate medical students, and the prevalence was between 21.1% and 69.93% (11). Other research conducted on students revealed that those studying health-related majors were characterized by more pronounced symptoms of orthorexia nervosa with evidently more pronounced symptoms among physicians, dieticians and athlete students (12).

We found that students with higher scores on orthorexia nervosa ate eggs and meat more frequently, avoided tobacco and alcohol, and had a higher level of physical activity compared to students with no orthorexia nervosa. The research identified no significant results between male and female students, with similar data being registered by other international studies (13).

The frequency at which physical activity is performed is an important indicator that has been identified in other studies. During the undergraduate period, the phys-

ical activity schedule is rather individual; there are no PE classes or other extracurricular activities organized systematically, and free time is rather spent watching TV or engaging in rather sedentary activities such as video games, or computer use (14, 15).

Moreover, during this period, parental involvements in healthy food choices and physical activity decreases, as only a small proportion of students still live with their parents. In addition, academic schedules are busy, further limiting contact between parents and students.

Students from lower socioeconomic backgrounds may face decreased access to nutritious food (16) as well as to the opportunity to attend gyms, which increases the risk of developing obesity. Economic constraints can lead to the consumption of cheap but energy-dense foods with low nutritional value.

Environmental factors such as the availability of unhealthy food options on or around campus and food marketing to students, coupled with the lack of safe and financially accessible spaces for physical activity, can influence students' weight (17, 18).

Last but not least, genetic predispositions influence an individual's susceptibility to weight gain and obesity. Hormonal imbalances, some medical conditions, and some pharmaceuticals can also contribute to weight-related problems among students (19).

It is important to note that these factors are bidirectional, demonstrating once again the complexity of the causes of obesity among students. Therefore, obesity needs to be addressed in a multidisciplinary manner and involve individuals, their families and friends, the university environment, the community, and policymakers to promote healthy eating, physical activity, and a supportive environment for students.

In terms of obesity among medical students, there are a few factors that differ from the general student population: the high level of stress and workload faced by medical students due to the demanding nature of medical studies, the long internship and lecture hours, and the intense workload. Poor stress management can lead to emotional eating, unhealthy food choices, and a lack of time for physical activity, potentially contributing to weight gain and obesity.

Medical students' irregular schedules, prompted by rigorous schedules and demanding medical school clinical rotations, can disrupt mealtimes and sleep patterns, and this irregularity can make it difficult to maintain a healthy eating routine and engage in regular physical activity, thereby increasing the risk of weight gain, especially through a preference for fast food options with low nutritional value.

Although medical students are knowledgeable about health, including the risks associated with obesity, the pressures and demands of medical school can lead to neglecting personal health and prioritizing academic activities over their own health.

Medical schools are characterized by a competitive environment and peer pressure, and consequently, long study hours and neglecting personal wellness to achieve academic success can contribute to stress eating, sedentary behaviors, and weight gain.

The lack of clinical exposure, which is prevalent in the early years of medical school, further contributes to the development of sedentary behavior as students spend more time in classes than in the clin-

ical environment.

Obesity has a significant impact on a person's quality of life at different stages of life. At the psychological level, negative body image, depression, and social stigma are commonly experienced by people with obesity, negatively affecting their social relationships, couple relationships, and quality of life (20, 21). At the same time, as their social life is also affected, they enter a vicious circle in which the person with obesity finds it increasingly difficult to form or maintain social relationships and adopts a social isolation that will further lower their self-esteem and deepen their depression. Economically, a person with obesity may face reduced productivity at work and increased medical expenses.

CONCLUSIONS

Addressing obesity among medical students requires specific attention and support from medical schools and institutions. Implementing programmes that promote healthy lifestyle behaviors, providing access to affordable nutritious food on campus, creating opportunities for physical activity, and offering free stress management support services could help medical students adopt healthy lifestyle habits despite the academic challenges they face.

CONFLICTS OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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COMPARATIVE MORPHOLOGIC AND CHEMICAL PRELIMINARY ASSESSMENT OF THREE ROMANIAN *AGASTACHE* SPECIES

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COMPARATIVE MORPHOLOGIC AND CHEMICAL PRELIMINARY ASSESSMENT OF THREE ROMANIAN *AGASTACHE* SPECIES (Abstract): Phenolic compounds, such as flavonoids and phenolic acids, are classified as secondary metabolites in plants. The increasing interest in utilizing these components in traditional medicine for the prevention or treatment of diverse pathologies based on their antioxidant properties. **The objectives** of this study were to identify the microscopic characteristics and to quantify the levels of total phenolics and flavonoids of three different *Agastache* species: *A. foeniculum*, *A. mexicana*, and *A. urticifolia*. **Material and methods:** Microscopy followed 10th European Pharmacopoeia (Ph. Eur.) provision regarding the evaluation of plant products. The quantification of phenolic content was conducted using the Folin-Ciocalteu method, while the determination of total flavonoids was conducted using the aluminum chloride and sodium nitrate method. **Results**: Microscopic features showed similar structures for leaves and flowers. Differences were given by the density of stomata and glandular or surface trichomes. The total phenolic content of the extracts varied from 213 to 642 mg % and the maximum was determined for *A. foeniculum*. Also, the measured total flavonoid content was found to be between 29.4 mg % and 58 mg %, the same sample having the highest amount. **Conclusions:** The methanolic extracts of this plant exhibits a notable concentration of phenolic and flavonoid compounds, suggesting its potential as a therapeutic agent for various diseases. However, further investigation is required to fully understand its efficacy and safety profile. **Keywords**: MICROSCOPY, TOTAL PHENOLS, TOTAL FLAVONOID CONTENT.

The taxonomic classification of the plant genus *Agastache* situates it within the botanical family *Lamiaceae*, encompassing a collective assemblage of 22 distinct species of enduring aromatic herbs that possess notable medicinal characteristics. The *Agastache* genus is known for its notable prevalence of phenylpropanoid and terpenoid specialized metabolites, a trait frequently observed within the *Lamiaceae* botanical family. One classification of chemical compounds encompasses flavonoids, free phenolic acids and depsides, alongside lignans, while the other significant classification is based on terpenoids, which can be discerned as both volatile and nonvolatile fractions (1, 2).

Agastache foeniculum, known by vari-

ous common names such as anise hyssop, blue giant hyssop, fragrant giant hyssop, or the lavender giant hyssop, is indigenous to the north-central and northern regions of North America. It possesses a chemical composition that includes volatile oils, phenolic compounds (specifically phenolic acids and flavonoids), as well as derived flavonoids, alcohols, cinnamic aldehydes, terpenoids, and other similar substances, with concentrations ranging from 0.9 g % to 1.8 g % (3, 4, 5). Various components of the plant are utilized for the management of cardiovascular, nervous system, and gastrointestinal ailments, in addition to their application in the treatment of common colds, fevers, and bodily discomfort. Additionally, the plant exhibits antivomitive, antibacterial, and antifungal properties (6, 7).

Agastache mexicana, a vascular plant indigenous to Mexico, exhibits the characteristic morphology of the *Lamiaceae* family, including opposite, petiolate leaves, a four-angled stem, and abundant trichomes. This plant has been traditionally recognized in Asia for its culinary and medicinal properties. Research findings have demonstrated that certain components of the plant exhibit properties associated with antioxidant, antifungal, anti-inflammatory, antiobesity, and anti-photoaging effects (8, 9).

Agastache urticifolia is a perennial plant that is strongly aromatic, and it is known by wo varieties of the species var. *urticifolia* and var. *glaucifolia*, however, only native populations of the former exist in Utah mountain ranges (10, 11). Though native to the Intermountain West (USA), this species has been cultivated throughout the world and even used for ethnobotanical practices in Bangladesh (12, 13).

The majority of the published studies primarily concentrate on the analysis of essential oils. Additionally, a considerable number of scholarly articles have been published documenting the process of isolating and comprehending the molecular structure of diverse phytochemical compounds (14, 15, 16, 17).

The aim of this study was to conduct a comparative analysis of three *Agastache* species, namely *A. foeniculum*, *A. mexicana*, and *A. urticifolia*, following microscopic features of the leaves and flowers and the total polyphenolic and flavonoid compounds obtained from methanolic extracts.

MATERIAL AND METHODS

Plant material and reagents: Agastache foeniculum (AG1) comes from Cluj-Napoca 46°46′42.24″ N, 23°37′01.92″ E, *Agastache foeniculum* (AG2) and *Agastache mexicana* (AGM) were harvested from Brașov (Dacia Plant), 45°39′ N 25°36′E, while *Agastache urticifolia* (AGU) was harvested from the Republic of Moldova, Chisinau, 47°01′0″N 28°52′0″E. All chemicals and reagents used for extraction and characterization were of analytical grade, provided by Sigma Aldrich Chemical Co. (Germany) and were used without further purification.

For each of the three species, the air-dried plants (5 g) were pulverized into a fine powder using a mill. The resulting powder was then subjected to extraction using 50 mL of 50% methanol (V/V) at 70°C for 1 hour under reflux. Following the cooling process, the extracts underwent filtration. The isolated products were subsequently rinsed with a solution consisting of 10 mL of 50% (V/V) methanol. The resulting filtrates were combined in a volumetric flask and further diluted to a final volume of 100 mL using the same solvent. A portion of 80 mL was taken from each extract and was concentrated using a rotary

evaporator (Buchi R 210, Switzerland) in order to eliminate the solvent. The residues were subjected to a drying process and subsequently stored at a temperature of 4°C in order to facilitate further analysis.

Microscopic investigation: The powdered plant material was clarified with chloralhydrate (80% in water) directly on the microscopic plate. All steps were according to the indications of Ph. Eur. 10 (18). The visualization and photography of the observed elements were realized with an Optika (WF10X-11MM) microscope with built-in camera (Canon PS A450).

Determination of total phenolic content: The determination of the total phenolic content of each extract of *Agastache* was conducted using the Folin-Ciocalteu reagent, following the method proposed by Singleton and Rossi (19), with gallic acid serving as the standard. A standard curve was generated by determining the concentrations of gallic acid within the range of 3-6 µg/mL. The outcomes were quantified in terms of gallic acid equivalents (GAE), specifically measured as milligrams of gallic acid per 100 g dry plant material (20, 21).

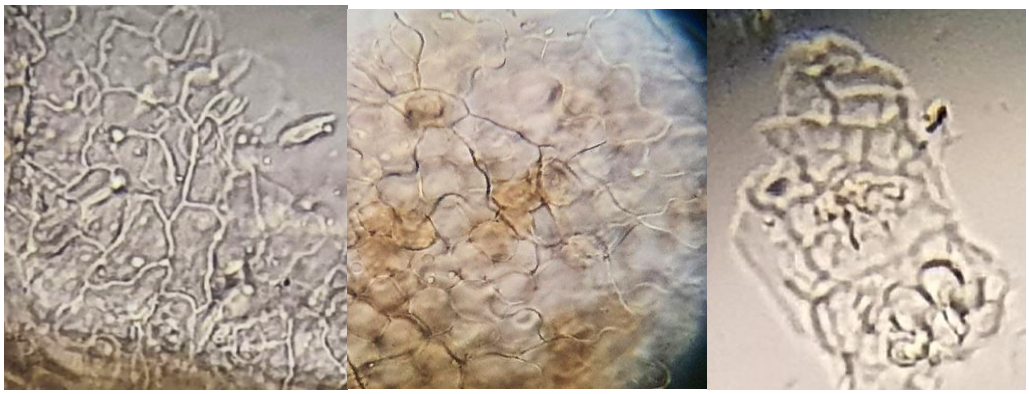
Determination of flavonoid content: The quantification of the overall flavonoid content was conducted using a colorimetric assay as previously (20, 21). The quantification of the total flavonoid content in the extract was denoted as rutoside (mg rutoside (RUE)/100 g dry plant product).

For all determinations, samples were analyzed in triplicate.

RESULTS AND DISCUSSION

Morphologic characterization

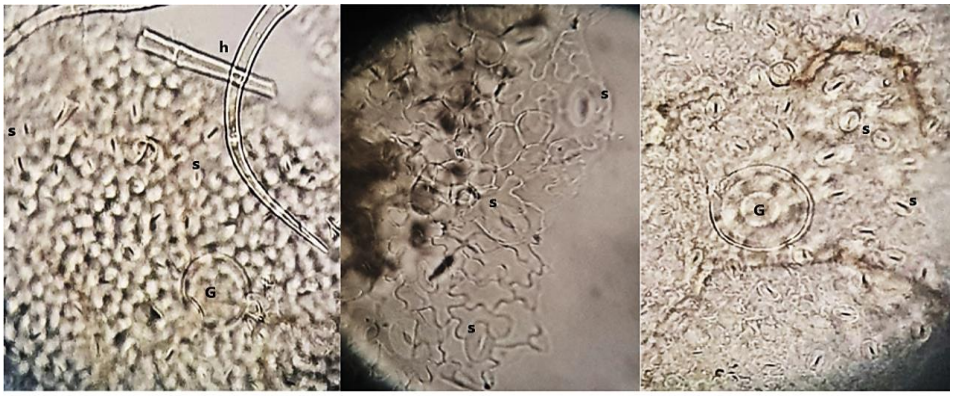
Our observations indicated the presence of common tissue (upper and lower epidermis, stomata, vascular bundles) and similar structures (octocellular glandular trichomes and surface hairs) with taxonomic relevance in all investigated samples, as indicated in fig 1-3. Such microscopic characteristics prove that the investigated species belong to Lamiaceae family (22).



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

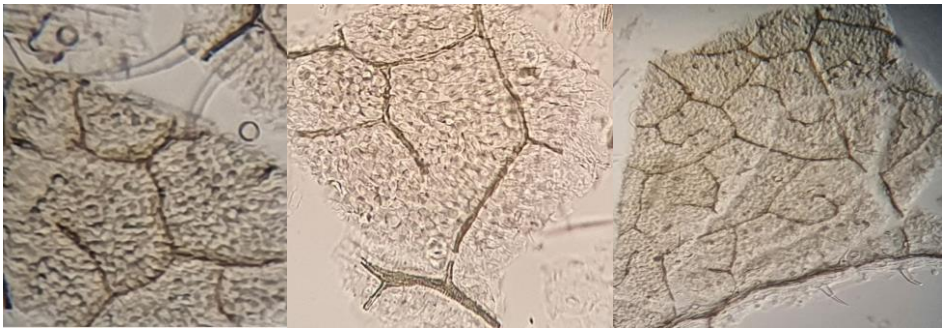
Fig. 1. Front view of upper epidermis (adaxial surface) showing elongated cells with thicker and almost straight walls in the investigated *Agastache* samples

Numerous small stomata were present in *A. foeniculum* and *A. urticifolia* abaxial surface, whereas bigger and fewer stomata were noted in the *A. mexicana* sample (fig. 2).



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

Fig. 2. Front view of lower epidermis showing cells with thin and wavy walls: s - stomata, G - octocellular glandular trichomes, h - pluricellular surface trichomes



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

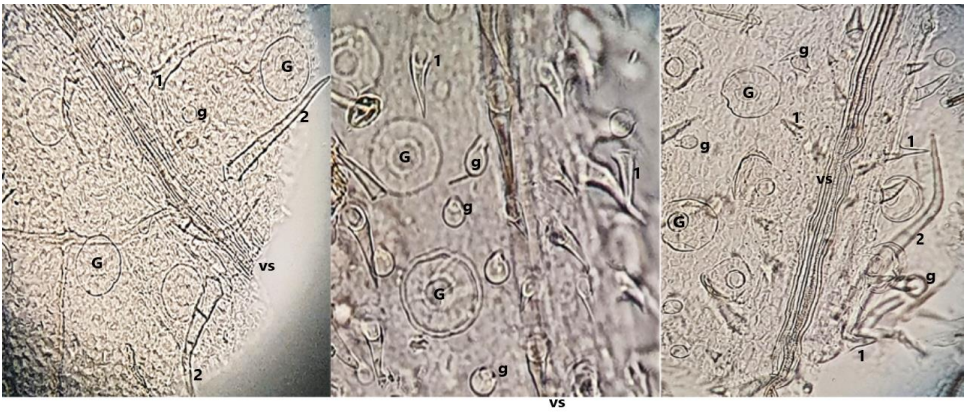
Fig. 3. Front view of leaf mesophyll with vascular bundles (vascular network is represented by the dark brown veins

For all three species, the microscopic examination highlighted the presence of numerous short, single cell surface hairs, monocellular head and sessile octocellular glands denser on the flower epidermis and on the abaxial surface of the leaves (fig. 4). Moreover, long pluricellular (3-5 cells) surface hairs were noted in *A. foeniculum* and *A. urticifolia*, but not as frequent as the short trichomes.

Although the information regarding leaf and flower morphology of *Agastache* species is scarce, similar to our results but using scanning electron microscopy previous researchers have revealed that the upper surface of the leaf comprises both simple non-glandular and glandular (capitate and subsessile) trichomes, which possess the capability to yield essential oil through extraction processes. The essential oil samples of *A. urticifolia* from cultivated (Russia) and native (Oregon, USA) populations, which have been previously examined, have demonstrated a predominant composi-

tion of volatile compounds, namely limonene, menthone, and pulegone. It is worth noting that these compounds exist as enantiomers in the natural environment (23, 24).

In our samples, pollen grains were visible in two of the investigated samples (fig. 5).



Agastache foeniculum *Agastache mexicana* *Agastache urticifolia*

Fig. 4. Front view of flower epidermis: G - octocellular glandular trichomes, g - monocellular head glands, 1 - monocellular surface trichomes, 2 - pluricellular surface trichomes, *vs*. – vascular bundles (viewed by transparence)

Agastache foeniculum *Agastache mexicana*

Fig. 5. Pollen grains and pollen sacs indicate the presence of flowers in the powdered plant material

Secondary metabolites quantification

Total phenols content varied between 213 ± 3.21 mg% to 642 ± 3.12 mg%, *Agastache foeniculum* samples being the richest in such metabolites. Table I represents the analytical data for phenolics and total flavonoid content of the methanolic extract of the three *Agastache* species.

Plant extracts' total phenolic content has been linked to antioxidant activity due to their redox properties, which allow them to function as reducing agents, hydrogen donors, and singlet oxygen quenchers. For our samples, the most promising samples seem both *A. foeniculum* species, the best being AG2, the sample harvested from Brasov. The lowest content was detected for AGM, a species which originates from

Mexico and most probably the environmental conditions do not offer proper conditions for a better biosynthetic capacity of the plants grown in our country. Similar results were obtained by Balanescu *et al.* for ultrasonicated ethanolic and methanolic extracts obtained from *A. foeniculum* cultivated in Romania (25). A better extractability was noted for the methanolic extracts as compared to ethanolic extracts.

TABLE I.
Total phenolic content for the three species of *Agastache*

Sample name	Total polyphenols (mg GAE %)	Total flavonoids content (mg RUE %)
AG1	455 ± 2.02	53 ± 1.78
AG2	642 ± 3.12	58 ± 2.43
AGM	213 ± 3.21	29.4 ± 1.08
AGU	396 ± 2.01	49.9 ± 1.11

In regard to the flavonoid content, the same sample, AG2, had the richest content of flavones, whereas *A. mexicana* registered the lowest amount. It was notable that the same variability was registered for flavonoids and total phenolic content. Taking into account that the extracted amounts were high, we can state that 50 % methanol was a good solvent for these classes of metabolites.

Currently, there are few studies pertaining to the phytochemical compositions and components found in *Agastache* species. Furthermore, a comprehensive comparative study is necessary in order to thoroughly understand the distinctions between these species. Furthermore, the characterization of primary and secondary metabolic compounds in *Agastache* species remains incomplete in terms of comparative study, with particular emphasis on the influence of flowering stage and cultivation time. Additionally, there is a lack of quantitative research that has investigated the alterations in composition throughout various stages of flowering, as well as the significance of each stage within the flowering process (7, 9, 14, 17).

CONCLUSIONS

The utilization of herbal products for the treatment of diseases has garnered significant attention in recent years. This is primarily attributed to the wide range of phytometabolic components found in these products, which possess diverse chemical structures and exhibit various biological activities. The utilization of *Agastache* species in traditional medicine for the treatment of diverse ailments has been prevalent for an extended period of time.

Plants exhibit a substantial presence of polyphenols and flavonoids which was demonstrated in this paper for *Agastache foeniculum*, *Agastache mexicana* and *Agastache urticifolia*, which contribute to their robust antioxidant capabilities. Consequently, these properties enable plants to engage in diverse defensive mechanisms and combat diseases effectively. Phenolic compounds are regarded as significant constituents of plants due to the presence of one or more hydroxyl groups on their aromatic ring, making them secondary metab-

olites. Due to the absence of adverse effects on human health, there has been a rise in the utilization of plant species with elevated levels of phenolic compounds within the food industry, with the objective of enhancing the overall quality of food products.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

Mihaela-Magdalena Flutur and Denisa Marin-Batir, had equal contribution and should be regarded as main authors of the manuscript.

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TRANSLATION, RELIABILITY AND VALIDITY OF THE ROUMANIAN VERSION OF ORTHOGNATHIC QUALITY OF LIFE QUESTIONNAIRE (OQLQ)

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TRANSLATION, RELIABILITY AND VALIDITY OF THE ROUMANIAN VERSION OF ORTHOGNATHIC QUALITY OF LIFE QUESTIONNAIRE (OQLQ) (Abstract): Orthognathic surgery is an essential treatment for addressing dentofacial anomalies which significantly affect the psychological and social aspects of a patient's life. This study aimed to translate, adapt, and validate the OQLQ questionnaire into Romanian language, specifically designed to measure the quality of life of individuals with dentofacial anomalies. **Materials and methods**: The validation process of the OQLQ questionnaire in Romanian language involved adaptation, translation, and validation methodologies. The Romanian version was administered to 18 patients undergoing orthognathic surgery. **Results:** The adapted and validated version demonstrated satisfactory psychometric properties in term of reliability, sensitivity, and validity. The average Cronbach's alpha coefficient was 0.92, with a slightly lower value (0.79, p=0.046) observed for the Oral function domain. The Social aspects domain exhibited the highest value, recording an average Cronbach's alpha coefficient of 0.96. **Conclusions:** Dentofacial anomalies detrimentally impact the quality of life of orthognathic patients. The Romanian version of the OQLQ has proven its validity in assessing the quality of life among Romanian orthognathic patients. **Keywords:** DENTO-FACIAL ANOMALIES, ORTHO-GNATHIC TREATMENT, OQLQ.

The lives of individuals dealing with dentofacial abnormalities are significantly affected, and orthognathic surgery boasts a high success rate as a viable treatment. However, this positive outcome relies on various subjective factors such as patient expectations, anxiety levels associated with the condition, and the secondary psychological stress it causes, in addition to the defined goals of the medical team (1, 2).

People with dentofacial anomalies may experience both physical and mental challenges based on their condition and how they perceive their appearance, as well as how others perceive them. The primary reasons cited for seeking treatment by these patients often revolve around aesthetic and social concerns, which can profoundly impact their mental well-being (3).

Individuals experiencing dentofacial disfigurements commonly express heightened levels of stress, reduced self-esteem,

and increased challenges in navigating social interactions. Some evidence indicates that the severity of skeletal misalignment correlates with the prevalence of mental health issues (4). Tangible concerns stand as the second most frequently cited reasons prompting this population to seek treatment. These concerns encompass reduced occlusal (bite) function, speech difficulties, airway obstruction problems, and temporo-mandibular joint-related pain (5).

The physical and mental challenges faced by individuals with dentofacial deformities can significantly impact their overall quality of life. "Quality of life" is a broad term that encompasses how individuals perceive their circumstances concerning health, finances, and other aspects deemed significant in their lifestyle (6). Within the healthcare sector, there has been a growing emphasis on Health-Related Quality of Life (HRQOL), which evaluates the influence of an illness or its treatment on a patient's ability to perform everyday tasks and lead a normal life (7).

The Orthognathic Quality of Life Questionnaire (OQLQ), devised by Cunningham et al., stands as a notable example of a tool designed to measure Oral Health-Related Quality of Life (OHRQoL) specifically for assessing the impact of dentofacial deformities on individuals' lives. This questionnaire serves as a "condition-specific" instrument tailored to evaluate the quality-of-life outcomes in patients with these specific deformations (8). The Orthognathic Quality of Life Questionnaire (OQLQ) has been employed to assess the quality of life of patients with dentofacial deformities and to measure the impact of surgical interventions. This instrument has demonstrated greater sensitivity compared to conventional surveys. Furthermore, it has been translated into and validated in various languages (9). The aim of this study was to translate and validate the Orthognathic Quality of Life Questionnaire (OQLQ) for the population in Romania requiring orthognathic treatment.

MATERIALS AND METHODS

The Orthognathic Quality of Life Questionnaire (OQLQ) comprises 22 items, graded on a scale from 0, representing "no bother at all," to 4, signifying "a lot of bother." The OQLQ evaluates how an individual's dentofacial anomaly influences their quality of life across four domains: facial aesthetics (items 1, 7, 10, 11, and 14; scoring range 0 to 20), oral function (items 2 to 6; scoring range 0 to 20), awareness of dentofacial aesthetics (items 8, 9, 12, and 13; scoring range 0 to 16), and the social impact of dentofacial deformity (items 15 to 22; scoring range 0 to 32). Higher scores on the OQLQ suggest a lower quality of life. This questionnaire has been employed to assess the quality of life in individuals with dentofacial anomalies and the effects of surgical interventions (8).

Questionnaire translation

The validation process followed specific methodological steps. We have obtained the author's consent for the use of the questionnaire and its validation. The English OQLQ was translated into Romanian by two independent bilingual translators, both being native Romanian speakers. This resulted in two separate Romanian translations. Any disparities or differences in word choices between the two translations were deliberated upon by a consensus committee. This committee consisted of the translators and the principal investigator. Through discussion, discrepancies were addressed, and a common synthesized Romanian translation was created. The synthesized Romanian translation was then back translated into English by a native English speaker who was unaware of the original version. This step aimed to ensure the accuracy and con-

sistency of the translated questionnaire. All versions - original, translated, and back-translated - were consolidated and scrutinized by an expert panel comprising two orthognathic specialists and a methodologist. This panel offered feedback and modifications were made to the translations based on their suggestions and insights. The feedback from the expert panel was incorporated to refine and establish the final Romanian version of the OQLQ.

Patients' selection

Approval was secured from the institutional review board of the University of Medicine and Pharmacy Grigore T. Popa Iasi (no. 202/12.06.2022) prior starting the study. The study participants were selected from the Department of Orthodontics, University of Medicine and Pharmacy Grigore T. Popa Iasi. Inclusion criteria of participants were as follows: voluntary participation, aged 18 years or above, individuals with dentofacial anomalies who need orthognathic treatment, absence of diagnosed cognitive impairment, native Romanian speakers. Exclusion criteria were as follows: individuals with diagnosed with micrognathia, hemifacial microsomia deformities due to trauma, tumor and cleft lip and / or palate.

Informed consent was acquired from all participating individuals before their involvement in the study.

The information was processed in a specially designed database. The statistical study, depuration and recoding of the database was all undertaken with IBM SPSS Statistics 24.0. The statistical process of validation was done by study of the viability, reliability, sensitivity, and validity. The reliability was evaluated by analysis of the internal consistency using Cronbach's alpha. The sensitivity to change was evaluated by use of the effect size index and bivariate tests of statistical significance (Wilcoxon). The construct validity was evaluated by doing a factorial analysis to measure the concordance of the dimensions with the original model.

Statistical analysis

To assess internal consistency, Cronbach's alpha coefficient was calculated for each direction of the questionnaire. A Cronbach's alpha value exceeding 0.7 is considered suitable for comparing different patient groups using the questionnaire (12,13). For construct validity, a principal components analysis was conducted, and its validity was confirmed through Bartlett's test of sphericity and the Kaiser-Meyer-Olkin (KMO) measure (considered acceptable with values above 0.5). Repeatability was measured using the intra-class correlation coefficient (ICC). A strong correlation was determined when the ICC value exceeded 0.71, while a moderate correlation fell between 0.51 and 0.70. Statistical significance was defined as p-values less than 0.05 (two-sided).

RESULTS

The sample consisted of 18 participants, 11 of them being female. 83.3% of the participants came from urban areas, and 60.1% of them had completed university or postgraduate studies (tab. I).

The results of the statistical analysis for internal consistency, intra-class correlation, and Cronbach's alpha coefficient for each item are depicted in Table 1. The findings reveal that, for the domain of *aesthetic facial aspects*, the average Cronbach's alpha coefficient was 0.92, with item values ranging between 0.89 and 0.95 (p=0.010). Conversely, a lower average value (0.79, p=0.046) was observed for the Oral function domain. The highest value was recorded in the *social aspects'* domain, with an average Cronbach's alpha coefficient of 0.96; likewise, the intra-class correlation coefficient displayed the same value (p=0.000) (tab. II).

TABLE I.

The demographic characteristics of the study sample

	No.	%
Age	28.33±7.74 years	
Gender		
Female	11	61.1
Male	7	38.9
Residence		
Urban	15	83.3
Rural	3	16.7
Level of studies		
Secondary schools (8 classes)	1	5.6
High schools (12 classes)	3	16.7
post-secondary	1	5.6
Postgraduate (PhD)	2	11.1
University (Bachelor)	6	33.3
University (Master's)	5	27.8

TABLE II.

General Description, Internal Consistency, intra-class correlation coefficient of the OQLO

Domain / item	Cronbach`s alpha / item	Mean value	SD	Cronbach`s alpha/ domain	Intra-class correlation coefficient	p
Aesthetic facial aspects						
1. I am self-conscious about the appearance of my teeth	.95	2.22	1.43	.92	.93	0.010
7. I don't like seeing a side view of my face	.89	1.83	1.61			
10. I dislike having my photo-graph taken	.90	2.17	1.65			
11. I dislike being seen on video	.89	1.94	1.62			
14. I am self-conscious about my facial appearance	.90	2.00	1.49			
Oral function						
2. I have problems biting	.70	2.17	1.38	.79	.79	0.046
3. I have problems chewing	.71	2.11	1.23			
4. There are some foods I avoid eating because the way my teeth meet makes it difficult	.80	1.78	1.30			
5. I don't like eating in public places	.78	1.83	1.42			
6. I get pains in my face or jaw	.76	1.44	1.14			

Domain / item	Cronbach`s alpha / item	Mean value	SD	Cronbach`s alpha/ domain	Intra-class correlation coefficient	p
Concern about deformity						
8. I spend a lot of time studying my face in the mirror	.88	1.89	1.60	.88	.88	0.027
9. I spend a lot of time studying my teeth in the mirror	.85	2.17	1.50			
12. I often stare at other people's teeth	.86	2.39	1.65			
13. I often stare at other people's faces	.81	2.44	1.65			
Social aspects						
15. I try to cover my mouth when I meet people for the first time	.96	1.22	.80	.96	.96	0.000
16. I worry about meeting people for the first time	.95	1.33	1.18			
17. I worry that people will make hurtful comments about my appearance	.95	1.33	1.28			
18. I lack confidence when I am out socially	.95	1.61	1.29			
19. I don't like smiling when I meet people	.96	1.78	1.26			
20. I sometimes get depressed about my appearance	.95	1.67	1.32			
21. I sometimes think that people are staring at me	.95	1.78	1.21			
22. Comments about my appearance really upset me, even when I know people are only joking	.95	1.56	1.42			

The intra-class correlation coefficients (ICCs) for all subcategories, with a 95% confidence interval (CI), were as follows: 0.93 for *Aesthetic facial aspects*, 0.79 for *Oral function*, 0.98 for *Concern about deformity*, and 0.96 for *Social aspects*. The lowest recorded value was observed in the Oral function category (tab. II).

The Bartlett's sphericity tests yielded significance ($p = 0.016$), and the Kaiser-Meyer-Olkin measure was calculated at 0.921. These outcomes facilitated the execution of a factor analysis based on the correlation matrix. Six factors were identified, collectively explaining 75% of the variance. Given the interrelation among these factors, an orthogonal rotation method was utilized. Concerning *Aesthetic facial aspects* (5 items), the recorded values ranged from 0.77 to 0.88. In the instance of *Oral function* (5 items), values were observed between 0.70 and 0.88. For *Concern about deformity* (4 items), values ranged from 0.75 to 0.84. Regarding *Social aspects* (8 items), values fell within the range of 0.79 to 0.88 (tab. III).

TABLE III.

Bartlett's sphericity test and Kaiser-Meyer-Olkin measure

Domain / item	Mean value
Aesthetic facial aspects	
1. I am self-conscious about the appearance of my teeth	.82
7. I don't like seeing a side view of my face	.79
10. I dislike having my photograph taken	.88
11. I dislike being seen on video	.77
14. I am self-conscious about my facial appearance	.80
Oral function	
2. I have problems biting	.70
3. I have problems chewing	.81
4. There are some foods I avoid eating because the way my teeth meet makes it difficult	.88
5. I don't like eating in public places	.81
6. I get pains in my face or jaw	.74
Concern about deformity	
8. I spend a lot of time studying my face in the mirror	.79
9. I spend a lot of time studying my teeth in the mirror	.81
12. I often stare at other people's teeth	.84
13. I often stare at other people's faces	.75
Social aspects	
15. I try to cover my mouth when I meet people for the first time	.79
16. I worry about meeting people for the first time	.85
17. I worry that people will make hurtful comments about my appearance	.82
18. I lack confidence when I am out socially	.86
19. I don't like smiling when I meet people	.87
20. I sometimes get depressed about my appearance	.88
21. I sometimes think that people are staring at me	.82
22. Comments about my appearance really upset me, even when I know people are only joking	.83

DISCUSSION

One of the medical concerns significantly impacting the quality of life in the dental field relates to dentofacial issues. The primary reasons individuals seek treatment for their dentofacial abnormalities stem from developmental and chronic conditions leading to social hindrances, such as unconventional facial appearances. Although functional issues also contribute to the necessity for treatment, they are often overshadowed by the social repercussions stemming from the patient's facial appearance. Consequently, this is closely intertwined with the issue of Quality of Life (QoL) (14-16).

To generalize the results to different populations and to ensure that a study be-

comes a valuable international tool, it is crucial to use validated and properly adjusted questionnaires. Prior to implement a survey in a different population and social context than the one in which it was originally developed, it needs to undergo a process of transcultural adaptation. Given that a simple direct translation might overlook certain cultural subtleties, the objective is to construct an instrument that closely reflects the original (9, 12, 13).

The most widely used method for semantic and cultural adaptation involves a process known as back-translation, carried out by bilingual experts. This is followed by a thorough analysis of the resulting version, which is then refined to assess its psychometric properties such as reliability, internal consistency, and sensitivity. Reliability refers to an instrument's capability to generate consistent results each time it is used. Internal consistency, which assesses the similarity of responses related to the same concept, is used to assess this reliability. In our study, using Cronbach's alpha, we obtained values exceeding 0.7 for all domains, indicating that the new version demonstrates adequate internal consistency. Sensitivity depends on the nature of an instrument to recognize genuine cases.

The OQLQ has been demonstrated as a straightforward and user-friendly language questionnaire that can be easily understood by individuals. This validation further confirms the reproducibility, reliability, validity, and comparability of the OQLQ when compared to its original form (17-20).

The reliability of the OQLQ was excellent, with a high Cronbach's alpha value ranging between 0.89 and 0.95. Specifically, the Cronbach's alpha value for the overall score exceeded 0.9, while the intraclass correlation coefficient was higher than 0.8 for three out of four domains, with Oral function being slightly lower than 0.8. Most validated versions of the OQLQ, including the original scale (20), show Cronbach's alpha values similar to the results observed in the Romanian adaptation of the OQLQ. This outcome suggests that the study demonstrated reliability for repeated assessments and maintained high consistency over time.

CONCLUSIONS

Romanian version of OQLQ demonstrated validity, reliability and sensitivity in evaluating the quality of life of patients who require orthognathic treatment, the results being consistent and comparable with those of the original version.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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PREDICTORS OF ADMISSION/NON-ADMISSION INTO THE HOSPITAL, HEALTH EFFECTS, AND METEOROLOGICAL FACTORS RELATED TO A VIOLENT STORM IN TIMISOARA, ROMANIA

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PREDICTORS OF ADMISSION/NON-ADMISSION INTO THE HOSPITAL, HEALTH EFFECTS, AND METEOROLOGICAL FACTORS RELATED TO A VIOLENT STORM IN TIMISOARA, ROMANIA (Abstract): The aim of the performed study is to investigate predictors of admission/non-admission into the hospital, health effects and meteorological factors related to a violent storm that happened on the 17th of September 2017, in Timisoara, Romania. **Materials and methods:** Case-study with a 6-day retrospective investigation (15th-20th September) of admissions/non-admissions, all health effects and storm-trauma health effects, and meteorological factors on a sample of 1,156 patients (54.93% males, 45.07% females) who came to the ERU, sample that included a "storm-trauma" sample of 71 patients (60.60% males, 39.40% females). **Results:** Three subgroups of the entire sample were considered: before storm-no effects (15th-16th), during and after the storm-immediate effects (17th-18th and after the storm-delayed effects (19th-20th of September). Traumatic health effects (16.7% of trauma, 13.3%-due to the storm) and meteorological factors values (air temperature-31.6^{0}C, air pressure-732 mmHg, gust wind speed-106.92 Km/h-violent storm) were recorded on the 17th of September. **Discussion:** Main predictors for admission into the hospital were covariate: mean air temperature (OR=1.924, P<0.001), relative humidity and age increase, and categorical: cardiac diseases (OR=0.436, P<0.001), fractures (OR=0.375, P<0.001), and urban/rural environment. A primary categorical predictor for non-admission into the hospital was contusions. A subgroup of population exposed to the storm (17th-18th of September) recorded significant differences in term of being admitted into the hospital in comparison with the other two subgroups. **Conclusions:** there is a relation between admissions/non-admissions into the hospital and personal characteristics (age, rural/urban), health effects (cardiac diseases, fractures, contusions), and meteorological factors (air temperature, humidity, pressure) in the context of the violent-storm. **Keywords:** ADMISSION, HOSPITAL, HEALTH EFFECTS, METEOROLOGICAL FACTORS, STORM.

INTRODUCTION

On the 17th of September 2017 at 3.40 p.m. a violent storm happened in the Western region of Romania. Under the conditions of a particularly strong thermal contrast (the quick decrease of the air temperature from 31-32°C to 16°C) the base of the formed clouds was very close to the ground (500-600 meters), while their peak reached 10,000-13,000 meters and caused the pro-

duction of these particularly violent phenomena. An orange code was issued at 3.38 p.m. (1), but since it was a Sunday, people were outdoors and relaxing. Severe health effects and environmental damages followed the storm: 8 people died and 67 were wounded (2). It was a mid-latitude storm, with around 100 Km^2 surface area (Western region of the country- large disaster) and small number of deaths (8- small disaster) (3, 4). Several trees and several buildings were damaged. People were also injured as a result of trying to repair the damage done to buildings and trees. The Emergency Receiving Unit (ERU) of the County Emergency Clinical Hospital performed the reception, consultation and referral of patients to hospitals' departments. Current research that has been carried out on the impact of climate change at global and regional level reviewed vulnerabilities of the natural world and human societies to adapt to climate change and extreme weather events (5). Older persons and their families employed predictive and adaptive strategies to avoid and minimize extreme weather impacts (6). Vital lessons could be learned from extreme weather events and a management strategy for wind disasters is important for planning in advance a well prepared and prompt response to potential damages involving health (7). Certain studies on extreme weather events in the area have described a historic afternoon pattern of tornadoes in the Eastern region of the country (8) and an inappropriate behavior of the population during and immediately following a disaster (9). A lack of knowledge is observed regarding health effects of extreme weather events in the Western region of the country. A framework consisting of rapid needs assessments, health surveillance, tracking, registries and epidemiological investigations, including risk factors and health outcome studies and evaluation of interventions need to be applied throughout a disaster management cycle. Epidemiological methods (case-studies) have to be routinely integrated into disaster response and fully communicated to response leaders (10). The aim of this study is to investigate predictors of admission/non-admission into the hospital, the health effects, and the meteorological factors that are related to a violent storm.

MATERIALS AND METHODS

The study was performed on a "retrospective 6-day investigation" sample of 1,156 patients (54.93% males and 45.07% females, aged 8-93 years) with an included "storm-trauma" sample of 71 patients (60.6% males and 39.4% females, aged 9-91 years), patients who were received at the Emergency Receiving Unit (ERU) of the County Emergency Clinical Hospital, in the context of a violent storm that happened on the 17th of September 2017. The "retrospective 6-day investigation" sample consisted of patients who were received at the ERU over the course of 6 days (15th-20th September, 2017) considering all health effects. The "storm-trauma" sample consisted of patients received at the ERU from the 17th of September at 4 p.m. to the 18th of September at 8 p.m., considering traumatic health effects due to the storm. The daily distribution of patients received at the ERU can be seen in tables I and II.

The method consisted of a case-study with a retrospective 6-day investigation (15th -20th of September) using:

Primary medical evidence: patients who went or were taken to the ERU; patients who were/not were admitted into the hospital; all health effects and storm related

trauma health effects;

The meteorological factors: temperature-^{o}C, relative humidity-%, precipitations-liters/m^2, pressure-mmHg, wind speed-Km/h, sunshine-hours) established at the Regional Meteorological Center.

The storm happened on the 17th of September and lasted 20 minutes (from 3:40 to 4:00 p.m.), all the population in the area were exposed to the specific meteorological factors. In order to separate the population exposed to the storm from the population not exposed the author considered: 15th-16th of September corresponding to before storm- no effects; 17th-18th of September - during and immediate effects of the storm; 19th -20th of September - after the storm- delayed effects.

Statistical analysis (frequency, Kruskal Wallis, Mann-Whitney with Bonferroni correction, Chi square, ANOVA one way and binary logistic regression) was performed with the aid of *SPSS 20* software (11). Storm trauma effects were analyzed considering the frequency due to the small size of the sample. Statistical comparison between 2-day distribution series of patients was performed by the aid of Kruskal Wallis, Mann-Whitney with Bonferroni correction and *Chi square* analysis for categorical variables (gender, rural/urban, diagnostics, admission/non-admission into the hospital) and ANOVA one way for covariate variables (age, meteorological factors).

Personal predictors (gender, age and urban/rural environment), time predictors (date and hour of patients' reception at the ERU), established diagnostics and meteorological factors (temperature, humidity, precipitations, wind speed, pressure and sunshine) as possible predictors were analyzed for the entire sample relative to admission/non-admission of patients into the hospital sections or the other medical units from the ERU, using binary logistic regression. This type of regression uses the dependent variable consisting of only two nominal categories (admission/non-admission into the hospital), and any score (covariate: age, temperature, humidity, wind velocity and pressure values) or nominal (categorical: gender, rural/urban environment, daily and hourly presentation to the ERU, diagnostics) variables can be used as the predictors. The reference category is the non-admissions into the hospital for categorical variables. A similar analysis was performed for admitted patients with the main associated diagnostics (presence/absence of diagnostic) as dependent variable related to personal characteristics, time, and meteorological possible predictors.

The author asked for and received from the County Hospital Leadership the agreement to access primary evidence while keeping this evidence confidential and respecting the personal data protection law. The medical data were already collected for medical and statistical purpose, with the requested and received individual consent of the patients. The initial outcome of all health effects data registration was for medical and statistical purpose, and the initial outcome of the traumatic health effects due to the storm data collection was to report to local and national authorities. The agreement (no. 8/22.03.2019) of the University of Medicine and Pharmacy's Ethical Commission was requested and obtained (for this statistical clinical study).

RESULTS

Daily and hourly presentation of patients to the ERU

Daily presentations of patients to the ERU recorded the highest frequencies on

the 17th (17.39%), 18th (18.94%) and 19th (17.48% of patients) of September, 2017 (tab. I). The highest frequency of patients received at the ERU due to storm related trauma was recorded on the 17th of September, 2017 (76.06% of patients) (tab. II).

Hourly evolution of the all causes related trauma and of the storm related trauma emergencies observed during the 17th and 18th of September, 2017 reveals the greatest numbers of patients with traumatic health effects due to the storm on the 17th of September between 4 p.m. and 9 p.m. (48 patients) and on the 18th of September between 12 a.m. and 8 p.m. (17 patients) in the context of all trauma health effects (fig. 1).

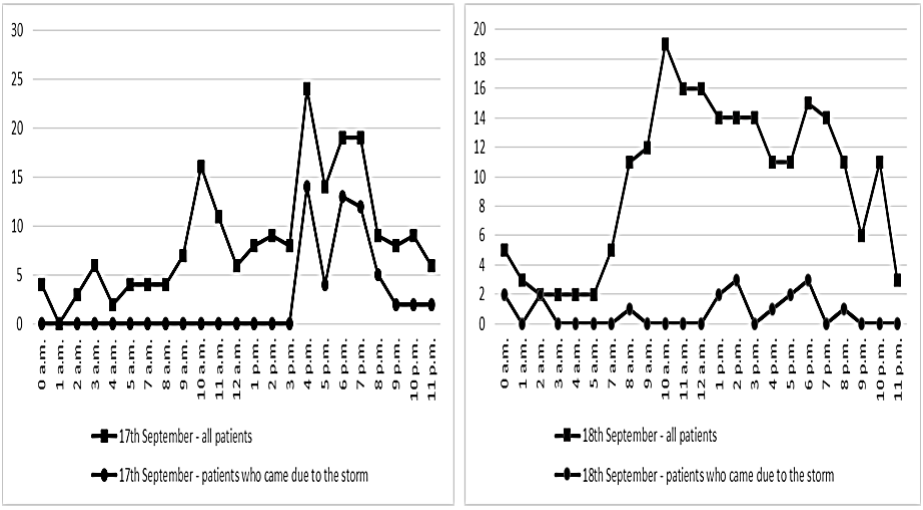

Fig.1. Hourly-evolution of all patients and patients who came at the ERU due to the storm (17th-18th September)

Personal features (gender, age, and rural/urban environment)

Patients who went or were taken to the ERU during the retrospective 6-day investigation for all health effects were more frequently males (54.9%) than females (45.1%) with a peak on the 18th of September for males (57.5%) (tab. I). Patients who went or were taken to the ERU due to the storm related trauma were more frequently males (60.6%) than females (30.4%) with peaks on the 17th of September for both genders: males-32 patients and females-22 patients (tab. II).

Age groups distribution of patients who went or were taken to the ERU with all health effects from the 15th to 20th of September 2017 recorded the greatest frequencies at ages 60-70 (16.9%), 20-30 (15.2%) years (tab. I). For patients who arrived at the ERU with storm related trauma on the 17th and 18th of September the highest frequencies were recorded for ages 50-60 (26.8%) and 20-40 (38%) years (tab. II).

Between the 15th and 20th of September, the patients from urban area came or were taken to the ERU more frequently than patients from rural area (tab. I). During the 17th-18th of September period, the frequency of patients who came from rural area increased (from 43.8% to 52.1%, respectively) and the frequency of patients who came from urban area decreased (from 56.2% to 47.9%, respectively). On the 18th

of September the frequency of patients from rural areas (52.1%) was higher than patients from urban areas (47.9%) (tab. II). Patients who were directly affected (with trauma) by the storm came to the ERU more frequently from rural (38, 53.5%) than from urban (33, 46.5%) areas (tab. II).

There was no statistically significant difference (Kruskal-Wallis, Mann-Whitney with Bonferroni correction) in the personal characteristics of patients who came or were taken to the ERU before, during and after the storm 2-day samples.

Health effects

All diagnostics distribution in the patient population received at the ERU during the retrospective 6-day investigation (15th-20th September) can be seen in first table. An increase of frequencies was recorded for neurologic (199; 13.7%), digestive (162; 11.2%) diseases, contusions (159; 11%), urinary diseases (112; 7.7%), fractures (100; 6.9%) and cardiac diseases (94; 6.5%). There was no statistically significant difference (Kruskal-Wallis, Mann-Whitney with Bonferroni correction) in the diagnostics of patients who came or were taken to the ERU before, during and after storm.

Traumatic health effects

The greatest numbers of trauma diagnostics were recorded on the 18th (166 diagnostics, 28.7%) and 17th (97 diagnostics, 16.7%) of September 2017 (tab. I). During these 2 days 45.8% of all trauma were observed, 19.3% (112 diagnostics) of them due to the storm, according to the patients' statements (tab. II).

Traumatic health effects due to the storm

Traumatic health effects due to the storm could be seen in comparison with all trauma diagnostics on the 17th (amputations-1/1, contusive wounds-21/21, cut wounds-5/5, CCT-cranial-cerebral trauma-17/17 and fractures-15/15) and 18th of September (contusions-7/47, CCT-4/13, fractures-9/26, and sprains-5/13) (fig. 2).

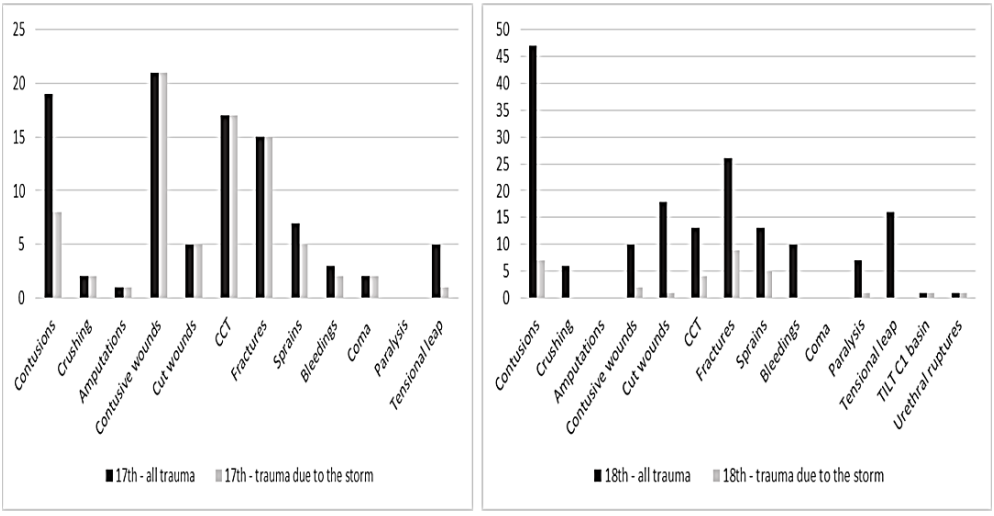

Fig. 2. All traumatic health effects and traumatic health effects due to the storm, seen comparatively (17th-18th September)

Admissions/non-admissions into the hospital

Over the period from the 15th to the 20th of September, the patients who were received, consulted and treated at the ERU were sent home (829; 71.7%), or they were admitted to the hospital sections (327; 28.2%) as follows: surgery (71; 6.1%), neurosurgery (36; 3.1%), neurology (43; 3.7%), orthopedics, traumatology and politraumatology (32; 2.8%), gastroenterology (20; 1.7%), cardiology (17; 1.5%), infectious diseases (15; 1.3%) (fig. 3).

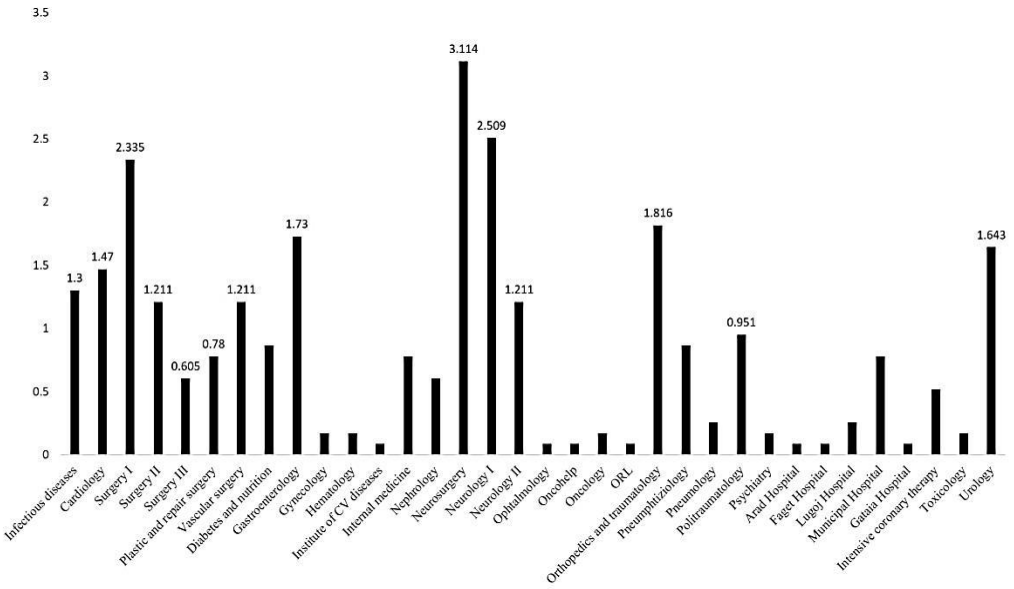

Fig. 3. Patients' distribution (%) according to hospital sections to which they were sent from the ERU

Daily admission/non-admission into the hospital can be seen in tables I and II. Admission of patients into the hospital was the highest in term of frequency in the 17th and 18th of September for all diagnostics (22.3% and 24.8%, respectively) and for trauma due to the storm diagnostics (23.9% and 15.5%, respectively).

A statistically significant difference (Chi Square=25.97, P<0.001) of admitted patients between time series (15th-16th, 17th-18th and 19th-20th of September) was found as follows: between 15th-16th and 17th-18th (Mann-Whitney: z=-4.87, P<0.001) and between 17th-18th and 19th-20th (Mann-Whitney: z=-3.306, P=0.001). No difference (Mann-Whitney: z=-1.674, P=0.094) of admitted patients was recorded between 15th-16th and 19th-20th of September.

Patients affected directly by the storm were more frequently admitted (28 patients, 39.4%) into the hospital than patients from the 6-day investigation sample (327; 28.2%). Most frequently admitted into the hospital due to the storm were male patients (26.7%), age group 50-60 years (11.2%), from the rural environment (25.4%), with fractures (12.7%), on the 17th of September (23.9%), when the violent storm happened.

TABLE I.

Descriptive statistics of 6 days investigation sample, all variables

Variables		6 days investigation sample n[1] (%)						
		15.09	**16.09**	**17.09**	**18.09**	**19.09**	**20.09**	**Total**
Daily presentation to the ERU		166(14.4)	186(16.1)	201(17.4)	219(18.9)	202(17.5)	182(15.7)	**1156 (100%)**
Gender	Male	94(56.6)	100(53.8)	106(52.8)	**126(57.5)**	114(56.4)	97(53.2)	**635(54.9)**
	Female	72(43.4)	86(46.3)	**95(47.2)**	93(42.5)	88(43.6)	87(47.8)	**521(45.1)**
Age	0-10 y.o.	1(0.6)	0	2(1)	1(0.5)	0	0	4(0.3)
	10-20 y.o.	3(1.8)	1 (0.5)	8(4)	11(5)	11(5.45)	3(1.65)	37(3.2)
	20-30 y.o.	23(13.9)	35(18.8)	31(15.4)	27(12.3)	31(15.35)	29(15.9)	**176(15.2)**
	30-40 y.o.	21(12.7)	33(17.7)	30(14.9)	30(13.7)	26(12.9)	23(12.6)	163(14.1)
	40-50 y.o.	22(13.3)	27(14.5)	23(11.4)	37(16.9)	32(15.8)	23(12.6)	164(14.2)
	50-60 y.o.	22(13.3)	15(8.1)	32(15.9)	26(11.9)	20(9.9)	30(16.5)	145(12.6)
	60-70 y.o.	36(21.7)	33(17.7)	34(16.9)	28(12.8)	35(17.3)	30(16.5)	**196(16.9)**
	70-80 y.o.	17(10.2)	24(12.9)	22(10.9)	31(14.2)	28(13.9)	22(12.1)	144(12.5)
	80-90 y.o.	8(4.8)	12(6.5)	12(6)	12(5.5)	9(4.5)	12(6.6)	65(5.6)
	> 90 y.o.	1(0.6)	0	1(0.5)	0	3(1.5)	2(1.1)	7(0.6)
	Missing[2]	12(7.2)	6(3.2)	6(3)	16(7.3)	7(3.5)	8(4.4)	55(4.8)
Environment	Rural	68(41.0)	78(41.9)	**88(43.8)**	**114(52.1)**	80(39.6)	84(46.2)	**512(44.3)**
	Urban	98(59.0)	108(58.1)	113(56.2)	**105(47.9)**	122(60.4)	98(53.8)	**644(55.7)**
Diagnostics	Neurologic	27(12.4)	35(16)	27(12.2)	50(13.9)	27(11.7)	33(16.3)	**199(13.7)**
	Digestive	26(12)	27(12.4)	24(10.8)	24(6.6)	34(14.7)	27(13.3)	**162(11.2)**
	Contusions[3]	19(8.7)	21(9.6)	19(8.6)	47(13)	29(12.6))	24(11.9)	**159((11)**
	Urinary	21(9.7)	14(6.4)	19(8.6)	23(6.4)	15(6.5)	20(9.9)	**112(7.7)**
	Fractures[3]	13(6)	18(8.3)	15(6.8)	26(7.2)	16(7)	12(5.9)	**100(6.9)**
	Cardiac	23(10.6)	12(5.5)	11((5)	20(5.5)	19(8.2)	9(4.5)	**94(6.5)**
	CCT[3]	8(3.7)	14(6.4)	17(7.7)	13(3.6)	9(3.9)	6(3)	67(4.6)
	Contusive wounds[3]	10(4.6)	5(2.3)	21(9.5)	10(2.8)	14(6)	3(1.5)	63(4.3)
	Tensional leap[3]	14(6.4)	8(3.7)	5(2.3)	16(4.4)	4(1.7))	8(4)	55(3.8)
	Respiratory	1(0.5)	13(6)	9(4.1)	13(3.6)	9(3.9)	7(3.4)	52(3.6)
	Cut wounds[3]	6(2.8)	5(2.3)	5(2.3)	18(5)	10(4.3)	5(2.5)	49(3.4)
	Sprains[3]	6(2.8)	8(3.7)	7(3.1)	13(3.6)	6(2.6)	5(2.5)	45(3.1)
	Others ([3])	43(1) (19.8)	38(4) (17.4)	42(8) (19)	88(23) (24.4)	39(1) (16.9)	43(4) (21.3)	293(41) (20.2)
	Total[3]	77(13.3)	83(14.3)	97(16.7)	166(28.7)	89(15.4)	67(11.6)	579(100)
Total diagnostics		217(15)	218(15)	**221(15.2)**	**361(24.9)**	**231(15.9)**	202(14)	**1450(100)**
Admission/ non-admission into the hospital		36(11)/ 130(15.7)	37(11.3)/ 149(18)	**73(22.3)/ 128(15.4)**	**81(24.8)/ 138(16.6)**	53(16.2)/ 149(18)	47(14.4)/ 135(16.3)	**327(100)**/ 829(100)

[1]n - number of patients; [2]Patients did not declare age; [3]Trauma diagnostics.

TABLE II.

Descriptive statistics: 17th-18th September (investigation and trauma storm) variables

Variables		17-18.09.2017			17-18.09.2017		
		Investigation sample n[1] (%)			Storm trauma sample n[1] (%)		
		17.09	18.09	Total	17.09	18.09	Total
Daily presentation to the ERU		**201(17.4)[5]**	**219(18.9)[5]**	**420(36.3)[5]**	**54(76)**	**17(24)**	**71(100)**
Gender	Male	106(52.8)	126(57.5)	232	32(59.3)	11(64.7)	43(60.6)
	Female	95(47.2)	93(42.5)	188	22(40.7)	6(25.3)	28(39.4)
Age	0-10 y.o.	2(1)	1(0.5)	3(0.7)	1(1.8)	0	1(1.4)
	10-20 y.o.	8(4)	11(5)	19(4.5)	1(1.8)	0	1(0.1.4)
	20-30 y.o.	31(15.4)	27(12.3)	58(13.8)	10(17.9)	3(17.6)	13(18.3)
	30-40 y.o.	30(14.9)	30(13.7)	60(14.3)	12(21.4)	2(11.8)	14(19.7)
	40-50 y.o.	23(11.4)	37(16.9)	60(14.3)	4(7.1)	3(17.6)	7(9.8)
	50-60 y.o.	32(15.9)	26(11.9)	58(13.8)	14(25)	5(29.4)	19(26.8)
	60-70 y.o.	34(16.9)	28(12.8)	62(14.8)	4(7.1)	2(11.8)	6(8.4)
	70-80 y.o.	22(10.9)	31(14.2)	53(12.6)	2(3.6)	1(5.9)	5(7)
	80-90 y.o.	12(6)	12(5.5)	24(5.7)	4(7.1)	0	4(5.6)
	> 90 y.o.	1(0.5)	0	1(0.2)	1(1.8)	0	1(1.4)
	Missing[2]	6(3)	16(7.3)	22(5.2)	3(5.4)[4]	1(5.9)[4]	4(5.6)[4]
Environ-ment	Rural	88(43.8)	114(52.1)	202	26(48.1)	12(70.6)	38(53.5)
	Urban	113(56.2)	105(47.9)	218	28(51.9)	5(29.4)	33(46.5)
Diagnos-tics	Neurologic	27(12.2)	50(13.9)	77(13.2)	0	0	0
	Digestive	24(10.8)	24(6.6)	48(8.2)	0	0	0
	Contusions[3]	19(8.6)	47(13)	66(11.4)	8(10.4)	7(20)	15(13.4)
	Urinary	19(8.6)	23(6.4)	42(7.2)	0	0	0
	Fractures[3]	15(6.8)	26(7.2)	41(7)	15(19.5)	9(25.7)	24(21.4)
	Cardiac	11((5)	20(5.5)	31(5.3)	0	0	0
	CCT[3]	17(7.7)	13(3.6)	30(5.2)	17(22.1)	4(11.4)	21(18.7)
	Contusive wounds[3]	21(9.5)	10(2.8)	31(5.3)	21(27.3)	3(8.6)	24(21.4)
	Tensional leap[3]	5(2.3)	16(4.4)	21(3.6)	0	2(5.7)	2(1.7)
	Respiratory	9(4.1)	13(3.6)	22(3.8)	0	0	0
	Cut wounds[3]	5(2.3)	18(5)	23(4)	5(6.5)	1(2.8)	6(5.3)
	Sprains[3]	7(3.1)	13(3.6)	20(3.5)	5(6.4)	5(14.3)	10(8.9)
	Others ([3]) (%)	42(8) (19)	88(23) (24.4)	130(32) (22.3)	6(7.8)	4(11.4)	10(8.9)
	Total [3]	97(16.7)[5]	166(28.7)[5]	263(45.4)[5]	77(13.3)[6]	35(6)[6]	112(19.3)[6]
	Total diagnos-tics	221(15.2)[5]	361(24.9)[5]	582(40.1)[5]	77(68.8)	35(31.2)	112(100)
Admission/ non-admission into the hospital		73(22.3)/ 128(15.4)[5]	81(24.8)/ 138(16.6)[5]	154(47.1) 266(32)[5]	17(23.9) 36(50.7)	11(15.5) 7(9.9)	28(39.4) 43(60.6)

[1]n - number of patients; [2]Patients did not declare age; [3]Trauma diagnostics;
[4]Not considered into trauma sample;[5] % from 6 days investigation sample; [6] % from total trauma diagnostics.

Meteorological factors

The highest values of the air temperature (mean-21.2ºC, maximum-31.6ºC and minimum-16.1°C) and of the wind speed (mean-7.2 Km/h, maximum-19.08 Km/h, and at gust-106.92 Km/h) were recorded on the 17th of September. Large variation of air temperature (difference between maximum-31.6°C and minimum-16.1°C) during the same day could be noticed. Low values of air pressure (mean-735.5 mmHg, maximum-737 mmHg and minimum-732.1 mmHg) were recorded on the 17th of September (tab. III) in association with precipitations (6 liters/m^2) and only 3.4 hours of sunshine. Gust wind speed indicated a violent storm on the Beaufort scale (level 11) on the 17th of September (fig. 4).

Table III.

Descriptive statistics: meteorological factors

Variables		6 days investigation meteorological factors						
		15.09	**16.09**	**17.09**	**18.09**	**19.09**	**20.09**	**Total**
Temperature (0C)	mean	18.1	19.6	21.2	16.5	17.6	14.4	17.9
	maximum	23.6	30.8	**31.6**	23.5	25.0	21.3	25.9
	minimum	14.4	12.0	**16.1**	11.0	10.4	11.6	12.6
Relative humidity (%)	mean	53.5	60.0	66.0	77.5	68.0	93.8	69.8
Air pressure (mmHg)	mean	738.8	736.7	735.5	739.4	735.1	735.1	736.8
	maximum	740.9	739.0	**737.0**	740.5	739.3	735.5	738.7
	minimum	735.0	735.6	**732.1**	732.1	732.8	734.2	733.6
Wind velocity (Km/h)	mean	6.84	5.76	7.2	3.6	5.4	5.4	5.7
	maximum	20,52	17.28	19.08	9.72	13.32	12.96	15.5
	at gust	51.12	33.12	**106.92**	22.68	27.72	33.12	45.8

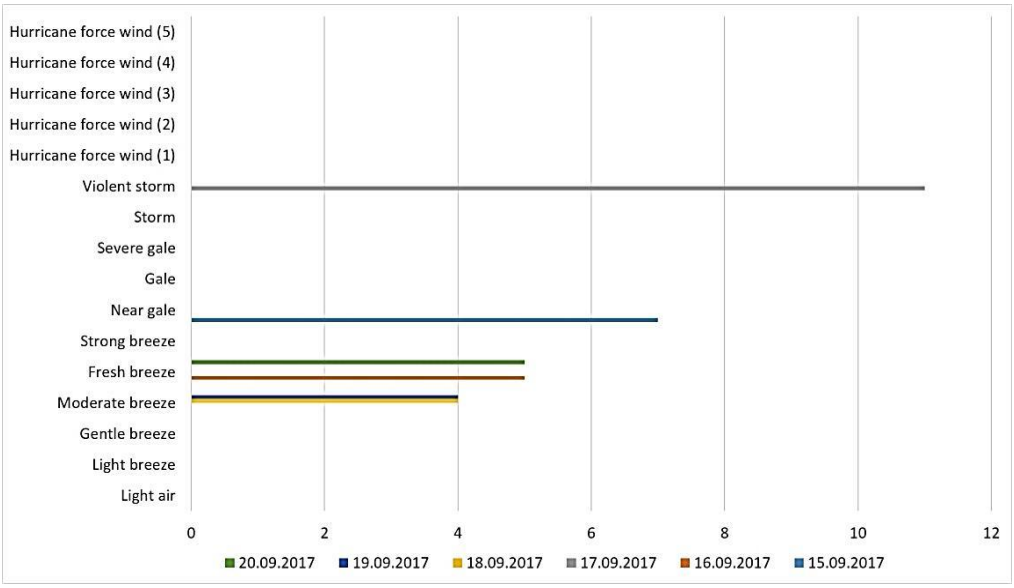

Fig. 4. Wind speed at gust over the investigated 6-day period on the Beaufort scale

There was a statistically significant difference (ANOVA) between air temperature recorded on 19th-20th of September and the other 2 time series 15th-16th (P<0.001) and 17th-18th September (P<0.001) as follows: mean air temperature (F=317.402, P<0.000), maximum air temperature (F=201.204, P<0.000) and minimum air temperature (F=240.641, P<0.001).

Mean air pressure recorded a statistically significant difference between the three time-series (ANOVA: F=467.596, P<0.001) with post hoc: 19th-20th and 15th-16th of September (P<0.001); and 19th-20th and 17th-18th of September (P<0.001). Air pressure maximum presented a statistically significant difference (ANOVA: F=203.901, P<0.001) between each of the three time-series.

Wind speed mean (F=79.355, P<0.001) and wind speed maximum (F=277.885, P<0.001) presented significant statistical differences between 15th-16th of September, and 17th-18th and 19th-20th series (*post hoc*, P<0.001).

Predictors of admission/non-admission into the hospital

Most relevant predictors of admission/non-admission into the hospital were established for the entire 6-day investigation sample of patients who came to the ERU.

Personal predictors (urban/rural environment, age)

An interesting result was obtained for the urban/rural environment categorical predictor related to admission/non-admission into the hospital. Reference for admission/non-admission was the last category (non-admission) for categorical variables. Patients who came from urban areas were more frequently non-admitted (B=0.563-good influence of predictor, P=0.001, Exp(B)= 1.755=Odd Ratio (OR) - an increase of non-admission of patients from urban area). The change in-2 Log Likelihood= 16.778= Chi square (χ^2) was significant (P=0.001) and supported the prediction (tab. IV). As a complementary result, patients who came to the ERU from the rural areas were more frequently admitted to the hospital than the patients who came from urban areas.

Age resulted as a covariate predictor significant for admission/non-admission into the hospital (B=0.033, OR=1.033 (95% CI: 1.026-1.041), χ^2=85.677, P<0.001) (tab. IV). An increase of admission into the hospital is predicted by an increase of patients' age. The model is 70.7% accurate.

Time predictors (date, hour)

Date and hour at which the patients were at the ERU were not found as predictors or influencing factors on admission/non-admission into the hospital.

Established diagnostics as predictors

Main diagnostics that resulted as categorical predictors for admission/non-admission of patients into the hospital were: cardiac diseases (B=-0.831, OR=0.436, 95% CI: 0.279-0.681, χ^2=12.997, P <0.001), fractures (B=-0.982, OR=0.375, 95% CI: 0.239-0.589, χ^2=17.612, P <0.001), and digestive diseases (B=-0.589, OR=0.555, 95% CI: 0.387-0.794, χ^2=10.171, P=0.001) (tab. IV). Negative values for B and Exp(B)=OR<1 indicate a decrease of non-admission (reference-last category=non-admission) into the hospital and a complementary increase of admission of patients into the hospital.

Other diagnostics that resulted as categorical predictors for admission/non-admission of patients into the hospital were: contusions (B=0.740, OR=2.096, 95% CI: 1.333-3.298, χ^2=11.419, P=0.001),

sprains (B=1.337, OR= 3.807, 95% CI: 1.337-10.838, P=0.012, χ^2=8.773), cut wounds (B=1.211, OR= 3.357, 95% CI: 1.306-8.625, P=0.012, χ^2=8.449) and psychiatric diseases-panic attacks (B=1.518, OR=4.564, 95% CI: 1.376-15.137, P=0.013, χ^2=9.101). Positive values for B and OR>1 indicate an increase of non-admission (reference-last category-non-admission) into the hospital (tab. IV). The model was 70.3% accurate.

Meteorological factors as predictors

Meteorological factors found as covariate predictors for admitted/not admitted patients who went or were taken to the ERU were: average air temperature (B=0.654, OR=1.924, 95% CI: 1.403-2.638, χ^2=17.408, P<0.001), maximum air temperature (B=-0.228, OR=0.796, 95% CI: 0.698-0.909, χ^2= 12.217, P<0.001) and relative humidity (B=0.065, OR=1.067, 95% CI: 1.035-1.101, χ^2=17.165, P<0.001) (tab. IV). Positive values of B and Exp(B)>1, 95% CI values>1 indicate an increase of hospital admission when the covariate predictors (mean air temperature between 16^0C-20^0C and relative humidity between 50%-80%) increase. Negative values of B and Exp(B)<1 indicate an increase of admissions into the hospital when the covariate predictor (maximum air temperature between 26°C-20°C) decreases. The model was 71% accurate (tab. IV).

TABLE IV.

Predictors of admission/non-admission into the hospital (n=327/n=829)

Personal predictors		B	Adjusted OR (95% CI)	*P*-value	Chi square	*P*-value
Step 1[a]	Age	0.033	1.033 (1.026-1.041)	0.000	85.677	0.000
	Gender (1)	-0.251	0.778 (0.592-1.022)	0.071	3.280	0.070
	Urban/Rural (1)	0.563	1.755 (1.339-2.301)	0.000	16.778	0.000
	Constant	-2.734	0.065	0.000		
Diagnostics		b	Adjusted OR (95% CI)	*P*-value	Chi square	*P*-value
Step 1[b]	Cardiac (1)	-0.831	0.436(0.279-0.681)	0.000	12.997	0.000
	Digestive (1)	-0.589	0.555(0.387-0.794)	0.001	10.171	0.001
	Contusions (1)	0.740	2.096(1.333-3.298)	0.001	11.419	0.001
	Contusive wounds (1)	0.796	2.217(1.023-4.806)	0.044	4.766	0.029
	Cut wounds (1)	1.211	3.357(1.306-8.625)	0.012	8.449	0.004
	Fractures (1)	-0.982	0.375(0.239-0.589)	0.000	17.612	0.000
	Sprains (1)	1.337	3.807(1.337-10.838)	0.012	8.773	0.003
	Psychiatric (1)	1.518	4.564(1.376-15.137)	0.013	9.101	0.003
	Constant	-4.144	0.016	0.000		
Meteorological factors		b	Adjusted OR (95% CI)	*P*-value	Chi square	*P*-value
Step 3[c]	Temperature air mean	0.654	1.924 (1.403-2.638)	0.000	17.408	0.000
	Temperature air maximum	-0.228	0.796 (0.698-0.909)	0.001	12.217	0.000
	Relative humidity	0.065	1.067 (1.035-1.101)	0.000	17.165	0.000
	Precipitation	-0.074	0.929 (0.874-0.987)	0.017	5.662	0.017
	Constant	-11.084	0.000	0.000		

a. Variable(s) entered on step 1: age, gender, and urban/rural environment. b. Variable(s) entered on step 1: cardiac, digestive diseases, contusions, and contusive wounds, cut wounds, fractures, sprains, psychiatric.

Predictors for main diseases of the patients admitted into the hospital

An interesting result was obtained for predictors of cardiac diseases and fractures.

Predictors of cardiac diseases

For cardiac diseases the main predictors were the age (B=0.038, OR=1.038, P<0.001, χ^2=42.516), and air pressure maximum (B=0.195, OR=1.215, P=0.002, χ^2=10.326, P=0.001). An increase of age and air pressure maximum (between 739 mmHg-741 mmHg) determines an increase in the number of patients with cardiac diseases. The model was 91.8% accurate. Both limits of air pressure maximum are situated under 760 mmHg (normal value at the sea level).

Predictors of fractures

For fractures the main predictors was the air pressure maximum (B=0.160, OR=1.173, and P=0.01, χ^2=7.213, P=0.007). An increase of air pressure maximum (between 739 mmHg-741 mmHg) relates to an increase of the frequency in which fractures occurred.

It is interesting that maximum pressure values between 739 and 741mmHg were recorded on the 18th of September. This means one day after the storm happened, when resulted an increase in the rate of these 2 diseases. The models were 74% accurate.

DISCUSSION

In the study performed the health effects in patients who went or were taken to the ERU due to common causes and due to a violent storm were retrospectively investigated. Use of the emergency health information offered to the author the possibility to analyze all health effects and health effects due to the violent storm, time evolution of patients received at the ERU, personal characteristics and admissions/non-admissions into hospital. The entire process that was analyzed happened in the context of population exposure to specific meteorological factors before, during and after the storm. The retrospective approach of extreme weather phenomena related to health effects recorded at emergency department is frequently mentioned in research literature in order to investigate the impact on population in real situations (12) and to assess the risk as an implementation strategy to reduce the negative impact on the population exposed (13).

Age distribution of the patients who were received most frequently at the ERU during the performed case-study indicates two age groups: old people (60-70 years) and young adults (20-30 years). Research in the field describes length of time spent in the emergency department for patients over 70 years (14) and an association between wind disasters and emergency department visits by age and causes (injuries, cardiac diseases for people over 65 years and renal diseases for people between 18-64 years) (15).

During the retrospective 6-day investigation the gender distribution of patients who were taken to the ERU was higher for males than for females, and the difference between the two genders distribution increased when the main reason for their needing medical attention at the ERU was injury due to the storm. The role of gender in emergency research and practice was mentioned in certain studies that consider trauma diseases (16) or cardiovascular diseases (17).

Patients who arrived at the ERU during

the retrospective 6-day investigation came more frequently from urban than rural areas. During the 17th and 18th of September, the days when the patients affected directly by the storm came to the ERU along with the other patients, those affected by the storm came more frequently from rural than from urban areas. The primary care system capacity in rural areas could be inadequate for responding to storm (18). Research literature regarding rural emergency management of public health crisis identifies as solutions: permanent institutions; improving interventions at the pre-disaster, disaster and post-disaster stages; investments and a reserve system of resources (19). On the other hand, the attitude and the response capacity of primary care medical staff in a rural area proved to be poor in another study (20).

Many patients who were affected by the storm went or were taken to the ERU between 4 p.m. and 9 p.m. on the 17th of September and between 12 a.m. and 8 p.m. on the 18th of September. The greatest percentage in terms of frequency of patients who arrived at the ERU was recorded there from the 17th to the 19th of September. An important aspect is that this violent storm happened on Sunday, during the weekend. In the performed study, the days of the week and the hours did not impact significantly the activity of ERU. In other research studies, on the other hand, the day of the week was shown to be an important factor (especially during the weekend) that could impact the Emergency Department efficiency: more time caring for emergency patients, less median specialist intensity or higher mortality at admission (21).

Health effects diagnosed in patients who came to the ERU were most frequently neurologic diseases, digestive diseases, contusions, urinary diseases, fractures and cardiac diseases. The number of admitted conditions (15) resulted in another research was similar to the study performed (17, if all trauma conditions are considered trauma), although they seem to be of a different order in terms of magnitude (22). Fractures and cardiac diseases most frequently resulted in hospital admissions. The research literature mentions acute myocardial infarction as one of the main causes of mortality in emergency department for non-traumatic diseases (23).

When traumatic health effects were analyzed over the entire 6-day period, resulted that the greatest frequency of trauma happened during the two day-period of the 17th to the 18th of September. Moreover, certain traumatic diseases were frequent and directly connected with the storm during these 2 days, such as: contusive wounds, CCT, fractures, sprains, contusions and cut wounds. Another study of trauma showed that women mainly experienced fractures and contusions/abrasions, whereas men experienced open wounds (24). Traumatic brain injuries found in the performed study were also investigated in research literature with the purpose of improving the efficiency of the emergency departments' management of patients with mild traumatic brain injuries (25).

An important aspect pursued in this case-study with the retrospective 6-day investigation was the admission and non-admission to the hospital (sending the patients home). A great proportion of patients (2/3 - 3/4) of the total number were received, consulted, treated at the ERU and sent home (26). The hospital sections that most frequently received patients who were admitted to the ERU were: surgery, neurosurgery, neurology, orthopedics and trau-

matology and politraumatology, cardiology and infectious diseases. Current research demonstrated that a proposed mobile emergency medical center (MEMC) facilitated transportation of patients with 2 lines ambulances which transported patients in critical condition: between MEMC and disaster, and from the MEMC to the requested hospitals for further treatment (27). In another study polytrauma was seen in the majority of patients and the affected hospital had the uphill task of treating hospitalized patients as well as disaster victims (28). A significant difference was found in the performed study when admissions into the hospital during and immediately after the storm (17th-18th of September) were compared to admissions before the storm (15th-16th), and to admissions after the storm (19th-20th of September). Concerns about weather-related injury were noticed in another study (29).

Meteorological factors investigated during the retrospective 6-day investigation (15th-20th September) indicated the maximum, minimum and the greatest difference between maximum and minimum temperature in the same day, 17th September 2017. Low pressure values were associated with precipitation on the 17th and 20th of September. Gust wind speed surpassed 100 Km/h and indicated a violent storm on the 17th of September. Wind disaster, one of the hazards included in classification of meteorological disasters, was investigated in China (30).

When time series (15th-16th, 17th-18th and 19th-20th of September) of meteorological factors were considered, there was a significant difference between 19th-20th of September and the other 2 series for air temperature and air pressure and a significant difference between the 15th-16th of September and the other 2 series for wind speed. In other research studies outdoor air temperature and related meteorological parameters were associated with stroke attack (30) or with behavioral change with temperature increase (32). Different kinds of incidents in Europe with extreme weather events demonstrated effects of meteorological factors and climate change on public health (33).

Although, no statistically significant differences were found between time series of patients (15th-16th - not exposed, 17th-18th - exposed with immediate effects; and 19th -20th of September - exposed with delayed effects) regarding personal characteristics (gender, age, rural/urban) and pathology, there were significant differences in term of being admitted into the hospital.

Main predictors of admission/non-admission into the hospital of patients from the ERU were:

•Age increase, cardiac diseases, digestive diseases, fractures, mean air temperature and relative humidity increase, which predict admission in hospital.

•Urban environment, contusions, contusive wounds, cut wounds, and maximum air temperature increase, which predict non-admission into the hospital.

Another study (28) demonstrated that there was an interrelation of risk factors or a risk factors cascade triggered by individual or a cluster of risk factors. Therefore, extraordinary events (such as violent storms) or a crisis ordinarily have the so-called cascade effect. An example of this may be windstorm, resulting in a power outage and affecting hospitals, where it is essential for acute care clients to have a regular intake of electrical energy to ensure their life functions (33). By reducing hu-

man vulnerability to disasters, we can lessen--and at times even prevent their impact (34). Communities resilient to the health risks of climate change anticipate risks; reduce vulnerability to those risks; prepare for and respond quickly and effectively to threats; and recover faster, with increased capacity to prepare for and respond to the next threat (35). Preparing for anticipated effects of disasters can help reduce the public health and economic burden (36). Technical and operational synergies are identified to link disaster risk research and communities' adaptation in health (37).

In the performed study main predictors of the main diseases (cardiac and fractures) which led to admissions into the hospital were: age increase for cardiac diseases and air pressure maximum increase for cardiac diseases and for fractures. In research literature the increased incidence rate of ischemic stroke was consistent with the daily lowest and highest air pressure, highest air temperature and presence of hurricanes or storm (38). When confronting changing weather conditions, thinking in ecological terms about the determinants of health, disease and death in human population was considered critically important in another study (39).

The retrospective 6-day investigation offered us valuable information on the efficiency of the ERU, the pathology they usually encounter and the pathology they face in an extreme weather event. A limit of this study is that it is an observational one.

CONCLUSIONS

A relevant prediction of admission/non-admission into the hospital was found for the entire investigation sample. Main predictors of admissions into the hospital related to the storm are: rural environment, increase in age, cardiac diseases, digestive diseases, fractures, average air temperature and relative humidity increase. Main predictors of sending patients home are: urban environment, decrease in age, contusions, cut wounds, sprains, psychiatric diseases (panic attacks) and maximum air temperature increase. Main predictors for diseases with which patients were admitted to the hospital are age increase (cardiac diseases) and air pressure maximum increase 739-741 mmHg (cardiac diseases and fractures). There is a significant difference between admissions into the hospital during 17th-18th of September and the other two time series. Traumatic health effects were the most frequently recorded at the ERU during the 18th and 17th of September (mainly due to the storm). The greatest variation of air temperature, the gust wind (violent storm on the Beaufort scale) and the lowest values of air pressure were recorded during the storm, on the 17th of September. There is a relation between admission or non-admissions of people into the hospital and personal predictors, health effects, and meteorological factors in the context of the violent storm.

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STATEMENT OF ETHICS

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The Ethical Agreement No. 8/22.03.2019 was requested and obtained from the "Victor Babes" University of Medicine and Pharmacy Timisoara's Ethical Commission, for this statistical clinical study.

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The author declares that the research was conducted in the absence of any commercial or financial relationships that could be considered as a potential conflict of interest.

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CONSIDERATIONS REGARDING THE QUALITY OF LIFE IN PATIENTS WITH TRAUMA ADMITTED TO AN EMERGENCY DEPARTMENT

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CONSIDERATIONS REGARDING THE QUALITY OF LIFE IN PATIENTS WITH TRAUMA ADMITTED TO AN EMERGENCY DEPARTMENT (Abstract): Quality of life (QoL) is an important outcome indicator in assessing interventions and medical treatments, in order to understand the burden of disease and identify health issues. **The study aim** was to assess the QoL of patients admitted in an Emergency Department, with polytrauma, by applying the SF-12 validated questionnaire. **Materials and methods:** The study group consisted of 101 subjects, aged 15-17, admitted in the "Sf. Ioan" Children's Emergency Hospital, Galati. **Results:** The Cronbach alpha coefficient was 0.707; mean age 16.43 ±0.88; 69.3% males; 56.4% from urban areas; traumas diagnosed in 19.8% of patients. The mean SF-12 score was significantly lower in patients with sprain (score=89.20) or polytrauma (score=89.90) (p=0.018); slightly lower in patients whose physical and emotional health greatly affected social activities. The degree of impairment in the patient who felt a lot of change in physical and/or emotional health reduced the SF-12 score by 20% (p=0.829). Very intense body pain was associated with a 24% lower SF-12 global score (p=0.308). The mean level of SF-12 scores differed significantly depending on the general health status (p=0.001): on the total study group, a reduced impairment of the QoL was revealed, the mean score was 91.95±7.67, representing 85.9% of the maximum value recorded; the lowest mean value was recorded in patients with a poor general condition (86.67±11.93). **Conclusions**: The QoL in pediatric patients admitted with various types of trauma did not differ significantly between age groups, sexes or residential environments. The global SF-12 score revealed a decrease in the QoL, due to by the very intense bodily pains that significantly affected the patients' physical and emotional health. **Keywords**: CHILD, EMERGENCY, QUALITY OF LIFE, QUESTIONNAIRE.

The concept of quality of life (QoL) was included as one of the basic principles in the Constitution of the World Health Organization (WHO) through the very definition of the notion of health as "a state of physical, mental and social well-being and not merely the absence of disease or infirmity". Currently, there is an increasing interest in the field of QL and specific attention is paid to traumatic brain injury, orthopedic injuries, family stress and post-traumatic stress disorder (1). Studies have noted difficulties with variability in participant characteristics, health-related quality of life measures, and duration of patient follow-up across cohort studies over longer

periods of time (2).

The QoL in children significantly affects that of the parents and the family. Vast pediatric conditions, from common viral and bacterial infections (3), tuberculosis (4) from minor trauma to polytrauma, have an important impact on the QoL in pediatric patient and their families. There are a number of questionnaires available to assess the quality of pediatrics, which are versions of: The Child Health Questionnaire (CHQ); the assessment questionnaire of the quality of life of the child and parents (Child Quality of Life Parent and Child Forms - TACQOL), etc. (5). The Quality of Life Questionnaire, Short Form (SF-12) was developed by the Medical Outcomes Study. The abbreviated form survey instrument provides a solution to the problem faced by many medical investigators of having to restrict the length of the survey. The instrument was designed to reduce respondent burden while achieving minimum standards of accuracy for the purpose of group comparisons involving multiple dimensions of health (6).

The study aim was to assess the QoL of patients admitted in an Emergency Department (ED), with various polytrauma, from Galati county, by applying a validated questionnaire.

MATERIALS AND METHODS

The study group consisted of 101 subjects, aged between 15 years and 17 years and 11 months, admitted in the ED, „Sf. Ioan" Children's Emergency Hospital, Galati.

Questionnaire method. The SF-12 (Short Form-12) questionnaire was applied to assess the health status of pediatric patients aged between 15 and 17 years and 11 months, admitted to the ED with trauma. The informed consent was applied for each patient and for the relative (adult), later he freely consented to answer the questions.

Questionnaire validation. The Cronbach alpha coefficient was calculated = 0.707 and respectively the value based on the standardized items 0.716., so this questionnaire was validated for the Romanian language for our group of patients.

Statistical methods

The data were also processed with the help of statistical software *MS Excel 2010* and *SPSS version 18.0*. The significance threshold used was 95% ($p < 0.05$). The statistical normality of the series of values and checking whether they are contained was checked with Skewness or Kurtosis tests. The mean, median, minimum and maximum values were considered as primary indicators, as well as the standard deviation, the coefficient of variation as dispersion indicators. Nonparametric chi-square tests or the Kruskal Wallis test were used to compare two or more frequencies within the same study group.

RESULTS

Demographic data

Regarding the age group, it varied from 15 to 17.9 years; mean age 16.43 years ±0.88; 69.3% were male subjects (78.3%; 63.6%, respectively 68.9%; p=0.542); 56.4% from urban areas (65.2%; 60.6%, respectively 48.9%; p=0.172). The most frequent diagnosis was represented by sprains (29.7%), especially in 33.3% of 16-year-old children and contusions (21.8%), more frequently in the 17-year-old age group (26.7%). Traumas were identified in 19.8% of patients, more frequently at 16 years (27.3%), and fractures at 13.9% of the total, more frequently at 15 years (17.4%) and 17 years (17.8%) (tab. I).

TABLE I

Distribution of cases according to diagnosis and age groups

Diagnosis	15 years (n=23)		16 years (n=33)		17 years (n=45)		Total (n=101)	
	n	%	n	%	n	%	n	%
Arthralgia	-	-	1	3.0	-	-	1	1.0
Contracture	1	4.3	-	-	-	-	1	1.0
Avulsion	-	-	1	3.0	-	-	1	1.0
Contusion	2	8.7	8	24.2	12	26.7	22	21.8
Sprain	7	30.4	11	33.3	12	26.7	30	29.7
Fracture	4	17.4	2	6.1	8	17.8	14	13.9
Injury	3	13.0	9	27.3	8	17.8	20	19.8
Polytrauma	4	17.4	1	3.0	5	11.1	10	9.9

Analysis of the questionnaire, with the selective presentation of the items

Item 1. General health status. Depending on the diagnosis (p=0.039), the subjects with trauma assessed their general health status as "good" (55%); the subjects with polytrauma rated their general health stats as "good" (70%) (fig. 1).

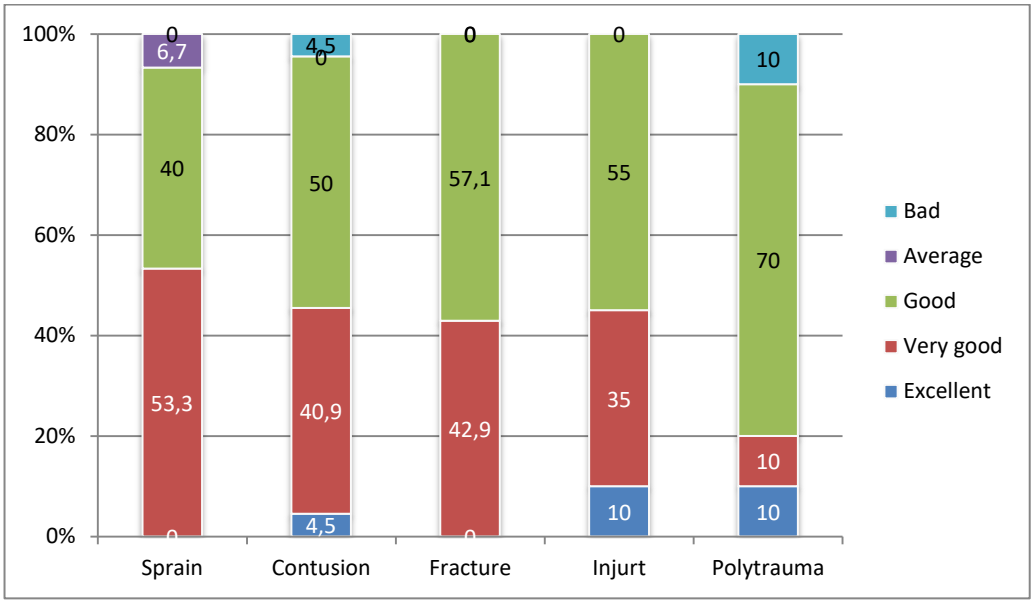


Fig. 1. General health status according to diagnosis

Item 3. Limitation of physical activities depending on the current health status. The activities with the greatest limitation were strenuous activities (52.5%); moderate activities (49.5%) (p=0.001).

Item 10. Affecting social activities by the state of physical or emotional health. 10.9% of subjects believe that physical or emotional health sometimes affected social activity, and 45.5% rarely; most of the

time. Depending on the diagnosis, it was highlighted as follows (p=0.880): in patients with sprains - most of the time (6.7%); most of the time (64.3%) in patients with fractures; most of the time (15%) in trauma patients (fig. 2).

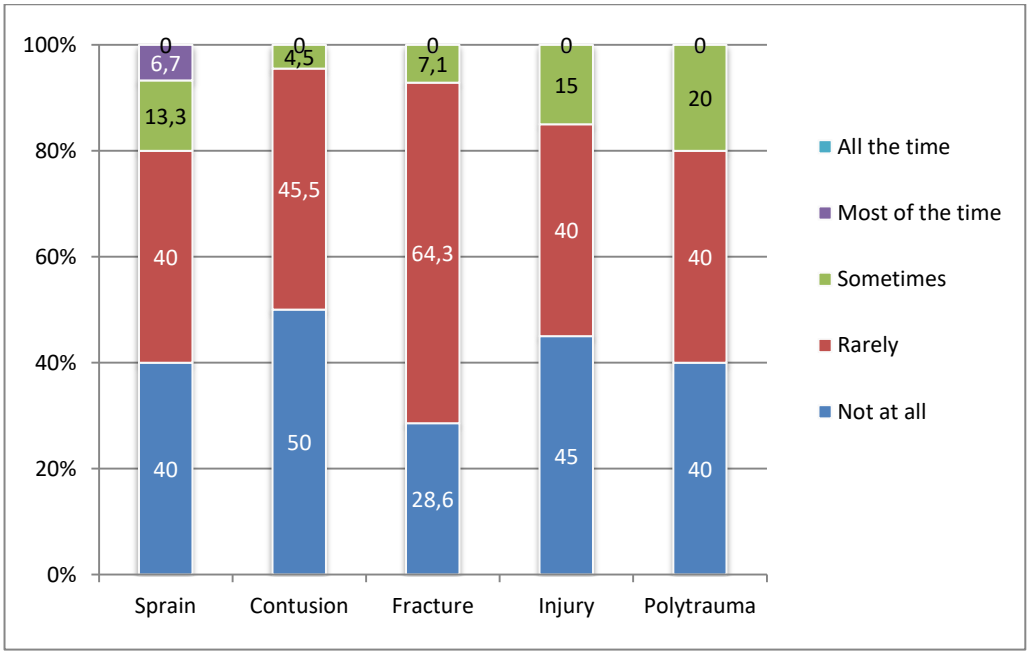
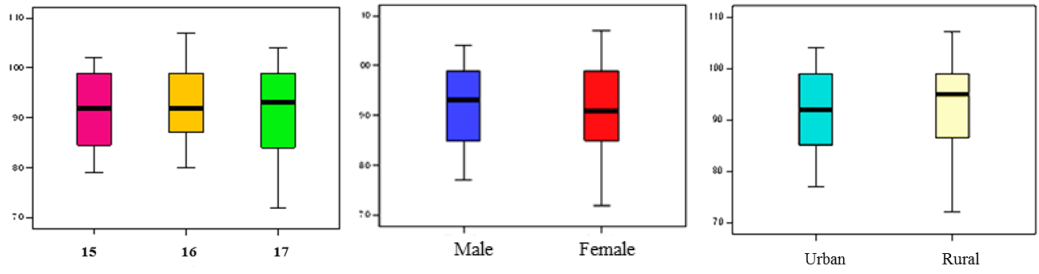


Fig. 2. The influence of social activities by the state of physical health or emotional depending on the diagnosis

Evaluation of scores for the applied questionnaire

The mean SF-12 score was slightly higher in the 16-year-old age group (91.61; 92.61; respectively 91.64; p=0.839). The mean SF-12 score was slightly lower in females (92.33 and 91.10 respectively; p=0.460). The mean SF-12 score was slightly lower in urban patients (91.23 vs. 92.89; p=0.284) (fig. 3).



a. **b.** **c.**

Fig. 3. The average level of the SF-12 score compared by age groups (a.) and compared by gender (b.) and residence areas (c.)

The mean SF-12 score was significantly lower in patients with sprain (score=89.20) or polytrauma (score=89.90) and significantly higher in patients with contusions (score=96) (p=0.018) (tab. II).

The multivariate analysis revealed that the limitation of physical activities due to the health status (R square =0.046; p=0.339), accompanied or not by emotional limitations (R square =0.060; p=0.699) did not significantly affect the QoL (tab. III).

TABLE II.

Descriptive indicators of SF-12 score by health status

Gender	N	Mean	Std. deviation	Std. error	Confidence interval 95%		Min	Max	F test (ANOVA) p
					-95%CI	+95%CI			
General health status									
Bad	3	86.67 p=0.884	11.93	6.888	57.03	116.30	77	100	0.484
Good	50	91.56 p=0.999	7.48	1.058	89.43	93.69	78	104	
Very good	44	92.45 p=0.999	7.64	1.152	90.13	94.78	72	107	
Excellent	4	95.25 p=0.999	8.18	4.090	82.23	108.27	83	100	
Total	101	91.95	7.67	0.763	90.44	93.47	72	107	
Current health status									
Much better now	3	91.00 p=0.984	13.86	8.000	56.58	125.42	83	107	**0.019**
Something better	17	94.53 p=0.882	6.22	1.508	91.33	97.73	80	101	
About the same	43	93.86 p=0.038	7.33	1.118	91.60	96.12	78	104	
Something worse	32	88.75 p=0.104	7.19	1.272	86.16	91.34	72	104	
Much worse	6	88.50 p=0.038	8.36	3.413	79.73	97.27	82	104	
Total	101	91.95	7.67	0.763	90.44	93.47	72	107	

TABLE III

Global score according to physical and emotional activities limited by the health status

Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	,214[a]	,046	,006	7,650	,046	1,149	4	96	,339
2	,245[b]	,060	-,011	7,713	,014	,477	3	93	,699

a. Predictors: (Constant), I4d, I4a, I4b, I4c

b. Predictors: (Constant), I4d, I4a, I4b, I4c, I5c, I5b, I5a

DISCUSSION

QoL measurement is currently an important outcome indicator in evaluating interventions and medical treatments (7), in understanding the burden of disease, in identifying health inequalities, in allocating health resources and in epidemiological studies (8) and health surveys (9). In clinical practice, it has been suggested that QoL tools can be useful in identifying and prioritizing health problems for patients (10), facilitating communication between patients and medical staff, identifying hidden or unexpected health problems (11), as decision aids and in monitoring changes in patients' health or detecting responses to treatment (12).

In our study, the SF-12 health-related quality of life score was slightly lower in patients whose physical and emotional health greatly affected social activities. The degree of impairment in the patient who felt a lot of change in physical and/or emotional health reduced the SF-12 score by 20% (p=0.829). Very intense body pain in the past four weeks was associated with a 24% lower SF-12 global score, but the difference was not statistically significant (p=0.308). The difference between the mean levels of SF-12 global score was not statistically significant according to the impairment of usual work by the pain felt in the last four weeks. The degree of impairment in patients who experienced a lot of pain decreased the SF-12 score by 14% (p=0.706). Physical and emotional health status that affected social activities was not significantly associated with SF-12 global score. The degree of impairment in patients who felt most of the time the change in social status decreased the SF-12 score by 17% (p=0.898).

The mean level of SF-12 scores differed significantly depending on the general health status (p=0.001): on the total study group, a reduced impairment of the QoL was revealed, the mean score was 91.95±7.67, representing 85.9% of the maximum value recorded; the lowest mean value was recorded in patients with a poor general condition (86.67±11.93), without significant differences compared to the mean level recorded for the other responses (p=0.884); the highest mean value was recorded in patients with an excellent general condition (95.20±8.18) without significant differences compared to the average level recorded for the other responses (p=0.999).

As compared to the previous year, the mean level of SF-12 scores registered significant differences according to the current health status (p=0.019): the lowest mean value was registered in patients with a much worse current state (88.50 ±8.36), significant difference compared to the average level recorded for the other responses (p=0.038); the highest average value was recorded in patients with a general condition currently approximately the same as the previous year (93.86±7.33) (p=0.038).

As shown by other recently published studies, the decrease in the health-related quality of life of children after trauma contributes to the increase in caregiver stress and family burden. Posttraumatic stress correlates with changes in QoL and with significant impact on the child's physical, social and family activities. The development of QoL instruments for children and adolescents has continued in recent years, especially with regard to specific questionnaires (13).

Since QoL is subjectively evaluated through questionnaires (14), further studies would be necessary to follow the evolution of QoL over time, in these patients, which we intend to do in the near future.

CONCLUSIONS

The QoL in pediatric patients admitted in ICU with various types of trauma did not differ significantly between age groups, sexes or residential environments. The global SF-12 score revealed a decrease in the QoL, generated by the very intense bodily pains that significantly affected the patients' physical and emotional health.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no funding.

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EFFECTS OF EXOSOMES DERIVED FROM MSC-iPSC ON FIBROBLASTS *IN VITRO* IN THE PRESENCE OF PLATELET-RICH PLASMA

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EFFECTS OF EXOSOMES DERIVED FROM MSC-iPSC ON FIBROBLASTS *IN VITRO* IN THE PRESENCE OF PLATELET-RICH PLASMA (Abstract): Mesenchymal stem cells, as well as mesenchymal stem cells-induced pluripotent stem cells, can definitely induce alterations of fibroblasts and osteoblasts micro medium, stimulating the development of bone around the dental implants. **Aim**: The purpose of the current study was represented by the following of the effects of stimulation and inhibition of multiple intracellular functional pathways of senescent gingival fibroblasts in culture when exosomes released from pluripotent stem cells reprogrammed from mesenchymal stem cells and platelet-rich plasma were used. Materials and m**ethods**: We used flow cytometry to measure the production of the beta-galactosidase level by gingival fibroblasts in culture after 30 passages and ultraviolet treatment. **Results**: Reduction of β-galactosidase concentrations in senescent gingival fibroblasts was evident upon administration of platelet-rich plasma, exosomes and exosomes co-administered the same time with: LY-294002, nutlin-3, berberine, resveratrol, collagen, thrombin, and fibronectin. On the other side, lipopolysaccharide (which might induce a pro-inflammatory profile on iPSC-MSC) enhanced the production of β-galactosidase. The most intense effects were associated with collagen, thrombin, fibronectin, resveratrol, and berberine. **Conclusions**: Exosomes isolated from pluripotent stem cells derived from mesenchymal stem cells, administered in the culture medium of senescent gingival fibroblasts, can modify the degree and evolution of the senescence of these last cells by potentiating, activating, or stimulating some intracellular pathways of biological signal transduction. **Keywords:** MSC, MSC-iPSC, GINGIVAL FIBROBLASTS, EXOSOMES, LY294002, NUTLIN-3, BERBERINE RESVERATROL, LPS, COLLAGEN, THROMBIN, FIBRINOGEN, PLATELET-RICH PLASMA.

INTRODUCTION

Large vertical and horizontal alveolar ridge deficits in mandibular and maxillary bone remain a barrier for doctors practicing implant dentistry. One of the disadvantages of porous blocks for bone repair in big lesions in the oral cavity and in the musculoskeletal system is that the fibrin clot does not adequately cover the interior pores and, the same time, does not persist long enough

to accommodate cell migration into the center of the block (1).

Platelet concentrates, namely platelet-rich plasma, fibrin, or concentrated growth factors, represent cost-effective autologous preparations comprising platelet-derived growth factor, transforming growth factor β, insulin-like growth factor 1, as well as vascular endothelial growth factor. As a result, they are commonly employed in regenerative medicine to repair wounds, nerve damage, cartilage, and bone abnormalities. Unfortunately, following administration, these preparations rapidly release growth factors, which quickly lose their effectiveness. As a result, the therapy must be repeated, which causes the patient more pain and anguish. Recent studies indicate that mixing platelet concentrates with biomaterials solves this problem by releasing growth factors in a more sustainable manner. Furthermore, this notion fits with the most recent advancements in tissue engineering, including biomaterials, bioactive agents, and cells (2).

The progress made in tissue engineering is the result of a thorough investigation of cell-tissue interactions. The selection of a specific biomaterial in Tissue Engineering is crucial because it serves as an interface for adherent cells in the establishment of a microenvironment suited for cell proliferation and differentiation (3).

Lesions linked with medication-related osteonecrosis of the jaws (MRONJ) are resistant to many therapeutic options. As a result, alternative treatments capable of enhancing patient outcomes should be investigated. Leukocyte- and platelet-rich fibrin (LPRF) is a second-generation platelet concentrate composed of natural autologous fibrin matrix. It exhibits anti-infective activity via immunological modulation and accelerates the angiogenesis and multiplication of fibroblasts and osteoblasts; as a result, it promotes soft tissue repair and reduces alveolar bone exposure in the oral cavity. As a result, this could be a noninvasive, rapid, and novel technique to managing bone exposure. The LPRF membrane contributes to a favorable outcome by acting as a physical barrier against microorganisms, preventing secondary infections (4).

Cellular therapy is a new treatment option in regenerative endodontics. Allogeneic mesenchymal stromal cells were tested for their ability to stimulate dental pulp and apical bone regeneration in an endodontically treated tooth. After 14 months after MSC transplantation, the tooth being studied demonstrated sensitivity to cold and electric pulp testing. Radiographic and cone-beam computed tomography images revealed improved periapical bone density, healing of the periapical lesion, and nearly complete apical remodeling. Thus, allogeneic MSCs could be a first-line therapy in regenerative endodontics (5).

The purpose of our research was to investigate the effects of stimulating and inhibiting multiple intracellular functional pathways of senescent gingival fibroblasts in culture, in the presence of microvesicles released by pluripotent stem cells derived from mesenchymal stem cells and platelet-rich plasma.

MATERIALS AND METHODS

The laboratory research aimed to quantify the evolution of the degree of senescence of gingival fibroblasts (senescent cells) by evaluating β-galactosidase through flow cytometry (6, 7) in a biculture, in the presence of platelet-rich plasma and pluripotent stem cells, repro-

grammed from mesenchymal stem cells (in culture), or under the action of exosomes/micro vesicles released from MSC-iPSCs, in the form of concentrated supernatant, administered in the culture medium.

Storage rat gingival fibroblasts (cell line no. 1) were cultivated under conventional laboratory conditions and analyzed for β-galactosidase using flow cytometry after passages 1, 10, 20, and 30. To obtain senescent gingival fibroblasts, they were treated with ultraviolet (λ=315 nm, 80 mJ/cm^2) and multiplied through 30 consecutive passes.

To produce MSC-iPSCs, we used the episomal technique with iPSC reprogramming vectors and the appropriate Stemline culture media (Invitrogen), in accordance with the manufacturer's instructions. We picked this method since the usage and manipulation of potentially genetic material, which is mentioned in the "dangerous" chapter, is completely prohibited. The growth and multiplication of pluripotent stem cells reprogrammed from mesenchymal stem cells were carried out utilizing an Alvetex 3D bio support -a gift from Reinnervate-, as previous investigations revealed the highest efficiency for this form of support (8).

The platelet-rich plasma (PRP) gel was prepared as previously described (9) and delivered at a concentration of 1%.

For exosome separation, the culture medium of pluripotent stem cells produced from rat aortic smooth muscle cells was centrifuged at 100,000 x g (10).

The following compounds were employed in tests and protocols with senescent gingival fibroblasts: 1 µM LY294002, 1 µM nutlin-3, 1 µM berberine, 1 µM resveratrol, 1 µM lipopolysaccharide (LPS), 1 µg/ml collagen, 1 µM thrombin, and 1 µg/ml fibrinogen.

All experimental results obtained during the current study were processed entirely by statistical methodologies, using a number of well-known and optimized tests over time and for medical research, among which the One-Way ANOVA test should be mentioned, which was also supplemented, on occasion, with the Student-Newman-Keuls test.

All the experiments were approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy from Iaşi.

RESULTS

The best qualitative and quantitative development in the case of pluripotent stem cells experimentally reprogrammed from mesenchymal stem cells, beginning with the second passage in culture, was obtained when we multiplied them in a preconditioning medium containing only 1% O2 (8), as previously demonstrated. We always assumed a 100% increase (n=5 experiments per set, data not shown, personal observation).

In addition, the most important β-galactosidase reduction effects in senescent gingival fibroblasts in monoculture were observed in the presence of exosomes/micro- vesicles at a concentration of 0.01%, obtained in concentrated form by ultracentrifugation of the MSC-iPSC supernatant. These latter cell types were also developed in a medium preconditioned with 1% O2. We observed a 20% drop in β-galactosidase levels in senescent gingival fibroblasts under these circumstances, consistent with earlier studies.

In the first series of tests, senescent gingival fibroblasts were treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet-rich plasma.

Previously, MSC-iPSCs were treated with 1 μM LY294002, a highly selective PI3K kinase inhibitor. In senescent gingival fibroblasts, we observed an average 15% drop in β-galactosidase activity (fig. 1).

In the second series of tests, a separate batch of senescent gingival fibroblasts was treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet rich plasma. Previously, MSC-iPSCs were treated with 1 μM nutlin-3, which effectively stabilized the non-genomic activities of p53 (MDM2/p53 pathway). Senescent gingival fibroblasts showed an average 13% drop in β-galactosidase levels (fig. 1).

In the third series of tests, a large number of senescent gingival fibroblasts were treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet rich plasma. MSC-iPSCs were treated with 1 μM berberine, which inhibits ROS-dependent and JNK-driven cell death via PI3K/Akt. We observed a 23% drop in β-galactosidase levels in senescent gingival fibroblasts (fig. 1).

In the fourth series of studies, a completely separate batch of senescent gingival fibroblasts were treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet-rich plasma. MSC-iPSCs were treated with 1 μM resveratrol, potentially improving their survival, self-renewal, lineage commitment, and anti-aging benefits. In senescent gingival fibroblasts, we observed an average 52% drop in β-galactosidase levels (fig. 1).

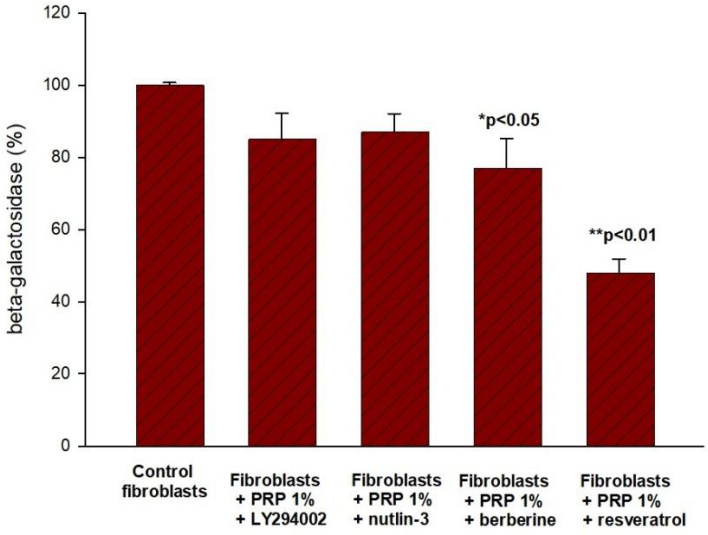

Fig. 1. Determination of β-galactosidase in senescent gingival fibroblasts, when cultured with 0.01% exosomes obtained from the supernatant of MSC-iPSC and 1% platelet-rich plasma. Previously, MSC-iPSCs have been treated with 1 μM LY294002, nutlin-3, berberine, and resveratrol. (*p<0.05 as compared to the normal culture medium. **p<0.01 as compared to the normal culture medium)

In the fifth series of tests, senescent gingival fibroblasts were treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet-rich plasma. Previously,

MSC-iPSCs were treated with 1 μM lipopolysaccharide (LPS), which induces a proinflammatory profile on iPSC-MSC. We observed a 37% drop in β-galactosidase levels in senescent gingival fibroblasts (fig. 2).

In the sixth series of studies, a separate batch of senescent gingival fibroblasts was treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet rich plasma. Previously, MSC-iPSCs were treated with 1 μM collagen, which increased chemokine and growth factor production by MSCs. We observed a 61% drop in β-galactosidase levels in senescent gingival fibroblasts (fig. 2).

In the seventh series of tests, a large number of senescent gingival fibroblasts were treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet rich plasma. MSC-iPSCs were treated with 1 μM thrombin, which increases fibronectin production through protease-activated receptor-mediated signaling pathways. In senescent gingival fibroblasts, we observed an average 58% drop in β-galactosidase levels (fig. 2).

In the eighth series of trials, a completely separate batch of senescent gingival fibroblasts was treated with 0.01% exosomes derived from MSC-iPSC supernatant and 1% platelet rich plasma. Previously, MSC-iPSCs were treated with 1 μM fibrinogen, which promotes self-renewal by stabilizing bFGF and activating autophagy. We observed a 48% drop in β-galactosidase levels in senescent gingival fibroblasts (fig. 2).

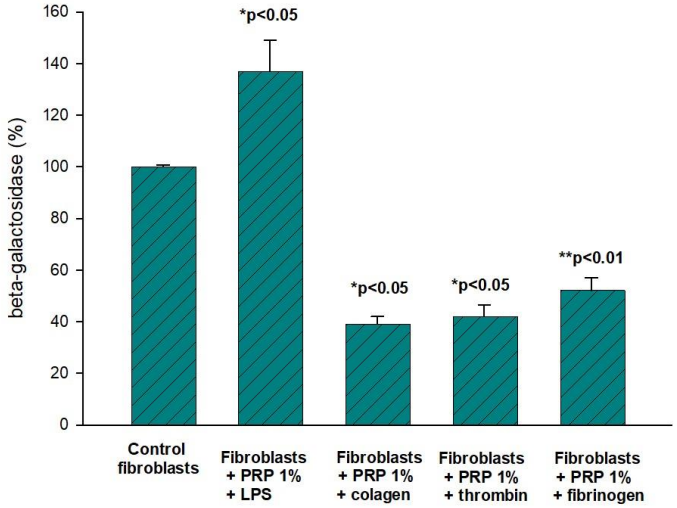

Fig. 2. Measurement of β-galactosidase in senescent gingival fibroblasts, when developed in the presence of 0.01% exosomes obtained from the supernatant of MSC-iPSC and 1% platelet-rich plasma. Foregoing, MSC-iPSCs have been treated with 1 μM LPS, collagen, thrombin, and fibrinogen. (*p<0.05 as compared to the normal culture medium. **p<0.01 as compared to the normal culture medium)

DISCUSSION

There are studies underway to investigate platelet-rich plasma (PRP) effects in human mesenchymal stem cells, which may support or limit the clinical use of MSCs in conjunction with PRP. The effects of PRP

on proliferation, migration, stemness, the preservation of MSC immune-modulatory capabilities, and the emergence of the senescence phenotype were investigated. Overall, PRP promotes MSC proliferation, maintains multipotency, and does not impede with lineage differentiation. PRP (platelet lysate or releasate) protects MSCs' immune-privileged potential and may postpone the onset of the senescent phenotype. Currently, there is little evidence tying specific chemicals to biological mechanisms. Several knowledge gaps must be addressed in order to gather enough usable information for translation (11).

Platelet-rich plasma is defined as an enriched platelet suspension in plasma. In addition to its clinical applications in numerous orthopedic illnesses and beyond, PRP and platelet lysate (PL) have received attention in the field of tissue engineering. PRP has been used for a variety of tissue engineering applications, including bone, cartilage, skin, and soft tissue repair, with the majority of studies focused on bone tissue engineering. These methods take advantage of either the release of chemo attractive, angiogenic, proliferative, and potentially pro-regenerative growth factors from PRP, or the hydrogel properties of activated PRP, which make it suited as a cell delivery vehicle. Many of these researches combine PRP with biomaterials, cells, and, in some cases, recombinant growth factors. Although the experimental design frequently does not allow for judgments on the pro-regenerative effect of PRP, the majority of articles show positive results when PRP is introduced to the tissue-engineered construct. Furthermore, it was established that the release of growth factors from PRP can be customized and regulated by combining PRP with growth factor-capturing materials. Platelet-derived preparations (PRP and PL) are a promising source of autologous growth factors that can be used as a cell culture supplement or to induce regeneration in tissue-engineered constructions. Furthermore, activated PRP shows promise as an autologous cell carrier. However, research exploring PRP in these circumstances frequently provide inconsistent results, which are most likely due to a lack of standardized preparation methods, notably in terms of platelet composition and donor variance. Finally, the use of PRP must be customized for each application (12).

Soft tissue abnormalities can be treated using autologous fat grafts or adipose-derived stem cells (ASCs). However, the results are inconsistent and can include tissue resorption and necrosis. This could be owing to poor vascularization. Platelet-rich plasma (PRP) contains concentrated autologous platelets. Platelet-derived growth factors and cytokines can promote angiogenesis. The concomitant use of PRP may boost the regeneration potential of fat grafts. The ideal ratio has yet to be identified. PRP preparation produces platelet-poor plasma (PPP). PRP and PPP can both be utilized to increase ASCs in cell culture without the use of xenogeneic chemicals. A PRP concentration greater than 20% exerts inhibitory effects (13).

Platelet-rich plasma (PRP) is a biological therapy that stimulates biological processes like cell growth. The extent of PRP's effect is determined by a variety of parameters, the most important of which is the composition of PRP. The study aimed to investigate the correlation between cell proliferation and growth factors (namely IGF-1, HGF, PDGF, TGF-β, and VEG) in platelet-rich plasma. First, PRP's composi-

tion and effect on cell proliferation were compared to platelet-poor plasma (PPP). The association between each PRP growth factor and cell proliferation was then investigated. Cell proliferation was greater in cells treated with PRP lysates than in cells treated with PPP lysates. PRP showed considerably greater levels of PDGF, TGF-β, and VEGF. When the PRP growth factors were examined, only IGF-1 showed a significant correlation with cell proliferation. IGF-1 levels were the only ones that did not link with platelet levels. The extent of PRP's effect depends not only on platelet count, but also on platelet-independent molecules (14).

Platelets produce platelet growth factors (as PDGF, IGF-1, EGF-, HGF, TGFβ, bFGF, and VEGF) that regulate wound healing at all stages. These chemicals are derived from platelet-rich plasma. Over the last five decades, there has been a substantial growth in interest and utilization of platelets' regenerative qualities in a variety of medical sectors around the world. PRP and PRF derived preparations are used in dentistry, sports medicine, as well as dermatology, and cosmetology to treat alopecia, further hair reconstruction (FGF-7, HGF), acne scars, skin so in rejuvenation and regeneration, chronic wounds, burns, and acquired vitiligo. Another important domain is represented by gynecology and reproductive medicine (as treatment of infertility, but also erectile dysfunction - using PDGF-β, TGF-β, IGF-1, sexual dysfunction - PDGF, vaginal atrophy). Ophthalmology is becoming more and more important from the point of view of using platelet-rich plasma derivatives (as healing of corneal epithelial wounds, dormant corneal ulcers, dry eye syndrome and, last but not least, corneal surface reconstruction). Treatments in neurology are involving also substances obtained from platelet-rich plasma (speaking about regeneration of neurons, pain relief, and clinical symptoms -through the use of TGF-β 1, IGF-1, PDGF, VEGF, and FGF). So, platelet-rich plasma therapy is a very interesting alternative and supplement to established treatment procedures. However, the potential use of platelets is not entirely known. Many factors influence the composition of platelet-rich plasma, which can have an impact on its efficacy and clinical advantages. Additional study is required to standardize PRP delivery preparation protocols and strategies for a specific disease entity or clinical instance (15).

Mesenchymal stem cells (MSC) added to bone replacement materials can aid in bone repair. Bone development in bio composites is highly reliant on the type of biomaterial, its pre-treatment, and the cells used. Supplements that may be immunogenic or infectious, such as fetal calf serum (FCS), should not be used in cell expansion media. Cells cultured in two different mediums were examined on several biomaterials for bone forming capacity following ectopic implantation in vivo, as well as growth rate and differentiation capacity in vitro. The replacement of FCS with PRP removed the hazards associated with the use of xenogeneic supplements. It increased the expansion of MSC and retained their differentiation and in vivo bone forming potential in an environment suitable for autogenous usage (16).

Implant therapy is a popular therapeutic option in dentistry and orthopedics, however it is frequently accompanied with an increased risk of microbial contamination of the implant sites, which can cause bone tissue damage. This work intends to create two silver-enriched platelet-rich plasma (PRP) multifunctional scaffolds that can

prevent implant-associated infections while also boosting bone repair. In vitro tests were conducted on both commercial silver lactate (L) and freshly synthesized silver deoxycholate: β-Cyclodextrin (B). Initially, the antibacterial activity of the two silver soluble forms and PRP supplemented with the two silver forms was investigated on microbial planktonic cells. At the same time, the biocompatibility of silver-enriched PRPs was evaluated using an MTT test on human primary osteoblasts (hOBs). Following that, a study was done to assess the efficacy of various concentrations and types of silver-enriched PRPs in suppressing microbial biofilm formation and boosting hOB differentiation. PRP-L (0.3 μg/mm2) and PRP-B (0.2 μg/mm2) inhibit planktonic cell proliferation and biofilm formation in Staphylococcus aureus, Staphylococcus epidermidis, and Candida albicans, while retaining hOB viability and differentiation capability. As a partial conclusion, the results obtained suggest that L- and B-enriched PRPs could constitute a promising preventive strategy against biofilm-related implant infections and can be used as a new silver formula that, in addition to increasing fibrin binding protecting silver into the truncated cone-shaped cyclic oligosaccharides, to be able to achieve comparable inhibitory results on prokaryotic cells, but with a lower concentration (17).

Regulatory authorities oppose the use of fetal bovine serum (FBS) as a cell culture supplement in order to reduce the danger of zoonotic and xenogeneic immunological reactions in the transplanted host. Furthermore, FBS production has come under fire because to animal welfare problems. Platelet derivatives have been proposed as FBS alternatives for the ex-vivo expansion of mesenchymal stem/stromal cells (MSCs), as platelet-derived growth factors can enhance MSC multiplication. Platelet-derived growth factors can be found in platelet lysate (PL), which is generated by freezing and thawing platelet-rich plasma repeatedly or by adding physiological stimuli such as thrombin or CaCl2.PL-expanded MSCs have already been used in clinical trials, taking advantage of their quicker proliferation compared to FBS-expanded preparations. If PL is used to other biopharmaceutical drugs, demand is anticipated to skyrocket. The use of fresh platelet units in the manufacturing of PL poses problems because to the scarcity of platelet donors. Expired units could be an option, however further information is needed to determine safety, including pathogen decrease, and usefulness of the obtained PL. Furthermore, significant problems about the establishment of PL release criteria, such as the concentration ranges of certain growth factors in PL batches for different therapeutic reasons, must be addressed. We are still distant from a uniform definition of PL and standardized PL production due to our poor knowledge of the processes that drive PL-promoting cell proliferation (18).

Dental pulp stem cells (DPSCs) are intriguing candidates for therapeutic use due to their high proliferative capability, multipotent differentiation, immunomodulatory properties, and lack of ethical problems. Clinical research on DPSCs is still in its early phases. The failure to obtain clinically effective results may be due to issues with the DPSC production process. Cell properties may be impacted by differences in DPSC preparation procedures and reagent formulas, resulting in conflicting experimental outcomes. The preparation of clinical-grade DPSCs is far from complete. To reach therapeutic use, it is required to transition the manufacture of stem cells

from laboratory to clinical grade (19).

Human platelet lysate (PL) as well as human platelet lysate serum (PLS) are used as cell culture supplements instead of fetal bovine serum (FBS) due to ethical concerns, batch variability, and the possibility of introducing xenogenic pollutants. Some studies examined the content and efficiency of PL, PLS, as well as FBS as supplements during the culture and cryopreservation of human gingival fibroblasts, also Wharton's jelly-derived mesenchymal stem cells (WJ-MCS), and finally adipose tissue (AdMSC). Biochemical components, certain growth factors, and cytokines were examined in each of them; additionally, the cells were cultivated in media supplemented with 5% PL, 5% PLS, and 10% FBS and exposed to various freezing and thawing solutions containing the supplements under investigation. Biochemical parameters in PL and PLS were found to be similar to those in FBS, with some variations in fibrinogen and calcium concentrations. PL and PLS contained higher levels of growth factors and cytokines than FBS. Cell proliferation and morphology did not differ significantly between the three culture mediums. PLS and FBS provided better results for cell cryopreservation and thawing. To summarize, PL and PLS are an ideal choice to substitute the conventional supplement of animal origin (FBS) in the media necessary for the culture and cryopreservation of fibroblasts, WJ-MSC, and AdMSC (20).

CONCLUSIONS

Exosomes were found to reduce β-galactosidase concentrations in senescent gingival fibroblasts by around 20% on average. Exosomes were also delivered in combination with: LY-294002 (a non-selective inhibitor of phosphatidylinositol 3-Kinase (PI3K) pathway, α/δ/β, 15% on average); nutlin-3 (a potent and selective small-molecule MDM2 antagonist that activates p53, 13% on average); berberine (which protects against MSC apoptosis by preventing ROS-dependent, as well as JNK-driven cell apoptosis, in a PI3K/Akt-dependent manner, 23% on average); resveratrol (may improve the therapeutic effects of MSCs by enhancing their survival, as well as self-renewal, lineage commitment, and anti-aging effects, 52% on average); collagen (promotes the secretion of the chemokines and growth factors by MSCs, 61% on average); thrombin (promotes fibronectin secretion by MSCs via the protease-activated receptor mediated signaling pathways, 58% on average); and fibronectin (supports self-renewal of MSCs through stabilization of bFGF and activation of autophagy, 48% on average. On the other side, lipopolysaccharide (which might induce a proinflammatory profile on iPSC-MSC) enhanced the production of β-galactosidase (37% on average).

All of these findings suggest that exosomes derived from pluripotent stem cells induced from mesenchymal stem cells and administered in the culture medium of senescent gingival fibroblasts can influence the degree and evolution of senescence in these cells by potentiating, activating, or stimulating some intracellular biological signal transduction pathways, particularly those involving resveratrol, collagen, thrombin, and fibronectin.

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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21.

COMBINED RELUGOLIX TREATMENT FOR HIGHLY SYMPTOMATIC UTERINE LEIOMYOMA PATIENTS

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COMBINED RELUGOLIX TREATMENT FOR HIGHLY SYMPTOMATIC UTERINE LEIOMYOMA PATIENTS (Abstract): Many conservative treatments for symptomatic uterine leiomyoma have been proposed but in the large majority of cases these are often limited by the associated side effects. A relative novel treatment with GnRH antagonists has been reported in the literature with good results. The primary goal was to assess the degree of reduction of uterine global volume and largest uterine fibroma volume at the end of 24 weeks of combined treatment with Relugolix 40 mg, 1 mg estradiol and 0.5 mg norethindrone acetate as well as at 12 weeks after. The secondary goal was to subjectively assess the reduction of vaginal bleeding during treatment and the evolution of hematologic parameters. **Materials and methods:** We analyzed 5 patients with uterine leiomyoma with heavy menometrorrhagia and severe anemia. 4 cases rejected the surgical treatment and 1 case was not suitable for surgery due to morbid obesity and associated pathology. All cases accepted the combined therapy with relugolix 40 mg, 1 mg estradiol and 0.5 mg norethindrone acetate for a period of 24 weeks. **Results:** In all cases, the reduction of uterine and largest leiomyoma volume was consistent after 24 weeks of treatment and maintained so even 12 weeks after. In addition, the severity of vaginal bleeding was also reduced, so that the hematologic parameters slowly improved. **Conclusions:** Combined therapy with relugolix 40 mg, 1 mg estradiol and 0.5 mg norethindrone acetate is a very good alternative especially in cases where surgery is not accepted or is not suitable due to associated morbidity. **Keywords:** LEIOMYOMA, MENOMETRORRAHAGIA, SEVERE ANEMIA, RELUGOLIX.

INTRODUCTION

Uterine leiomyoma, known in medical practice as uterine fibroid, is the most common form of benign tumor of the uterus. It is also known that the prevalence of this tumor reaches its maximum rates among women in the reproductive period (15-50 years old women) (1, 2) and its development and growth is highly dependent of hormonal factors; throughout

life, approximately two out of three women, especially between 30 and 50 years of age, will develop this type of pathology (3).

Cases of uterine fibroids in the prepubertal period have not been described in literature, and cases found among teenage girls have rarely been found. The maximum incidence is found among Afro-American women in their fifties. In Caucasian population, the incidence is reached by women the ages of 30 and 40. Currently, there is not enough information to clarify the rate of occurrence of uterine fibroids among Hispanic and Asian women (4).

The incidence of these tumors is firstly connected to the hormonal activity (5) and this is sustained by the association of this pathology with nulliparity/infertility, early menarche and late menopause. Heredity, race and age are also important risk factors (5). Other risk factors such as heart disease, especially hypertension, smoking, alcohol consumption, oxidative stress, have not been sufficiently documented.

This pathology is often neglected by patients, many of them are presenting with large pelviabdominal tumors, heavy vaginal bleeding and severe anemia.

Although the definitive treatment remains hysterectomy, conservative treatments are recently widely accepted, and these are mostly sustained by the desire of the patients to preserve the uterus and thereby fertility. The main goals of the conservative treatment are to reduce the symptoms and to diminish the size of the leiomyomas (6).

Despite being the first-line medical treatment for uterine fibroids, there is little evidence to support the use of contraceptives (7).

Although leuprolide acetate and other injectable long-acting gonadotropin-releasing hormone (GnRH) agonists (8) are effective, their duration of use is limited by hypoestrogenic sequelae, which may need the use of supplementary hormonal therapy to offset adverse effects.

The selective progesterone-receptor modulator - ulipristal acetate - is recommended by the European Medicines Agency to be used for the treatment of uterine fibroids only in premenopausal women in whom surgical procedures (including uterine fibroid embolization) are not appropriate or have not worked. However, its use must be very cautious since there are reported rare cases of serious liver injury (9).

The GnRH antagonist elagolix is licensed for the treatment of uterine fibroids for a period of 24 months and lowers excessive menstrual flow in women with uterine fibroids when taken with estradiol and norethindrone acetate (10). However, due to its short half-life, elagolix must be taken twice daily. Its use has been linked to negative effects on blood pressure, cholesterol and liver enzyme levels, and bone mineral density at one year (10, 11).

Relugolix and linzagolix are new GnRH antagonists which have been demonstrated high efficiency in the treatment of symptomatic leiomyoma patients (12, 13).

MATERIALS AND METHODS

This study reflects our experience with combined relugolix therapy for highly symptomatic uterine leiomyoma patients.

Our case series included five patients that addressed to Obstetrics and Gynecology 2nd Clinic, “Sf. Ap. Andrei” Clinical Emergency County Hospital Galati, between the 1st of November 2023 and 31st of March 2024 for heavy vaginal bleeding and

severe anemia due to large uterine leiomyomas. After initial assessment, in all cases dilatation and curettage (D&C) with hemostatic and bioptic indication was performed along with hematologic reanimation (in three cases blood transfusions were indicated).

Ultrasound endovaginally scan was performed in all cases using a *General Electric Voluson* S8 equipment. Uterine volume as well as the volume of the largest leiomyoma were noted in all cases.

Surgical treatment - total hysterectomy - was proposed and categorically refused by 3 patients. In the remaining two cases, due to morbid obesity sustained by BMI above 40, conservative treatment was our first choice.

All five cases in our study were treated with a combination of relugolix 40 mg, 1 mg estradiol and 0.5 mg norethindrone acetate for 24 weeks. The patients were clinically and ultrasonographical assessed at the beginning of the trial, at the end of the pharmacological treatment in week 24, and again 12 weeks from stopping the treatment.

RESULTS

The cases under investigation were white female, 42 to 50 years of age. Body mass index (BMI) was above 30 in all cases (mean 39.74 ± 5.03). In 2 patients, in which associated morbidity such as diabetes or cardiac disease were also noticed, the BMI was even above 40 (tab. I).

TABLE I.

Clinical and paraclinical features of the cases.

BASELINE CHARACTERISTICS	Case 1	Case 2	Case 3	Case 4	Case 5
Age(years)	42	48	50	46	49
Race	White	White	White	White	White
Region	Europe	Europe	Europe	Europe	Europe
BMI (kg/m^2)	33.4	36.7	45.8	39.2	43.6
MBL - (No. of pads/tampons)					
0 w	10	9	12	11	12
24 w	5	7	7	8	7
36 w	4	7	6	7	6
Uterine volume (cm^3)					
0 w	210.23	390.73	449.89	381.47	472.9
24 w	186.8	320.7	387.8	317.6	394.1
36 w	172.5	304.9	367.2	308.5	379.4
Volume of largest leiomyoma (cm^3)					
0 w	53.52	85.76	112.69	66.1	113.92
24 w	41.6	63.8	79.2	49.6	75.6
36 w	39.7	58.5	71.4	43.8	67.8
Hemoglobin concentration (g/dL)					
0 w	7.8	8.5	5.7	8.2	6.8
24 w	9.6	9.8	9.2	9.7	9.4
36 w	9.8	10.1	9.6	10.0	9.8

BMI - body mass index; MBL - menstrual blood loss.

All cases presented with a history of severe menstrual and/or intermenstrual bleeding, which was subjectively assessed by the number of absorbent pads used daily. In our group, the number of pads used in the heaviest day of vaginal bleeding was between 9 to 12 per day. During the combined treatment with relugolix, the number of pads used was progressively lower so that, at the end of 24 weeks of treatment this number ranged between 5 to 8. Moreover, 12 weeks after the initial treatment, vaginal bleeding was reasonable, with a number of absorbent pads between 4 to 7 daily (tab. I).

The reduction of vaginal bleeding along with the hematological treatment were the key factors for correction of severe anemia. At the start of the trial, the patient's hemoglobin levels ranged between 5.7-8.5 g/dL (mean 7.4 ± 1.14). After 24 weeks of combined treatment the hemoglobin values improved, ranged between 9.2-9.8 g/dL (mean 9.54 ± 0.24), and maintained above 9.6 g/dL (ranged between 9.6-10.1 g/dL, mean 9.86 ± 0.19) at 12 weeks after stopping the treatment (tab. I).

Positive outcomes were additionally noticed in terms of uterine global volume and the volume of the largest uterine leiomyoma (figs. 1, 2).

When combined therapy with relugolix was initiated, the global uterine volume of the cases ranged between 210.23 and 472.9 cm^3 (mean 381.04 ± 103.01) and the greatest leiomyoma's volume between 53.52 and 113.92 cm^3 (mean 86.39 ± 27.12). A decrease of the global uterus volume was recorded in all cases with limits between 37.73 and 93.5 cm^3. Additionally, compared to day 0, the greatest leiomyoma's volume was much smaller at week 36, with values between 39.7 and 71.4 cm^3, the range of recorded reduction being between 13.82 to 46.12 cm^3 (tabs. I, II, figs. 1, 2).

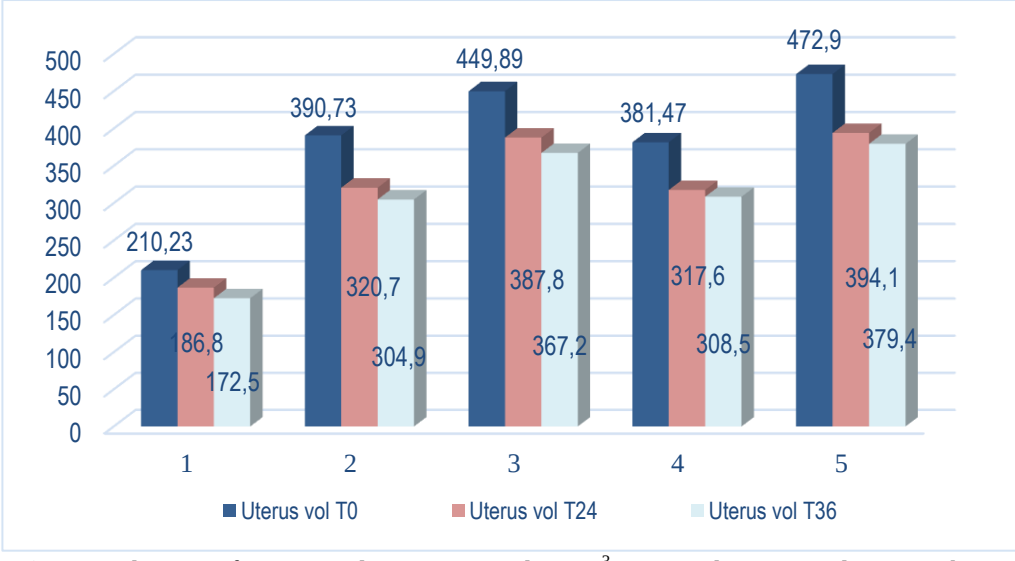

Fig. 1. Reduction of uterine volume expressed in cm^3 at 24 and respectively 36 weeks from the beginning (T0) of combined therapy with relugolix.

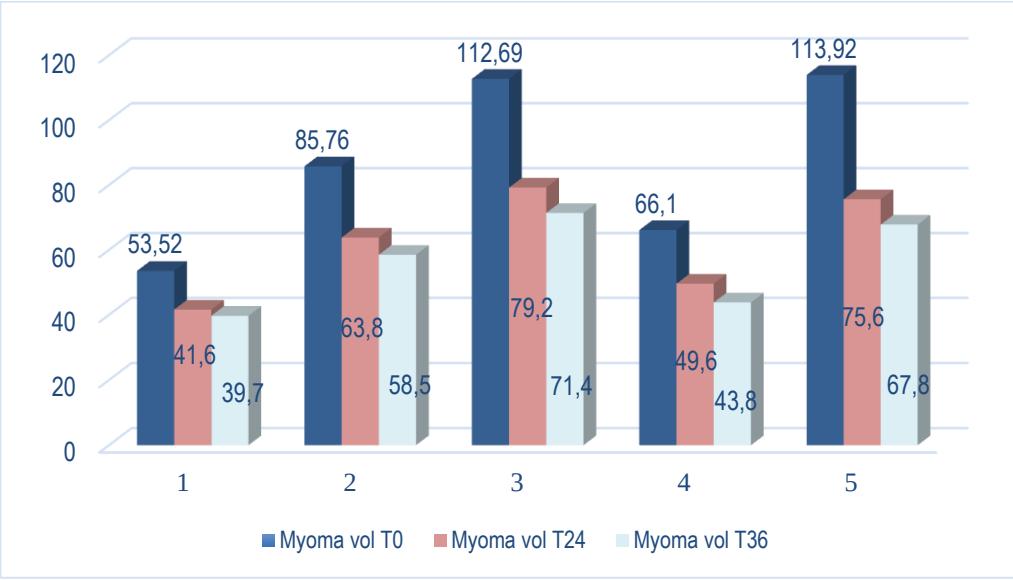

Fig. 2. Volume reduction of the largest uterine leiomyoma expressed in cm^3 at 24 and respectively 36 weeks from the beginning (T0) of combined therapy with relugolix.

TABLE II.

Study results evaluated at the beginning of treatment (T0), after 24 weeks (T24) of combined therapy, and after 36 weeks (T36) from the enrolment.

	TIME OF EVALUATION		
CHARACTERISTICS	**T0**	**T24**	**T36**
Uterine volume (cm^3)	381.04 ± 103.01	321.4 ± 83.4	306.5 ± 82.09
Volume of the largest uterine myoma (cm^3)	86.39 ± 27.12	61.96 ± 16.23	56.24 ± 14.11
Hemoglobin concentration (g/dL)	7.4 ± 1.14	9.54 ± 0.24	9.86 ± 0.19
Values are expressed as mean ± SD			

The treatment was well tolerated by the patients, none of them reported significant side effects.

DISCUSSION

Current studies regarding the conservative treatment of highly symptomatic uterine leiomyoma focus on the use of relugolix. This is a GnRH receptor antagonist that can be administered in daily doses and has the effect of blocking the binding and signaling of endogenous GnRH and thus leading to reversible, dose-dependent decreases in gonadotropin concentrations and subsequent suppression of the ovarian production of estradiol and progesterone.

Following two international studies, LIBERTY 1 and LIBERTY 2, that used combined therapy (relugolix, estradiol and norethindrone acetate) with daily administration, a significant decrease in menstrual bleeding was observed compared to the placebo group (12). Specifically, according to Al-Hendy *et al*, more than 70% of wom-

en with heavy uterine bleeding enrolled have reported a significant reduction of menstrual bleeding. Compared with placebo group, the mean reduction of menstrual blood loss was as much as 84.3% (12). Despite the fact that we did not used an objective method to quantify the amount of menstrual blood lost, the subjective assessment based on number of pads or tampons used in the heaviest day of menstrual bleeding suggests the same positive effect of the therapy.

The reduction of significant vaginal bleeding along with specific hematological treatment should eventually correct the anemic syndrome and thus, assuring a better physical condition for the patients. As we found in our study, other reports from the literature emphasize the significant increase in hemoglobin levels after relugolix combined therapy. In a recent observational study, Muzii *et al.* reported an increase in hemoglobin levels by 25% after 3 months of combined therapy, from 9.3 ± 1.1 to 11.6 ± 1.7 g/dL (14).

Moreover, after 24 weeks of relugolix combined therapy, patients should experience not only an improvement in the quality of life by reducing symptoms related to pelvic pain, heavy menstrual bleeding, oxidative stress but also an improvement in sexual life (12).

As in case of GnRH agonists (15, 16, 17), the use of GnRH antagonists with a concomitant hormonal add-back therapy has the same effect in reduction of uterine as well as myoma volumes but with less secondary effects associated with hypoestrogenism (14).

In a recent multicentric observational study published by Muzii *et al.*, the reduction of mean uterine volume after 3 months of combined relugolix therapy was as much as 26%. In addition, the reduction in diameter of the largest myoma was noted as much as 14% (14).

Although limited by the number of cases, our study reports near similar results. The mean uterine volume decreased from 381.04 ± 103.01 cm^3 at the enrolment to 321.4 ± 83.4 cm^3 after 24 weeks of combined treatment, corresponding to a 15.6% decrease, and again to 306.5 ± 82.09 after another 12 weeks of surveillance, corresponding to a 19.56% decrease. Moreover, the mean largest myoma volume decreased from 86.39 ± 27.12 cm^3 at the enrolment to 61.96 ± 16.23 cm^3 after 24 weeks of treatment, corresponding to a 28.27% decrease, and again to 56.24 ± 14.11 cm^3 after another 12 weeks of surveillance, corresponding to a 34.89% decrease. Despite the fact that reductions in uterine as well as in myoma`s volume were recorded in all patients, the most important degree of reduction noticed in our group was in patients with the largest uterus and largest myomas.

Moreover, the more significant degree of reduction of the uterine myomas when compared to the degree of reduction of global uterine volume could be explained by the very well-known facts that both estradiol and progesterone are essential for myoma development and these tumors are more responsive to sex steroids than normal myometrium (18).

We are in consensus with other recent reports from the literature on the fact that relugolix combined therapy could be used as a presurgical treatment as well, in order to reduce the volume of the uterus and myomas and thus to enable an improved surgical field, a less invasive surgical procedure or even postpone or amend the surgical treatment at all. In a recent study

from the literature, from the total number of 31 patients scheduled for surgery because of abnormal uterine bleeding associated with uterine myomas enrolled, in 4 patients (13%) the surgical procedure was shifted after 3 months of relugolix combined therapy to a less invasive approach (hysteroscopic myomectomy and laparoscopic hysterectomy) whereas in 8 patients (26%) the surgical treatment was temporarily avoided in favor of medical therapy for another 3 additional months (14).

Recent trials focus on efficacy and safety of long-term relugolix combined therapy. In a recent report published by Al-Hendy *et al*, from a total of 610 patients who were enrolled and completed LIBERTY trials, a number of 363 patients completed an additional 28 weeks of relugolix combined therapy (19). The reported results were very good and encouraging. 87.7% of patients with continuous 52 weeks of combined therapy had a sustained improvement in menstrual bleeding. Additionally, long-term combined therapy was also associated with a sustained reduction of other uterine leiomyoma symptoms and with a modest but continued reduction of uterine and leiomyoma volumes at week 52 when compared with week 24 of treatment (19). According to the same study, long-term relugolix combined therapy was generally well tolerated, headaches and hot flushes being the most frequent reported side effects (19).

CONCLUSIONS

Relugolix combined therapy proved to be very effective in patients with heavy vaginal bleeding associated with uterine leiomyomas. Clinical symptoms and anemic syndrome are significantly reduced after 24 weeks of treatment and maintained so even 12 weeks after. This therapy should always be taken into consideration in highly symptomatic leiomyoma patients who do not agree or are not suitable with surgical management or as preoperative medical treatment in order to reduce the uterine and myomas volumes and thus assuring a safer and perhaps more conservative approach. Despite our little number of cases, the results are similar to other reports from the literature. More studies have to be done in order to establish the efficacy and safety of long-term treatment.

CONFLICT OF INTEREST AND FUNDING

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A STUDY ON PHYSICIANS' ATTITUDES DURING THE RESOLUTION PROCESS OF MEDICAL MALPRACTICE COMPLAINTS

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A STUDY ON PHYSICIANS' ATTITUDES DURING THE RESOLUTION PROCESS OF MEDICAL MALPRACTICE COMPLAINTS (Abstract): Complaints filed against physicians represent an increasingly debated issue in both the medical and legal fields. In recent years, there has been a marked rise in the number of complaints submitted against healthcare professionals, which has contributed to a growing interest in the study of this phenomenon. The aim of our research was to explore and understand physicians' attitudes throughout the process of resolving medical malpractice complaints. **Materials and methods:** To achieve this objective, we conducted a qualitative study using semi-structured interviews. The participants were lawyers with substantial experience in handling medical malpractice cases. **Results:** A total of 14 lawyers participated in the study. Thematic analysis of the collected data led to the identification of 9 themes and 21 subthemes. This article focuses on the attitudes displayed by physicians during the malpractice complaint resolution process. According to the lawyers interviewed, physicians generally exhibited a negative attitude during procedures related to malpractice claims. This behavior is attributed to several factors, including the absence of a well-structured legal framework and the psychological pressure associated with involvement in judicial proceedings related to professional liability in healthcare. **Conclusions:** Our findings suggest the need for a mechanism that supports both the patient and the physician, enabling the latter to adopt a more open and constructive approach during professional liability procedures. Such a system could facilitate the resolution of medical malpractice complaints in a manner that minimizes the emotional and professional impact on all parties involved. **Keywords:** MEDICAL MALPRACTICE, PHYSICIANS, LAWYERS, QUALITATIVE RESEARCH.

INTRODUCTION

Medical errors are considered inevitable events within the practice of medicine (1), often generating consequences in the realm of professional liability. According to a study conducted in the United States in 2024, 38.2% of physicians surveyed admitted to having been involved in disputes related to medical malpractice within the past decade (2)

Legislation provides protection for patients (3). Safeguarding patient rights is a natural and necessary aspect of medical practice, as it reinforces physician accountability within the therapeutic relationship. At times, treatments may not yield the

expected outcomes, resulting in harm or injury to the patient.

Medical malpractice has increasingly become a subject of interest among scholars and professionals (4). According to data from the literature, involvement in legal proceedings is an especially stressful experience for physicians (5), which may help explain certain attitudes adopted by doctors during the resolution of malpractice complaints filed against them.

The aim of our study was to explore and identify the attitudes of physicians throughout the process of reviewing and resolving medical malpractice complaints submitted by patients or their families.

MATERIALS AND METHODS

To achieve the aim of this research, we employed a qualitative research methodology. Data were collected through semi-structured interviews. The target population consisted of lawyers who had handled at least one case involving a medical malpractice complaint.

Both male and female participants were included in the study, with no selection criteria based on years of experience in the profession, as this variable was not considered relevant to our research objectives.

Prior to participating, all respondents were informed about the purpose, objectives, and procedures of the study and provided written informed consent.

The initial part of the interview comprised introductory questions concerning the participants' legal specialization, their motivation for choosing this area of law, and their experience and number of cases handled in the field of medical malpractice litigation. The interview consisted of a set of 19 open-ended questions.

Among them were questions regarding physicians' attitudes during the resolution of malpractice complaints, as well as inquiries related to alternative dispute resolution methods in medical malpractice cases.

All interviews were fully transcribed and analyzed using *NVivo 14 software* (trial version). The responses were categorized into nine thematic areas, initially coded through a systematic review of the interview transcripts.

These codes were subsequently grouped into subthemes, which were identified based on their frequency and recurrence across multiple participants.

This study was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași, through Decision No. 135/23.01.2022.

RESULTS

A total of 14 lawyers participated in our study, 10 of whom were men and 4 women. Regarding their professional experience, 5 lawyers had less than 10 years of practice, 3 had between 11 and 15 years, while 6 had over 15 years of experience.

Out of the nine major themes identified through the analysis of the conducted interviews, this article focuses on participants' perspectives regarding physicians' attitudes throughout the process of reviewing and resolving medical malpractice complaints. Within this thematic category, five subthemes were extracted from the participants' responses, most of which reflected the perception of a predominantly negative attitude among medical professionals.

1. Denial and Lack of Accountability

Some respondents perceived that physi-

cians tend either to categorically deny any possibility of wrongdoing or attempt to shift responsibility onto others.

"The physician continuously rejected the accusations, without even attempting to engage in a dialogue to resolve the conflictual situation." (Participant A12)

2. Non-cooperation

Another perception expressed by the interviewed lawyers was that physicians often refuse to engage in the resolution process and instead adopt a collective defensive stance. This attitude may lead to litigation, which ultimately has negative consequences for both parties involved.

"The doctor isolates himself, doesn't respond to anything, refuses to communicate with anyone, and allows the institution to speak on his behalf. Inevitably, the case ends up in court, where things can become extremely complicated." (Participant A2)

3. Formal Attitude

Some participants described a formal, procedural attitude on the part of physicians and medical institutions-one that prioritizes strict adherence to protocols. Compliance with procedural rules is an essential component of any legal process. When legal norms are disregarded, sanctions may be imposed on the responsible parties. This procedural compliance is seen as a dual obligation, applying equally to both the complainant and the defendant.

"You receive a response on time; no one accuses you of suing a prominent physician. At least on a formal, statutory level, impartiality is maintained." (Participant A3)

4. Aggressive Attitude

Some lawyers reported having perceived the attitude of physicians or their legal representatives as overtly aggressive.

"The doctor's lawyer came to the mediation session and almost verbally assaulted my client. We were all present, and she told my client, 'You, shameless woman, you're faking illness just to get medical leave" (Participant A14)

5. Delay and Concealment of the Truth

Several respondents observed that the conduct of medical personnel sometimes approached the boundaries of legality, potentially obstructing the course of justice. While the right to defense includes the accused party's ability to invoke the right to remain silent, such rights are not absolute. In cases where evidence is destroyed or altered, legal mechanisms exist to ensure accountability.

"In other instances, the hospital supporting the accused doctor offered illegitimate assistance. 'Oh, you didn't fill out the medical chart properly? Let's fix that now.' And they start tampering with the patient's records, forgetting that the patient had obtained a copy a year earlier… which only leads them into deeper legal trouble." (Participant A6)

DISCUSSION

The interviewed lawyers generally perceived the attitude of medical professionals during the resolution process of malpractice complaints as negative. In a study conducted in the United States in 2000, it was found that 50% of the physicians surveyed believed they bore no responsibility for the harm claimed by the complainant. In other words, respondents stated that they had made no error (6). Other studies investigating physicians' attitudes during malpractice complaint procedures have focused on the emotional experiences of healthcare professionals, identifying reactions such as shock,

fear, anger, anxiety, and frustration (7).

1. Denial and Lack of Accountability

In line with the opinions expressed during our interviews, both the lawyers' testimonies and the existing literature emphasize that medical malpractice proceedings are subject to significant debate in many countries worldwide (8). Stressful emotional events play a substantial role in the vulnerability of physicians involved in malpractice complaints (7). A related form of avoidance of responsibility identified in the literature is the practice of defensive medicine, whereby physicians refuse to treat certain patients (9) due to fear of potential malpractice claims.

2. Non-cooperation

The issue of non-cooperation was repeatedly highlighted by the lawyers interviewed for this study. According to the literature, such behavior is common among physicians. Many justify their reluctance to share information based on a paradoxical fear that anything disclosed could later be used against them during malpractice proceedings (10). Furthermore, studies have noted that non-cooperation may stem from a reluctance among some doctors to critique the professional conduct of their colleagues (11).

The statements made by our interview participants are thus corroborated by existing research. Physicians tend to believe that their professional accountability should be framed differently from that asserted by legal professionals. This fundamental disagreement fosters non-cooperative behavior, ultimately undermining the resolution process of malpractice complaints (6). It is also worth noting that this attitude may manifest not only during legal proceedings but also throughout the broader physician-patient relationship (12).

3. Formal Attitude

The lawyers interviewed in our study stated that physicians tended to adopt a formal attitude during the process of resolving malpractice complaints, with a strong emphasis on procedural compliance. In line with academic literature, healthcare professionals often adopt a closed and hierarchical communication style, as they believe such an approach shields them from liability in cases of medical malpractice (13).

Moreover, the formal demeanor exhibited by physicians, as discussed in the scholarly literature, is frequently shaped by the inherent formalism of legal accountability mechanisms concerning unlawful acts (14).

4. Aggressive Attitude

According to the opinions expressed by the interviewed lawyers, physicians displayed an aggressive attitude during the resolution process of malpractice complaints, despite the fact that the scholarly literature advises against such behavior (15). In a study identified in the literature, among the emotions reported by physician's subject to malpractice claims were irritability and indignation (5); however, no reference was made to overt aggression.

5. Delay and Concealment of the Truth

With regard to the lawyers' claims concerning delays and the concealment of the truth, the academic literature suggests that the current medical culture tends to discourage disclosure (16). This tendency is largely attributed to prevailing professional pressures within the medical field (17). The concealment of truth by physicians is also addressed from the perspective of medical ethics, where such behavior is sometimes justified by the intention

to avoid causing anxiety in patients or creating confusion due to the complexity of the information that must be communicated (18).

Moreover, scholarly literature refers to a phenomenon known as the "conspiracy of silence" (19), which occurs when physicians refrain from disclosing information about medical errors. This behavior-characterized by postponement and non-disclosure-may also be the unintended consequence of the way certain legal norms are formulated. Ambiguities in legal interpretation may inadvertently encourage physicians to remain silent, thereby influencing the resolution process of complaints submitted by patients (20).

CONCLUSIONS

Our study highlighted that lawyers tend to hold a predominantly negative perception of physicians' attitudes during the process of resolving medical malpractice complaints. The analysis we conducted revealed a lack of openness and cooperation on the part of physicians, manifested in various ways depending on the specific case.

The shortcomings of the current legal framework do little to encourage physicians to adopt a different approach. Procedures related to medical malpractice liability are often complex and burdensome, placing considerable pressure on healthcare professionals.

Our findings suggest the need for a mechanism that benefits both patients and physicians-one that fosters a more transparent and constructive approach from medical professionals in professional liability proceedings. Such a mechanism would facilitate the resolution of malpractice complaints in a more efficient manner, while minimizing the psychological and procedural impact on all parties involved.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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INVESTIGATION OF JOB SATISFACTION AMONG PHARMACISTS IN IAȘI COUNTY, ROMANIA: A QUANTITATIVE ANALYSIS

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INVESTIGATION OF JOB SATISFACTION AMONG PHARMACISTS IN IAȘI COUNTY, ROMANIA: A QUANTITATIVE ANALYSIS (Abstract) **Aim**: Assessment of pharmacists' satisfaction with employers, working conditions, productivity, and their level of engagement in organizational activities. **Materials and methods**: The questionnaire, designed by academic staff of the Faculty of Pharmacy in collaboration with practicing pharmacists and pharmacy managers, comprised 23 questions with either open-ended or pre-formulated responses. It was completed by licensed pharmacists employed at various pharmaceutical units, both in public institutions and private ones, in Iași County. The questionnaire was administered online via a digital survey platform and in printed form to facilitate completion by pharmacists. **Results**: According to the obtained data, we found that the degree of agreement of the respondents regarding the level of job satisfaction is quite high (over 80%). A good communication in the pharmacy was indicated by 83.3% of the respondents, but 46% of the pharmacists consider that they are not remunerated at the desired level for the additional work performed, which may put the organization at risk in terms of workforce. Only 40% of respondents are satisfied with their choice of the pharmacist profession, which reflects the fact that there may be improvements regarding the possibility of increasing job satisfaction. **Conclusions**: Effective managerial communication is crucial for optimizing organizational structures and processes, adapting to dynamic markets and employee needs, and an annual internal communication audit could significantly enhance engagement and satisfaction within pharmaceutical units. **Keywords**: SATISFACTION, PHARMACIST, QUESTIONNAIRE, COMMUNICATION, MANAGEMENT, PATIENT.

INTRODUCTION

It is known that the medical-pharmaceutical field includes professions that rely heavily on communication, being one of the most relevant indicators of medical competence. The motivation for choosing this topic lies in the importance that must be given to the social side, the interaction between employees in the medical-pharmaceutical field. Studies in recent years show that good communication between employees can have a beneficial impact on the patient's health, as well as on increasing the degree of patient safety and satisfaction. However, education in Romanian Medical and Pharmaceutical Universities places too little emphasis on communication, and students/residents do not benefit from training in this regard (1-4).

The need for communication is obvious and necessary, as are the patients' demands to receive much better structured information. In this regard, it is important for medical personnel to practice more the

process of communication and relationships both among themselves and with patients. This involves knowledge, the ability to obtain information, and interpersonal relationship capacity (5-7).

The process of providing health care services must be in accordance with the patient's requirements. Achieving such a goal involves identifying all the connections between healthcare personnel and beneficiaries of medical services. Unfortunately, the environment in which the pharmaceutical and medical act is carried out is not always conducive to communication. The causes are numerous and include: lack of time, overcrowding, cultural differences, anxiety on the part of medical and pharmaceutical personnel and patients, fear of conflict and complaints, unrealistic expectations (8).

On the other side, employee satisfaction is the level of satisfaction that team members have with the roles they fulfill within the company, the activity they carry out, but also the satisfaction with certain professional relationships found in the organization (9-11). If you know how to increase or maintain employee satisfaction at a high level, organizational development can take place in the long term.

In this paper, we focused on job satisfaction of pharmacists in pharmaceutical units. In order to provide a broader perspective on the current state of the relationship between members of a pharmaceutical organization, we analyzed the factors that are considered important in the development of the communication process.

MATERIALS AND METHODS

We have created a questionnaire, through which we can observe the satisfaction of pharmacists with the employer, with working conditions, as well as the productivity and degree of involvement of pharmacists in organizational activities. A high degree of satisfaction of employees (internal customers of the organization) directly determines a high level of satisfaction from patients/consumers (external customers of the organization) (12-17).

The prospective cross-sectional study has as its research method a questionnaire-type survey carried out among pharmacists in community pharmacies in our geographical area, which was carried out over a period of 3 months (January-March 2025). This study was carried out in two phases: the development and validation of the questionnaire, and the evaluation of level satisfaction of the pharmacists. The time required to answer the questionnaire items was approximately 10 min. The questionnaire, designed by academic staff of the Faculty of Pharmacy in collaboration with practicing pharmacists and pharmacy managers, comprised 23 questions with either open-ended or pre-formulated responses. It was completed by licensed pharmacists employed in various pharmaceutical units, both in public and private institutions, in Iași County. The questionnaire was administered online via a digital survey platform and in printed form to facilitate completion by pharmacists (18-24).

Participants did not receive any material compensation for their participation in the research. The questions were designed to give us a comprehensive picture of pharmacists' job satisfaction. A total of 150 pharmacists of different ages, genders and training levels completed the questionnaire, all of whom completed the questionnaire were thus considered eligible. The results were analyzed and statistically interpreted. It was imperative to anonymized the collected data and adequately protect the identity of the subjects. Personal information was separated from the questionnaire responses to ensure confidentiality.

Subjects were thoroughly informed about the purpose of the study, how their responses would be used, who would have access to the data, and the duration of data retention. Additionally, explicit consent was obtained from participants for the collection and use of their data, with special emphasis on sensitive data. The entire study was conducted in accordance with the principles set forth in the Declaration of Helsinki, Law No. 190/2018, and Law No. 506/2004 regarding the processing of personal data and the protection of privacy in the electronic communications sector.

RESULTS

General characterization of the group. The study group is mainly composed of pharmacists who work in open-circuit pharmacies (82.7%) and who are permanently employed full-time (87.3%). The majority are pharmacists without additional specializations (71.3%), with over 6 years of experience in the same pharmacy (51.3%). About 64.6% of pharmacists have over 6 years of experience in the profession and three quarters of them (72.7%) have previously completed questionnaires about job satisfaction (tab. I).

TABLE I.
General characteristics of the study group

Question		n	%
1. Do you work in a pharmacy?	Closed-circuit	26	17.3
	Open-circuit	124	82.7
2. Do you work under contract in a pharmacy?	Full-time fixed-term	2	1.3
	Full-time permanent	131	87.3
	Part-time fixed-term	2	1.3
	Part-time permanent	6	4.0
	Temporary/Seasonal	9	6.0
3. Your position in the pharmacy is:	Pharmacist	107	71.3
	Pharmacist with a Ph.D.	3	2.0
	Primary pharmacist	3	2.0
	Resident pharmacist	13	8.7
	Specialist pharmacist	24	16.0
4. I have been working in this pharmacy for:	Less than 1 year	23	15.3
	1-5 years	50	33.4
	Over 6 years	77	51.3
5. My experience as a pharmacist is:	Less than 1 year	12	8.0
	1-5 years	41	27.3
	6-10 years	47	31.3
	Over 11 years	50	33.3
6. This is the first time I have completed a questionnaire about job satisfaction as a pharmacist:	No	109	72.7
	Yes	41	27.3
Total		150	100.0

The general analysis of the responses. This analysis shows that the degree of agreement of the respondents regarding the level of job satisfaction is quite high. The highest percentages of agreement with the proposed questions (over 80%) refer to the work schedule, with which 89.3% of the respondents agree, to the observance of the

rules imposed by the pharmacy superiors, with which 87.3% of the respondents also agree, and to the good communication in the pharmacy, indicated by 83.3% of the respondents. Over 70% of the respondents consider that they have good relations with the rest of the pharmacy staff, the tasks in the pharmacy are well explained to them, the mistakes they make are discussed and analyzed, they feel respected by their colleagues and the pharmaceutical unit is oriented towards the good of the patients. In contrast, pharmacists tend to be dissatisfied with the fact that they have not been promoted in the pharmacy where they work, 44% of them mentioning this aspect, and that the remuneration they receive is not equivalent to the work done (46% of respondents noted this aspect). Only 40% of respondents were rewarded for additional work in the pharmacy. The general degree of satisfaction of respondents with the profession of pharmacists is not very high, however only 40% of respondents are generally satisfied with the career choice made, 35.3% expressed their disagreement and a quarter (24.7%) are neutral on this subject (tab. II).

TABLE II.

Questionnaire responses-general results

Question	Agree		Disagree		Neutral		Total	
	n	%	n	%	n	%	n	%
7. I am satisfied with the profession of pharmacist that I perform.	92	61.3	36	24.0	22	14.7	150	100.0
8. My superiors appreciate my work in the pharmacy.	100	66.7	24	16.0	26	17.3	150	100.0
9. I consider that I have good relations with the rest of the pharmacy staff.	110	73.3	24	16.0	16	10.7	150	100.0
10. I have an adequate work schedule in the pharmacy where I work.	134	89.3	3	2.0	13	8.7	150	100.0
11. Communication in the pharmacy is good.	125	83.3	12	8.0	13	8.7	150	100.0
12. The tasks in the pharmacy are well explained.	110	73.3	24	16.0	16	10.7	150	100.0
13. I am periodically evaluated in the pharmacy.	98	65.3	22	14.7	30	20.0	150	100.0
14. The pharmaceutical unit is oriented to the benefit of the patient.	105	70.0	13	8.7	32	21.3	150	100.0
15. I am appreciated in the pharmacy.	98	65.3	12	8.0	40	26.7	150	100.0
16. I have been promoted in the pharmacy where I work.	37	24.7	66	44.0	47	31.3	150	100.0
17. The errors of the pharmacy staff are discussed.	114	76.0	16	10.7	20	13.3	150	100.0
18. The rules imposed by the superiors in the pharmacy are respected.	131	87.3	7	4.7	12	8.0	150	100.0
19. The remuneration for the pharmacist position is equivalent to the work performed.	59	39.3	69	46.0	22	14.7	150	100.0
20. I feel respected at the pharmacy by my colleagues.	113	75.3	12	8.0	25	16.7	150	100.0
21. I feel respected at the pharmacy by the patients.	99	66.0	12	8.0	39	26.0	150	100.0
22. I am rewarded for the additional work in pharmacy.	60	40.0	36	24.0	54	36.0	150	100.0
23. Overall, I am satisfied with the chosen profession of pharmacist.	60	40.0	53	35.3	37	24.7	150	100.0

Comparative study by type of pharmacy. In general, there are no statistically significant differences between pharmacists' responses regarding the degree of professional satisfaction in open-circuit pharmacies compared to closed-circuit ones. The degree of agreement differs by less than 5% between the responses in the case of items 7 and 15, and the only item for which statistically significantly different responses were recorded between the 2 categories of professionals was item 12, 75% of pharmacists working in open-circuit pharmacies consider that the tasks in the pharmacy are well explained to them compared to only 65.4% of those working in closed-circuit pharmacies. Of these, 7.7% consider that the tasks in the pharmacy are not properly explained to them and 26.9% are neutral, while in the case of open-circuit pharmacies only 7.3% are neutral, the remaining 17.7% considering that the tasks in the pharmacy are not properly explained to them. More than three-quarters of pharmacists working in closed-circuit pharmacies agree with the statements made in items 9, 10, 17, 18 and 20. Less than half agree with the statements made in items 16, 19, 22 and 23. Similarly, more than three quarters of pharmacists working in open-circuit pharmacies agree with the statements made in items 10, 11, 12, 17, 18 and 20, while less than half agree with the statements made in items 16, 19, 22 and 23. Regarding item 23, the general level of satisfaction with the chosen profession, it is relatively low, only 30.8% of pharmacists working in closed-circuit pharmacies are satisfied with their chosen profession, as are 41.9% of those working in open-circuit pharmacies, and the share of pharmacists dissatisfied with their chosen profession is 46.2% of those working in closed-circuit pharmacies, almost half, compared to only 33.1% of those working in open-circuit pharmacies, one third (tab. III).

TABLE III.
Questionnaire responses by type of pharmacy

Question		Pharmacy type				Pearson Chi-squared p-value
		Closed-circuit		Open-circuit		
		N	%	N	%	
7. I am satisfied with the profession of pharmacist that I perform.	agree	17	65.4%	75	60.5%	0.821
	disagree	5	19.2%	31	25.0%	
	neutral	4	15.4%	18	14.5%	
8. My superiors appreciate my work in the pharmacy.	agree	15	57.7%	85	68.5%	0.489
	disagree	6	23.1%	18	14.5%	
	neutral	5	19.2%	21	16.9%	
9. I consider that I have good relations with the rest of the pharmacy staff.	agree	20	76.9%	90	72.6%	0.442
	disagree	5	19.2%	19	15.3%	
	neutral	1	3.8%	15	12.1%	
10. I have an adequate work schedule in the pharmacy where I work.	agree	21	80.8%	113	91.1%	0.296
	disagree	1	3.8%	2	1.6%	
	neutral	4	15.4%	9	7.3%	
11. Communication in the pharmacy is good.	agree	19	73.1%	106	85.5%	0.108
	disagree	2	7.7%	10	8.1%	
	neutral	5	19.2%	8	6.5%	

Question		Pharmacy type				Pearson Chi-squared p-value
		Closed-circuit		Open-circuit		
		N	%	N	%	
12. The tasks in the pharmacy are well explained.	agree	17	65.4%	93	75.0%	0.009**
	disagree	2	7.7%	22	17.7%	
	neutral	7	26.9%	9	7.3%	
13. I am periodically evaluated in the pharmacy.	agree	18	69.2%	80	64.5%	0.439
	disagree	5	19.2%	17	13.7%	
	neutral	3	11.5%	27	21.8%	
14. The pharmaceutical unit is oriented towards the benefit of the patient.	agree	19	73.1%	86	69.4%	0.628
	disagree	1	3.8%	12	9.7%	
	neutral	6	23.1%	26	21.0%	
15. I am appreciated in the pharmacy.	agree	17	65.4%	81	65.3%	0.998
	disagree	2	7.7%	10	8.1%	
	neutral	7	26.9%	33	26.6%	
16. I have been promoted in the pharmacy where I work.	agree	5	19.2%	32	25.8%	0.727
	disagree	13	50.0%	53	42.7%	
	neutral	8	30.8%	39	31.5%	
17. The errors of the pharmacy staff are discussed.	agree	21	80.8%	93	75.0%	0.457
	disagree	1	3.8%	15	12.1%	
	neutral	4	15.4%	16	12.9%	
18. The rules imposed by the superiors in the pharmacy are respected.	agree	22	84.6%	109	87.9%	0.162
	disagree	0	0.0%	7	5.6%	
	neutral	4	15.4%	8	6.5%	
19. The remuneration for the pharmacist position is equivalent to the work performed.	agree	6	23.1%	53	42.7%	0.174
	disagree	15	57.7%	54	43.5%	
	neutral	5	19.2%	17	13.7%	
20. I feel respected at the pharmacy by my colleagues.	agree	21	80.8%	92	74.2%	0.344
	disagree	3	11.5%	9	7.3%	
	neutral	2	7.7%	23	18.5%	
21. I feel respected at the pharmacy by the patients.	agree	14	53.8%	85	68.5%	0.274
	disagree	2	7.7%	10	8.1%	
	neutral	10	38.5%	29	23.4%	
22. I am rewarded for the additional work in pharmacy.	agree	8	30.8%	52	41.9%	0.568
	disagree	7	26.9%	29	23.4%	
	neutral	11	42.3%	43	34.7%	
23. Overall, I am satisfied with the chosen profession of pharmacist.	agree	8	30.8%	52	41.9%	0.418
	disagree	12	46.2%	41	33.1%	
	neutral	6	23.1%	31	25.0%	
Total		26	100.0%	124	100.0%	

The degree of satisfaction of pharmacists working in open-circuit pharmacies. We investigated in this subgroup of 124 pharmacists working in open-circuit pharmacies their degree of job satisfaction.

General characteristics of the subgroup. The vast majority of pharmacists in this category are permanent full-time employees (87.1%), depending on the pharmacist (69.4%) and have over 5 years of experience in the profession (61.3%) and over 5 years in the same pharmacy (50.0%) (tab. IV).

TABLE IV.
General characteristics of the investigated subgroup

Question		n	%
2. Do you work under contract in a pharmacy?	Full-time fixed-term	2	1.6
	Full-time permanent	108	87.1
	Part-time fixed-term	2	1.6
	Part-time permanent	4	3.2
	Temporary/Seasonal	8	6.5
3. Your position in the pharmacy is:	Pharmacist	86	69.4
	Pharmacist with a Ph.D.	2	1.6
	Primary pharmacist	2	1.6
	Resident pharmacist	12	9.7
	Specialist pharmacist	22	17.7
4. I have been working in this pharmacy for:	Less than 1 year	20	16.1
	1-5 years	42	33.9
	Over 6 years	62	50.0
5. My experience as a pharmacist is:	Less than 1 year	11	8.9
	1-5 years	37	29.8
	6-10 years	36	29.0
	Over 11 years	40	32.3
6. This is the first time I have completed a questionnaire about job satisfaction as a pharmacist:	No	92	74.2
	Yes	32	25.8
Total		124	100.0

Comparative study by position in pharmacy. In a first stage, we investigated the degree of professional satisfaction compared to the current position in pharmacy, which differentiates pharmacists without specializations from those with primary and possibly Ph.D. degrees, specialists or residents. In general, no statistically significant differences were found between these 4 categories of professionals in terms of the degree of professional satisfaction. Three quarters of primary or Ph.D. pharmacists (75%) are satisfied with the profession, compared to, for example, only half (50%) of specialist pharmacists. Resident and specialist pharmacists feel the most appreciated by their superiors (more than three quarters of them). Specialist pharmacists also consider that they have the best professional relations with the rest of the pharmacy staff (77.3%), as do those without specialization (74.4%). The vast majority of pharmacists are satisfied with their working hours and also with the communication in the pharmacy, which seems to be slightly more deficient only in the case of primary or Ph.D. pharmacists, of whom 25% are not satisfied with this aspect. The vast majority of specialist pharmacists (86.4%) consider that the tasks in the pharmacy are well explained to them, compared to only 66.7% of residents. On the other hand, almost all residents (91.7%) consider that they are periodically evaluated in the pharmacy, compared to just over half of the other categories of pharmacists. The vast majority of specialist pharmacists (81.8%) also consider that the pharmacy in which they work is oriented towards the benefit of patients, unlike about half of the other categories of pharmacists, a difference that is at the limit of statistical significance. All primary or

Ph.D. pharmacists feel appreciated in the pharmacy, compared to only 33.3% of resident pharmacists. Half of primary or Ph.D. pharmacists were promoted, compared to a third of residents and only 4.5% of specialist pharmacists, a discrepancy that also lies at the threshold of statistical significance. Three quarters of pharmacists, regardless of their professional position, believe that the mistakes they make at work are discussed appropriately and all resident pharmacists, like primary or Ph.D. pharmacists, believe that the rules imposed by superiors are respected. 75% of primary or Ph.D. pharmacists believe that the remuneration they receive is proportional to the work performed, compared to only 18.2% of specialist pharmacists and about half of the others in the other 2 categories, a difference that is also important and located near the threshold of statistical significance. As expected, all primary or Ph.D. pharmacists feel respected by their colleagues, as do 3/4 of the other categories of pharmacists. About 3/4 of pharmacists also feel respected by patients, most of them being resident pharmacists, of whom 83.3% feel respected by patients compared to only 66.3% of pharmacists without additional specialization. Only a quarter of primary or Ph.D. pharmacists believe that they are rewarded for the additional work they do in pharmacy, compared to 58.3% of resident pharmacists. In terms of overall satisfaction, three quarters of primary or Ph.D. pharmacists feel satisfied with their chosen profession, as do half of residents and 40% of specialist or non-specialized pharmacists (tab. V).

TABLE V.

Questionnaire responses by position in pharmacy

Question		Your position in the pharmacy is:								Pearson Chi-squared
		Pharmacist		Primary pharmacist / with Ph.D.		Resident pharmacist		Specialist pharmacist		
		N	%	N	%	N	%	N	%	p-value
7. I am satisfied with the profession of pharmacist that I perform.	agree	54	62.8%	3	75.0%	7	58.3%	11	50.0%	0.470
	disagree	22	25.6%			4	33.3%	5	22.7%	
	neutral	10	11.6%	1	25.0%	1	8.3%	6	27.3%	
8. My superiors appreciate my work in the pharmacy.	agree	56	65.1%	2	50.0%	9	75.0%	18	81.8%	0.308
	disagree	13	15.1%			2	16.7%	3	13.6%	
	neutral	17	19.8%	2	50.0%	1	8.3%	1	4.5%	
9. I consider that I have good relations with the rest of the pharmacy staff.	agree	64	74.4%	2	50.0%	7	58.3%	17	77.3%	0.073+
	disagree	9	10.5%	1	25.0%	5	41.7%	4	18.2%	
	neutral	13	15.1%	1	25.0%			1	4.5%	
10. I have an adequate work schedule in the pharmacy where I work.	agree	80	93.0%	4	100.0%	11	91.7%	18	81.8%	0.435
	disagree	2	2.3%							
	neutral	4	4.7%			1	8.3%	4	18.2%	

Question		Your position in the pharmacy is:								Pearson Chi-squared
		Pharmacist		Primary pharmacist / with Ph.D.		Resident pharmacist		Specialist pharmacist		
		N	%	N	%	N	%	N	%	p-value
11. Communication in the pharmacy is good.	agree	73	84.9%	3	75.0%	11	91.7%	19	86.4%	0.786
	disagree	7	8.1%	1	25.0%	1	8.3%	1	4.5%	
	neutral	6	7.0%					2	9.1%	
12. The tasks in the pharmacy are well explained.	agree	63	73.3%	3	75.0%	8	66.7%	19	86.4%	0.531
	disagree	15	17.4%	1	25.0%	4	33.3%	2	9.1%	
	neutral	8	9.3%					1	4.5%	
13. I am periodically evaluated in the pharmacy.	agree	53	61.6%	2	50.0%	11	91.7%	14	63.6%	0.523
	disagree	12	14.0%	1	25.0%	1	8.3%	3	13.6%	
	neutral	21	24.4%	1	25.0%			5	22.7%	
14. The pharmaceutical unit is oriented towards the benefit of the patient.	agree	58	67.4%	2	50.0%	8	66.7%	18	81.8%	0.092+
	disagree	7	8.1%	2	50.0%	2	16.7%	1	4.5%	
	neutral	21	24.4%			2	16.7%	3	13.6%	
15. I am appreciated in the pharmacy.	agree	59	68.6%	4	100.0%	4	33.3%	14	63.6%	0.113
	disagree	6	7.0%			3	25.0%	1	4.5%	
	neutral	21	24.4%			5	41.7%	7	31.8%	
16. I have been promoted in the pharmacy where I work.	agree	25	29.1%	2	50.0%	4	33.3%	1	4.5%	0.067+
	disagree	34	39.5%			7	58.3%	12	54.5%	
	neutral	27	31.4%	2	50.0%	1	8.3%	9	40.9%	
17. The errors of the pharmacy staff are discussed.	agree	64	74.4%	3	75.0%	9	75.0%	17	77.3%	0.941
	disagree	10	11.6%	1	25.0%	2	16.7%	2	9.1%	
	neutral	12	14.0%			1	8.3%	3	13.6%	
18. The rules imposed by the superiors in the pharmacy are respected.	agree	75	87.2%	4	100.0%	12	100.0 %	18	81.8%	0.488
	disagree	4	4.7%					3	13.6%	
	neutral	7	8.1%					1	4.5%	
19. The remuneration for the pharmacist position is equivalent to the work performed.	agree	40	46.5%	3	75.0%	6	50.0%	4	18.2%	0.077+
	disagree	32	37.2%	1	25.0%	6	50.0%	15	68.2%	
	neutral	14	16.3%					3	13.6%	

Question		Your position in the pharmacy is:								Pearson Chi-squared
		Pharmacist		Primary pharmacist / with Ph.D.		Resident pharmacist		Specialist pharmacist		
		N	%	N	%	N	%	N	%	p-value
20. I feel respected at the pharmacy by my colleagues.	agree	61	70.9%	4	100.0%	9	75.0%	18	81.8%	0.394
	disagree	5	5.8%			2	16.7%	2	9.1%	
	neutral	20	23.3%			1	8.3%	2	9.1%	
21. I feel respected at the pharmacy by the patients.	agree	57	66.3%	3	75.0%	10	83.3%	15	68.2%	0.730
	disagree	6	7.0%			1	8.3%	3	13.6%	
	neutral	23	26.7%	1	25.0%	1	8.3%	4	18.2%	
22. I am rewarded for the additional work in pharmacy.	agree	36	41.9%	1	25.0%	7	58.3%	8	36.4%	0.217
	disagree	16	18.6%	2	50.0%	2	16.7%	9	40.9%	
	neutral	34	39.5%	1	25.0%	3	25.0%	5	22.7%	
23. Overall, I am satisfied with the chosen profession of pharmacist.	agree	34	39.5%	3	75.0%	6	50.0%	9	40.9%	0.692
	disagree	31	36.0%			4	33.3%	6	27.3%	
	neutral	21	24.4%	1	25.0%	2	16.7%	7	31.8%	
Total		86	100.0%	4	100.0%	12	100.0 %	22	100.0 %	

Comparative study based on seniority at the pharmacy where they currently work. As expected, pharmacists with over 6 years of experience at the pharmacy where they currently work are the most satisfied with their profession (62.9%), although almost 60% of the other categories of pharmacists are also satisfied with their profession. The most appreciated by their superiors are pharmacists with seniority at the pharmacy where they currently work between 1 and 5 years (76.2% of them) and the least appreciated by their superiors are pharmacists with seniority less than 1 year, respectively 60% of them. Regardless of seniority, three quarters of pharmacists consider that they have good relations with their colleagues and almost all consider that the working hours are adequate and also that communication in the pharmacy is good. Also, 3/4 of pharmacists, regardless of seniority, consider that the service tasks are well explained and about 65% consider that they are evaluated periodically. 90% of pharmacists with less than one year of experience at the pharmacy where they currently work believe that the pharmacy is oriented towards the benefit of the patient, compared to only 65% of those with more experience at that pharmacy. The degree of appreciation in the pharmacy is similar for all categories of pharmacists, and 40% of those with less than one year of experience at that pharmacy have been recently promoted, compared to only 1/4 of the others. 85% of pharmacists with less than one year of experience at that pharmacy believe that the errors they make are dis-

cussed in the pharmacy, compared to only 69% of those with between 1 and 5 years of experience. The vast majority believe that the rules imposed by superiors are respected and just over half of pharmacists with between 1 and 5 years of experience at the pharmacy where they currently work believe that the remuneration received is equivalent to the work performed (54.8%), compared to only 1/4 of those with less than one year of experience. Pharmacists with less than one year of experience at the pharmacy where they currently work feel the least respected, with only 60% of them considering themselves respected by their colleagues and patients (55%). On the other hand, 60% of them consider themselves rewarded for the extra work they do, compared to about 38% of pharmacists with more experience. Half of pharmacists with less than one year of experience at the pharmacy where they currently work are generally satisfied with their chosen profession, compared to just over a third of those with between 1 and 5 years of experience at the pharmacy where they currently work (35.7%) and 43.5% of those with more than 6 years of experience (tab. VI).

TABLE VI.

Questionnaire responses according to seniority at the pharmacy where they currently work

Question		I have been working in this pharmacy for:						Pearson Chi-squared
		Less than 1 year		1-5 years		Over 6 years		
		N	%	N	%	N	%	p-value
7. I am satisfied with the profession of pharmacist that I perform.	agree	12	60.0%	24	57.1%	39	62.9%	0.613
	disagree	6	30.0%	9	21.4%	16	25.8%	
	neutral	2	10.0%	9	21.4%	7	11.3%	
8. My superiors appreciate my work in the pharmacy.	agree	12	60.0%	32	76.2%	41	66.1%	0.562
	disagree	3	15.0%	6	14.3%	9	14.5%	
	neutral	5	25.0%	4	9.5%	12	19.4%	
9. I consider that I have good relations with the rest of the pharmacy staff.	agree	15	75.0%	31	73.8%	44	71.0%	0.342
	disagree	5	25.0%	5	11.9%	9	14.5%	
	neutral			6	14.3%	9	14.5%	
10. I have an adequate work schedule in the pharmacy where I work.	agree	19	95.0%	35	83.3%	59	95.2%	0.259
	disagree			1	2.4%	1	1.6%	
	neutral	1	5.0%	6	14.3%	2	3.2%	
11. Communication in the pharmacy is good.	agree	18	90.0%	35	83.3%	53	85.5%	0.553
	disagree			5	11.9%	5	8.1%	
	neutral	2	10.0%	2	4.8%	4	6.5%	
12. The tasks in the pharmacy are well explained.	agree	14	70.0%	33	78.6%	46	74.2%	0.501
	disagree	3	15.0%	8	19.0%	11	17.7%	
	neutral	3	15.0%	1	2.4%	5	8.1%	
13. I am periodically evaluated in the pharmacy.	agree	12	60.0%	27	64.3%	41	66.1%	0.921
	disagree	4	20.0%	5	11.9%	8	12.9%	
	neutral	4	20.0%	10	23.8%	13	21.0%	
14. The pharmaceutical unit is oriented towards the benefit of the patient.	agree	18	90.0%	28	66.7%	40	64.5%	0.204
	disagree	1	5.0%	3	7.1%	8	12.9%	
	neutral	1	5.0%	11	26.2%	14	22.6%	

15. I am appreciated in the pharmacy.	agree	12	60.0%	28	66.7%	41	66.1%	0.293
	disagree	4	20.0%	3	7.1%	3	4.8%	
	neutral	4	20.0%	11	26.2%	18	29.0%	
16. I have been promoted in the pharmacy where I work.	agree	8	40.0%	10	23.8%	14	22.6%	0.225
	disagree	10	50.0%	17	40.5%	26	41.9%	
	neutral	2	10.0%	15	35.7%	22	35.5%	
17. The errors of the pharmacy staff are discussed.	agree	17	85.0%	29	69.0%	47	75.8%	0.468
	disagree	1	5.0%	8	19.0%	6	9.7%	
	neutral	2	10.0%	5	11.9%	9	14.5%	
18. The rules imposed by the superiors in the pharmacy are respected.	agree	18	90.0%	37	88.1%	54	87.1%	0.993
	disagree	1	5.0%	2	4.8%	4	6.5%	
	neutral	1	5.0%	3	7.1%	4	6.5%	
19. The remuneration for the pharmacist position is equivalent to the work performed.	agree	5	25.0%	23	54.8%	25	40.3%	0.245
	disagree	11	55.0%	14	33.3%	29	46.8%	
	neutral	4	20.0%	5	11.9%	8	12.9%	
20. I feel respected at the pharmacy by my colleagues.	agree	12	60.0%	32	76.2%	48	77.4%	0.024*
	disagree	5	25.0%	2	4.8%	2	3.2%	
	neutral	3	15.0%	8	19.0%	12	19.4%	
21. I feel respected at the pharmacy by the patients.	agree	11	55.0%	33	78.6%	41	66.1%	0.388
	disagree	2	10.0%	3	7.1%	5	8.1%	
	neutral	7	35.0%	6	14.3%	16	25.8%	
22. I am rewarded for the additional work in pharmacy.	agree	12	60.0%	16	38.1%	24	38.7%	0.513
	disagree	3	15.0%	11	26.2%	15	24.2%	
	neutral	5	25.0%	15	35.7%	23	37.1%	
23. Overall, I am satisfied with the chosen profession of pharmacist.	agree	10	50.0%	15	35.7%	27	43.5%	0.671
	disagree	7	35.0%	16	38.1%	18	29.0%	
	neutral	3	15.0%	11	26.2%	17	27.4%	
Total		20	100.0%	42	100.0%	62	100.0%	

Comparative study according to seniority in the profession of pharmacist. Finally, we conducted the same study according to the general seniority in the profession of the pharmacists interviewed. In this case, no statistically significant differences were found between the categories of pharmacists interviewed. The level of satisfaction with the pharmacist profession is the lowest among pharmacists with seniority between 1 and 5 years (56.8%), being high over 60% among the other categories of pharmacists. Pharmacists with seniority between 1 and 5 years, on the other hand, feel the most appreciated by their superiors (73%), compared to only 54.5% of pharmacists with seniority under one year. On the other hand, almost all of this category (90.9%) consider that they have good working relationships with the rest of the pharmacy staff, compared to only 66.7% of pharmacists with seniority between 6 and 10 years. All pharmacists with less than one year of experience consider that their working hours are adequate and that the level of communication in the pharmacy is good, as do the vast majority of others. The highest degree of disagreement with these 2 statements was found among pharmacists with between 1 and 5 years of experience. Over

80% of pharmacists with less than one year or more than 11 years of experience consider that their job tasks are well explained to them, compared to about 70% of those with between 1 and 10 years of experience. Only 57.5% of pharmacists with more than 11 years of experience consider that they are evaluated periodically, compared to, for example, 72.7% of those with less than one year of experience. Of these, the majority consider that the pharmacy is oriented towards the benefit of patients (90.9%), while only 65% of those with more than 6 years of experience have the same opinion. The most appreciated are pharmacists with over 11 years of experience (72.5%), but those who were promoted are those with less than a year of experience (45.5%). Those promoted less often are pharmacists with between 6 and 10 years of experience (16.7%). Almost all pharmacists with less than a year of experience believe that mistakes at work are discussed in the pharmacy (90.9%), compared to only 63.9% of pharmacists with between 6 and 10 years of experience who share the same opinion. The vast majority of pharmacists, regardless of experience, believe that the rules imposed by superiors are respected. Only 18.2% of pharmacists with less than a year of experience believe that the remuneration received is proportional to the work done, compared to over 40% of pharmacists with more experience. Also, pharmacists with more experience feel more respected by their colleagues than younger ones. 80% of pharmacists with over 11 years of experience also feel respected by patients. On the other hand, only half of those with 6 to 10 years of experience or less than a year of experience feel respected by patients. Almost three-quarters of pharmacists with less than a year of experience believe that they are rewarded for the extra work they do in the pharmacy (72.7%), compared to just over a third of those with 1 to 10 years of experience and 45% of those with over 11 years of experience. Satisfaction with their chosen profession is, however, admitted by about a third of pharmacists with up to 5 years of experience and by about 45% of those with over 6 years of experience. About a quarter of pharmacists, regardless of experience, are neutral regarding this statement and about a third are not satisfied with their chosen profession (tab. VII).

TABLE VII.

Questionnaire responses according to seniority in the profession of pharmacist

Question		My experience as a pharmacist is:								Pearson Chi-squared
		Less than 1 year		1-5 years		6-10 years		Over 11 years		
		N	%	N	%	N	%	N	%	p-value
7. I am satisfied with the profession of pharmacist that I perform.	agree	7	63.6%	21	56.8%	22	61.1%	25	62.5%	0.963
	disagree	2	18.2%	9	24.3%	10	27.8%	10	25.0%	
	neutral	2	18.2%	7	18.9%	4	11.1%	5	12.5%	
8. My superiors appreciate my work in the pharmacy.	agree	6	54.5%	27	73.0%	26	72.2%	26	65.0%	0.856
	disagree	3	27.3%	5	13.5%	4	11.1%	6	15.0%	
	neutral	2	18.2%	5	13.5%	6	16.7%	8	20.0%	
9. I consider that I have good relations with the rest of the pharmacy staff.	agree	10	90.9%	27	73.0%	24	66.7%	29	72.5%	0.804
	disagree	1	9.1%	6	16.2%	6	16.7%	6	15.0%	
	neutral			4	10.8%	6	16.7%	5	12.5%	

10. I have an adequate work schedule in the pharmacy where I work.	agree	11	100.0%	31	83.8%	35	97.2%	36	90.0%	0.483
	disagree			1	2.7%			1	2.5%	
	neutral			5	13.5%	1	2.8%	3	7.5%	
11. Communication in the pharmacy is good.	agree	11	100.0%	30	81.1%	30	83.3%	35	87.5%	0.719
	disagree			3	8.1%	4	11.1%	3	7.5%	
	neutral			4	10.8%	2	5.6%	2	5.0%	
12. The tasks in the pharmacy are well explained.	agree	9	81.8%	26	70.3%	26	72.2%	32	80.0%	0.226
	disagree			10	27.0%	6	16.7%	6	15.0%	
	neutral	2	18.2%	1	2.7%	4	11.1%	2	5.0%	
13. I am periodically evaluated in the pharmacy.	agree	8	72.7%	23	62.2%	26	72.2%	23	57.5%	0.444
	disagree	1	9.1%	6	16.2%	6	16.7%	4	10.0%	
	neutral	2	18.2%	8	21.6%	4	11.1%	13	32.5%	
14. The pharmaceutical unit is oriented towards the benefit of the patient.	agree	10	90.9%	26	70.3%	24	66.7%	26	65.0%	0.321
	disagree	1	9.1%	1	2.7%	4	11.1%	6	15.0%	
	neutral			10	27.0%	8	22.2%	8	20.0%	
15. I am appreciated in the pharmacy.	agree	7	63.6%	22	59.5%	23	63.9%	29	72.5%	0.689
	disagree	2	18.2%	4	10.8%	2	5.6%	2	5.0%	
	neutral	2	18.2%	11	29.7%	11	30.6%	9	22.5%	
16. I have been promoted in the pharmacy where I work.	agree	5	45.5%	8	21.6%	6	16.7%	13	32.5%	0.363
	disagree	5	45.5%	17	45.9%	16	44.4%	15	37.5%	
	neutral	1	9.1%	12	32.4%	14	38.9%	12	30.0%	
17. The errors of the pharmacy staff are discussed.	agree	10	90.9%	25	67.6%	23	63.9%	35	87.5%	0.145
	disagree	1	9.1%	6	16.2%	5	13.9%	3	7.5%	
	neutral			6	16.2%	8	22.2%	2	5.0%	
18. The rules imposed by the superiors in the pharmacy are respected.	agree	10	90.9%	32	86.5%	30	83.3%	37	92.5%	0.615
	disagree	1	9.1%	1	2.7%	3	8.3%	2	5.0%	
	neutral			4	10.8%	3	8.3%	1	2.5%	
19. The remuneration for the pharmacist position is equivalent to the work performed.	agree	2	18.2%	17	45.9%	17	47.2%	17	42.5%	0.214
	disagree	6	54.5%	15	40.5%	12	33.3%	21	52.5%	
	neutral	3	27.3%	5	13.5%	7	19.4%	2	5.0%	
20. I feel respected at the pharmacy by my colleagues.	agree	7	63.6%	25	67.6%	28	77.8%	32	80.0%	0.111
	disagree	3	27.3%	4	10.8%	1	2.8%	1	2.5%	
	neutral	1	9.1%	8	21.6%	7	19.4%	7	17.5%	
21. I feel respected at the pharmacy by the patients.	agree	6	54.5%	28	75.7%	19	52.8%	32	80.0%	0.150
	disagree	1	9.1%	3	8.1%	5	13.9%	1	2.5%	
	neutral	4	36.4%	6	16.2%	12	33.3%	7	17.5%	
22. I am rewarded for the additional work in pharmacy.	agree	8	72.7%	14	37.8%	12	33.3%	18	45.0%	0.227
	disagree	1	9.1%	10	27.0%	7	19.4%	11	27.5%	
	neutral	2	18.2%	13	35.1%	17	47.2%	11	27.5%	
23. Overall I am satisfied with the chosen profession of pharmacist.	agree	4	36.4%	14	37.8%	16	44.4%	18	45.0%	0.986
	disagree	4	36.4%	12	32.4%	12	33.3%	13	32.5%	
	neutral	3	27.3%	11	29.7%	8	22.2%	9	22.5%	
Total		11	100.0%	37	100.0%	36	100.0%	40	100.0%	

DISCUSSION

In 2023, a total of 18,241 pharmacists were licensed for independent practice in Romania, distributed across 8100 officially recognized community pharmacies spread throughout the country's 41 counties. The average number of pharmacists per county is approximately 450. Of these, the 150 pharmacist respondents of our study, representing 30% of officially employed pharmacists, constitute a significant proportion. Notably, in Iași, these 30% of pharmacists report a job satisfaction rate of only 40%, indicating substantial issues in a context where over 70% of them are deeply committed to the pharmaceutical practice and prioritize patient satisfaction over their own. This situation highlights the need to reevaluate working conditions and employment policies to enhance professional satisfaction among pharmacists.

This study aimed to raise awareness of the need for knowledge and continuous improvement of pharmacist communication skills, in all areas of pharmaceutical practice, in order to provide adequate counseling to patients, but also at the organizational level. We found international comparisons in specialized studies. For example, in Vietnam, hospital pharmacists reported 80.4% overall satisfaction, notably higher than Ethiopia (approximately 53%) and Malaysia (52%) (25, 26). Also, community pharmacists from New Zealand (NZ) felt their environment was conducive to safe care (57%) and had sufficient staffing (45%), while pharmacists from Canada are less satisfied (47%, respectively 32%) (27). Global early-career pharmacists (92 countries) found that workplace climate, training opportunities, workload, autonomy, and remuneration significantly influenced job satisfaction (28). Our study revealed that about 40% of pharmacists have positive overall satisfaction, with strong perceptions of team collaboration and communication, but lower scores in remuneration and promotion.

In Vietnam, working conditions were the strongest predictor of satisfaction, followed by leadership and benefits (25). In NZ and Canada, pharmacists reported inadequate time for breaks and tasks; dissatisfaction and psychological distress remained high (27). Swedish pharmacists reported high workloads but maintained strong satisfaction, possibly due to good continuing professional development (29). Our study highlights positive responses regarding schedule, task clarity, and communication, suggesting decent working conditions-though pay and promotion lag behind, thus reflecting trends from Europe.

In Ethiopia and Saudi Arabia, over 80% of pharmacists were dissatisfied with salary (26). In Iraq, pharmacists were relatively satisfied with income compared to developed countries (30). Reddit discussions indicate high pay/respect for pharmacists in Switzerland, Australia, UK, and Canada (31). Our study showed that 39.3% pharmacists believe salary matches work, with 46% dissatisfied, mirroring trends in Ethiopia and Saudi Arabia.

Ethiopian and Zimbabwean pharmacists cited lack of promotion and training as major dissatisfaction causes (26). Early-career pharmacists globally emphasized training and autonomy as key to satisfaction. In Sweden, career satisfaction was tied to access to continuing professional development (29). In our study only about 25% of pharmacists feel promoted, many lack regular evaluations echoing global

concerns around career stagnation and professional growth.

In Vietnam, the leadership and colleague relationships were significant satisfaction factors (25). Global studies reinforce the importance of work climate, autonomy, and recognition (28). NZ pharmacists also complained about time pressure, but valued environment with good staffing (27). In our study we found highly positive results on peer respect (75%), supervisor appreciation (66.7%), and communication (83.3%) align with leadership/work climate being core satisfaction drivers.

CONCLUSIONS

Based on the obtained results we can draw a series of conclusions and recommendations.

Clear career development pathways should be established, allowing pharmacists to specialize or advance professionally through defined criteria and opportunities. More experienced pharmacists should be encouraged to mentor newer colleagues, creating a culture of knowledge sharing and professional support. A transparent promotion system is needed, including specific benchmarks for advancement and internal job mobility. Management should explore the root causes of dissatisfaction by organizing confidential interviews, focus groups, or feedback sessions, and regular, anonymous job satisfaction surveys should be introduced to make continuous improvements.

To maintain strong team cohesion, regular team-building sessions, communication workshops should be scheduled and peer recognition practices should be encouraged. An annual internal communication audit could significantly enhance engagement and satisfaction within pharmaceutical units.

Work schedules should remain flexible and tailored to pharmacists needs and loyalty programs or milestone rewards implemented to acknowledge long-term employees. All extra hours worked or additional responsibilities should be appropriately rewarded.

A review of the current remuneration system is needed to ensure salaries that reflect the actual responsibilities and workload of pharmacists. Performance-based bonuses or alternative rewards, flexible days off, or training sponsorship should be considered to boost motivation.

In light of the research results, we consider useful that pharmacists should be empowered to take on more advisory roles, offering personalized guidance to patients to reinforce the pharmacy's patient-centered mission.

CONFLICTS OF INTERESTS AND FUNDING

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VALIDATION AND ADAPTATION TO THE ROMANIAN LANGUAGE AND POPULATION OF THE EBBCAM SCALE OF PERCEIVED COMPETENCE IN THE FACE OF DEATH, IN NURSING AND MEDICAL STUDENTS

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VALIDATION AND ADAPTATION TO THE ROMANIAN LANGUAGE AND POPULATION OF THE EBBCAM SCALE OF PERCEIVED COMPETENCE IN THE FACE OF DEATH, IN NURSING AND MEDICAL STUDENTS (Abstract): We carried out the translation, validation and cultural adaptation in Romanian language of the EBBCAM Questionnaire for the measurement of Perceived Death Competence in Medical and Nursing students. **Materials and methods:** 117 medical and nursing students of the Faculty of Medicine, Grigore T. Popa University of Medicine and Pharmacy Iasi. **Results:** The Romanian translation of the Bugen Brief Bugen Coping Competence in the Face of Death (EBBCAM) questionnaire, which is a multidimensional instrument consisting of 16 items, was administered. Subsequently, a Confirmatory Factor Analysis was performed in order to verify the validity of the measurement model. Both the study of the reliability of the responses (f1: .902; f2: .824; f3: .882 y f4: .828) and the Confirmatory Factor Analysis showed that the four-factor model had an adequate fit. (NNFI: .882; CFI: .920; IFI: .825 y RMSEA: .117). **Conclusions:** The Bugen Brief Competence in the Face of Death (EBBCAM) scale is a suitable instrument for the measurement of Perceived Competence in the Face of Death in Romanian-speaking students of Health Sciences (Medicine and Nursery). **Keywords:** DEATH COMPETENCE, VALIDATION, SCALE, ATTITUDES TOWARD DEATH, MEDICAL OR NURSING STUDENTS.

INTRODUCTION

The research shows how many health care professionals and health science students have difficulties in dealing with death, both their own and that of the patients they are taking care for (1, 2). In this sense, inappropriate management of one's own emotions, as well as poor self-knowledge (Bermejo, 2011) will influence the attitudes we adopt towards our patients, especially if they are at the final stage of their lives (3).

Any medical or nursing professional will sooner or later face death. It does not matter the service or area in which they work. From primary care in health centers or specialty

centers where elderly people and others suffering from degenerative diseases and terminal processes are attended, as well as in nursing homes and in any hospital unit, such as Palliative Care Units, Intensive Care Units, Internal Medicine Units, but also in Gynecology, Otorhinolaryngology or Ophthalmology Units, to name a few, where death may seem to be absent.

Perceived competence can be defined as the perception that one is capable of interacting effectively with the environment (4). The study of competence in the face of death is, therefore, fundamental for the effective performance of the tasks and processes involved in end-of-life care (5-7). For this purpose, the translation, validation and cultural adaptation in Romanian language of the EBBCAM Questionnaire for the measurement of Perceived Death Competence in Medical and Nursing students has been carried out.

MATERIALS AND METHODS

Measurement instrument. The EBBCAM scale (Bugen Brief Scale of Competence in the Face of Death) is an adaptation of the EBAM scale made by Medrano (2017; 2020) (8, 9), it is composed of 16 statements that evaluate 4 dimensions, to each dimension belong some of these statements. The EBBCAM is composed of the following dimensions or factors:

f1: Assistance and communication;

f2: Post-mortem care;

f3: Self confidence

f4: Management of own fear.

The reliability for the present scale in each of its dimensions respectively is: .787, .766, .814, .702 respectively.

Methodology

1. To carry out the adaptation process (translation and content validity), the scale was first translated from Spanish into Romanian by three Romanian nationals, third- and fourth-year students of the Nursing Degree at the Catholic University of Valencia (Spain), and then corrected by a non-professional in the health sciences, a native speaker of Romanian and Spanish. Subsequently, the translated scale was revised by an official translator in Romania (Iasi).

2. Data collection was implemented through an online questionnaire developed by one of the authors (VP). The time to complete the questionnaires was estimated at less than 15 minutes.

3. To study the psychometric properties of the scale, a study of its validity and reliability was carried out. For this purpose, a structural equation model was specified, estimated and evaluated, and internal consistency estimates were calculated.

The equation model carried out, confirmatory factor analysis (CFA), is characterized by representing the causal processes under study by means of a series of equations, and these structural equations can be modelled pictorially to conceptualize more clearly the theory studied (10).

This procedure is appropriate when, as in this case, the researcher has prior knowledge about the relationships between the observable variables (items), in this case, the original structure proposed by the authors.

The CFA tested for the EBBCAM Questionnaire specified a structure of four correlated factors (Companionship and communication, Post-mortem care, Self-confidence and Self-fear management), which depart from Medrano's original model (8, 9) and which would explain the 16 items of the instrument (fig. 1).

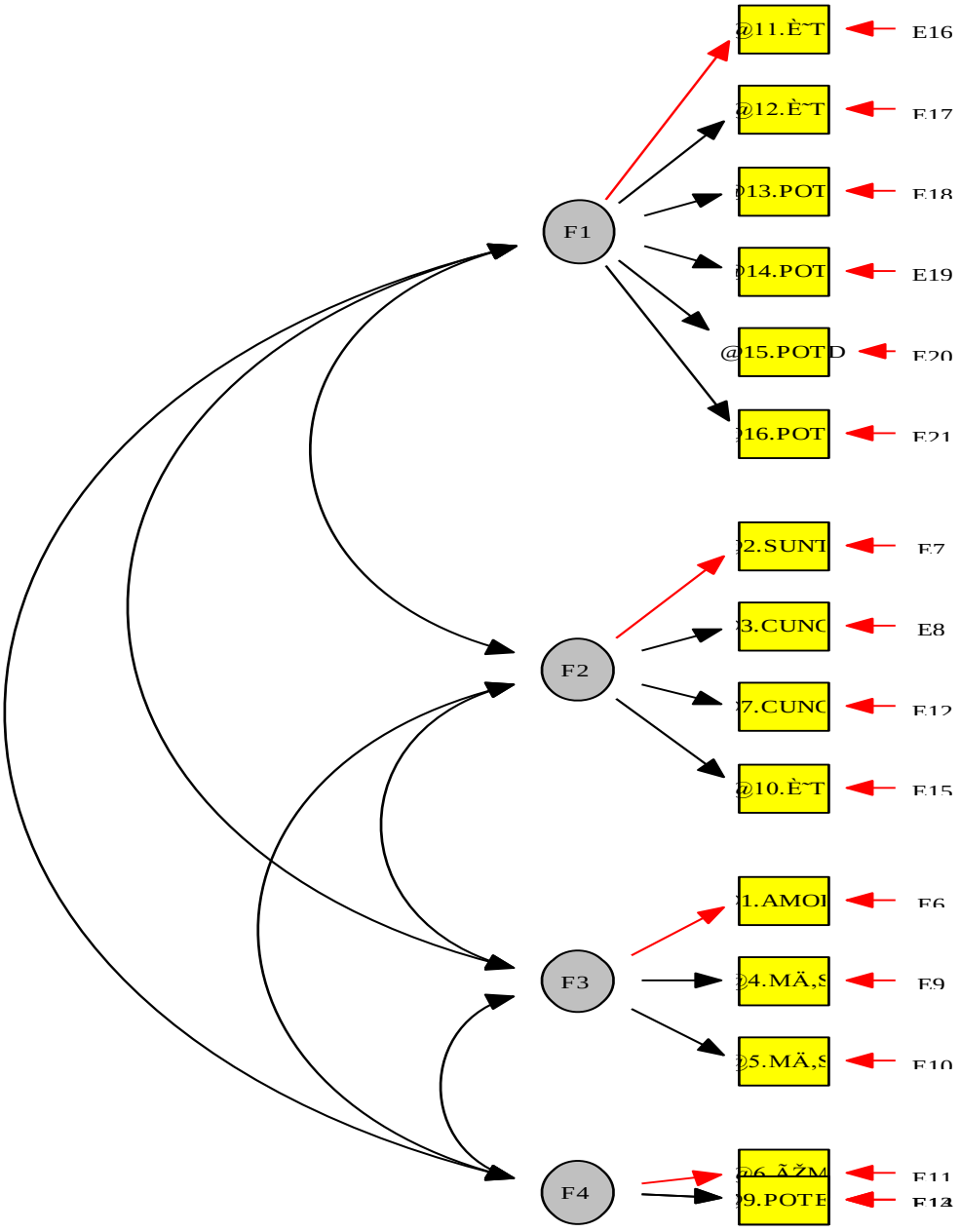

Fig. 1. Proposed confirmatory factor analysis of the EBBCAM Questionnaire

RESULTS

There is a high representation of the 5th and 4th courses, medium in the third and first grades and low in the second grade. Also, there is a high female participation and a low participation of male students.

Both groups (medicine and nursing) are optimally represented, although with a higher participation of medical students (tab. I).

TABLE I.
Basic sample data

N=117		
Average age		22.2 (SD: 3.01)
Age range		18-43
		N (%)
Sex	Male	17 (14.5)
	Female	100(85.5)
Marital Status	Single	108 (92.3)
	Married	9 (7.7)
Specialty	Medical	78 (66.6)
	Nursing	39 (33.3)
Year of study	**1º**: 12; **2º**: 6; **3º**: 15; **4º**: 58; **5º**: 25	

Table II shows that the four factors analyzed have high reliability (tab. II).

The confirmatory factor analysis shows an adequate fit to the theoretical dimensions from the original EBBCAM scale in Spanish (tab. III).

The confirmatory factor analysis shows an adequate fit to the theoretical dimensions from the original EBBCAM scale in Spanish (tab. III).

TABLE II.
Internal consistency for theoretical factors

	f1	**f2**	**f3**	**f4**
α	.902	.824	.882	.828

TABLE III.
Confirmatory Factor Analysis for the four-factor model

CHI²	**NNFI**	**IFI**	**RMSEA**
254.6233	.882	.825	.117

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

DISCUSSION

Based on recommendations in the litera-

ture (11, 12), the fit of the models to the data was assessed using various criteria, such as the chi-square (χ^2) statistic (13). This statistic, however, has been widely criticized for its sensitivity to sample size, the magnitude of correlations, and violations of multivariate normality assumptions, which has led researchers to rely on complementary indices of model fit. Consequently, several alternative fit indices have been developed to provide a more robust and nuanced evaluation of how well a model represents the observed data. Among these, the Comparative Fit Index (CFI) and the Root Mean Square Error of Approximation (RMSEA), both employed in the present study, are among the most widely accepted and frequently reported indicators in structural equation modeling. The CFI compares the fit of the hypothesized model with that of a null model that assumes zero covariances among the observed variables, thereby reflecting the relative improvement in fit achieved by the proposed model. It ranges from 0 to 1, with higher values indicating better model fit. Values above .90 are generally interpreted as acceptable, whereas values above .95 are considered indicative of excellent fit (9, 14). In this sense, the CFI provides a standardized measure of how well the specified relationships among variables reproduce the observed covariance structure.

The RMSEA, in contrast, evaluates the degree of approximation error of the model in the population rather than in the sample. It estimates how well the model would fit the population covariance matrix if it were available, thereby accounting for model complexity and degrees of freedom. Values of zero indicate a perfect fit, while values below .08 are typically interpreted as reflecting an adequate fit to the data (15, 16). Lower RMSEA values therefore suggest that the discrepancies between the hypothesized model and the observed data are small and within acceptable limits. Together, the CFI and RMSEA offer complementary perspectives on model fit, capturing both relative and absolute aspects of model adequacy. Using both indices provides a more balanced and rigorous evaluation than relying on a single statistic alone.

To evaluate the reliability of the scales, Cronbach's alpha was computed for each of the subscales. This coefficient is the most widely used index of internal consistency and reflects the extent to which the items within a scale measure the same underlying construct. Values between .70 and .79 are generally considered to indicate moderate reliability, whereas values of .80 or higher are interpreted as reflecting high reliability (17). In the present analyses, the reliability estimates not only met these accepted standards but also exceeded those reported in the original Medrano version of the instrument (9). This improvement suggests that the current adaptation demonstrates enhanced internal consistency and psychometric robustness in the studied sample.

CONCLUSIONS

The data show a good psychometric fit and high reliability, making the Romanian version of the Bugen Brief Death Competence Scale a reliable and valid instrument for application in the Romanian population. This scale is easy and quick to apply and offers useful data in specific areas related to the analyzed construct.

CONFLICTS OF INTERESTS AND FUNDING

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DEMOGRAPHIC AND CLINICAL FEATURES WITH SURGICAL CONSIDERATIONS IN ADULT CRANIOPHARYNGIOMAS: A ROMANIAN NEUROSURGICAL CENTER EXPERIENCE

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DEMOGRAPHIC AND CLINICAL FEATURES WITH SURGICAL CONSIDERATIONS IN ADULT CRANIOPHARYNGIOMAS: A ROMANIAN NEUROSURGICAL CENTER EXPERIENCE (Abstract): Craniopharyngiomas (CPs) are rare benign epithelial tumors of the sellar-suprasellar region that remain surgically challenging. **Aim**: To evaluate the clinical, imaging, pathological and surgical aspects of CPs diagnosed in adult patients as such previous analyses are rare. **Materials and methods:** We performed a retrospective study that included adult patients (≥18 years) with surgical treated and pathologically confirmed CP between January 2016 and December 2021. Eligible cases were analyzed regarding demographic data, clinical presentation, and surgical approach. **Results:** Eleven adult patients were included (mean age 46.6 ± 12.6 years), with a slight female predominance (54.5%). Two peaks in patients' age were noted: one in the fifth decade of life (40- to 49-year-olds) and another in the seventh decade of life (60- to 69-year-olds). Visual disturbances were the most frequent presenting symptom (81.8%), but headache (54.5%), endocrine disfunctions (36.36%), and neurocognitive symptoms (27.3%) were also identified. Open craniotomy was performed in 72.7% of cases, while extended endoscopic endonasal approach was realized in the rest of the cases. Subtotal resection was achieved in 63.6% of all cases. **Conclusions:** The findings of our study align with published data, while highlighting specific diagnostic peculiarities in Romanian adult patients with craniopharyngiomas. **Keywords:** CRANIOPHARYNGIOMA; ADULT PATIENTS; VISUAL DISTURBANCES; SURGICAL APPROACH.

INTRODUCTION

Craniopharyngiomas CPs) are rare intracranial epithelial tumors that develop from remnants of Rathke's pouch due to errors occurring in the process of differentiation during embryogenesis (1).

Even though, from a histologically point of view, CP was first described more than a century and a half ago and the first successful surgical resection of such a tumor was performed at the beginning of the 20th century (2,3), this entity is still considered "a challenging tumor of the sella" (4) due to its proximity to vital neurovascular structures and the difficulties in surgical interventions when surgeons try to reach the tumor.

Although both 2021 WHO 5th edition of Classification of CNS tumors (5) and the

latest 2025 WHO 5th edition Classification of Endocrine Tumors (6) assign grade 1 malignancy to craniopharyngioma, this tumor is associated with varied clinical manifestations, elevated recurrence, long-term postsurgical comorbidities, but also with a high risk of mortality (7-10).

In the United States, the incidence of pathologic-confirmed CP was 0.16 per 100,000 persons. This tumor has a bimodal distribution, with two peaks, one in childhood (5- to 9-year-olds) and another in adults (55 - to 69-year-olds) (1).

In adults, these tumors account for 1-3% of all primary intracranial tumors (11), but it is believed that the incidence could increase in the coming period due to widespread use of cranio-cerebral computed tomography (CT) and Magnetic Resonance Imaging (MRI) whenever a neurological or endocrinological symptoms appear (12).

In recent years, comprehensive studies on the demographic, clinical, imaging, pathological and surgical aspects of CPs are mostly review-type (11, 13-15), and those based on case series are quite rare, often analyzing a very small number of patients (16, 17). In addition, reports on CPs identified in children (12, 18, 19) are more numerous than those dedicated to craniopharyngioma diagnosed in adults (20) or studies that include both pediatric and adult patients (21).

Considering that some differences in tumor biology, clinical presentation and functional outcomes have been reported depending on age (22), in the present study, we performed a retrospective analysis of CPs diagnosed in adult patients and treated in the Prof. Dr. N. Oblu Clinical Emergency Hospital in Iași, Romania, which is a tertiary care center with a reference area of approximately 5 million inhabitants. The aim of this study was to evaluate the clinical, imaging, pathological and surgical aspects of CPs diagnosed and treated in this health institution in adult patients and to compare these data with data from similar studies in the literature, as such previous analyses are rare. Such data may provide a new perspective on this disease in adults, providing useful information for future treatments.

MATERIALS AND METHODS

The study was approved by the Ethic Committee of Prof. Dr. N. Oblu Emergency Clinical Hospital, Iași, Romania (No. 1333/ January 1st, 2023), as a quality control study. Informed consent was obtained from all patients included in this study.

A retrospective study of patients who underwent surgical intervention for CP for a period of 6 years (January 1, 2016 and December 31, 2021) in the Department of Neurosurgery, Prof. Dr. N. Oblu Emergency Clinical Hospital, Iași, Romania, was performed.

The inclusion criteria were as follows: (1) adult patients (aged 18 years and older) admitted in any Neurosurgery Departments during the study period within the Hospital and having pathologically confirmed diagnosis of a CP; (2) all clinical, imaging, pathological and surgical data available.

The exclusion criteria were as follows: (1) Pediatric patients (aged less than 18 years) admitted in any Neurosurgery Departments during the study period within the Hospital and having pathologically confirmed diagnosis of a CP; (2) absence of informed consent; (3) unavailability of relevant clinical data; (4) previous resection or radiotherapy for their CP.

Over the analyzed period, searching in

the hospital database identified 18 patients with pathologically confirmed CP. Seven patients were excluded after meticulous scrutiny based on exclusion criteria. Finally, 11 patients were found eligible for further analysis.

For these 11 patients, data were collected from medical records and included: patients' demographic data (gender and age), presenting symptoms at admission, tumor location identified on magnetic resonance imaging (MRI), as well as type of the surgery (transcranial approach *vs.* endonasal approach) and extent of tumor resection (total *vs.* subtotal).

The information was collected using a data collection sheet. Due to the small sample size, all correlations were analyzed descriptively; some values were reported as a number, or as a number and percentage, while others as a mean ± SD value or as a median value.

RESULTS

In 6 years, 11 adult patients with CP met the inclusion criteria for the present study. Their mean age at diagnosis was 46.6 ± 12.6 years (range 23-64), with a median age of 46 years. There was a slight female predominance (54.5%), the female-to-male ratio being 1.2: 1.

In the present series, the presence of two peaks in the identification of craniopharyngioma is noted: one in the fifth decade of life (40- to 49-year-olds) and another in the seventh decade of life (60- to 69-year-olds (fig. 1).

Because symptom reporting was heterogeneous, all of them were coded into standardized categories based on narrative descriptions (tab. I). Visual disturbances were the predominant presenting symptom, observed in 81.8% of all cases, including blindness, visual field defects, and decreased visual acuity.

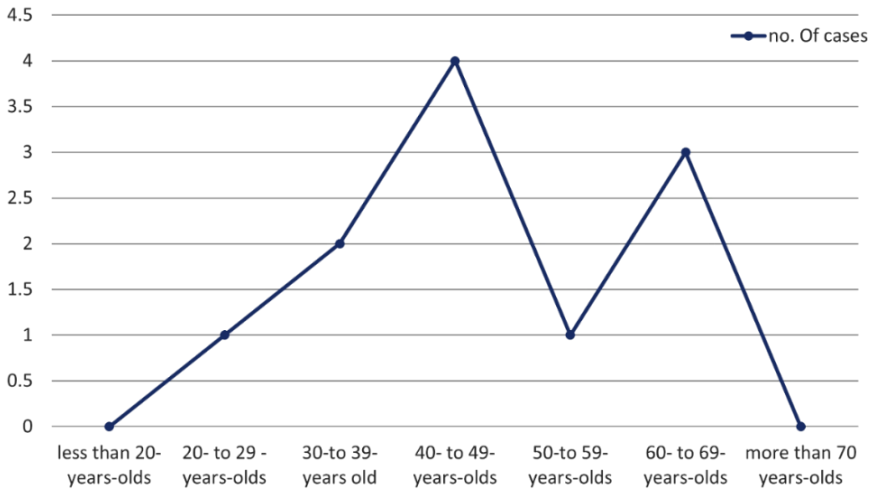

Fig. 1. Craniopharyngiomas distribution along the life decades in the study cohort.

Headache was reported by 54.5% of all patients, while endocrine disfunction was identified in 36.36% of all cases, diabetes insipidus being documented in 18.2% of all

cases. 27.3% of all cases exhibited neurocognitive symptoms such as confusion, or somnolence.

MRI tumor evaluation for CP location (tab. II) demonstrated a predominant suprasellar involvement, either isolated or combined with sellar extension (5/11 cases; 45.45%). Development of a CP in peri mesencephalic cistern was observed in a minority of cases (9.1%). Also, pure third ventricle (V3) location was rare (9.1%). Suprasellar tumor development with V3 extension was identified in 18.2% of cases. Prepontine tumor extension was recorded in 9.1% of cases. Wide tumor extension (prepontine and into the Sylvian fissure) was also identified in only 9.1% of cases.

Surgical resection (tab. III) was performed through open craniotomy in 8 cases (72.7%) (fig. 2) and by extended endoscopic endonasal approach in 3 cases (27.3%) (fig. 3). Gross total resection was achieved in 4 cases (36.4%) subtotal resection in 7 cases (63.6%).

TABLE I.

Clinical characteristics of the study cohort

MAIN PRESENTING SYMPTOMS	n	(%)
Visual disturbances (blindness, visual field defects, decreased visual acuity)	9	81.8%
Headache	6	54.5%
Endocrine disfunction	4	36.36%
• Diabetes insipidus	2	18.2%
• Pituitary FSH and LH deficiency	1	9.1%
• Pituitary GH and ACTH deficiency	1	9.1%
Neurocognitive symptoms (confusion, somnolence)	3	27.3%

TABLE II.

Craniopharyngioma locations identified on MRI scans

TUMOR LOCATION (MRI)	n	%
Exclusive development in suprasellar region	2	18.2%
Exclusive development in peri mesencephalic cistern	1	9.1%
Exclusive development in the V3	1	9.1%
Sellar and suprasellar development	3	27.3%
Suprasellar development with V3 extension	2	18.2%
Suprasellar development with prepontine extension	1	9.1%
Sellar and suprasellar development, with prepontine and Sylvian fissure extensions	1	9.1%

TABLE III.

Surgical management of patients with craniopharyngiomas, including type of surgical approach and extent of tumor resection in the present series (n = 11).

SURGICAL VARIABLE	n	(%)
Types of surgical approach		
• Extended endoscopic endonasal approach	3	27.3%
• Open craniotomy	8	72.7%
○ Trans callosal / trans ventricular approach	4	36.4%
○ Fronto-temporal/ pteryonal approach	4	36.4%
Extent of tumor resection		
• Gross total resection (macroscopic)	4	36.4%
• Subtotal resection	7	63.6%

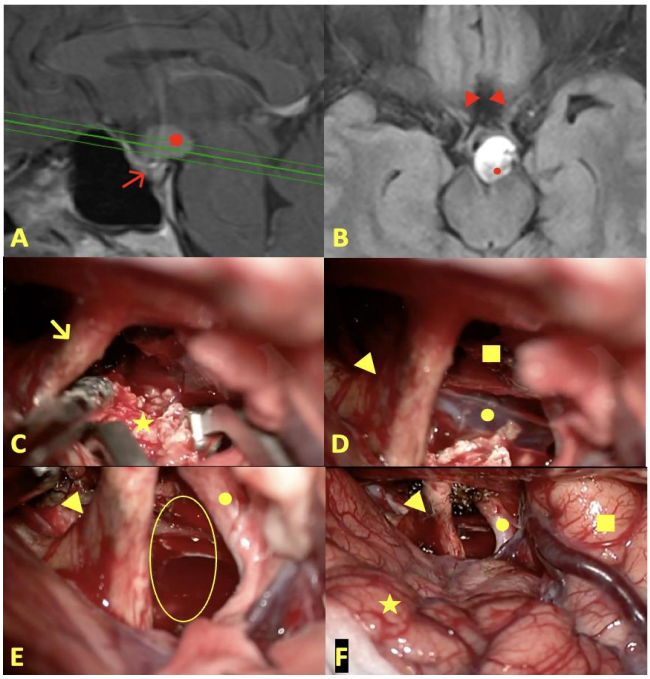

Fig 2. A) and B) images - preoperative MRI showing a suprasellar lesion with contrast enhancement (circle), the normal pituitary gland (arrow) and the optic nerves (arrow head) and a calcified portion (circle). C) To F) images: intraoperative pteryonal approach - showing on C) - the optic nerve (arrow) and the calcified portion of the lesion (star), D) - optic nerve and the optic chiasm (arrowhead), the pituitary gland (square) and the Lilliquist membrane (circle), E) - intraoperative aspect after tumoral resection showing the optic nerve (arrow head) right carotid artery (circle) and the optical-carotid window (oval); F) - same image at lower magnification.

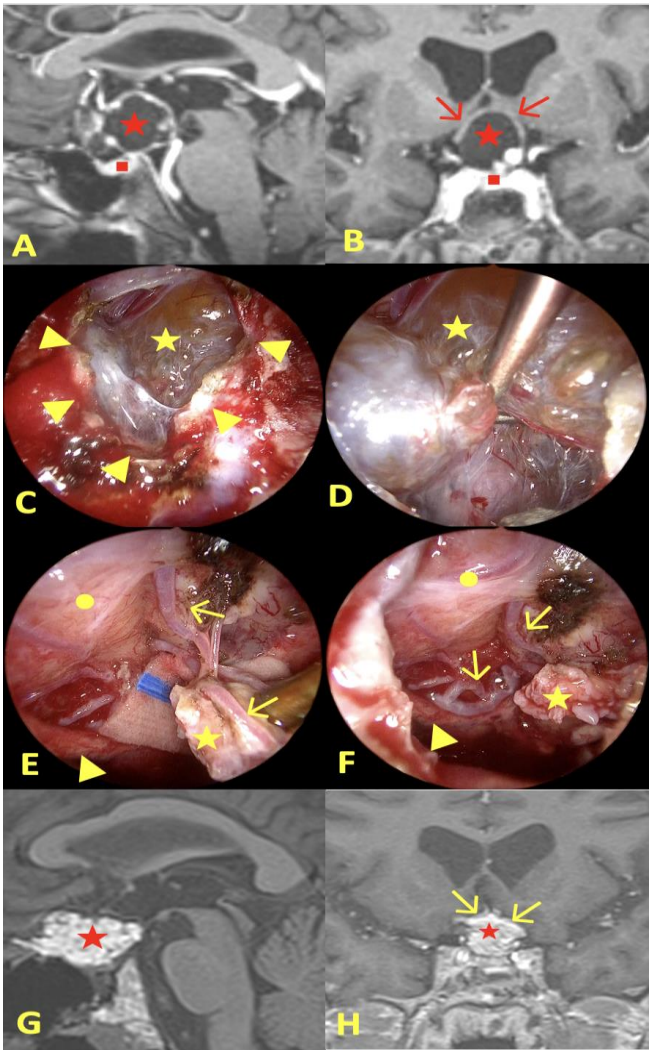

Fig 3. A and B MRI with contrast enhancement showing a cystic suprasellar lesion (star) the superior displacement of the optic chiasm (arrows) and the normal pituitary gland at the level of the sella (square). C-F intraoperative images (extended endoscopic transsphenoidal approach): C - at the level of the sphenoid sinus showing the opening of the dura (arrow heads) with exposing of the lesion (star), D - closer view of the tumor, E - intraoperative aspect after partial resection showing the tumor capsule (star) with close adhesion to the A2 segment of anterior cerebral artery (ACA) (arrow), the right optic nerve (arrow head), and the right basal frontal lobe (circle), F - final step of the resection showing the right optic nerve (arrowhead), the particular anatomic conformation (fenestration) of the anterior communicating complex (arrow) and a small tumor capsule remnant on the A2 segment of left ACA (star). G-H postoperative MRI showing the reconstruction of the skull base with fat plug (star), and the anatomic integrity of the anterior communicating complex (arrows).

DISCUSSION

Craniopharyngiomas diagnosis and management remain challenging because of their complex anatomy, heterogeneous imaging appearance, and frequent overlap with other sellar and suprasellar lesions (23).

The literature reports that CP has a bimodal age distribution with two peaks: one at age 5-14 years and the second at age 45-74 years (24), which may suggest the influence of certain underlying biological or environmental factors that contribute to the age-related incidence of the disease (25).

The present cohort, made up of adult patients (≥ 18 years old), included predominantly middle-aged adults (mean age 46.6 years) with a slight female predominance. Our data are different from those reported by others who found a slightly male predilection (26) or, more recently, no gender predilection (11). The demographic profile of the present cohort is also different from those reporting only one peak of CP incidence in adult patients, either in age group 40-44 years (26, 27) or 45-74 years (24). We found a small shift from the reported data because we identified two separate peaks (at 40-to 49 years-old and at 60- to 69-years-old). These new data can signify a change in tumor biology or a particularity for our study group.

From a clinical perspective, CP presentation in adults is often insidious with many and various symptoms that can be difficult for patients as well as for clinicians to identify early (16). Because CPs develop in close proximity to vital structures (optic pathways, hypothalamus, thalamus, brain stem), their clinical spectrum includes endocrine, visual, and neurological impairment, as well as hypothalamic syndrome and increased intracranial pressure syndrome (23, 28).

The high prevalence of visual disturbances in our cohort (81.8%) closely mirrors reported rates ranging from 60% to over 84% in adult and mixed populations (10, 27) reflecting the frequent suprasellar extension and optic chiasm compression characteristic of CPs (25). However, visual symptoms are common to multiple other sellar region tumors and therefore lack discriminatory value.

The identification of visual disturbances in a high percentage in the present series also reflects the fact that the patients presented late to the neurosurgeon when CP already developed as a bulky tumor, with wide extension into neighboring structures, including the optic chiasm and hypothalamus.

Increased intracranial pressure due to voluminous tumor itself or to the hydrocephalus resulting from compression of V3 determine headache, which can occur in 35% - 68% of cases (10, 27). In the present study, the identified value for headache falls between these values, which demonstrates that even though the cohort was small, the data obtained are correct and align with those reported by authors from other countries. Also, in the present cohort, the frequent association with headache and hydrocephalus further emphasizes the mass effect exerted by these lesions, particularly in tumors with intraventricular extension.

Compression of the pituitary gland by any lesion cause anterior pituitary dysfunction with variable deficiencies of Growth Hormone (GH), gonadotropins [Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH)], Adrenocorticotropic Hormone (ACTH), or Thyroid Stimulating Hormone (TSH). The literature reported that the most common deficiency encoun-

tered in CPs are those of GH (86% of cases) (27), gonadotropins (40%), ACTH (25%), and TSH (25%) (25). Compression of the pituitary stalk and/or hypothalamus, determine hypothalamic-pituitary dysfunction with vasopressin deficiency manifesting as diabetes insipidus that appear in 17-38% of cases (25, 27). In the present cohort, adult patients with CPs also presented at admission some endocrine dysfunction, but the percentages are much lower than those reported by other authors, probably because they were not properly investigated preoperatively given the diagnostic error upon admission to the hospital.

Cognitive deficits (such as impaired memory, attention, and reduced processing speed), or sleep disorders (somnolence) appear in 17% to 71.5% of CP patients, especially in those with voluminous tumors that compress the hypothalamus (16, 29). The values obtain in the present series align with these reported percentages.

CPs have exceedingly a heterogeneous topography because these tumors may arise anywhere on the path of embryonic cell migration (7). Most CPs are located in the sellar and parasellar regions (23), an aspect also identified in the present series.

CPs can extend in all the directions: superior, inferior, lateral, anterior and posterior. MRI plays a pivotal role in evaluating the complex patterns of extension of CPs and their relationship with adjacent neurovascular structures.

These tumors may extend superiorly from the suprasellar region with compression of the third or lateral ventricles, laterally into parasellar regions and cavernous sinuses, anteriorly toward the frontal lobes with displacement of the anterior cerebral arteries, or posteriorly into the peri mesencephalic cisterns, brainstem, and cerebellar spaces. Inferior extension to the sellar region, paranasal sinuses and nasopharynx may also be observed.

Accurate identification of these extension patterns is essential for surgical planning, guiding the choice between total and subtotal resection, and for differentiating craniopharyngiomas from other extensive skull-base lesions (30).

The tumor can also develop in the V3, either within the neural tissue of the infundibulum and tuber cinereum, which are components of V3, or strictly in V3, originating in its subependymal area (31). However, the incidence of purely intraventricular CPs accounts for 0.5 to 11% of all cases, as we also found in the present study. Most important is the fact that CPs located in V3 posse surgical challenges because of the complex and vital surrounding structures, including the hypothalamus, infundibulum, optic pathways, limbic system, and nearby vasculature (32).

Ectopic locations of CP have also been identified in adults, but they are extremely rare, published only as isolated case reports. As far as we know, CP location in the prepontine cistern, a situation in which there is no sellar involvement or endocrine dysfunction (33), in the temporal lobe with no connection to the craniopharyngeal duct (34), the nasopharynx, the posterior fossa, and the spine (7), or orbit (35) have been reported.

Although limited in terms of the small number of cases, two of these ectopic locations of the CP were also identified in the present series, namely in V3 and the peri mesencephalic cistern, which, prior to the pathological diagnoses, determined an erroneous imaging diagnosis and a real surgical challenge.

Surgical management remains the cor-

nerstone of CP treatment. The choice of the type of surgical approach is adapted to each individual case, but also in correlation with the equipment of each hospital and the presence of at least one neurosurgeon specialized in such surgical interventions. The goal of a quality surgical intervention is to achieve a radical resection while minimizing damage to the surrounding vital neurovascular structures, especially the hypothalamus (21).

In the present series, surgical treatment was performed predominantly through open craniotomy approaches, reflecting frequent large tumor extension into the surrounding neuroanatomical structures, while endoscopic endonasal surgery was reserved for selected midline lesions.

Recent literature has reported increasing use of endoscopic endonasal approaches in selected midline lesions; however, these approaches are generally reserved for tumors with favorable anatomy and limited lateral or ventricular extension, offering a direct midline corridor while minimizing brain retraction. Also, it has the advantage of a gross total resection ranging of up to 70% in some series (36, 37).

In the present study, due to the predominance of large tumors with adherence and large invasion into adjacent vital neural structures, the surgeon took the decision to make a subtotal resection in two thirds of cases in order to avoid postoperative morbidity. The introduction of the extended endonasal endoscopic approach and the existence of a well-trained neurosurgeon led to a reconsideration of this tactic in our hospital.

CONCLUSIONS

This study offers a detailed analysis of demographic, clinical, and surgical data showing their utility in the management decisions of patients with craniopharyngiomas. Overall, the present cohort shows strong concordance with published series, but also highlighted certain particularities that are probably related to the genetic pools of the patients studied and to the craniopharyngioma changing biology in the last years. We found out two peaks of the incidence of craniopharyngioma developing in adults, a slight predominance of affecting female patients, and some particular and rare locations of craniopharyngiomas, such as ventricle 3 and peri mesencephalic cisterns. The predominance of transcranial approaches in our cohort is comparable to many contemporary surgical series, particularly in craniopharyngiomas with large tumor volume and suprasellar or intraventricular extension.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest.

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FACTORS INFLUENCING THE LEVEL OF DEPRESSION AMONG FRESHMAN NURSING STUDENTS

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FACTORS INFLUENCING THE LEVEL OF DEPRESSION AMONG FRESHMAN NURSING STUDENTS (Abstract): **Aim**: To identify the level of depression among nursing students and the factors that influence it. **Material and methods**: A group of 89 nursing students were included in the research. Socio-demographic data (age, sex, marital status, and experience as a nurse) and four instruments were used *(The Beck Depression Inventory, Eysenck Personality Inventory, The Maslach Burnout Inventory* and *The Job Satisfaction Scale)* to identify the level of depression, burnout, the personality traits, job satisfaction scores and the relationship between the considered variables. Data have been processed using SPSS 17. **Results and discussion**: Almost 20% of first-year nursing students have symptoms of depression, ranging from mild to severe. Significant statistical correlations were identified between depression and age, experience as a nurse, emotional exhaustion, introversion, emotional instability and job satisfaction. **Conclusions**: Melancholic individuals are more prone to depression than other temperaments. The more emotionally exhausted individuals are, the higher their chances are of developing depression. Emotional instability is increasing the risks of depression. **Keywords:** NURSING STUDENTS, DEPRESSION, EMOTIONAL EXHAUSTION, PERSONALITY TRAITS, JOB SATISFACTION.

The academic years are usually very stressful for all medical students. Firstly, the distress level is determined by the large and diverse amount of disciplines and by the continuous assessment. Secondly, psychological distress is due to the interaction with the patients during their practice stages. Thirdly, the transition from adolescence to maturity and the changes in their personal lives also have consequences on their psychological well-being.

Apart from burnout, depression is one of the most important psychological problems experienced by nursing students during their academic years. The scientific literature provides data for the general population of students, about 33%. Regarding the presence of depressive symptoms among nursing students, the data presented are different, depending on the country: 52.4% in Greece (1), 22.9% in China (2), 30.2% in Brazil (3), 30.5% in Denmark and 43% in Saudi Arabia (4, 5).

Studies have also pointed out that re-

ports for depressive symptoms among medical students are higher compared to the general population, even before students begin their medical education (4).

Identifying and providing knowledge about depression and burnout among healthcare professionals is an important aspect of medical education. Professionals in the medical field are exposed to higher risks of depression and professional exhaustion, and their negative impact on work and successful results has been proved by the researches in the field: higher rates of errors and of difficulties in maintaining good relationships with patients or colleagues and low rates of job-related satisfaction (6, 7).

The aim of the present study is to evaluate the level of depression among first-year nursing students and to identify its relationship with personality traits, burnout, job satisfaction and socio-demographic variables. The article is part of an extended research focusing on psychological problems related to socio-demographic aspects and personality traits among freshman students from nursing studies.

MATERIAL AND METHODS

A group of 89 freshman students from the nursing specialty took part in the research. A fill-in paper recording socio-demographic data and 4 instruments were provided, after the students were informed about the goal of the research and the confidentiality of personal data.

Socio-demographic data like age, sex (male, female), environment (urban/rural), marital status (single, in a partnership), the number of children in the family of origin (singleton/siblings), having children (yes/no) and length of experience as a nurse were recorded.

Four instruments were used for the research to evaluate depression and the relationship with burnout syndrome, personality traits and job-related satisfaction:

1. The level of depression was evaluated by *The Beck Depression Inventory* (BDI). The instrument has twenty-one items using a four-point scale ranging from 0 (symptom is not present) to 3 (symptom is very intense). Ratings from 0 to 13 are considered in the minimal range, 14 to 19 mild, 20 to 28 moderate, and 29 to 63 severe depression (8) The Cronbach alpha (.85) shows good internal consistency.

2. *Eysenck Personality Questionnaire* (EPQ) was used to assess participants' personality traits (9). This instrument with 57 items evaluates psychoticism, extraversion, neuroticism and social desirability. Combining scores for the two subscales, the four types of temperaments are identified (sanguine, melancholic, choleric and phlegmatic). Extroverts are usually outgoing, talkative, and in need of external stimulation, while introverts are overstimulated and excitable; they need peace and quiet to achieve a good level of performance. People high on neuroticism experience high levels of negative affect, such as depression and anxiety, while emotionally stable people are calm and don't experience negative affect unless they are faced with major stressors. Psychoticism is associated with aggression and with chances of having a psychotic episode and Lie/Social Desirability measures the extent to which respondents try to be socially desirable in their answers. The instrument has good internal consistency, with a Cronbach's alpha coefficient of 0.81.

3. The burnout syndrome was evaluated using *The Maslach Burnout Inventory* (MBI). The instrument contains three

scales which have nine, five and respectively eight items. MBI evaluates e*motional exhaustion* (referring to the feeling of being emotionally spent and fatigued by work), d*epersonalization* (describing coldness in responses towards the beneficiaries of one's work) and *personal achievement* (related to feelings of competence and achievement in working with other people). The higher scores on *emotional exhaustion* and depersonalization subscales are related to higher degrees of burnout and lower scores on the *personal achievement* subscale correspond to higher rates of burnout (10). For the three subscales we obtained the following Cronbach's alpha scores: .828, .591 and .829.

4. Because more than half of the students are working in the medical field, we have also wanted to evaluate the level of job-related satisfaction with *The Job Satisfaction Scale* (JSS). The instrument contains 4 subscales with a total of 32 items: *payment and promotions* (assessing someone's satisfaction with rewards like payment, promotion etc.), *management and interpersonal relationships* (the employee's satisfaction with the social setting, the relationships he/she has with colleagues/manager), *organization and communication* (the employee's satisfaction with work organization and the way work is performed) and the total score for the scale - *overall job satisfaction*-providing a total score for satisfaction with all aspects of the work (11). The Cronbach alpha scores for the subscales are: .77, .61 respectively .62 and for overall job satisfaction the score is .86.

The data were processed using *SPSS 17.0* for Windows. We used descriptive statistics to point out the results obtained for each instrument used (mean and standard deviation). For the comparative analysis we used one-way ANOVA and Independent Samples *t* Test. For the correlational analysis we used Pearson correlations and for the regression analysis we used multiple linear regression.

RESULTS AND DISCUSSION

Descriptive analysis

Socio-demographic data papers and scales to be filled in were distributed among first-year nursing students in a university in north-eastern Romania. A number of 89 fully filled-in questionnaires were returned in time. The students come from 6 counties from this region (Bacău, Botoșani, Iași, Neamț, Suceava, Vaslui). Socio-demographic data are presented in Table I.

The research focused on depression, temperamental traits, burnout and job satisfaction scores. The results obtained using the four instruments are presented in Table II.

Depression was evaluated for all participants. The analysis of scores proves that 9.5% of the participants have mild depression, 6.7% have moderate depression, and 2.2% have severe depression.

Regarding burnout subscales, it has been identified that 15.7% of students have high levels of emotional exhaustion, for the other two subscales the scores are low.

Eysenck scores focused on revealing introvert/extrovert traits and the emotional in/stability. The analysis of data proved that 42% of participants are introverts and 58% are extroverts, while 53.4% are emotionally stable and 46.6%, emotionally unstable. The temperamental categories are: 28.1% of students are sanguine, 25.8% are phlegmatic, 14.6% melancholic, and 31.5% choleric.

TABLE I
Socio-demographic data

Variables		N (%)
Gender	Male Female	8 (9) 81 (91)
Environment	Urban Rural	55 (62.50) 33 (37.50)
Age (25.52 ± 9.42)	Minimum age Maximum age	19 48
Relationship status	Single (single or divorced) In partnership (couple or married)	58 (65.2) 31 (34.8)
Children	Have children No children	19 (21.3) 70 (78.7%)
Experience as nurse	Yes No	24 (24.7) 65 (75.3)
Length of employment as a nurse (3.31 ± 6.52)	Minimum length Maximum length	1 year 26 years
Family of origin	Singleton Siblings	14 (15.7%) 75 (84.3)

TABLE II
Scores for BECK, Eysenck, JSS, MBI

Instruments	Scale/subscale	Mean ± Standard deviation
BECK		10.08 ± 8.70
EYSENCK	introversion - extraversion	11.43 ± 3.41
	stability - instability	12.34 ± 4.40
	social desirability	3.60 ± 1.82
MBI	emotional exhaustion	19.54 ± 10.08
	personal achievement	12.79 ± 8.56
	depersonalization	6.57 ± 5.41
JSS	payment and promotion	3.23 ± .70
	management-interpersonal relationships	4.05 ± .72
	organization-communication	4.28 ± .60
	overall satisfaction	3.80 ± .58

Correlational analyses

Pearson correlations were used for the investigated variables in the present study. A positive relation was identified between age and depression (meaning that the higher the age is, the higher the scores on depression are) and between the length of employment as a nurse and depression (the more nursing experience participants have, the higher they score on depression).

TABLE III

Results regarding correlations between depression and other variables

	Variables	Depression
SOCIO-DEMOGRAPHIC DATA	Age	**R = .256*, p = .019**
	Number of children	R = .036, p = .745
	Nurse experience	**R = .347*, p = .001**
EYSENCK	Introversion-extraversion	**R = -.271*, p = .013**
	Stability-instability	**R = .309**, p = .005**
MBI	Emotional exhaustion	**R = .362**, p = .002**
	Personal accomplishment	R= -.098, p = .417
	Depersonalization	R= -.145, p =.230
JSS	Payment-promotion	**R = -.257*, p = .032**
	Management-interpersonal relationships	**R= - .317**, p = 007**
	Organization-communication	**R = - .333**, p = .004**
	Overall job satisfaction	**R = - .354**, p = .003**

A negative correlation between introversion-extraversion and depression were identified, meaning that higher scores on this subscale of the *Eysenck Personality Questionnaire* correspond to lower levels of depression and a positive correlation appears to exist between the stability-instability subscale and depression. More exactly, the higher the scores on stability-instability, the higher the scores on depression; the lower the scores on stability-instability, the lower the scores on depression.

The relationship between depression and subscales of MBI was analyzed. Positive correlations were pointed between the *emotional exhaustion* subscale and depression, meaning that higher scores on *emotional exhaustion* are associated with higher scores on depression, while lower scores on *emotional exhaustion* are associated with lower scores on depression.

Negative correlations were identified between depressions and factors of job satisfaction (including the overall scores). The more satisfied participants were on *payment-promotion*, the lower they scored on depression. When considering the *management-interpersonal* relationships subdomain, the higher subjects scored on this variable, the lower their scores on depression. The more individuals were satisfied with the *organization-communication* aspect of job satisfaction, the less depressed they felt. Finally, the higher the score is for *overall job satisfaction*, the lower the depression value is. It is important to point that the results prove that depression affect all job-related aspects.

Comparative analysis

For the variables where we have three or more independent (unrelated) groups (temperament, burnout), we used the one-way analysis of variance (ANOVA) in order to establish if there are any statistically significant differences between the means of these groups. For the variables where we have two independent groups (nursing experience, presence of children, relationship status), we used the Independent Samples *t* Test. This is a parametric test comparing the means of two independent groups and determining if the means are significantly different.

The number of male students is too low to proceed with a comparative analysis, to analyze if there are some differences between men and women, considering this variable. Studies from the literature point out some higher scores for depression for female students, compared to males (12).

To determine whether the type of temperament has an influence on depression, we took into consideration the four known types: sanguine, phlegmatic, melancholic, and choleric. One-way ANOVA results (F (3.80), p = .005) show that there is a difference in depression among participants, according to their temperament. Multiple/post-hoc comparisons showed that sanguine individuals have lower scores on depression compared to melancholic individuals. Another difference that has emerged is that phlegmatic people have lower scores on depression compared to melancholic people. Finally, melancholic individuals have higher scores on depression compared to choleric individuals.

While the comparison between introverts and extroverts reveals no statistically significant differences (t [81] = 1.733, p = .087), the Independent Samples *t* Test shows that there is a difference between stable and unstable individuals on depression: t [81] = -2.134, p = .037. More specifically, stable individuals have lower scores on depression compared to unstable individuals.

For analyses concerning burnout subscales, we divided the variable into three groups on each of the three subscales: emotional exhaustion, personal achievement, and depersonalization. The results of one-way ANOVA showed that only emotional exhaustion influences depression (F (2.68) = 4.126, p = .020). Multiple/post-hoc comparisons showed that individuals with low scores on emotional exhaustion have lower scores on depression compared to those with high scores on emotional exhaustion.

For analyses concerning nursing experience, we divided the participants into two groups: no nursing experience and with nursing experience. The statistical analysis showed no differences in depression between participants with or without nursing experience (t [82] = -1.579, p = .129).

The results of the comparison between participants with and without children showed no differences concerning depression (t [82] =.013, p = .990).

When comparing single individuals with the ones involved in a relationship, the results of the Independent Samples *t* Test (t [82] = -.698, p = .487) showed no differences regarding the depression level between single students and those involved in a partnership. The analysis of results from the scientific literature provides different results about the scores for depression when it is about the marital status. The study of Sharokani et al. (13) mentioned that single students are more susceptible to higher rates of depression compared to married students. Contrary to these results, Bayram et al. (14) reported lower rates for single students.

Regression analysis

The table of correlations revealed the influence of some variables (age, nursing experience, introversion/extraversion, stability/instability, emotional exhaustion, payment-promotion, management interpersonal relationships, organization communication, and overall job satisfaction) on depression. To identify the most effective model for estimating the predictors for depression, we used multiple linear regression by the hierarchical method.

Of the 9 models in which the depression criterion was tested, the fifth model explains 30% of its variance. Among the 5 predictors of the model, only stability/

instability and emotional exhaustions are significant predictors of depression (p = .016, respectively p = .027), their effect on the criterion being positive (b = .613, beta = .277, respectively b = .253, beta = .266). Results are presented in Table IV.

TABLE IV
Regression analysis

	Model	Adjusted R^2	F	R^2 change
Step 1	Age	.063	5.041 (p =.029)	.079
Step 2	Age, nurse experience	.120	5.106 (p =.009)	.071
Step 3	Age, nurse experience, introversion/extraversion	.167	4.997 (p =004)	.059
Step 4	Age, nurse experience, introversion/extraversion, stability/instability	.247	5.928 (p =.000)	.089
Step 5	Age, nurse experience, introversion/extraversion, stability/instability, emotional exhaustion	.300	6.132 (p =.000)	.060
Step 6	Age, nurse experience, introversion/extraversion, stability/instability, emotional exhaustion, payment-promotion	.287	5.025 (p =.000)	.000
Step 7	Age, nurse experience, introversion/extraversion, stability/instability, emotional exhaustion, payment-promotion, management-interpersonal relationships	.303	4.731 (p =.000)	.026
Step 8	Age, nurse experience, introversion/extraversion, stability/instability, emotional exhaustion, payment-promotion, management-interpersonal relationships, organization-communication	.291	4.085 (p =.001)	.001
Step 9	Age, nurse experience, introversion/extraversion, stability/instability, emotional exhaustion, payment-promotion, management-interpersonal relationships, organization-communication, overall job satisfaction	.295	3.793 (p =.001)	.015

CONCLUSIONS

The research proves that almost 20% of first-year nursing students have symptoms of depression, ranging from mild to severe. The factors influencing the level of depression are temperament, emotional exhaustion and emotional stability. Melancholic individuals are more prone to depression than sanguine, choleric and phlegmatic individuals. The more emotionally exhausted individuals are, the higher their chances are of developing depression. Regarding emotional stability, we identified that unstable individuals present higher risks of developing depression. The results are important for both students and teachers, to be aware that personality traits and coping strategies are important for the personal and professional life.

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NEWS

A NEW HYPOTHESIS OF THE AETIOLOGICAL FACTORS IN DENTINE HYPERSENSITIVITY

Dentine hypersensitivity is a relatively common dental complaint, derived from the exposure of dentine. A recent study of O'Toole and Bartlett analyzed 600 adults, with the mean age of 44, using a questionnaire that included data of the tooth hypersensitivity, acidic intake habits and toothbrushing habits. Results showed that the intake of the acidic drinks between meals, the habit of sipping, swishing or holding drinks in the mouth prior to swallowing, consuming fruit in more than 10 minutes at a single sitting, the use of a soft brush and the presence of severe erosive tooth wear were connected with dentine hypersensitivity. The authors concluded that the contact time of the acid inside the oral cavity might be a more important factor for the dentine hypersensitivity compared to frequency of the acid intake or frequency of toothbrushing. (O'Toole S, Bartlett D. The relationship between dentine hypersensitivity, dietary acid intake and erosive tooth wear. *J Dent*; 2017; Available from: http://dx.doi.org/10.1016/j.jdent.2017.10.002)

Emilia Bologa

ETIOLOGICAL AND CLINICAL-PARACLINICAL IMPLICATIONS OF ACUTE ABDOMINAL PAIN IN CHILDREN

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ETIOLOGICAL AND CLINICAL-PARACLINICAL IMPLICATIONS OF ACUTE ABDOMIMAL PAIN IN CHILDREN (Abstract): Abdominal pain is the most important symptom of digestive diseases in children, being one of the most frequent reasons for their coming to the general practitioner, pediatrician or emergency room. The etiological possibilities for abdominal pain are numerous and the condition is difficult to manage even in the best of clinical circumstances, but especially in children who cannot tell a precise history or locate the pain. In such circumstances, the most important role of the doctor is to distinguish between benign self-limiting conditions, and life-threatening diseases, which are true surgical emergencies. Thorough anamnesis and full physical exam of the child guides the diagnosis, and paraclinical tests targeted to the most common group of diseases depending on the child's age will lead in most cases to setting the positive diagnosis and thus allow the timely initiation of an adequate therapy, either medical, or surgical. This review aims to provide an update on the etiology and the main diagnostic methods of acute abdomen, helping discriminate between those patients who can be managed entirely in primary care, with recommendations based on the most currently accepted concepts in the management of acute abdomen in children. **Keywords**: ABDOMINAL PAIN, ACUTE ABDOMEN, CHILDREN.

The term "acute abdomen" designates any traumatic or non-traumatic condition with fast onset and digestive pain and symptoms, which requires urgent hospitalization in a surgical ward for monitoring and possibly surgical therapy, if necessary.

Abdominal pain is the third most common chief complaint in emergency departments and is a difficult condition to diagnosis even in the best of clinical circumstances, and especially in children who cannot tell a precise history or locate the pain (1). The etiological possibilities are numerous, but abdominal pain is the most important symptom of digestive diseases and it occurs in most abdominal conditions (involving the guts, the peritoneum, the vessels and nerves, the mesentery, the omentum). When the pain is very sharp, accompanied by other signs and symptoms, and especially when it occurs suddenly, the children are brought by their parents directly to the emergency room of the hospital (2). In this case, the most important role of the doctor is to distinguish between benign self-limiting conditions, such as acute viral

gastroenteritis, constipation or infant's abdominal colic, and life-threatening diseases, which are true surgical emergencies. This review aims to provide an update on the etiology and the main diagnostic methods of acute abdomen, helping discriminate between those patients who can be managed entirely in primary care, with recommendations based on the most currently accepted concepts in the management of acute abdomen in children.

Patient assessment and anamnesis

The process of making a triage decision always begins by reassurance that the child is clinically stable. After this, patient assessment should start with a thorough anamnesis about the child's age, personal medical history, possible history of physical injury or psychological trauma, presence or absence of appetite (for instance, a child who is hungry is less likely to have appendicitis), pain onset and chronic nature, and associated symptoms, and it should be followed by a detailed physical exam. The child's anamnesis and physical exam are essential for diagnosis setting and for choosing the right therapeutic approach, and especially for developing a relationship of trust between the doctor and the patient, and the family, considering the usually difficult circumstances of the exam (in the emergency room, while the child is in great pain and the family is deeply distressed). Anxiety increases even more when a possible surgical condition is suspected and it reaches its peak in the family when the surgeon comes. Therefore, it is extremely important for the surgeon performing the anamnesis to be infinitely patient and calm, and to allow the family and then the child (if he/she is older than 2-3 years) to tell what the problem is, while carefully observing the patient's movements when he/she undresses and is asked to lie on his/her back.

The **child's age** can provide important information. In neonates, congenital malrotation of the small bowel can result in volvulus, causing bowel obstruction with biliary, fecaloid or bloodstained vomiting. Hirschprung's disease is also a potential diagnosis in neonates that present with enterocolitis. In children aged between 2 months and 1 year, intussusception is the most common cause of bowel obstruction. Puberty may be the cause of vague abdominal pain in girls with the first menstruation. Just remember that in any girl of child-bearing age pregnancy should always be excluded. Also for adolescents, check for adnexal torsion in girls and testicular torsion in boys.

Pain onset may provide useful information. For example, a sharp sudden pain spreading quickly throughout the whole abdomen may be suggestive of gastrointestinal perforation, adnexal torsion or ruptured extra-uterine pregnancy in female adolescents. Extreme agitation of the patient suggests the involvement of an ischemic mechanism, the pain being stabbing, accompanied by paleness, reflex vomiting and perspiration (vagal signs), like in intussusception, volvulus or adnexal torsion. Any pain with insidious onset and increasing intensity may suggest an infectious (acute appendicitis, Meckel diverticulitis), obstructive (bowel obstruction) or neoplastic process. The pain onset seat, when it may be set, namely in children older than 5-6 years, may provide precious information: a pain starting in the epigastrium and then moving towards the right iliac fossa is suggestive of acute appendicitis, a pain in the right hypochondrium irra-

diating in the shoulder is specific to irritation of the diaphragm (sub phrenic abscesses after acute peritonitis for which surgery was recently performed) or to acute cholecystitis, a pain in the left iliac fossa and left hypochondrium is characteristic to enterocolitis, whereas a diffuse periumbilical pain is suggestive of intestinal parasites. When investigating abdominal pain, it is important to ask about pain elsewhere in the body as this can also give clues towards the cause of the disease (referred pain). Common examples are scapular pain due to biliary colic, shoulder pain due to blood or infection irritating the diaphragm (Kehrs sign: left shoulder tip pain is a classical symptom of a ruptured spleen).

The second most common symptom of acute abdomen is **vomiting**, which may be reflex, in case of ischemia or peritoneal irritation (acute volvulus, strangulated hernia, acute appendicitis), or may be the consequence of an obstruction, which is initially food-related and subsequently becomes bilious and then fecal. Impaired intestinal gas and feces transit, or, on the contrary, diarrhea (especially in small children) may both be signs of acute surgical abdomen.

Finding the cause of acute abdomen in children younger than 5 years is extremely difficult since the child is neither able to express the type or intensity of the pain, nor to locate it, since the ability to locate abdominal pain appears after the age of 4-5 years, with the maturity of the central nervous system. Sometimes, atypical and not very noisy symptoms, or even the absence of physical signs, are another impediment. For instance, a 2-year child with peritonitis may be simply lethargic, complaining of abdominal distention and diarrhea. Therefore, on the first exam, acute appendicitis is mistaken for something else in almost 100% of the patients up to 2 years of age and in 28-57% of the children below the age of 12 (3). The key to early diagnosis is a high level of clinical suspicion, based on the clinical experience of the first doctor examining the child. Given the difficulties encountered when assessing young children with abdominal pain, the ruptured appendix rate is much higher in children than in adults, ranging between 30 and 65% (4).

Clinical Assessment

Clinical assessment should be done using the systematic process of inspection, auscultation, palpation and percussion. Therefore, it will start by a general exam of the body, with special focus on the abdomen, to detect any abdomen rigidity or distention, skin rashes (Henoch-Sconlein purpura), postoperative scars, possible sings of injury (fig. 1). Palpation is used to detect any areas of guarding, tenderness or any masses and should start away from the area of tenderness. The abdomen should be palpated gently, while observing the patient's facial expression and respecting the little patient's hand that will push the doctor's hand away from the painful spot. Percussion is also a good way of assessing for peritonism and will elicit pain even if the patient has received strong analgesia. Abdomen percussion may reveal hyper sonority in case of pneumoperitoneum or dullness in case of fluid effusions, and auscultation may detect bowel sounds in case of bowel obstruction or abdominal silence in peritonitis or paralytic ileus. One must always ensure that the inguinal and scrotal areas are examined for strangulated hernias, testicular hydrocele in tension or acute scrotum syndrome.

Fig. 1. Sign of injury – bike handlebar blow – in a child with post-injury jejuna perforation

All physical exams for acute abdomen should end with a **rectal exam**, which may provide extremely important data: pain in the pouch of Douglas in case of appendicitis or peritonitis, palpation of an ovarian tumor or of an extra-uterine pregnancy; also, rectal exam may detect the presence of a fecalith or rectal polyp, may set a diagnosis of infant's intussusception in the presence of specific "red currant jelly" stool or "meat juice" bleeding (fig. 2).

Fig. 2. Rectorrhagia after rectal examination – in an infant with intussusception

However, rectal exam may induce severe discomfort, defined as major crying or screaming, in nearly one fourth of children presenting with possible appendicitis, and mild discomfort, defined as facial grimacing, was reported in another 37% (5). That is why the information gained from the rectal examination must be weighed against the potential of induced physical discomfort.

Paraclinical Assessment

Complete blood count and erythrocyte sedimentation rate, coagulation tests, biochemical exam, urine and stool tests are the routine laboratory tests that should be performed. For adolescent girls, pregnancy testing may also help narrow the differential diagnosis. The **blood count** and coagulation tests may reveal the presence of hemorrhagic anemia (in case of abdominal injury, for instance), abnormally high inflammatory test findings are suggestive of acute appendicitis or another infectious process, high amylase and lipase levels may point to acute pancreatitis. **Hematuria** is specific to a renal condition like lithiasis, but it may also occur in case of irritated ureter by an inflamed retrocecal or mesoceliac appendix. Diarrhea is specific to acute enterocolitis, yet it may also occur in the peritoneal irritation syndrome, whereas blood present in the stool may point, together with perineum and rectal examination, to anal fissures due to constipation, rectal polyp or intussusception (fig. 2).

Among the imaging tests, **ultrasound scanning** is usually the first method that is recommended due to its accessibility and non-invasive nature, as well as to its ability to contribute precious information for diagnosis setting and therapeutic approach. The ultrasound may reveal:

- the presence of free fluid in the peritoneal cavity – blood in case of spleen or liver injuries, gastric or intestinal fluid in

digestive perforations, reaction fluid or puss in acute appendicitis or peritonitis (6);

- the presence of inflamed aperistaltic cecal appendix, possibly with an inner appendicolith, with a diameter exceeding 6 mm in case of acute appendicitis;
- the presence of intraperitoneal abscesses – appendicular, subhepatic, peri splenic and pouch of Douglas abscesses;
- signs of acute cholecystitis – thickened gallbladder wall or small wall abscesses, pericholecystic abscess, gallbladder lithiasis;
- the presence of parenchymatous organ lesions in case of injury - hematoma or ruptured spleen, liver, kidney;
- diminished intestinal peristalsis, thickened-wall fluid-filled intestinal loops – for instance, in peritonitis or acute enterocolitis;
- distended intestinal loops, which are filled with fluid and air – in mechanical obstructions;
- specific "target-like" or "loop-in-loop" signs – in case of intussusception, in which case the ultrasound allows the real-time watching of loop disinvagination when air is blown in or a serum or contrast agent enema is performed (7);
- the presence of abdominal or retroperitoneal tumors;
- the state of the girls' inner genitalia, the presence of ovary cysts or pathological ovarian torsion – ovarian teratoma, hemorrhagic ovarian cyst (8);
- the state of the urinary system – hydronephrosis, kidney, ureter or gallbladder lithiasis, kidney or gallbladder tumors, full bladder.

If there is any suspicion of occlusive syndrome (hydroaeric levels revealed by abdominal X-ray), gastric or intestinal perforation (pneumoperitoneum) or ingestion of foreign bodies, a **thoracic-abdominal X-Ray** of the standing patient is recommended (fig. 3 and 4). Thoracic X-ray may reveal the presence of broken ribs, in case of injury, hydro or pneumothorax, or it may reveal basal pneumonia with symptoms such as occasionally sharp abdominal pain, vomiting, poor general condition resembling acute surgical abdomen.

Fig. 3. Occlusive syndrome – hydraeric levels

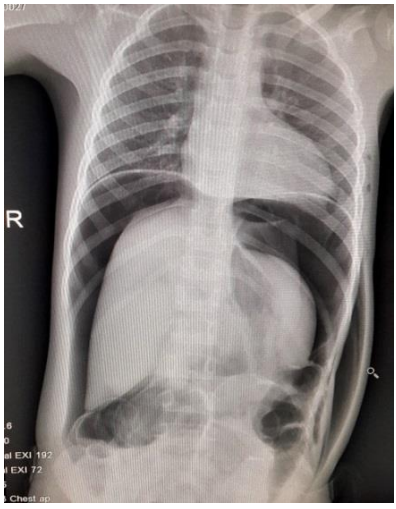

Fig. 4. Pneumoperitoneum – colic perforation

Due to the high radiation dose involved and to the need of young child sedation, **computed tomography** (CT) diagnosing acute abdomen in children should be recommended with caution, only when the children are obese, in case of uncertain diagnosis or when the ultrasound scan is inconclusive. However, CT is the preferred diagnosis setting method in case of abdominal injury, as it accurately reveals both solid organ lesions and possible cavity organ perforations. It is also recommended when assessing acute pancreatitis or intestinal inflammatory diseases, whereas in case of abdominal tumors nuclear magnetic resonance (NMR) is preferred, despite it being rarely accessible in the emergency room and despite the drawback of its requiring general anesthesia (9). Tests like angiography, upper or lower digestive endoscopy, paracentesis in case of fluid in the peritoneal cavity (which may reveal the presence of blood, intestinal fluid, pus, ascites fluid, urine) are more rarely used in children in the emergency room.

Laparoscopy has a special place among diagnosis setting exams, being used more and more often lately including in children, due to its many advantages: very small incisions, the possibility of probing the whole abdomen and of flushing it using physiological saline solution, the possibility of peritoneal fluid sampling or biopsy sampling for biological and pathological anatomy tests, and especially the possibility of solving the cause of acute abdomen (ovarian torsion solving and fixation or excision, appendectomy and peritoneal lavage, ileum perforation suture, peritoneal adhesion lysis, hernia reduction and hernia gap closing).

Causes of Acute Abdomen in Children

Here are the most common **medical** caused of acute abdomen in children: constipation, intestinal parasites, acute gastroenterocolitis, dyspepsia, allergy to cow milk proteins, esophagitis due to gastroesophageal reflux, irritable bowel syndrome, urinary tract infection and ureter colic, virus A hepatitis. Also, consider acute mesenteric lymphadenitis in children presenting after a viral illness or new onset diabetes presenting with abdominal cramps, which are associated with diabetic ketoacidosis. Acute gastroduodenitis, cholecystitis, acute idiopathic or toxic pancreatitis are causes of acute abdomen which usually require drug treatment, although they may sometimes get complicated and require surgery.

Recurrent abdominal pain is a prevalent disorder affecting 10% to 15% of school-age children (10, 11). In many cases, recurrent abdominal pain in children has psychogenic (**functional**) origins, without having a specific organic cause (infection, inflammation, tumor, obstruction). The big difficulty with recurrent abdominal pain is the fear of missing significant or serious underlying pathology. Table I gives a brief overview of how to differentiate functional from organic abdominal pain (12).

Psychogenic pain may be accompanied by symptoms such as anorexia, nausea, vertigo, cephalalgia, paleness, constipation and, more rarely, vomiting or diarrhea. Psychogenic pain occurs in sensitive overprotected children, with a family history of depression or anxiety, and it is associated with a series of stress factors like a history of frequent diseases, lactose or other hydrocarbon intolerance, drug administration, aerophagia, constipation, and especially with psychosocial factors like the death of

a parent or of another family member, arguments, divorce or separation from the family for other reasons, jealousy after the birth of a sibling, school or financial problems. Psychogenic abdominal pain is sometimes hard to diagnose, it is attributed to various organic conditions and treated with drugs. Drugs should not ordinarily be given because may aggravate the family's panic and the concept of the pain, creates an unnecessary dependency, and are almost always unrewarding. The treatment is useless unless the psychological cause is found and removed.

TABLE I.
Differentiating organic from functional abdominal pain

Clinical feature	Organic	Functional
Site of pain	Loin, suprapubic, right upper and lower quadrants	Central
Family history of abdominal pain, headache and depression	Less likely	More likely
Anxiety	Less likely	More likely
Headache	Less likely	More likely
Alarm symptoms	Persistent or significant vomiting, chronic diarrhea, fever, gastrointestinal blood loss	Less likely
Poor growth/weight gain	Yes	No
Abnormal full blood count, erythrocyte sedimentation rate or urinalysis	Yes	No

False acute abdomen (extra-abdominal pain causes) may be caused by: basal pneumonia, myocardial infarction, diabetic ketoacidosis, pericarditis, spine conditions, and central nervous system tumors.

The **surgical** causes may be grouped in four syndromes:

- The peritoneal irritation syndrome and acute peritonitic abdomen, respectively: acute appendicitis and appendicular peritonitis, more rarely primitive acute peritonitis, Meckel diverticulitis, perforated gastric ulcer in adolescents; in the specialty literature, the acute appendicitis is the most common cause of surgical acute abdomen in children, being diagnosed in 1-8% of the children coming to the emergency room complaining of abdominal pain (13, 14);
- Occlusive syndrome: intussusception, malrotation, inner hernia, intestinal obstructions due to adhesions or roundworms, strangulated inguinal hernia; intussusception is the most common cause of acute surgical abdomen in infants under the age of one, its incidence rate peaking between the age of 6 and 11 months (3);
- Hemorrhagic syndrome: intestinal polyposis, ruptured intestinal hemangioma, bleeding ulcer in Meckel's diverticulum;
- Visceral ischemia syndrome: enteromesenteric infarction (rare in children), normal or pathological ovarian torsion, gastric volvulus and intestinal volvulus, torsion of a pedicle abdominal tumor;
- Acute traumatic abdomen: abdominal injuries with ruptured solid organs or perforated cavity organs; as part of acute surgical abdomen, injuries have had an in-

creasingly higher occurrence rate due to the higher number of causing events, such as road accidents, aggressions, sporting events (fig. 1).

The etiological diagnosis may sometimes not be accurately set before the surgery, doctors only being able to determine the type of acute surgical abdomen that it belongs to. What is important is to decide whether surgery is required, as the procedure should be performed in due time, usually within a few hours, after a short hydro-electrolytic, acidic-basic rebalancing, abdominal decompression if necessary (naso-gastric drainage), antibiotic therapy or blood transfusions (on which the patient's life is sometimes dependent – for instance, in internal hemorrhage due to ruptured spleen or liver). Older literature recommended, in principal, not to administer analgesics to children with abdominal pain until setting the diagnosis or at least until the surgical examination, since the analgesics may conceal the progress of an acute surgical process. However, once the diagnosis is set and the therapeutic approach is decided on, ruling out any surgical cause, analgesics should be administered immediately. Recent research has proven that the use of analgesic drugs in children with abdominal pain does not increase the risk of diagnosis errors and it should therefore be administered immediately, even before the surgical exam (15).

DISCUSSION

Abdominal pain is one of the most common reasons for the children's coming to the general practitioner or pediatrician. The child's anamnesis and physical exam usually help set an accurate diagnosis, whereas paraclinical tests targeted to the most common group of diseases depending on the child's age will lead in most cases to setting the positive diagnosis. When determining the cause of abdominal pain and acute abdomen and subsequent therapeutic approach, communication is in most cases "the key to success"; both the questions asked and the explanations given to the family should be layman versions, spoken in a calm and reassuring voice and even repeated if necessary; direct communication with the patient, even when he/she is only a few years old, decreases the child's and family's stress and anxiety levels and strengthens their trust in the healthcare system. For instance, for children who are about to undergo surgery, there are there things which should be emphasized since they are essential: the fact that the child will not feel any pain during the procedure because he/she will be asleep, the fact that when he/she wakes up his/her parents will be there and the fact that he/she will then feel better and be able to go home (16). It is also important for the family to have time to think about and process the information received before giving their informed consent for the surgical procedures. This time will be used by the surgeon to analyze the biologist test results and imaging findings.

Time is of the essence for the doctor as well, since the clinical-paraclinical dynamic reassessment of a child with unclear clinical picture, or the child's reexamination 1-2 hours later, after his/her hydro-electrolytic and acid-basic rebalancing and after pain therapy administration, may reveal new signs. Biological tests and a few imaging procedures should be performed during this time interval, depending on the age of the child and initially suspected diagnosis. Monitoring the patient closely means that if intervention is required, this

can be done in a timely fashion, reducing post-operative complications.

CONCLUSIONS

Thorough anamnesis and full physical exam of the child suffering from acute abdomen who comes to the emergency room, followed by a set of tests and imaging investigations targeted depending on the patient's age and initial clinical suspicion, will in most cases distinguish medical from surgical etiology of acute abdomen of the child, and thus allow the timely initiation of an adequate therapy.

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HEAD INJURIES IN INFANTS (0-1 YEARS)

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HEAD INJURIES IN CHILD (0-1 YEARS) (Abstract): Head injuries are one of the major causes of morbidity and mortality in children. The most common causes are falls from the same level. Boys are twice more prone to head injuries than girls. The traumatic pathology of the first 3 years of life is completely different when compared to that of adults. **Material and methods:** Retrospective study of all infants aged 0-1 years admitted for head injuries to the pediatric neurosurgery departments of "Bagdasar-Arseni" Hospital and "Sanador" Hospital in Bucharest. This 17-year study (January 1st, 2000-December 31st, 2016) included 509 patients aged 0 to 3 years. **Results**: Of these, 111 (21.8 %) were aged under 1 year, presented to the Emergency Department (ED), were hospitalized for at least 24- hour observation, and were diagnosed with at least one traumatic head injury visible on the imaging studies. The level of consciousness in infants was assessed by using a variant of the Glasgow Coma Scale (GCS) adapted to this age (0-1 years), namely Pediatric Glasgow Coma Scale (PGCS). CT with Bone-window is the first investigation in case of a head injury. **Conclusions:** Infants show a different pathology from that at other ages. Head injuries in infants pose multiple difficulties related to diagnosis, complications and follow-up. The data obtained from unenhanced CT scans constitute an emergency assessment. **Keywords:** HEAD INJURY, CHILD 0-1 YEARS, CT SCAN, PEDIATRIC NEUROSURGERY, GLASGOW COMA SCALE, GLASGOW PEDIATRIC COMA SCALE

Head injuries (HI) are one of the most important causes of mortality, morbidity and transient or permanent disability in the general population, both in adults and in children. In the USA, the incidence of HI among the children aged 0-4 years is about 1256/100,000 population (1, 2).

The clinical manifestations of pediatric and adult HI differ even when they share a common cause. Raimondi (1998) emphasized the importance of the differences between adult and pediatric pathology concluding that" the newborn is not a baby, the baby is not a school child", just "the teenager is not a child" and even more "adults are completely different from children" (3).

The most common causes of HI in infants are accidental falls from the same level or from height, traffic accidents in which children may be involved both as passengers and as pedestrians, accidents with various objects and child abuse (4).

When an infant presents to the Emergency Department with head injury, unenhanced CT brain scan is the initial imaging modality of choice. This is the "gold-standard" investigation for head injuries, and is preferred due to its accessibility and good visualization of bleeding injuries, solutions of continuity in calvaria with or without dural or cerebral expansion in the bone gap or cerebral edema lesions (5).

MATERIAL AND METHODS

All patients with HI aged 0 to 3 years admitted to the departments of pediatric neurosurgery of "Bagdasar-Arseni" Hospital and "Sanador" Hospital in Bucharest between January 1st, 2000 and December 31st, 2016 were included in this 17-year study. Of the 509 pediatric patients, 111 (21.8 %) were aged under 1 year. In this study group, we encountered a category of injuries caused by obstetrical trauma related to fetal dystocia or instrumental delivery.

All patients included in the study were admitted for at least 24-hour observation, and were diagnosed with at least one post-traumatic injury visible on imaging studies.

To analyze patients' state of consciousness, the authors used the Pediatric Glasgow Coma Scale (PGCS), a variant of the Glasgow Coma Scale (GCS) adapted for pediatric patients (6) (tab. I).

TABLE I

Pediatric Glasgow Coma Scale (6)

Best verbal response	Best eye response	Best motor response
No verbal response (1p)	No eye opening (1p)	No motor response (1p)
Inconsolable, Agitated (2p)	Eyes open to pain (2p)	Extension to pain (2p)
Inconsistently inconsolable, Moaning (3p)	Eyes open to speech (3p)	Abnormal flexion to pain (3p)
Cries but consolable, abnormal interactions (4p)	Eyes open spontaneously (4p)	Infant withdraws from pain (4p)
Smiles, orients to sounds, follows objects (5p)	N/A	Infant withdraws from touch (5p)
N/A	N/A	Infant moves spontaneously (6p)

RESULTS

The level of *consciousness* determined with PGCS in the 0-1-year group is presented in Table II.

As to the *etiology* of HI in infants the most common causes in this study group were falls from the same level or from another level and traffic accidents. A special category is represented by the injuries caused by physical aggression (tab. III).

TABLE II

PGCS evaluation of the level of consciousness in the 111 infants

GPCS	No. of cases	Percentage
15-13 points	79	71.1
12-9 points	20	17.9
<8 points	12	10.8
Total	111	100.00

TABLE III

Causes of head injuries in infants (0-1 years)

Cause	No. of cases	Percentage
Fall from the same level	41	36.9
Fall from another level	35	31.5
Road accidents	23	20.7
Accidents at play	5	4.5
Aggression (Child abuse)	4	3.
Other causes	3	2.7
Total	**111**	**100**

HIs are extremely varied, a frequent finding pallor, encountered in all study cases. Infants who were in pain were agitated and anxious. Another clinical category was the symptoms of increased intracranial pressure expressed by the anterior fontanelle bulging, vomiting, convulsive events, motor deficiencies or anisocoria and alteration of consciousness in various degrees (tab. IV).

TABLE IV

Clinical Symptomatology reported at Emergency Room

Clinical symptomatology	No. of cases	Percentage
Pallor skin	111	100
Irritability with agitation	60	54.1
Irritability and drowsiness	42	37.8
Anterior fontanelle bulging	39	35.1
Vomiting	26	23.4
Convulsive seizures	21	18.9
Motor deficits	15	13.5
Anisocoria	9	8.1
Impaired consciousness/coma state	12	10.8

The post-traumatic injuries seen on CT scans most frequently encountered in the hospitalized patients (111 cases; 21.8%) were: skull fractures of several types, cephalohematoma, extradural hematomas and DAIs (tab. V).

TABLE V

Head injury types on CT-scan examination in patients aged 0-1 year

Lesion type	No. of cases	Percentage
Cephalohematoma	15	13.5
Linear skull fracture	58	52.2
"Ping-pong" fracture	11	9.9
Progressive fracture	5	4.5
Intrusive fracture	6	5.4
Extradural hematoma	7	6.3
Subdural hematoma	1	0.9
Intraventricular hemorrhage	1	0.9
Diffuse axonal injury	7	6.3
Total	**111**	**100**

DISCUSSION

All head injuries in child 0-1 years should be very carefully assessed, and if craniocerebral injury is suspected native CT-scan is mandatory. The first CT-scan in the emergency department is very important for the following neurological, neurosurgical steps. Complementarily, brain MRI can also be used to detect traumatic injuries in the adjacent soft tissue and brain substance. Unfortunately, access to this type of examination is limited, examination time is longer, general anesthesia is required, and bone lesions are poorly visualized (7).

A distinct category of post-traumatic cerebro-ventricular injuries encountered in pediatric patients is represented by diffuse cerebral edema and diffuse axonal injury (DAI). These types of injuries significantly affect patient's consciousness.

As in adults, physical examination of pediatric HI cases should include the assessment of vital signs, level of consciousness, focal post-traumatic injuries, possible cervical spine injuries and damage to other systems and organs (polytrauma), if the case. Neurological examination is aimed at identifying the possible motor or sensory segmentation deficits, and signs and symptoms suggestive of cranial nerve dysfunction, etc.

The use of the Glasgow Coma Scale (GCS) is extremely difficult in the pediatric patients, particularly in preverbal children. For these reasons, over time, several coma scores have been developed specifically for children in attempt to compensate for their differences in verbal and motor capabilities, and the last and most useful is Pediatric Glasgow Coma Scale (GPCS) (Ong et al., 1996) (6). Other two pediatric coma scales used in practice are CHOP Infant Coma Scale (Durham et al., 2000) (8) and Starship Infant Neurological Assessment Tool (Birse, 2006) **(**9).

We will present clinical manifestations and treatment of traumatic lesions in infants:

Characteristic types of injuries encountered in patients younger than 1 year are the bleeding injuries-*cephalohematomas* (15 cases, 13.5%), caput succedaneum and subgaleal hematoma and fractures - progressive diastatic fracture and "ping-pong" fracture.

Cephalohematoma is a post-traumatic collection of blood located between the skull and periosteum. The most clinically important feature is that it is limited by suture lines. The causes are related to obstetrical traumas or difficult vaginal deliveries when there is incompatibility between maternal pelvic size and fetal head size. Usually, cephalohematomas are detected during the first days of life and are reabsorbed spontaneously in few weeks. Sometimes, they are also associated with underlying linear fractures. They occur predominantly at parietal level. Surgery is indicated in case of ossified cephalohematoma for esthetic reasons (7), and all cases were operated with favorable neurological and aesthetic results.

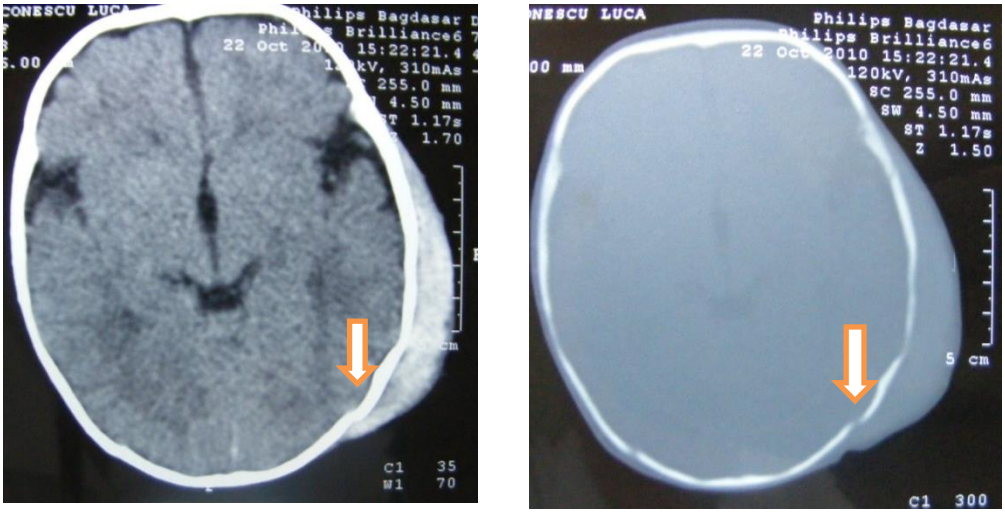
Among the types of fractures encountered there were *linear fractures* (58 cases, 52.2%) or *intrusive comminuted fractures* (6 cases, 5.4%) and particular types of fractures occurring only at this age, *diastatic fractures* due to the fact that suture fusion has not happened yet (5 cases) and *"the ping-pong" fracture* (11 cases, 9,9%), as the equivalent of" greenstick" fracture in the long bones. All the 11 cases were operated, immediately after diagnosis, with satisfactory results.

In these "ping-pong fractures", the individualized fracture paths cannot be detect-

ed, not even by imaging methods.

Solely skull deformation can be detected. In all "ping-pong" skull fractures it is recommended elevation of depressed calvaria soon as possible (10,11). In rare cases, this type of fracture may recover spontaneously (12).

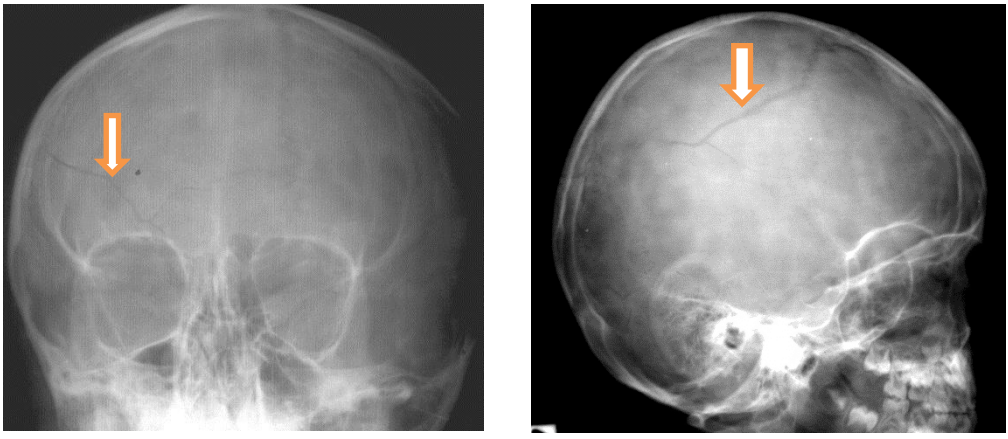
In case of *linear fractures*, hospitalization is required to monitor the patients. Linear fractures not associated with other injuries do not require surgical treatment, but they do require monitoring as an extradural hematoma may develop subsequently (fig. 1a and b).



a. CT- scan b. CT with bone window

Fig. 1. Liniar Fracture Left Parietal Bone

A different type of calvaria fracture is represented by the *diastatic fracture*, meaning the fracture involving the suture lines of the skull (5 cases) **(**fig. 2a and b).



a. Anteroposterior view b. Sagital Incidence

Fig. 2. Right fronto-parietal-diastatic fracture

One of the risks associated with a linear fracture, especially diastatic, is the potential transformation into a "*growing skull fracture*" **(**fig. 3).

Fig. 3. Left parietal progressive skull fracture

In this cases, meningeal structures and brain tissue could interfere between the edges of the fracture, preventing the consolidation process of the fracture. This process causes brain herniation between bone edges. Growing skull fracture needs neurosurgical extensive approach, removing gliotic tissue, CSF restoration, closing dura, cranio plasty. All the cases were operated with this procedure, before CT-scan evaluation, and MRI- scan. There were no death patients, but 3 from 5 cases remained with neurological deficits and seizure (13,14).

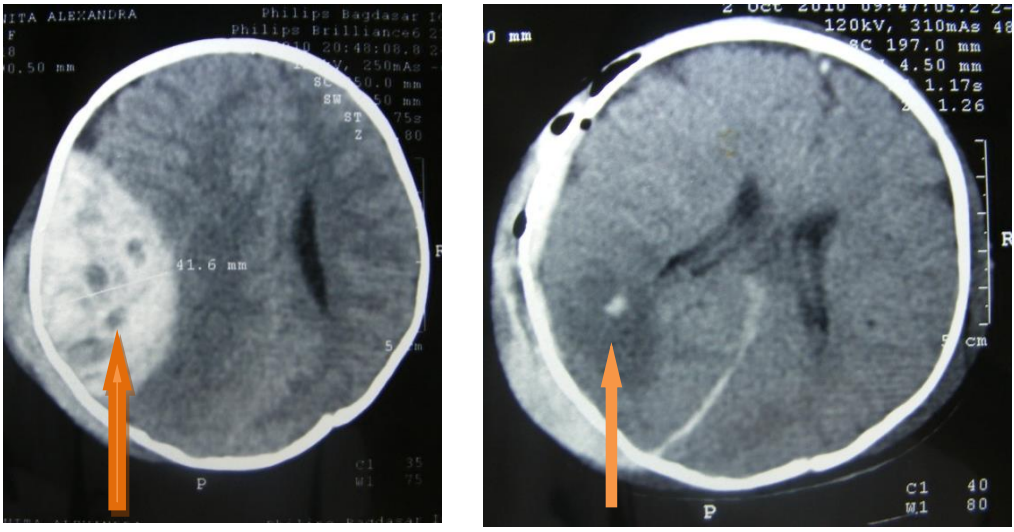
Comminuted skull fractures, with or without depressed bone fragments require surgery to reposition the fragments and to treat the underlying traumatic injuries, most likely related, because these fractures occur in case of high-energy trauma. Special attention is given to the management of comminuted fractures that are adjacent to or above the dural sinuses because of the major risk of important bleeding and hemorrhagic shock (15). All 6 cases (5.4%) were operated through a broad approach restoring cerebral structures, dural and skull, with favorable neurological outcomes; there was no postoperative death.

Post-traumatic head injuries that may be encountered are hemorrhagic brain contusion, diffuse brain injury and extradural, subdural, subarachnoid and intraventricular hematic collections. Any extra cerebrally hematic collection with compressive effect requires surgical evacuation and an adequate hemostasis to reduce intracranial hypertension that develops secondarily, with important consequences for the neurological status of patients. In our study, were 7 cases (6.03%) of *extradural hematoma* diagnosed and operated in time (7).

Results: 1 death patient, at the admission, CGS=3pts., with all the entire evacuation of the hematoma and intensive care measures could not be resuscitated, but rest of 6 cases (4 with good recovery and 2 with moderate disabilities (fig. 4).

Acute Subdural hematoma was present in one case (0.9%) but neurosurgical intervention was one time and postoperative evolution was favorable. Convulsive syndrome preoperatively, was treated with appropriate therapy.

In case of diffuse brain damage (DAI-diffuse axonal injury) (7 cases- 6,3%), which is usually associated with a poor neurological status and impaired consciousness, the aim of the initial treatment is to preserve the vital functions and minimize intracranial hypertension through nonsurgical methods. Unfortunately, these types of diffuse injuries have an extremely poor prognosis (with 2 died), despite all therapeutic measures taken (7).



a. preoperative appearance b. postoperative appearance

Fig. 4. CT-scan: Extradural hematoma in a 4-month-old infant

The Glasgow Outcome Scale (GOS) at 6 months was used for the assessment of functional outcome and prognosis, a favorable score (GOS = 5) being obtained in 65 (57.8%) of the cases (tab. VI).

The other 3 patients (2.8%) died because of extremely serious diseases: (2 death cases involving Diffuse axonal injury) and 1 case extradural hematoma

The authors draw attention on the unpredictability of clinical course in these patients, thus requiring a more complex and unitary therapeutic approach to maximize the probability of a favorable course due to the great functional resources of this patient population (**7**). Also, they emphasize that head injuries in infants is an important public health problem (4, 9).

TABLE VI

Outcome in Head Injuries - 111 of cases studied at 6 months

Glasgow Outcome Scale (GOS)			Number of cases	Percentage
5	GR	Good-recovery	65	57.8
4	MD	Moderate disability	25	22.3
3	SD	Severe Disability	13	12.3
2	VS	Vegetative state	5	4.8
1	D	Death	3	2.8
Total			**111**	**100**

CONCLUSIONS

From the point of view of therapeutic solutions, difficulties arise regarding the patients' youthful age, with multiple difficulties regarding diagnosis and monitoring of injuries, as patients have limited ways of expressing themselves. On the other hand, it is also necessary to adopt procedures that

limit blood loss, given the low capital of these patients, without neglecting the risks of significant neurologic deficits.

Concerning the laboratory investigations, brain CT scan is the gold standard in case of emergency examinations, with a high degree of identification of lesions in a reduced examination time.

Therefore, prevention of these head injuries by effectively supervising children and sending them in due time to a neurosurgical service, in case of signs and alarm symptoms, plays a very important role in achieving the most favorable results in terms of vital and functional prognosis of this category of patients.

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SUCCESSFUL AGING IS INFLUENCED BY FRAILTY AND HEALTH-RELATED QUALITY OF LIFE IN COMMUNITY-DWELLING SENIORS

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SUCCESSFUL AGING IS INFLUENCED BY FRAILTY AND HEALTH-RELATED QUALITY OF LIFE IN COMMUNITY-DWELLING SENIORS (Abstract): Successful aging at maintaining and enhancing elders' health and functioning for if possible. Frailty, a new concept in medicine, was found to be the main cause of disability and death, therefore it has an important influence on quality of life in these patients. **Aim**: To identify the prevalence of frailty in home-dwelling elderly and its influence on quality of life. **Material and methods**: The study was conducted on a group of non-hospitalized elderly (>65 years) without no prior hospitalization in the past 3 months who volunteered to take part in a geriatric assessment occasioned by the Day of Older Persons on October 1, 2016. Frailty was assessed using Fried phenotype, Groningen Frailty Indicator (GFI) and comprehensive geriatric assessment. SF-36 was used to assess the quality of life, and the participants were also asked to provide information on their medical history and medical treatments. **Results**: The study showed a low prevalence of frailty in non-hospitalized elderly people (27.77%), with higher scores recorded on GFI, but given the small sample size the correlations were not statistically significant. Quality of life was significantly decreased in the frail subjects both in terms of physical and mental function, with lower scores on the physical component. **Conclusions:** These findings suggest that early detection and intervention are essential for improving quality of life of frail elderly, one of the main elements of successful aging. **Keywords:** SUCCESFUL AGING, FRAILTY, QUALITY OF LIFE, ELDERLY

Increasing life expectancy is a well-known global phenomenon, which has led to the increase in the elderly quota, that is of people over 65 years of age; unfortunately, this phenomenon is not accompanied by a proportional increase in the youth quota, in order to keep harmonious proportions between the active and the retired population, and therefore, over the last thirty years, we have been witnessing a new demographic phenomenon known as population aging. This situation has numerous consequences, on many different levels: economic, social, medical, spiritual, implicitly requiring the generation of new projects and policies to address the health of the elderly.

Successful aging is one of these projects, which aims at preserving, for as long as possible, an acceptable level of health for the elderly (1). To achieve this goal, each older person should be assessed by a

geriatric multidisciplinary team that would develop an individual plan of measures to ensure a smooth transition and, if possible, without the interference of diseases, through the various stages of the physiological aging process. These individually tailored therapy plans have as end-goal the preservation of the quality of life and the physical, mental, spiritual, financial and social independence for if possible, trying to shorten to the maximum the final period burdened by suffering, sickness, disability and dependence (2, 3).

The prevalence and definitions of successful aging vary among studies (4); however, there is a consensus regarding its multidimensional nature, and one of the most important components is quality of life.

Quality of life (QOL) has an individual, multifactorial and dynamic nature. It is constantly related to past experiences and influenced by future expectations, which means that for an older person the quality of life changes its significance while aging (5). The Short Form-36 (SF-36) is a multidimensional scale and a well-established instrument that measures health concepts and self-reported quality of life (6).

Successful aging must also consider the degree of frailty of the elderly, an inherent phenomenon of progressive deterioration in the function of all body organs and systems, thus increasing the degree of vulnerability to various stress factors. Thus, frailty becomes a key element in ensuring successful aging. Initial studies focused on physical frailty because this was the most visible aspect in the physiological decline process, also having severe consequences on physical independence, increase in sedentary time and metabolic and cardiovascular diseases, increasing the degree of disability and the need for high-cost medical care (7). Subsequently, attention was drawn more to the phenomenon of mental and social frailty, which, in their turn can induce physical frailty (8). Evaluation of all elements of frailty is preferred in all recent studies.

Systematically applying the frailty scores to the elderly population would lead to the identification and classification of frailty in terms of stages; early intervention to combat this syndrome can make the difference between autonomy and dependence, between active and bedridden and, finally, between an optimal quality of life and the "burden of old age". The authors of recent studies still consider the best assessment and classification of frailty as being the result of a comprehensive geriatric assessment (9, 11, 12).

The impact of frailty syndrome on the quality of life of the elderly has become a subject of interest. Recent studies focus on determining the degree of negative impact of frailty syndrome on the quality of life of the elderly, but also on determining the manifestation with the highest impact on the decrease in QOL (10, 11, 12).

MATERIAL AND METHODS

The study was conducted on a group of 36 elderly persons (aged over 65) registered with family doctors and who volunteered for a geriatric assessment occasioned by the Day of Older Persons on October 1, 2016. All participants signed an informed consent and were free to withdraw from the observational study at any time. This group included all elderly persons with no acute diseases at the time of the presentation and/or who were not hospitalized for acute events over the past 3 months.

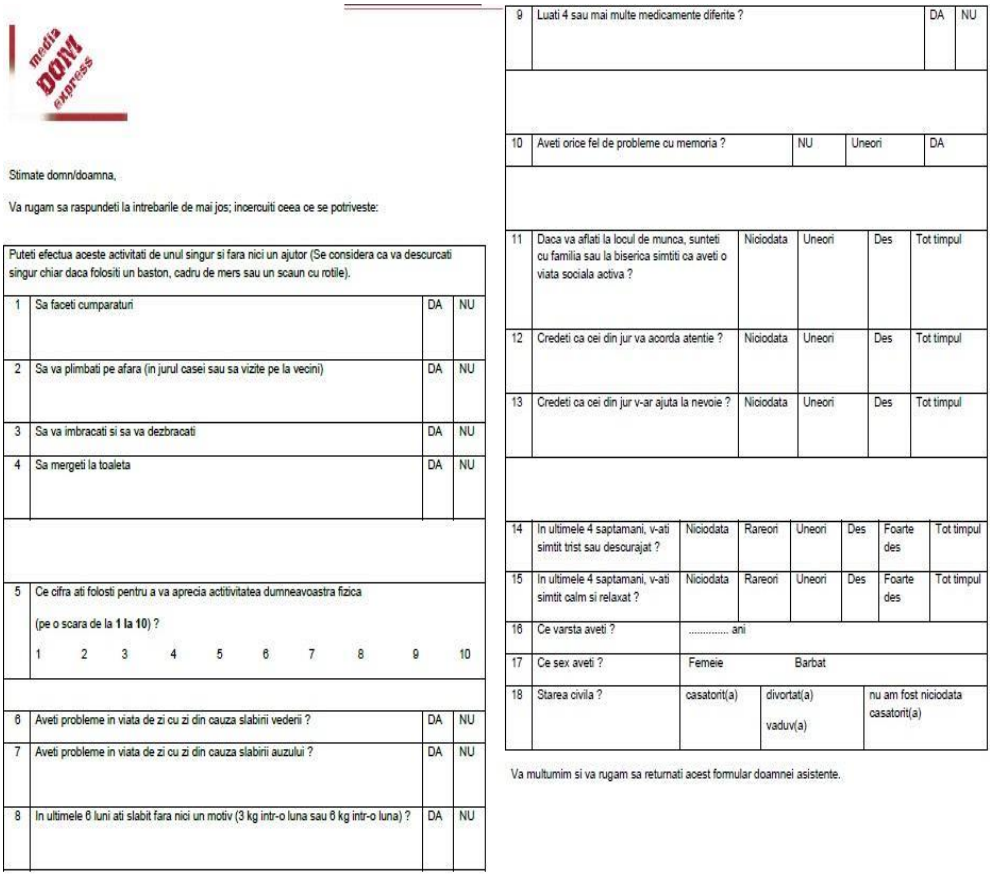
The geriatric assessment tried to capture various aspects of each participant in order to achieve an appreciation that is as close as possible to reality and which to generate an individualized therapeutic plan. In this regard, the participants filled in the following questionnaires: SF-36 (for assessing the

QOL), EASY (for the assessment of frailty through the Groeningen test) (fig.1), MMSE scale (assessment of cognitive status), MNA (assessment of nutritional status), GDS depression scale (assessment of emotional status), ADL and IADL scales (assessment of the ability to participate in daily activities). Besides these questionnaires, the participants were asked to provide information on their past medical history and the medication they take at home (including OTC); also, the parameters that define sarcopenia were assessed and anthropometric measurements were performed to calculate the Fried fragility score (tab. I).

TABLE I

Fried phenotype

Weakness	Grip strength <20% in the dominant hand
Slowness	<20% for time to walk 4.5 m
Low level of physical activity	<20% for calories expenditure
Exhaustion, poor endurance	Self- reported
Weight loss	Unintentional loss 4-5kg in 1 year



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Stimate domn/doamna,

Va rugam sa raspundeti la intrebarile de mai jos; incercuiti oeea ce se potriveste:

Puteti efectua aceste activitati de unul singur si fara nici un ajutor (Se considera ca va descurcati singur chiar daca folositi un baston, cadru de mers sau un scaun cu rotile).			
1	Sa faceti cumparaturi	DA	NU
2	Sa va plimbati pe afara (in jurul casei sau sa vizite pe la vecini)	DA	NU
3	Sa va imbracati si sa va dezbracati	DA	NU
4	Sa mergeti la toaleta	DA	NU
5	Ce cifra ati folosti pentru a va aprecia actitivitatea dumneavoastra fizica (pe o scara de la **1 la 10**) ? 1 2 3 4 5 6 7 8 9 10		
6	Aveti probleme in viata de zi cu zi din cauza slabirii vederii ?	DA	NU
7	Aveti probleme in viata de zi cu zi din cauza slabirii auzului ?	DA	NU
8	In ultimele 6 luni ati slabit fara nici un motiv (3 kg intr-o luna sau 6 kg intr-o luna) ?	DA	NU
9	Luati 4 sau mai multe medicamente diferite ?	DA	NU
10	Aveti orice fel de probleme cu memoria ?	NU	Uneori	DA
11	Daca va aflati la locul de munca, sunteti cu familia sau la biserica simtiti ca aveti o viata sociala activa ?	Niciodata	Uneori	Des	Tot timpul
12	Credeti ca oei din jur va acorda atentie ?	Niciodata	Uneori	Des	Tot timpul
13	Credeti ca oei din jur v-ar ajuta la nevoie ?	Niciodata	Uneori	Des	Tot timpul
14	In ultimele 4 saptamani, v-ati simtit trist sau descurajat ?	Niciodata	Rareori	Uneori	Des	Foarte des	Tot timpul
15	In ultimele 4 saptamani, v-ati simtit calm si relaxat ?	Niciodata	Rareori	Uneori	Des	Foarte des	Tot timpul
16	Ce varsta aveti ?	..... ani					
17	Ce sex aveti ?	Femeie	Barbat				
18	Starea civila ?	casatorit(a)	divortat(a) vaduv(a)	nu am fost niciodata casatorit(a)			

Va multumim si va rugam sa returnati acest formular doamnei asistente.

Fig.1. Groeningen test (25)

Using SF-36 to define successful aging is an acceptable tool because it assesses the overall health status in two directions: the physical component summary and the mental component summary, which are generally used to define health status.

The calculation of the Fried frailty score led to the division of the group into 3 subgroups:

- Subgroup 1- robust elderly persons (20 people) - no Fried criteria;
- Subgroup 2 - pre-frail elderly persons (6 people) - 1-2 Fried criteria;
- Subgroup 3 - frail elderly persons (10 people) - 3 or more Fried criteria.

The data were loaded and processed with the help of statistical functions in *SPSS 18.0*: ANOVA test - descriptive indicators of the monitored parameters (minimum, maximum, average, standard deviation, standard error, variance, confidence interval 95%), FANOVA - quantitative test used to study the significant difference between mean values in two or more groups, the χ^2 test - qualitative test that compares two or more frequency distributions in the same population.

RESULTS

The analysis of the demographic data indicated that patient age ranged from 65 to 89 years, mean age 75.5 years; most subjects (72.22%) were women, both in the initial group and in the 3 subgroups: 66.66 % in subgroup 1, 100% in subgroup 2 and 60% in the subgroup with frailty. These results support the data in the literature showing a significantly increased prevalence of frailty among female population (13,14). But, other studies report no significant gender differences in the prevalence of fragility, but emphasize a higher risk of mortality in men with clinically manifest frailty (15).

The conducted study showed a low prevalence of frailty among the non-hospitalized elderly people (27.77%), but the small size of the study group does not allow us to determine statistically significant data.

High Groningen scores were recorded both in the subgroup of robust elderly people (50%) and in the subgroup of frail elderly people (80%). No statistically significant correlation was found between the two used screening methods ($p = 0.285$). By using two screening tools for frailty, different degrees of impairment in the study group were identified. Thus, we found a slight overvaluation of frailty by using the Groeningen test. This tool is based on self-evaluation from the patient and is thus influenced by his/her perceived health status. The Fried score, though more succinct, includes quantifiable objective elements (muscle strength, walking speed decrease). Also, the ability to identify pre-frail subjects by using the Fried score extends its clinical utility, the measures for inducing the reversibility of frailty are believed to have the best result in the stage of pre-frailty.

The analysis of the questionnaire responses showed that the elderly enrolled in the study had no cognitive deficit and have a high degree of independence in carrying out daily activities. The GDS depression score was not significantly correlated with frailty ($p=0.184$), but there was noted the prevalence of the depressed people in the subgroup of frail subjects (40%), which confirms the role of the emotional status in the emergence and aggravation of frailty syndrome, element often omitted by the screening tools currently available, but easily identifiable by a comprehensive

geriatric assessment. The depressive syndrome in the elderly has a negative impact on QOL, nutrition, hygiene and therapeutic compliance, but once identified it benefits from specialized treatment and counselling (16).

The analysis of the SF-36 questionnaires showed that the QOL score for the physical component ranged from 25.2 to 93 with a mean score of 65,022. No significant correlation with the Fried score was found, but a higher QOL level was found in the subgroup without frailty, and a significant decrease in those with frailty (fig. 1, 2). The score for the mental component ranged from 20.70 to 98.00, with a mean score of 69.58. There was no significant correlation with frailty, but there can be noted a low level QOL in the subgroups of pre-frail and frail subjects (fig. 3, 4).

Fig. 1. Evolution of QoL physical component scores and FRIED score

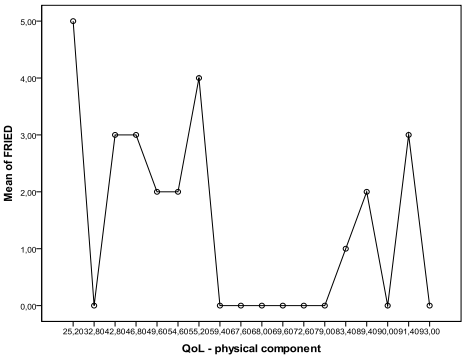

Fig. 2. Relationship between QoL physical component and FRIED phenotype

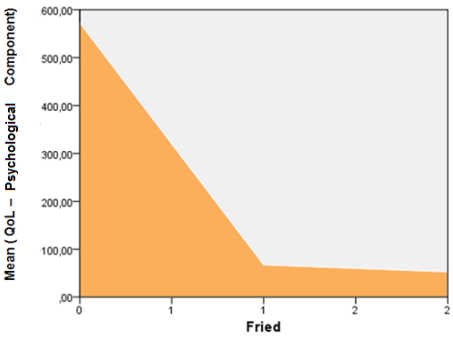

Fig. 3. Evolution of QoL psychological component scores and FRIED score

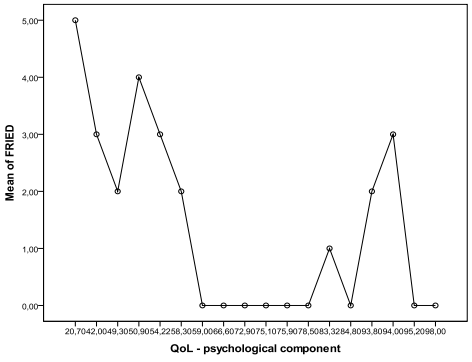

Fig. 4. Relationship between QoL psychological component and FRIED phenotype

DISCUSSION

Frailty syndrome was initially considered the prerogative of older people, being associated with and sometimes overlapping disability, the presence of concomitant chronic diseases and a high degree of de-

pendence. However, the results of our study show no correlation between age and the increase in the degree of frailty, thus making a clear distinction between physiological aging and frailty syndrome, supporting the data in the literature (17,18).

Most authors (9, 10, 11) consider that there are three stages of frailty. The first stage, preclinical, corresponds to a state in which the body has sufficient physiological reserves to respond to external or internal aggression factors, such as the aggravation of a disease, trauma or stress. Preclinical frailty is completely reversible, being the stage during which full recovery is possible, especially if the elements requiring intervention are identified.

The second stage is that of clinically manifest frailty, corresponding to a state in which the available functional reserves are insufficient. During this stage, recovery is incomplete, but a well-thought-out therapeutic plan, adapted to the patient's profile can favor the transition towards the pre-frail stage, with an obvious improvement of health and quality of life.

The final stage of frailty is that of frailty-related complications and is related to severe physiological vulnerability and reduction of functional reserves. The body is no longer able to cope with aggressions, leading to an increased risk of falls, disability, polypharmacy, prolonged hospitalizations, severe infections, institutionalization and death.

The initial findings of Fried et al. (19), supporting an increase in the prevalence of frailty syndrome once every five years, were confirmed by subsequent studies, one of the most important being conducted by Gill et al. (20), as part of a longitudinal study which observed and confirmed the transition between the three stages of frailty during the 4.5 years of follow-up. This transition is directly dependent on patient's initial frailty state, supporting once again the opportunity for screening and early diagnosis of frailty (21). Progression towards a more severe stage of frailty is more common than transition towards a lower stage, one of the causes being also the defensive medicine practice, addressing the frailty-related complications: treatment of falls, cognitive decline or immobilization. The association of sensory and cognitive deficits along with polypharmacy and the tendency towards therapeutic and diet non-compliance participate in the aggravation of chronic diseases, with repeated decompensations, frequent hospitalizations and the progression of frailty (22).

The assessment of the degree of frailty remains an intensely discussed topic, numerous studies conducted over the past 20 years suggesting various scales and tests. Using different measurement tools led to different results, hard to compare in identifying frailty, which led to the conclusion that the use of at least two tools for measuring frailty are necessary (22). Our study used the Groningen and the Fried scale; the results show that the Groningen test could not determine the pre-frail stage in the study group, along with an over-diagnosis of frailty in the group of robust older persons. By analyzing these data, we conclude that the comprehensive geriatric assessment remains the golden standard method for the diagnosis of frailty, provided that it is conducted by a team with experience in drafting the questionnaires and interacting with elderly persons. These findings are consistent with the numerous studies carried out throughout the world in recent years (23, 24). These results underline the need to adopt a tool for diagnosing the stages of frailty that is easy

to apply within primary health care or to facilitate the access of the elderly to geriatric centers with experience in the field.

Quality of life was poorer in the subgroup of frail compared with robust persons. The physical component has recorded lower scores than the mental component, which leads to the need of determining the other influencing factors, as well as the impact of frailty on each element related to quality of life. The multiple special aspects of QOL in the elderly require a more specific assessment, including the features of the individual in terms of expectations, social or family environment.

Disabilities, dependence and repeated hospitalizations experienced by elderly people due to frailty, adversely have a negative impact on quality of life. In this age segment, physical health has a more important role in the quality of life, thus the implementation of measures aimed at reversing the frailty syndrome can maintain the state of wellbeing and ensure successful aging.

CONCLUSIONS

We presented the preliminary results of a prospective study that shows that frailty has a significant influence on the quality of life of the elderly, but medical, psychological and social particularities in this age group require a specific QOL assessment tool.

The comprehensive geriatric assessment remains the most appropriate method for an accurate diagnosis of frailty, but it requires time and the expertise of a geriatrician, thus making it difficult to use as a screening method in the primary medical practice.

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NEWS

USE OF PCR TECHNIQUE FOR IDENTIFICATION OF *MICROSPORUM* SPECIES

The identification of species in the *Arthroderma otae* complex is necessary in order to determine the source of infection and reduce the risk of transmission. *Microsporum canis* is a zoophilic species, whereas *M. audouinii* and *M. ferrugineum* are anthropophilic. In a paper published by Kobylak *et al* an alternative method is proposed to allow species-specific identification of both anthropophilic and zoophilic species. For this purpose, two PCR assays (both traditional and real-time) were designed based on differences in the DNA fragment encoding β-tubulin and were applied using DNA extracted from pure culture. The two assays were able to identify *M. canis* and *M. audouinii/M. ferrugineum* with 100% sensitivity and specificity. These methods can be applied in routine laboratory praxis and are also useful for epidemiological studies (Kobylak N, Bykowska B, Kurzyk E, Nowicki R, Brillowska-Dąbrowska A.PCR and real-time PCR approaches to the identification of *Arthroderma otae* species *Microsporum canis* and *Microsporum audouinii/Microsporum ferrugineum*. *J Eur Acad Dermatol Venereol.* 2016 Jun 15. doi: 10.1111/jdv.13681. [Epub ahead of print])

Teodora Vremera

CONTROVERSIES ON TRANSVAGINAL ULTRASOUND SCREENING FOR ENDOMETRIAL CANCER IN ASYMPTOMATIC POSTMENOPAUSAL WOMEN

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CONTROVERSIES ON TRANSVAGINAL ULTRASOUND SCREENING FOR ENDOMETRIAL CANCER IN ASYMPTOMATIC POSTMENOPAUSAL WOMEN (Abstract): Endometrial cancer (EC) in postmenopausal women is usually associated with abnormal vaginal bleeding. However, asymptomatic cases of EC have been reported. The incidence of EC has been estimated to 1.3-3.05 per 1,000 screened postmenopausal asymptomatic women, the most significant ultra-sonographic feature being the thickened endometrium. We reviewed the literature on the accuracy of endometrial thickness measurement by transvaginal sonography (TVS) in predicting EC in asymptomatic postmenopausal women. The use of endometrial thickness as a sole screening test was found not to be of high predictive value and it should be combined with the evaluation of EC risk factors and followed by hysteroscopy and biopsy in suspicious cases. The lack of a universal cut-off value for endometrial thickness that could be correlated to EC is pointed out. **Keywords:** ENDOMETRIAL CANCER, ENDOMETRIAL THICKNESS, TRANSVAGINAL ULTRASOUND.

Endometrial carcinoma (EC) is the most common malignancy of the female genital tract in developed countries with over 60,000 new cases and over 10,000 deaths from the disease in each year (1). Endometrial cancer affects mainly postmenopausal women. The mean age of women diagnosed with endometrial cancer is 60. It is uncommon in women under the age of 45 (2). Adenocarcinoma of the endometrium is the most common histologic type of uterine cancer.

Postmenopausal vaginal bleeding is the presenting sign in more than 90% of postmenopausal women with EC and is associated with an up to 10% risk of EC (3), the most common cause of postmenopausal bleeding being endometrial atrophy (4).

Risk factors for EC are nulliparity, early menarche age, obesity, diabetes, hypermenorrhea, tamoxifen therapy and hirsutism-polycystic ovary syndrome. Measurement of endometrial thickness by transvaginal sonography (TVS) is a valuable test for estimating the risk of EC in women with postmenopausal vaginal bleeding (5). TVS is a non-invasive and painless procedure, has no complications, requires no special preparation, may be more sensitive in detecting carcinoma than blind biopsy, has a high cancer detection rate, is widely available and the cost is like biopsy (6, 7). Color and spectral Doppler ultrasound examination of uterine and the sub endometrial arteries can aid in the differential diagnosis of benign and malignant endometrium (8, 9). Seven to seventeen percent of postmenopausal women diagnosed with EC have no symptoms at the time of diagnosis**.** The incidence of asymptomatic EC has been reported to range from 1.3 to 1.7 per 1000 screened postmenopausal women (10, 11). The value of endometrial thickness measurement in healthy, asymptomatic postmenopausal women has not been documented yet. The general need for EC screening in these women is questionable and there is lack of consensus regarding the clinical significance of endometrial thickness measured by TVS (12-14).

The aim of this review is to determine the utility of endometrial thickness cut-off value for endometrial sampling in asymptomatic postmenopausal women who do not use Hormone Replacement Therapy (HRT).

TRANSVAGINAL SONOGRAPHIC EVALUATION OF THE ENDOMETRIUM IN ASYMPTOMATIC POSTMENOPAUSAL WOMEN

Endometrial thickness is measured as double-layer thickness at its thickest part in the longitudinal plane; homogeneity and echogenicity should also be mentioned. Values of 2-3 mm are considered normal atrophy, whereas a thickened endometrium may represent malignancy, hyperplasia or polyps. Also, to be considered 'normal', the endometrial lining must be regular and clearly visible over the totality of the uterine cavity (15). In symptomatic postmenopausal women, a cut-off value for endometrial thickness of 4-5 mm has been established. Although advanced EC has been identified in cases without noticeable endometrial thickness on TVS, the probability of endometrial cancer below this value is 1% (16, 17).

Little is known about endometrial thickness in healthy asymptomatic postmenopausal women. Universal **s**creening for endometrial malignancy is not considered necessary. The mean endometrial thickness in postmenopausal women without vaginal bleeding ranges between 3-5 mm (18-20). The risk of EC in women with endometrial thickness less than 5mm is approximately 1% compared to nearly 20% if the measurement is above this threshold (21, 22). A recent meta-analysis suggested that endometrial thickness > 11mm carries a risk of 6.7% for EC in asymptomatic postmenopausal women (23). The risk of malignancy was found to be small (1%) when a low cut-off value of 4mm was used (24). In a retrospective study among asymptomatic postmenopausal women with endometrial thickness ≥ 5mm, 0.9% was diagnosed with EC after endometrial sampling and 12.2% were diagnosed with atypical hyperplasia. For a cut-off value of 5mm, 106 endometrial samplings needed to be performed for the diagnosis of one case of EC (25). For the detection of all intrauterine pathologies the best cut-off value was found to be >8mm with a positive predictive value of 10.05. The sensitivity for EC was 100% at a cut-off value >10

mm (26). In patients referred to hysteroscopy due to thickened endometrial lining, TVS had a total false positive rate of 93.2% and a positive predictive value of 0.7% for EC (27). Retrospective studies of postmenopausal asymptomatic women with TVS endometrium thickness > 5 mm who underwent diagnostic hysteroscopy and biopsy found less than 1% atypical hyperplasia, with most findings (>80%) being atrophy, simple hyperplasia and polyps (28, 29). Endometrial cancer can be diagnosed early by TVS in a prognostic ally better stage in asymptomatic patients.

Another meta-analysis concluded that the use of TVS endometrial thickness as a screening method in asymptomatic postmenopausal women not on HRT is not justified because of the low incidence of endometrial cancer in the observed population and the insufficient data from the published studies to calculate an optimal threshold for endometrial thickness based on the sensitivity and specificity (30, 31).

The cut-off values for endometrial thickness in asymptomatic postmenopausal women determined by various research groups are presented in Table I (32-35).

TABLE I

Endometrial thickness and cancer findings in postmenopausal asymptomatic women

Author	Endometrial thickness (mm)	Number of women	Number of cancer cases	Negative of predictive value%
Osmers et al. 1990	≥ 4	283	10	96.50
Osmers et al. (1994)	≥ 8	88	18	79.55
Abu Hmeiden et al. 1992	≥ 10	24	3	87.50
Varner SE et al. 1991	≥ 4	20	1	95.84
Brooks SE et al. 1993	≥ 5	66	0	100.00
Vuento MH et al.	≥ 4	78	4	94.88
Bourne TH et al. 1991	7	104	1	99.10
Sheth S et al. 1993	≥ 5	35	5	85.80
Tsuda et al. 1997	≥3	16	1	93.70
Tsuda et al. 1993	≥ 2	147	2	98.59
Gambacciani et al. 2004	≥ 4.5	148	1	99.30
Fleischer et al 2001	>6	1750	1	99.95
Bakos et al. 1995	>6	106	1	99.00

Controversies also exist regarding the significance of intrauterine fluid collection in postmenopausal women. It can be considered as an ominous sign associated with malignancy even in the presence of thin endometrium, or it may represent fluid transuded from an atrophic endometrium which accumulates in the uterine cavity due to cervical stenosis**.** Small series of such patients reported endometrial mommas,

polyps, hyperplasia and rarely endometrial carcinoma, especially if endometrial thickness was greater than 4 mm (36).

DISCUSSION

TVS is being used in the last years as an accurate tool to identify endometrial pathology, including endometrial carcinoma in both symptomatic and asymptomatic postmenopausal women. In asymptomatic women diagnosed incidentally with a thick endometrium, a clinical dilemma concerning the case management arises. While the main presenting symptom is abnormal uterine bleeding, 7% to 17% of endometrial cancers are found in asymptomatic postmenopausal women. Despite the established association between a thick endometrium and endometrial carcinoma in postmenopausal women with vaginal bleeding, there is a lack of consensus regarding the asymptomatic postmenopausal women. Most of the researchers agree that in postmenopausal asymptomatic women endometrium thickness < 3 mm strongly reduces the probability of endometrial pathology, while endometrium thickness > 10 mm is correlated with pathologic histology. But there is a 'gray area' between 4 and 10 mm that needs further investigation, as sometimes EC is found. Most cases of EC in postmenopausal asymptomatic women have endometrial thickness measured with TVS over 5 mm.

The recommendations of the Society of Obstetricians and Gynecologists of Canada (37) state that TVS should not be used as a screening method for endometrial cancer in asymptomatic postmenopausal women not using HRT. In women with incidentally observed thickened endometrium and other positive ultrasound findings (increased vascularity, inhomogeneous endometrium, intrauterine fluid, thickening>11mm) together with endometrial cancer risk factors, case-by-case decisions and further investigation such as endometrial sampling is recommended.

In conclusion, transvaginal sonography (TVS) is a reliable, non-invasive method for the evaluation of the endometrial thickness and morphology in asymptomatic postmenopausal women. Universal endometrial screening is controversial because of the lack of evidence of increased 5-year survival rate for EC patients. The diagnostic success of this method also depends on examiner's training and experience. The difficult issue of how to manage an asymptomatic postmenopausal woman with an incidentally discovered thick endometrium has yet to be resolved. We believe that TVS should be combined with clinical signs and risk factors for endometrial cancer to achieve the optimal management and avoid unnecessary invasive interventions, procedural complications and psychological stress.

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IMPLICATIONS OF HYSTERECTOMY ON VAGINAL ROUTE IN GYNECOLOGIC PATHOLOGY

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IMPLICATIONS OF HYSTEROCTOMY ON VAGINAL ROUTE IN GYNECOLOGIC PATHOLOGY (Abstract): Vaginal hysterectomy was mentioned before our era by Soranus of Ephesus, and it is one of the most frequent surgical interventions in gynecologic practice; performed for the first time by Sauter of Constance, in 1822, who practiced a vaginal hysterectomy without ligation of vessels, carrying out the hemostasis with a swab dipped in alum. The essential argument for vaginal surgery is the advantages it brings. Current indications and contraindications encourage the frequent use of vaginal hysterectomy in benign pathology of the uterus, while the indication (Crossen, Rouhier, Campbell techniques) depends most of the times on the surgeon's experience and preference rather than on the critic evaluation of results. **Material and methods:** The study group included 84 patients, hospitalized from January 2013 to December 2015 in the Third Obstetrics Clinic. For the evaluation of results, we used longitudinal retrospective clinical-statistical method. **Results and discussion:** Practicing the surgery on vaginal route is mainly indicated in patients with severe anemias and different organic tares. When removal of the cervix is not indicated, or when there is an enlarged uterus or one with a small diameter, it is recommended to use laparoscopic hysterectomy. **Conclusions**: Vaginal hysterectomy is a technique that allows rapid removal of the uterus, with a minimal impact on the patient, indicated in the pathology of benign uterine tumors (uterine fibromatosis associated with metrorrhagia, fibromatous uterus with cervical dysplasia, or fibromatous uterus associated with different degrees of uterine prolapse).
Keywords: VAGINAL HYSTEROCTOMY, BENIGN TUMORAL CONDITIONS, HEMORRHAGE.

When the size of the uterus is not very big, hypogastrium does not have many adherences, there are no pathological changes and if the diameter of the vagina allows, hysterectomy on vaginal route is possible, either under epidural anesthesia – with a total limitation of mobility and senses –, or under general anesthesia (1).

For patients, it is more comfortable, while the operatory time, hospitalization days and recovery time are shorter than in cases of abdominal interventions.

Vaginal hysterectomy is an accepted method in patients with uterine descensus, whose basins allow vaginal surgery, whose tumors are small enough to allow the vaginal technique, and if the patients consent to be operated via vaginal route (1,2).

When there are large fibromas, pretreatment with a GnRH analogue will allow

the vaginal surgery, which was previously impossible (3,4).

Removing the uterus and cervix, also the stability of the vagina is reduced, as well as the future onset of a vaginal prolapse by applying special sutures; rarely there can be inflammations and/or bleedings, which can be shortly resolved by medical treatment or short local vaginal treatments (6,7).

In November 2009, ACOG (The American Congress of Obstetricians and Gynecologists), through the Committee on Gynecologic Practice, published an opinion that recommended vaginal hysterectomy as the safest and the most cost-effective method of uterus removal for non-neoplastic indications. Without changing the eligibility criteria for vaginal hysterectomy issued in 1989, the authors pointed out the fact that the surgeons can decide, depending on their technical skills, to use VH (vaginal hysterectomy) even when there are apparent contraindications (8).

The paper aims at highlighting particularities on the place and value of hysterectomy on vaginal route in resolving the most frequent surgical gynecologic conditions (uterine fibroma, conditions of genital apparatus associated with chronic diseases, septic states, advanced age, and organic tares).

MATERIAL AND METHODS

The study group of the retrospective longitudinal study included 84 patients, hospitalized from January 2013 to December 2015 in ”Elena Doamna” Clinical Hospital of Obstetrics and Gynecology of Iasi, under the service of Third Clinic of Obstetrics-Gynecology.

The inclusion criterion was preoperative indication of hysterectomy for benign pathology.

The information was collected from the patients’ observation charts, surgical interventions register and histopathological records. We monitored the features of the field – age, environment of origin, number of cases of vaginal hysterectomy for the associated uterine pathology (benign tumors) and the diseases of the adnexa’s, as well as the advantages and disadvantages of the method.

RESULTS AND DISCUSSION

Although hysterectomy is a surgical routine, its costs and morbidity in relation to this intervention had a significant economic impact on the health and social systems (8).

Within the period of time included in the study, 84 cases of vaginal hysterectomy were performed (tab. I), with various distributions on age groups (tab. II).

TABLE I

Yearly distribution of the cases of vaginal hysterectomy

Year	Vaginal hysterectomy	
	No.	%
2013	21	25.00
2014	34	40.47
2015	29	34.53
Total	84	100.00

TABLE II

Structure on age groups

Group (years)	No.	%
Under 30	3	3.57
31 - 35	14	16.66
36 - 40	9	10.73
41 - 45	22	26.19
46 - 50	10	11.90
51 - 55	9	10.72
56 - 60	13	15.47
→61	4	4.76

The frequency of obstetrical antecedents showed insignificant differences in relation with the number of pregnancies (tab. III).

TABLE III
Obstetrical antecedents of the study group

Frequency of births in antecedents	No.	%
1	**25**	29.76
2	31	36.90
3	28	39.34

Similar to other authors (9,10), we evaluated in our study the possible risks related to the nature of obstetrical and gynecologic antecedents, presence of comorbidities, drug allergies and ongoing therapies.

Vaginal hysterectomy in benign tumor pathology needs lab investigations: CBC (Complete Blood Count), blood type, glycaemia, urea, creatinine, coagulogram, urinalysis, urine culture, as well as cytological and bacteriologic examination of vaginal discharge. In some cases, it is possible to practice electrocardiogram, pulmonary radioscopy, echography with vaginal ultrasound probe, or intravenous urography. To eliminate neoplastic pathology, we performed biopsy curettage a month prior to surgical intervention, and to prevent post operatory infection, we administered second generation cephalosporin intra operatory, or 500 mg metronidazole, intravenous.

When cervix removal is not indicated, or there is an enlarged uterus, or a small diameter of vagina, laparoscopic hysterectomy is recommended. Thus, on one hand, the pathogenic agents in vagina don't reach the abdominal organs, and on the other hand, the stability of hypogastrium is maintained. When choosing the vaginal route, we considered the anatomical condition of each case, mobility of the uterus and the vaginal caliber (11,12).

A special situation arises when the polyfibromatous uterus is associated with different degrees of genital prolapse and stress urinary incontinence (tab. IV, fig.1).

TABLE IV
Some indications of vaginal hysterectomy

Main indications	Cases	
	No.	%
Uterine fibromatosis associated with different degrees of uterine prolapse	19	22.61
Uterine fibromatosis associated with metrorrhage	27	32.14

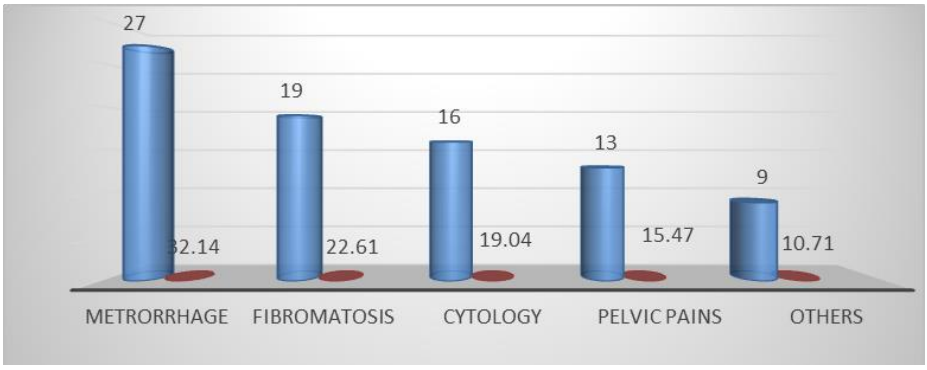

Fig.1. Indications for vaginal hysterectomy

We obtained special results in cases with prolapse; hysterectomy was supplemented by a suspension of the bladder and vaginal stump through the straps of the adnexa and ligaments, plus anterior colporraphy and posterior colpoperineorraphy.

Although other authors, too (10,11) admit that the main complications after vaginal hysterectomy can be stump hematoma, local infection, bladder and intestinal wounds, the most important complications are intra- or post operatory bleedings, infection, lesions of neighboring organs, thromboembolic complications etc.

The surgeon may decide, according to its technical possibilities of the use VH even in the presence of an apparent contraindication.

During the vaginal hysterectomy, predictable bleedings occurred in 3(3.57%) cases (bleeding from the vaginal trance, difficulties of detachment from myometrium from myometrium); 2 (2.38%) cases with accidental losses of blood due to incorrect ligatures, their secondary sliding and accidental rupture of vascular pedicles; 11(1.14%) patients presented delayed post operatory bleeding, in the third week, caused by the active bleeding from the vaginal stump. The complications we faced consisted of one case with vaginal stump abscess and two cases with bladder lesions due to close adherences; 6% needed surgical intervention.

TABLE V

Post operatory complications occurring in the study group

	Cases	
Complication	**No**	**%**
Bleeding	6	7.14
Bladder lesions	2	2.30

We evaluated the results post operatory one month, two months, four months and one year after the surgery.

Vaginal hysterectomy makes that the main changes arising post operatory are determined by the absence of the uterus from the pelvis, with redistribution of abdominal pressure on the pelvic floor. As any other type of hysterectomy, it can cause emotional changes which can be easily prevented by means of post operatory discussions and adequate treatment, including hormonal therapy (tab. V).

DISCUSSION

Vaginal route allows wide access of intrapelvic genital organs, wide array of gynecologic surgeries of the uterus and adnexa, from the simplest ones, such as myomectomy, salpingectomy, tubal ligation, to total hysterectomy and enlarged hysterectomy for cervical cancer.

According to ACOG, the upper limit of the size of the uterus is indicated to be extracted via the vaginal route is corresponding to a load of 12 weeks, which corresponds to a weight of about 280 g.

While some authors such as Donald-Fothergill, Manchester (quote,11,13), are still using vaginal surgery procedures which involve saving the uterus, most of the authors prefer radical surgeries, removing the uterus in order to avoid post operatory relapses, or the risk of the onset of benign or malignant tumors on the remaining uterus (3,7).

Kovac (13), one of the best specialists in vaginal surgery, has dedicated a great part of his career in redefining the indications of vaginal hysterectomy from modern perspectives; he knocks down, step by step, based on scientific evidence of wide personal series, most of traditional contraindications.

Lamman and Krige (quote 13) take uterus removal on vaginal route as a major indication. In 1984, Guillemin and Cavalhier (5, 12) proposed vaginal hysterectomy as an optimal treatment method.

Vaginal surgery can be performed with multiple anesthesia methods, the choice of the type of anesthesia depending on the anesthesiologist's preference and the anesthetic risk. Post operatory infection in total hysterectomy has a low incidence as a result of intra operatory prevention with antibiotics (2)

Worldwide, there is an increase demand for total hysterectomy with adnexectomy, a demand that is more and more frequent in Romania as well (15).

CREST study was performed, in which indications for abdominal hysterectomy were distributed as follows: uterine fibroma (40%), chronic pelvic pain and endometriosis (22%); for vaginal hysterectomy, indications were: pelvic relaxation (30%), bleeding (28%), cervical dysplasia (21%), and uterine fibroma (only 7% of the cases) **(**9).

Another national study was carried out is VALUE, which describes the hysterectomies performed in Great Britain for 2 years (1994 and 1995), and which finds that the most frequent indication for hysterectomy is dysfunctional uterine bleeding (46%), followed by prolapse (19.79%), uterine fibroma (18.51%), endometriosis (7%), pelvic masses (4.23%), and other indications (4.47%) (16).

Querleu (9) believes that mobilization of the bladder is difficult in patients with C-section antecedents, regardless of the hysterectomy route and believes that C-section antecedents are not a contraindication for vaginal approach.

Although the VH is a routine surgical intervention, its costs and morbidity have a significant economic impact on health and social systems.

CONCLUSIONS

Our evaluations brought new evidence in supporting the vaginal hysterectomy with uterus removal in a short period of time, with minimal impact on the patient, with indication in benign tumoral pathology of the uterus, among which uterine fibromatosis associated with metrorrhagia, fibromatous uterus with cervical dysplasia, or fibromatous uterus associated with different degrees of uterine prolapse; it is a technique which uses a natural route, has a limited invasion and allows a rapid recovery of the patients.

In all cases, we took into consideration the onset of surgical menopause, which asks for prevention of emotional and sexual disorders, as well as disorders of other functions.

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NEWS

IMMUNOSUPPRESSIVE THERAPIES FOR IGA NEPHROPATHY: A CONTINUOUS CHALLENGE

Immunoglobulin (Ig) A leads to end-stage renal failure in a significant proportion of patients. Immunosuppressive therapy, including steroids, is widely used to induce disease remission; however, it can cause serious side effects and therefore their use must be reduced. In this study the authors included pacients younger than 70 years, diagnosed with IgA nephropathy by renal biopsy; they had tonsillectomy. They were divided into two groups: Group A who's treatment was a single-course steroid pulse combined with mizoribine and Group B who received a three-course steroid pulse. In both groups, proteinuria decreased significantly after treatment. The glomerular filtration estimation rate (eGFR) increased after treatment in Group A, and tended to decrease in Group B. Group A also had a higher preservation of eGFR. In the **conclusion**, single-course steroid pulse therapy combined with mizoribine was considered to be superior with a protective renal effect in IgA nephropathy (Kaneko T, Arai M, Ikeda M et al. Comparison of immunosuppressive therapies for IgA nephropathy after tonsillectomy: three-course versus one-course steroid pulse combined with mizoribine. *Int Urol Nephrol*. 2015;47(11):1823-1830).

Anca Roxana Hîrja

COMPARISONS BETWEEN THE NON-PROLIFERATIVE AND PROLIFERATIVE THERAPY IN FIBROCYSTIC MASTOSIS

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COMPARASIONS BETWEEN THE NON-PROLIFERATIVE AND PROLIFERATIVE THERAPY IN FIBROCYSTIC MASTOSIS (Abstract) **Aim**: Fibrocystic mastosis (FCM) is the most frequent benign breast lesion. Most treatments for fibrocystic mastosis are: hormonl, with beneficial results and non-hormonal, with fluctuating results. **Material and methods:** A number of 210 cases were studied, which were divided into 7 groups. The study lasted for 9 months and it was carried out on the basis of a personal examination sheet. The following were monitored: age groups, mastodynia, reducing breast nodules, a significant reduction in the volume of the mastosic cysts, reducion of the fibrous tissue, medication tolerance. **Results**: Mastodynia has declined by 90% in the cases treated with Tamoxifen and Danazol, by 70% in the case of Lynestrenol and Bromocriptine, by 50% in the 15 patients who were given Utrogestan. Knowing the advantages and disadvantages of drugs (contraindications, side effects), age category, breast pain reduction, antiproliferative activity, tolerability, relapse allow us to assess the benefit-risk. Even in those circumstances that remained incompletely clarified for objective reasons, related to the inaccurate/incorrect reporting by the patients, there is a significant difference ($p < 0.05$) between the frequency of relapses following the treatment with Tamoxifen and the other categories of drugs who were administered. **Conclusions:** Our study shows that in the groups that were administered Logest, Utrogestan and Bromocriptine, only antalgic effects were achieved (disappearance or only decrease of mastodynia) and no anti-proliferative effects were obtained. Basically, hormone treatment should be made based on a histopathological examination. **Keywords**: BENIGN BREAST DISEASE, PROLIFERATIVE, MASTOSIS.

Histopathologically, the fibrocystic mastosis (FCM) is defined as all the dystrophic changes in the breast tissue, the gathering under the form of fibrosis of the epithelial, cystic, metaplasic and hiperplasic modifications. The disease classification is different; the French authors consider it as mammary distrophy: fibrocystic mastosis (FCM), while the American authors consider it as a benign breast disease (benign breast disease). There is no current consensus on the content of this notion.

Both the diagnosis and the treatment of the benign breast diseases remain a challenge, and the indication for surgery is not clearly defined.

Any of the 3 categories of histopathological lesions has a different risk with regard to the possibility of occurrence of the breast cancer in the next 15 years, so that the non-proliferative do not increase the risk of a breast cancer, even if there is a family history of breast cancer, the proliferative lesions without epithelial atypia are risk around 2 for the appearance of a breast cancer, taking into account the associated epidemiological factors (1).

In order to assess the therapeutic value of a medicine we must take into account the risk/benefit ratio, and the risk is determined according to the contra-indications and adverse side effects(8).

Hormonal treatments are justified by hormonally dependent character, and possibly regressive, of the parenchymatous breast proliferations: cysts, apocrine metaplasia, ductal epithelial hyperplasia (epiteliosis), lobular epithelial hyperplasia (adenosis) (7).

For some drugs with hormonal action it has been demonstrated that they have an anti-proliferative activity, e.g.: Lynestrenol (which is a norsteroid), Tamoxifen and Danazol.

Cupceancu reported that 48 women with benign breast disease (BBD) were treated with Tamoxifen + Lynestrenol, and that the combination of these medicines has reduced substantially the size of adenomas, fibroadenomas, cysts and dysplastic lesions. Thus it was showed that Tamoxifen also had an antiproliferative action (2). Hurst et al. (3) argued that Tamoxifen produces significant regression of the breast cysts. Viviani et al (4) in the study "Ultrasound evaluation of the volume of breast fibroadenoma in women treated with Tamoxifen" conclud that this drug significantly reduces the volume of fibroadenoma when administered for 50 days at a dose of 20 mg per day.

Tan Chiu et al. (5) stated that, in 1998, the National Surgical Adjuvant Breast and Bowel Project had demonstrated that the treatment with Tamoxifen reduces the incidence of noninvasive and invasive breast cancer in the woman at high risk for this disease (thus Tamoxifen is recognized as having a prevention effect on the breast cancer).

The same authors had found that Tamoxifen reduced not only the risk of noninvasive and invasive breast cancer, but it also reduced the risk of benign breast disease relapses (BBD) and morbidity and the costs of repeated diagnoses and treatments that are associated with them. Thus, Tamoxifen is recognized as having a prevention action of the BBD relapses, including FCM.

Pricop et al. (6) states that Danazol can accomplish the disappearance of the cysts smaller than one inch in diameter, but that doesn't seem to diminish the fibrous tissue. Le Franc et al. (1990) specifies that, for the objective amelioration of the FCM "tumour plaques", 6 months are required and these results are not superior to those who followed a treatment with progestagens or with birth control pills, with progestagen predominance.

On the other hand, progestagens and bromocriptine are effective in the treatment of mastodynia (especially in cyclical mastalgia) and, to some extent, in fighting against breast nodes.

Currently there is no encoding in the use of these broad categories of hormonal medications: oral contraceptives, progestagens (Utrogestan), norsteroids (Lynestrenol), antiprolactinics (Bromocriptine), antigonadotrope drugs (Danazol, LHRH

agonists) and antiestrogens (Tamoxifen).

However, in the literature (1) it was mentioned that, because of the adverse side effects of the norsteroids, in practice it is recommended to start preferably with progesterone, locally and/or general, and in the event of inefficiency to resort to other treatments, first the norsteroids (Lynestrenol), thenn Danazol or antiestrogens (Tamoxifen).

MATERIAL AND METHODS

A number of 210 cases were studied, which were divided into 7 groups. The study lasted for 9 months and it was carried out on the basis of a personal examination sheet (fig. 1).

The data collected as study material and considered refer to: the total number of cases under investigation and diagnosed with fibrocystic mastosis, the division of cases into age groups, admission reasons, clinical examination, pathological personal history of clinical relevance for the basic disease (main diagnosis), family history relevant for the basic disease, anatomo-pathological diagnosis, the treatment followed.

The local administration of Diclofenac gel in the first 10 days after menstruation has been justified by the chronic inflammatory process due to the breaking up of the cysts and the release of their contents in the surrounding stroma.

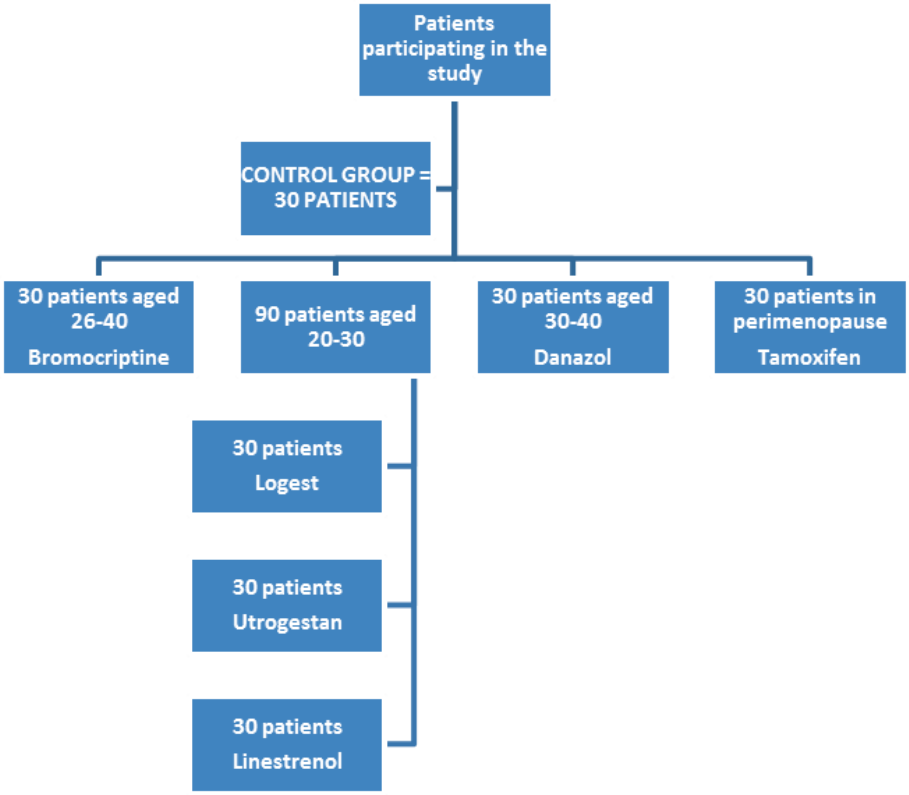

Fig. 1. Structure according to categories of patients included in the study

In 6 of the lots (of 30 patients each) a local background treatment with diclofenac gel and progestogel. Lot 7 was made of patients who refused any treatment (control group).

Each of the 6 groups were given, in addition to diclofenac gel and progestogel, one of the following drugs: Logest- 21 cp per month, 6 months; Utrogestan 10 days per month, 6 months; Lynestrenol 10 days per month, 3-6 months; Bromocriptine (parlodel), 3 months; Danazol 200 mg/day - 3 months; Tamoxifen 6 months. We have not had cases with LHRH (gonadotropin releasing hormone) treatment.

They were studied under this treatment, at first, after 3 months and after 6 months, by imaging (ultrasound and mammography) and FNAC - fine-needle aspiration cytology (sporadically). Further on, we studied, at 9 months, the possible relapses and some histo-pathological examinations after these hormonal treatments.

The following were monitored: age groups, mastodynia, reducing breast nodules, a significant reduction in the volume of the mastosic cysts (which has been made by ultrasonography), reducion of the fibrous tissue, medication tolerance (frequency of relapses; risk/benefit ratio).

RESULTS

The FCM incidence is estimated in over 60% of women. It is frequent in women of ages between 30 and 60 years. For the data in our study, we calculated the FCM incidence signalled in relevant personal history to 20%; obviously, values were lower after referring to the entire batch under study, namely 6.68%.

Most part of the patients who attended our unit came from the urban environment (66.9%), and the ones from the rural environment were only 1/3 of the reported cases (33.1%).

Depending on the age of the lot, the women aged between 20-30 years were administered, in an equal number of cases, namely 30 cases (out of a total of 90 cases), Logest or Utrogestan or Lynestrenol , and the women aged 26-40, i.e. 30 cases, were given Bromocriptine. In other 30 cases, the women aged between 30-40, were given Danazol, and Tamoxifen was administered to 30 patients placed in perimenopause, although Tamoxifen is recommended as the first-line treatment.

Mastodynia has declined by 90% in the cases treated with Tamoxifen (27 patients) and Danazol (27 patients), by 70% in the case of Lynestrenol (21 patients) and Bromocriptine (21 patients), by 50% in the 15 patients who were given Utrogestan. In only 9 patients (30%), mastodynia was reduced in case of the treatment with Logest.

Another apect that was had in view was the reduction of the nodules. This reduction was present in 80% of cases treated with Tamoxifen and Danazol in 50% of the cases treated with Lynestrenol, 40% of the cases receiving Bromocriptine, 30% of the cases treated with Utrogestan and 20% of those treated with Logest.

The volume of mastosic cysts, according to the diameter of the cysts measured by ultrasound, was reduced in a percentage of 80% in the cases treated with Tamoxifen and in those treated with Danazol. In the case of the treatment with Tamoxifen, the volume of the mastosic cysts was reduced by 50% in the case of the cysts with the diameter greater than 1 cm and reduced in full in the case of the cysts with a diameter smaller than 1 cm. As for the Brocriptine and Logest, in none of the cases there has been a reduction of the volume of the mas-

tosic cysts.

According to the reduction of the fibrous tissue under therapy, the influences (actions) of the physiotherapy treatments (ultra-rays, ultrasound, etc.) on the fibrous tissue have not been studied. No hormonal drug has reduced the fibrous tissue. This is an argument for the usefulness (or necessity) to prevent the formation of the fibrous tissue, via the early treatment of the fibrocystic mastosis. The fibrous tissue is not hormone-dependent.

The case distribution according to the tolerability of the treatment was of 100% in the cases treated with Logest and Utrogestan, with a very good degree of tolerability. The degree of tolerability is good in the cases treated with the other treatments.

In terms of the frequency of relapses, the results we obtained were as follows: for patients receiving treatment with Logest, Progestogel, Utrogestan (Duphaston) or Bromocriptine, the frequency of relapses was of 70%. In the case of the treatment with Lynestrenol, we noticed a presence of relapses in 50% of the cases. Danazol caused a lower frequency of relapses in only 30% of the cases. The best results were achieved following the treatment with Tamoxifen for which relapses were mentioned in only 20% of the cases.

After the experience of Pricop et al.-2003 and of Tan Chiu et al, 2003, and also after our personal experience, the risk-benefit ratio is most favorably inclined towards Tamoxifen, that does not represent any hazard at the dose of 10 mg/day (3 months).

DISCUSSION

Knowing the advantages and disadvantages of drugs (contraindications, side effects), age category, breast pain reduction, antiproliferative activity, tolerability, relapse allow us to assess the benefit-risk.

In order to assess the risk/benefit ratio, we must know the risks, which are represented by the contraindications and adverse side reactions.

It is known that if a drug, at a specific dose, has a favorable action in a certain direction but it has adverse side effects of high intensity, the risk/benefit ratio is low and the use of such drug is more limited.

In these cases the therapeutic strategy means associating more drugs with the same desired beneficial action, but with different adverse side effects (which are not cumulative). In this way drugs can be administered in low doses because the beneficial effects are cumulative and the adverse side effects are different and of low intensity, and they are not cumulative because they are not identical. The combination of drugs that have the same adverse side effects or similar side effects is contraindicated, as they will be cumulative.

Therefore, in treating FCM, the association of Lynestrenol with Danazol is not indicated because they have virilising side effects and adverse effects (on the liver) in liver diseases. The association of Danazol and Tamoxifen is not found in the literature, likely because both can cause prolonged amenorrhoea (though perhaps not by the same mechanism) and due to weight gain (fluid retention).

Moreover, except for the combination of Progestogel with an oral progestagen, and the combinaton of Tamoxifen with Lynestrenol (2), we have not found in the literature any other drug combination for the treatment of FCM.

Since the administration of Progestogel is relatively efficient and the most anodyne, we maintained the administration preceded

by Diclofenac gel in all cases, whether we associated it to Utrogestan, Lynestrenol, Bromocriptine, Danazol or Tamoxifen (the latter two at the minimum effective dose to reduce the adverse side effects that limit their use).

Another problem is the main drug choice based on age. Thus, in young patients, progestins were used, like simple Progestogel or combined with Utrogestan, or in rebel cases, Utrogestan was replaced by Lynestrenol (norsteroid progestagen).

Over time, the mastosic cysts can increase in size and number, accompanied by fibrous elements whose provenance is insufficiently known. This is why in our series the treatment, in all the groups, maintains the local use of Diclofenac gel.

In patients over 30-35 years old and in premenopause patients we used Danazol or Tamoxifen. By administrating Utrogestan, Bromocriptine, Danazol, Tamoxifen, under the same conditions, we have created the opportunity to compare their effectiveness.

The fibrous tissue prevents the access of drugs to the mastosic cysts and it is more resistant to treatment, if not even far from any effect, therefore much more effective drugs are necessary (even if they have more important adverse side effects) (9).

Even in those circumstances that remained incompletely clarified for objective reasons, related to the inaccurate/incorrect reporting by the patients, there is a significant difference ($p < 0.05$) between the frequency of relapses following the treatment with Tamoxifen and the other categories of drugs who were administered.

The intent of obtaining histopathological results after the hormonal treatments administered for 3-6 months has not been achieved because the patients refused the biopsy and even the fine needle aspiration cytopunction (10).

Following these results, there is the issue of choosing, between these drugs, the most beneficial hormonal treatment, taking into account the benefit/risk ratio, or possibly the individualization of these treatments.

CONCLUSIONS

Our study shows that in the groups that were administered Logest, Utrogestan and Bromocriptine, only antalgic effects were achieved (disappearance or only decrease of mastodynia) and no anti-proliferative effects were obtained.

In correspondence to the literature data, we found that, in a number of cases, relapse occurred.

In the group under Bromocriptine, tolerability was average, while it was very good in the other two groups. In the groups that were administered Lynestrenol, Danazol and Tamoxifen anti-proliferative effects were achieved (beside the disappearance of mastodynia).

Therefore, under Lynestrenol, the nodules (tumour plaques) disappeared, along with some of the mastosic cysts under 1 cm, and the mastosic cysts larger than 1 cm reduced their size. Tolerability was good.

Under Danazol, the nodules (tumor plaques) disappeared, along with many of the mastosic cysts under 1 cm, and the mastosic cysts larger than 1 cm reduced their size. Tolerability was average.

Under Tamoxifen, the nodules (tumor plaques) disappeared, along with many of the mastosic cysts under 1 cm, and the mastosic cysts larger than 1 cm reduced their size. Tolerability was good.

Further on, we found that after the treatment with Tamoxifen the relapses were the fewest.

Before beginning the hormone treatment for FCM, malignancy should be excluded, as we must not forget (1) that a malignant lesion can simulate a benign one.

According to our personal experience, and to the experience of Pricop et al. (6) and Tan Chiu et al. (5), the most favorable benefit-risk ratio refers to tamoxifen, which in the dose of 10 mg/day (3 months) is not dangerous for the patient.

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ATYPICAL FEMORAL FRACTURES AFTER LONG-TERM BISPHOSPHONATES THERAPY: CASE REPORT

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ATYPICAL FEMORAL FRACTURES AFTER LONG-TERM BISPHOSPHONATES THERAPY: CASE REPORT. (Abstract). We present a 77 years-old woman with no history of trauma, or associated with low-energy trauma, admitted to our clinic after three weeks of a left femoral fracture threated in Orthopedic Clinic. The patient was in treatment with bisphosphonates over 10 years for osteoporosis. **Discussion and conclusions:** The causal relationship between prolonged bisphosphonate use and the occurrence of atypical femoral fractures (AFF) has not yet been established. For the patient at high risk of fracture, it may be beneficial to continue bisphosphonate treatment beyond five years. The absolute risk of atypical femoral fractures is low (about 100 cases per 100,000 person-years among long-term users). For most people with osteoporosis, the proven fragility-fracture risk-reduction benefits of bisphosphonates outweigh the risks of AFF. **Keywords:** ATYPICAL FEMORAL FRACTURE; BISPHOSPHONATES; OSTEOPOROSIS.

Bisphosphonates are a class of widely prescribed drugs that are proven to be effective in reducing common bone fractures in people with osteoporosis and those at a high risk of fractures. Concern among doctors and patients has arisen following recent media reports that cite a possible association between unusual and unexpected (atypical) fractures of the upper thigh bone and bisphosphonate use. Recent published studies (1) examining this potential association are conflicting regarding the existence and strength of this association. Bisphosphonate-related proximal femoral fractures are an example of insufficiency fractures, although the direct causative link remains somewhat controversial (1). According to a task force report from the American Society for Bone and Mineral Research (ASBMR), this relationship has not yet been shown to be causal. All current evidence indicates that atypical femoral fractures (AFF) represent a rare subset of subtrochanteric and femoral shaft fractures (2). Femoral shaft fractures had an incidence of 2.5 to 9.9 per 100,000 person-years, but 75% occurred with high-energy trauma and the majority were comminuted and had a spiral configuration (3). A review of radiographs by

Schilcher et al. (4) found an incidence of 55 atypical fractures per 100,000 person-years among bisphosphonate users compared with 0.9 per 100,000 person-years among those with no bisphosphonate use. Characteristics of AFF are: a transverse, noncomminuted fracture at the subtrochanteric or femoral shaft region with a medial cortical spike at the fracture area. Other features include prodromal pain and generalized thickening of the femoral cortices on radiographs. For the clear criteria to define atypical femoral fractures, the ASBMR task force established major and minor features for both complete and incomplete atypical fractures of the femur (2). All of the major features should be present to designate a fracture as atypical: No history of trauma, or associated with low-energy trauma; Fracture located anywhere from distal to the lesser trochanter to proximal to the supracondylar area; Transverse or short oblique fracture configuration; Noncomminuted fracture; Medial spike in complete fractures, incomplete fractures involve only the lateral cortex. The minor features have also been described in association with atypical femoral fractures, but they are not required for diagnosis. Localized periosteal thickening of the lateral cortex; Generalized thickening of the femoral cortices; Prodromal symptoms; May be associated with bilateral fractures or symptoms; Evidence of delayed fracture-healing; Comorbid conditions or the use of some medications.

CASE REPORT

A 77 years-old woman, weight 62 kg and height 1.58 was admitted to Endocrinology clinic after three weeks of a left femur fracture in mid-thigh, threated in Orthopedic Clinic (fig. 1).

Fig. 1. The patient after orthopedic surgery

Orthopedic Surgery underwent the closed reduction and internal fixation with intra medullary hip screw (fig.2 b). The patient was in our clinical observation since 1983 for hypothyroidism after thyroidectomy in treatment with 100 mcg LT4. In 2002 the patient was diagnosed with osteoporosis following the DXA examination. The bone mineral density (BMD), measured by dual-energy X-ray absorptiometry (DXA) showed T-score at total lumbar spine: - 4.0, total hip: -2.1, and left femur neck: -3.0. 25-OHD3 level varied between 17.69 ng/mL and 5.54 ng/mL (normal>30 ng/mL). Thyroid Stimulating Hormone, thyroxin and triiodothyronine level was normal. Has been established treatment with risedronicum acidum 35 mg/week (Actonel), Alfacalcidol 1 mcg/day and calcium 1000 mg/day. Annual DXA-BMD shows a slight increase the density of the bone; patient medical treatment has been changed in zoledronicum acidum and then on alendronicum acidum.

In 2013 there is a decrease of BMD DXA, but our patient require the continuation of the therapy with a drug with large intervals administration .Ibandronicum acidum has been recommended. After surgical orthopedic treatment were discussed with the patient, the risks and benefits of each option. The new anteosteoporotic treatment was strontium ranelate in association with Alfacalcidol and calcium. An association between bisphosphonate long-term use and the occurrence of AFF has been suggested. This diagnostic has been supported and by the x-ray. The radiographic features of an atypical femoral fracture: femoral shaft region (fig. 2a), transverse fracture configurations, absence of comminution, a medial spike, localized periosteal thickening of the lateral cortex, and generalized thickening of the femoral cortices.

a b c

Fig. 2 a. transverse fracture configurations, absence of commination, a medial spike, localized periosteal thickening of the lateral cortex, and generalized thickening of the femoral cortices. b. Post-operative radiograph after surgical fixation. c. Post-operative radiograph after 3 month.

DISCUSSION

AFF have been associated with various factors, including: bilateral fractures, prodromal pain, the use of glucocorticoids and proton pump inhibitors, delayed fracture-healing, presence of rheumatoid arthritis or diabetes mellitus as well as vitamin-D3 deficiencies (5, 6). A post hoc analysis of data from three randomized trials of bisphosphonates for postmenopausal osteoporosis identified twelve subtrochanteric or diaphyseal femoral fractures in ten of the 14,195 study participants, yielding a rate of 23 per 100,000 person-years. Reanalysis of the data in three major randomized controlled trials showed no statistically significant increase in the risk of subtrochanteric femoral fracture in patients treated with bisphosphonates for as long as ten years (2). Given the mixture of results and the different methodologies used in these studies, it is difficult to demonstrate consisten-

cy of this association. Studies (7) have shown an increased risk of atypical femoral fractures in patients taking bisphosphonates for five or more years, suggesting a dose-response relationship. The ASBMR task force reported an incidence of 2 per 100,000 cases per year after two years of bisphosphonate use, increasing to 78 per 100,000 cases per year after eight years of use. Management of patients with AFF: Stop bisphosphonates. Consider an anabolic agent such as teriparatide. Calcium and vitamin-D supplementation (6). Identify any underlying metabolic bone diseases by laboratory investigations such as serum calcium, phosphate, 25-hydroxyvitamin D, intact parathyroid hormone, thyroid-stimulating hormone, bone turnover markers, and 24-hour urine calcium. Intramedullary nailing is the preferred method of fixation. Assess the contralateral femur. If the patient has an incomplete fracture, a period of conservative therapy may be considered. Five of six patients had progression of the lesion to complete fracture at a mean of ten months. Management of patients with prolonged bisphosphonate therapy: If a patient sustains an atypical femoral fracture, bisphosphonates must be stopped. Once administered, bisphosphonates accumulate in the bone and continue to be released for months or years after treatment is discontinued. Regarding over suppression of bone turnover with prolonged bisphosphonate therapy, it is recommended that osteoporosis treatment with bisphosphonates be stopped after a period of five years to provide patients a so-called ‘‘drug holiday’’. Park-Wyllie et al. performed a nested case-control study to explore the association between bisphosphonate use and femoral fractures, and they reported that bisphosphonates treatment of more than five years was associated with an increased risk of atypical subtrochanteric or femoral shaft fractures (7). Therefore, it may be appropriate to consider a drug holiday in patients with a cumulative duration of bisphosphonate treatment of more than five years. The absolute risk of atypical femoral fractures is low (3.2 to 50 cases per 100,000 person-years among short-term bisphosphonate users and about 100 cases per 100,000 person-years among long-term users). Consequently, for most people with osteoporosis, the proven fragility-fracture risk-reduction benefits of bisphosphonates outweigh the risks of AFF (8).

CONCLUSIONS

Patient confirms the risk of atypical femoral fractures in prolonged bisphosphonate therapy, but let us not forgets benefits of bisphosphonates.

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NEWS

SHED - BASIC STRUCTURE FOR STEM CELL RESEARCH

Stem cells are defined as clonogenic cells capable of both self-renewal and multi lineage differentiation. Stem cells have been differentiated from variety of body parts and have shown great promise in the management of variety of diseases in medicine. Recently, stem cells have been isolated and grown from: Human permanent dental pulp tissue stem cells (DPSCs), Stem cells from human exfoliated deciduous teeth (SHEDs), and Periodontal ligament apical papilla of immature teeth (PDLSCs). If we have in hand population of stem cells that reproducibly reform bone, cementum, dentin, and perhaps even periodontal ligament; it is possible to envision complete restoration of the hard tissues in the oral cavity using the patient's own cells, thereby avoiding issues of histocompatability. This would be a more biological approach rather mere mechanical one. It has been reported that the stem cells obtained from the above said sources can generate dentin like tissue both in vitro and in vivo studies in animals. In addition transplanted skeletal or dental stem cells have the potential to "rephrase" repair craniofacial defects and repair/regenerate teeth. This review study showed that stem cells from human exfoliated deciduous teeth are distinct from dental pulp stem cells by virtue of their proliferation rate, increased cell population doublings and osteoinductive capacity in vivo. It also demonstrated that human exfoliated deciduous teeth stem cells may not be a single-cell type, may well be a heterogenous population of cells from the pulp (Kashyap R. SHED - Basic Structure for Stem Cell Research. *Journal of Clinical and Diagnostic Research*. 2015 Mar, Vol-9(3): 7-9).

Doina Butcovan

HEAD CIRCUMFERENCE REFERENCES FOR SCHOOL AGE CHILDREN IN WESTERN ROMANIA

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HEAD CIRCUMFERENCE REFERENCES FOR SCHOOL AGE CHILDREN IN WESTERN ROMANIA (Abstract)**: Aims:** To provide head circumference references for school-aged children in western Romania, and compare them with references from other European countries. **Methods:** A total of 2742 children, aged 6-19 years, from Timis county, were examined by medical students, between February 2010-June 2011. Head circumference references were constructed by Cole's LMS method with LMSChartMaker software. The Romanian 3rd, 50th and 97th percentiles for head circumference were compared with recent references from Belgium and Germany. **Results:** Generally, boys show significantly larger head circumference compared to girls at any age. The head circumference increments between 6 and 19 years are < 1 cm/year. Head circumference increments decrease in increasing age of the children. In girls, adult head circumference is reached at the age of 16 years, whereas head circumference growth continues, in boys, slowly until 18 years. The comparison of Romanian head percentiles with those from Belgium and Germany revealed a smaller head circumference in Romanian children (both girls and boys). **Conclusions:** Comparing head circumference references from Romania to those from Germany and Belgium, we found lower median head circumference in Romanian boys and girls, that could be explained by a taller stature of boys and girls in Germany and Belgium compared to Romania. **Keywords**: HEAD CIRCUMFERENCE, CHILDREN, PERCENTILES, WESTERN ROMANIA.

Head circumference (HC) measuring and head growth evaluation constitute the most simple, inexpensive and readily available tools to assess the development of the central nervous system and identify neonates at risk of neurodevelopmental disorders (1). Furthermore, head circumference is an accurate indication of abnormal brain volume in later age, used mainly to screen for macro- or microcephaly and their various underlying pathologies (2). With knowledge of age-dependent head circumference-to-brain volume relationship, head circumference (in relation to age) can be an accurate indication of normalcy of brain size and growth in a clinical setting (3). Several studies also reported a direct relation between head size and intelligence, learning, nutritional status and brain development (4–6). Moreover, some studies suggest a relation between

head circumference and autism (7,8). A genetic study showed suggestive evidence of association between head circumference and adult intracranial volume, Parkinson's disease and other neurodegenerative diseases, indicating that a common genetic variant in the chromosome 17q21 region might link early brain growth with neurological disease in later life (9).

As research progresses, the clinical impact of HC measurements may become even more important. However, normative data for head circumference over the childhood and adolescence period are lacking for a Romanian population.

Therefore, the aim of this paper was to provide head circumference references for Romanian school-aged children and to compare them with references from other European countries.

MATERIAL AND METHODS

Studied population:

Timis is the largest county in the west part of Romania. According to the National Institute of Statistics, Timis county has an area of 8,697 km^2.(10) The city of Timisoara is the largest in the county. When the present study started, in 2010, in Timis there were 67146 children attending school. We randomly chose 5 schools from Timisoara (enrolling 5676 students) to collect data for this study. The response rate was 50 %. Some students failed to be measured due to various reasons, including school absenteeism and conflicting school activities/responsibilities (certain classes were not interrupted for the measurements). A total of 2742 children, aged 6-19 years were examined by medical students, in the period February 2010 - June 2011.

Exclusion criteria:

Exclusion criteria were: genetic syndromes, cancer, or other chronic disease influencing growth, including children diagnosed with growth hormone deficiency, diseases affecting bone metabolism, cystic fibrosis, renal disease, congenital adrenal hyperplasia, and congenital heart defect with impairment of physical fitness.

Anthropometric techniques:

Prior to each data collection round, all examiners were trained in taking accurate measurements, according to international measurement techniques (11). Each child underwent anthropometric assessments (height, weight and head circumference) that were carried out during one day session. Head circumference was measured with a non-stretchable glass fiber tape measurer. The tape measurer was placed in a straight line from the supra-orbital position to the largest protuberance at the back of the head. Caution was taken to clear the auricles before measuring. The head circumference was recorded to the last completed millimeter. The age of children was rounded down when child's age was years and 1-6 months and rounded up when the age was years and 7-12 months.

Statistical analysis

Growth reference construction

Data were cleaned and validated with SPSS, PC program (version 19.0 - SPSS Inc., USA). Outliers were checked carefully for possible mistakes of data recording. None of the outliers was considered to be biologically implausible. Descriptive statistics were run on all the variables. Statistical significance was considered when $p<0.05$. Growth references for head circumference were constructed with the lambda, mu, sigma (LMS) method and LMSChartMaker Light software (12). The LMS method (lambda, mu, sigma) summarizes the

changing distribution by three curves representing the median (mu), coefficient of variation (sigma) and skewness (lambda), the latter expressed as a Box-Cox power (13). We decided to fit the following percentiles: 3rd, 15th, 25th, 50th, 75th, 85th and 97th, for head circumference in relation to age. We made this choice in order to enable comparison with head circumference references from other European countries.

References for height, weight and body mass index for western Romania, from the collected data are presented in another article (14).

Comparison with references from other countries for head circumference

Comparison with the chosen head circumference references was based on comparability with regard to statistical methods of constructing the reference. The 3rd, 50th and 97th percentile for head circumference were selected and compared with the data from: 2009 References for growth and pubertal development from birth to 21 years in Flanders, Belgium (15) and 2011 German head circumference references for infants, children and adolescents - KiGGS study (16).

Ethical considerations:

Because of the noninvasive nature of the protocol, consisting only of measurements that are a routine for school examinations (height, weight, head circumference), we used a passive consent or opt out method. Parents were fully informed about all study procedures through a school information meeting. Parents who refused the participation communicated this tot the school principal. Prior to the measurement, the child was also asked whether s/he agrees with it. The children's names were not included in the electronic data files. Examiners ensured the basic principles of confidentiality, privacy and objectivity throughout the process. The children were measured in a private room, boys and girls separately. The approval of the University's Ethical Advisory Commission is in accordance with the Helsinki Declaration.

RESULTS

Demographics

After applying the exclusion criteria, 12 children were taken out of the database because they had either growth disturbances as a result of a primary disease (malignancies, endocrine diseases, etc.) or as a consequence of treatment (growth hormone, steroids). We also excluded 9 children that were older than 19 years. A total of 2742 children, aged 6-19 years were examined between February 2010-June 2011, from 5 public schools in Timisoara. The study evaluated 1071 boys (39.1%) and 1671 girls (60.9%), with a male/female ratio of 1:1.5. The mean values and SDs for body weight, height and head circumference in relation to age are shown in tab. I. The mean age was 12.99 years (SD=3.49); with a symmetric distribution (skewness = -0.098; kurtosis= -1.075).

The data showed a strong Pearson correlation between head circumference and height (r=0.583, p=0.000) and an even stronger correlation with age (r=0.815, p=0.000).

Smoothed percentiles for head circumference in centimeters, constructed with the LMS method, for boys and girls separately, are shown in tab. II and tab. III, respectively.

Graphical representation of smoothed percentiles for head circumference in centimeters, constructed with the LMS method, for boys and girls separately, are shown in figure 1 and 2, respectively.

TABLE I

Descriptive Statistics

	All (n=2742)		Girls (n=1671)		Boys (n=1071)		
Variable	Minimum-	Mean	Minimum-	Medie (SD)	Minimum-	Medie (SD)	**p**
Age (years)	6-19	12.9(3.5)	6-19	13.4(3.5)	6-19	12.3(3.3)	0.000
Weight (kg)	18-127	49.5(16.1)	18-127	48,3(13.5)	19-123	51.3(19,2)	0.000
Height (cm)	107-197	155(16)	107-188	154.6(14.1)	108-196	156.4(18.5)	0.007
BMI kg/m^2	8-45	19.9(3.9)	11.2-45.4	19.8(3.6)	8-38.7	20.2(4.3)	0.015
Head circum-	44-64	52.94(2.2)	44-64	52.6(2.0)	46-64	53.4(2.3)	0.000

TABLE II

Smoothed percentiles for head circumference (cm) for boys (n=1071)

Age	L	M	S	3rd	15th	25th	50th	75th	85th	97th
6	-2.028	50.329	0.032	47.6	48.4	49.3	50.3	51.5	52.5	53.7
7	-2.139	50.919	0.033	48.1	48.9	49.8	50.9	52.1	53.2	54.4
8	-2.254	51.488	0.034	48.5	49.4	50.4	51.5	52.7	53.9	55.1
9	-2.364	51.983	0.034	49.0	49.9	50.8	52.0	53.2	54.5	55.8
10	-2.404	52.384	0.035	49.3	50.2	51.2	52.4	53.7	54.9	56.3
11	-2.271	52.738	0.036	49.5	50.5	51.5	52.7	54.1	55.3	56.7
12	-1.909	53.160	0.036	49.9	50.9	51.9	53.2	54.5	55.8	57.1
13	-1.456	53.651	0.036	50.3	51.3	52.4	53.7	55.0	56.3	57.6
14	-1.012	54.118	0.036	50.7	51.8	52.8	54.1	55.5	56.7	58.0
15	-0.672	54.534	0.035	51.1	52.2	53.3	54.5	55.9	57.1	58.4
16	-0.478	54.902	0.035	51.5	52.5	53.6	54.9	56.2	57.4	58.7
17	-0.373	55.182	0.034	51.8	52.8	53.9	55.2	56.5	57.7	58.9
18	-0.290	55.392	0.034	52.0	53.1	54.1	55.4	56.7	57.9	59.1
19	-0.217	55.576	0.033	52.2	53.3	54.3	55.6	56.8	58.0	59.2

TABLE III

Smoothed percentiles for head circumference (cm) for girls (n=1671)

Age	L	M	S	3rd	15th	25th	50th	75th	85th	97th
6	2.169	49.371	0.032	46.3	47.3	48.3	49.4	50.4	51.4	52.3
7	1.860	50.097	0.033	46.9	47.9	49.0	50.1	51.2	52.2	53.1
8	1.539	50.806	0.034	47.5	48.6	49.6	50.8	52.0	53.0	54.0
9	1.155	51.470	0.035	48.1	49.2	50.3	51.5	52.7	53.7	54.8
10	0.619	52.034	0.035	48.7	49.7	50.8	52.0	53.3	54.4	55.5
11	-0.003	52.467	0.035	49.1	50.2	51.2	52.5	53.7	54.9	56.0
12	-0.632	52.776	0.034	49.5	50.5	51.6	52.8	54.0	55.2	56.4
13	-1.214	52.951	0.034	49.8	50.8	51.8	53.0	54.2	55.4	56.6
14	-1.723	53.043	0.033	50.0	50.9	51.9	53.0	54.3	55.5	56.7
15	-2.135	53.125	0.033	50.1	51.0	52.0	53.1	54.4	55.5	56.8
16	-2.419	53.231	0.033	50.2	51.1	52.1	53.2	54.5	55.7	57.0
17	-2.532	53.332	0.034	50.3	51.2	52.2	53.3	54.6	55.8	57.1
18	-2.541	53.412	0.034	50.3	51.2	52.2	53.4	54.7	56.0	57.3
19	-2.517	53.452	0.035	50.3	51.2	52.2	53.5	54.8	56.1	57.4

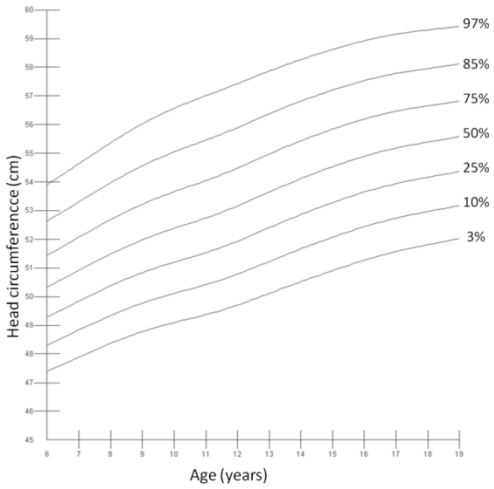

Fig. 1. Head circumference graph percentile for boys age 6-19 years

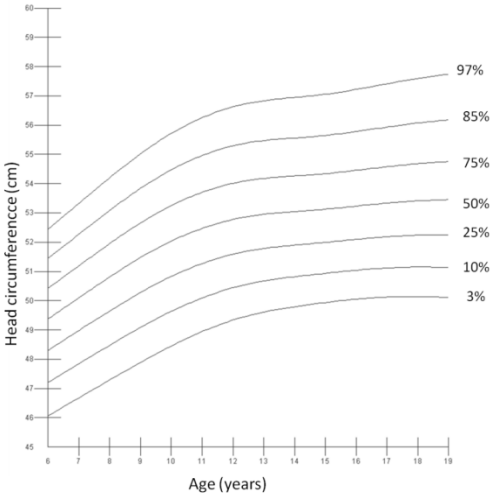

Fig. 2. Head circumference graph percentile for girls age 6-19 years

The head circumference increments between 6 and 19 years are < 1 cm/year. Head circumference increments decrease with older age from 0.96 to 0.01cm at age 18 years. Between ages 9 and 11 years in boys, the annual increments in head circumference increase again from 0.35 to 0.49 cm and thereafter decelerate gradually until age 17 years. In girls, the decrease in increment is progressive with older age, with increments < 0.2 cm after 9 years. From age 16 years onwards, the head circumference increments are still measurable in boys with at least 0.3 cm per year, but not in girls (<0.1 cm). At 19 years age, a median head circumference of 55.4 cm in boys and 53.5 cm in girls is attained. In general, boys show significantly larger head circumference compared to girls at any age.

For the purpose of comparison, we ana-

lyzed the 3rd, 50th and 97th percentile for head circumference for Romanian (presented data), German KiGGS study (15) and Belgian (14) children age 6-19 years, as shown in figures 3., for boys and in figure 4., for girls.

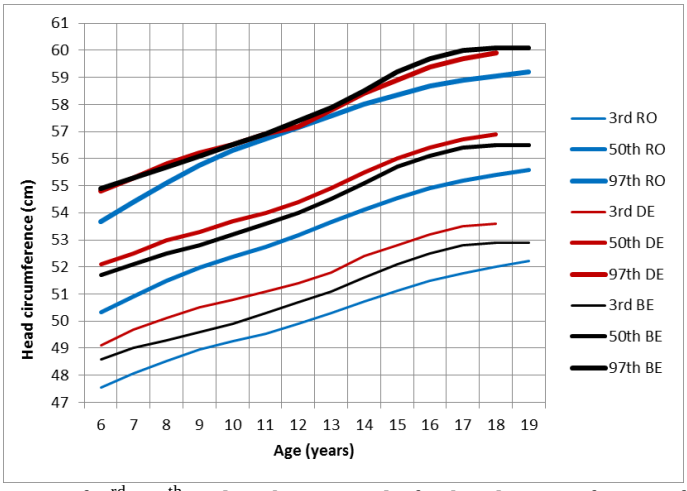

Fig. 3. Comparison of 3rd, 50th and 97th percentile for head circumference for boys age 6-19 years from Romania – in blue, Germany in red (16) and Belgium in black (15)

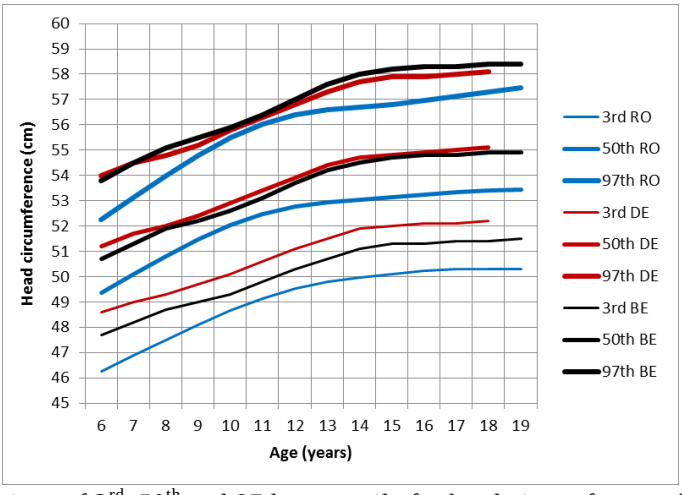

Fig. 4. Comparison of 3rd, 50th and 97th percentile for head circumference for girls age 6-19 years from Romania in blue, Germany in red (16) and Belgium – in black (15).

The comparison of 3rd, 50th and 97th percentile for head circumference in, both, boys and girls, age 6-19 years from Romania, Germany (16) and Belgium (15), shows that Romanian children have a smaller head circumference when compared to their peers in Germany and Belgium. Mean difference between German and Romanian head circumference in children is 1.4 cm for boys (2,56% of median

German head circumference) and 1.4 cm girls (2,61% of median German head circumference), while between Belgian and Romanian head circumference it is 1cm (1,84% of median Belgian head circumference) in boys and 1.2 cm in girls (2.24% of median Belgian head circumference). Greater mean differences (>1cm) in head circumference are seen between the Romanian compared to German and Belgian children especially after the age of 14 years in boys and after 13 years in girls.

Considering that the differences in head circumference in children between countries could be due to different genetics, we looked at height differences between children from these countries. Height data from Belgian study (15) show that Belgian boys are taller that Romanian peers with an average of 1 cm, while Belgian girls are taller with 1.2 cm in average, when compared with Romanian girls (14). When correlating the difference in height with difference in head circumference between Belgian and Romanian children, data revealed a strong Pearson correlation for girls ($r=0.834$, $p=0.000$) and poor correlation for boys ($r=0.291$, $p=0.000$).

DISCUSSION

The sex-specific head circumference-for-age percentiles presented here are the first reference values from a sample of Romanian children, for the age range 6 through 19 years. In general, boys show significantly larger head circumference compared to girls at any age. As expected, the head circumference increments between 6 and 19 years are small (< 1 cm/year) (15,16). Head circumference increments decrease with growing age. In girls, adult head circumference is reached at the age of 16 years, whereas head circumference growth continues, in boys, slowly until 18 years. This finding is consistent with previous studies (15,17,18). However, a study from the Netherlands showed that head circumference marginally increases until age 21 years in boys (19).

After 6 years of age until age 9 years, in boys, head circumference growth occurs in relatively constant increments per year, however, a growth spurt in head circumference follows, from 9 to 11 years and appears to be closely related to the puberty. Similar findings were published in the German KiGGS study (15) and a Japanese longitudinal study from birth to 15 years of age, who found an adolescent growth spurt in head size (20). However, in our study we did not detect similar findings for girls, but a steady decrease in head circumference increment after the age of 9 years.

The comparison of Romanian head percentiles with those from Belgium and Germany revealed a smaller head circumference in Romanian children (both boys and girls). The pattern of head size growth is particularly different in Romanian girls when compared with their peers from Germany and Belgium, showing the slowing of growth for the Romanian girls, after 13 years, when compared with the growth pattern of their peers. Considering that the differences between the 50th and the 3rd percentile, represent 6,11% of median head circumference, the difference between German, Belgian and Romanian peers (ranging from 1.8-2.6% of median head circumference) would represent approximately a 15 percentiles distance, that is significant. A plausible explanation for this discrepancy could be due to genetic factors. Height has a good correlation to head circumference (20). Therefore, we looked at height differences between populations and

found that Belgian boys and girls are taller than Romanian peers, with 1 cm, in average. However, observing correlation between differences in height and in head circumference we found inconclusive results: a good correlation for girls and poor correlation for boys. A clear conclusion could not be drawn regarding the relation between height and head circumference, thus, further studies might be needed.

Study limitations

The sample of children in this study is representative for the west part of Romania; however, it cannot be extended for the entire population of the country. Another limitation of the study is that it does not provide data from birth to 6 years age group. Head circumference can be influenced by multiple factors: gestational factors (such as, mother nutrition (22), socioeconomic status (4), period of gestation, iodine intake during pregnancy (23)), and genetic syndromes, or factors influencing puberty etc. These factors were not evaluated in this study.

The genetic background has a major role in determining both height and head circumference in a population; however, we did not investigate the ethnic background of the studied population. Results from the latest census of population, in 2011, in the Timisoara 81.4% are Romanian, 4.9% are Hungarians, 0.7% are Rroma, 0.2% are Ukrainians, 1.3% are Germans, 1.5% are Serbians and 10% have other ethnicity (10). Although, the genetics of these populations are different and have an impact on head circumference, the percent of different ethnicities is not high enough to influence the study results. Further studies are needed to elucidate the impact of ethnic background on head circumference.

CONCLUSION

The study shows sex specific head circumference-for-age references for a Romanian population, thus allowing a description of the head size growth from 6 to 19 years. Comparing references from Romania to those from Germany and Belgium, we found a significantly lower median head circumference in both Romanian boys and girls, that could be explained by a taller stature of boys and girls in Germany and Belgium compared to Romania. A more widespread study should be carried out by including children from different regions of Romania.

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THE RELEVANCE OF SOME MOLECULAR MARKERS IN RECURRENT PANCREATIC CANCER: FOCUSING ON CA 19-9 AND CYTOKERATINS

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THE RELEVANCE OF SOME MOLECULAR MARKERS IN RECURRENT PANCREATIC CANCER: FOCUSING ON CA 19-9 AND CYTOKERATINS (Abstract). Lately it is generally believed that the incidence of pancreatic neoplasm has increased exponentially all over the world, and especially in the last decade, by even becoming the fifth cause of death by cancer. However, the vast majority of the patients present to the clinic in the advanced unresectable stages, while significant risk factors for pancreatic cancer are still insufficiently studied. In addition, when it comes to the recurrence of the pancreatic cancer, there are also many unknowns regarding the mechanisms and the factors which are implicated. In this way, in this mini-review we want it to make a concise description and analysis for the relevance of some molecular factors in recurrent pancreatic cancer, by mainly focusing on CA19-9 and cytokeratins, besides the other classical clinical factors which are relevant in the diagnostic of the pancreatic cancer recurrence. **Keywords:** RECURRENT PANCREATIC CANCER, MOLECULAR MARKERS, CA 19-9, CYTOKERATINS.

The incidence of pancreatic neoplasm has increased over the last years in many parts of the world, by becoming the fifth or even sixth cause of death by cancer in the Western countries. In fact, pancreatic ductal adenocarcinoma is the most common malignant disease of the pancreas, with the origin in the ductal epithelium. With an incidence of 10-11 per 100.000 inhabitants in the Western countries, it is one of the most aggressive human tumors (1).

In this way, in 2.000 there were diagnosed 216.400 new cases and 215.500 deaths caused by this disease worldwide, while in 2004 in the United States there were predicted 31.860 new cases and 31.270 deaths, which indicates that, due to a very low survival rate, the incidence and mortality are similar (2).

Moreover, the fatality rate correlated with the age varies between 3.1 and 20.8 per 100.000 inhabitants with a corresponding peak for the patients between 60 and 80 years (3).

Unfortunately, 95% of the patients present to the doctor in the advanced unresectable stages, and despite the fact that over the past few decades the development and

advance of the surgical methods and techniques have improved; only morbidity and hospital postoperative mortality are becoming better, without significant impact on survival.

Thus, the only way of establishing a diagnosis in the early stage, potentially curable, would be a rigorous anamnesis that would reveal the early symptoms to 'send' the patient to the doctor in the early stage.

Despite this, most of the classical studies carried out have not been able to identify the significant risk factors for pancreatic cancer, but several risk factors were described as being involved: smoking, chronic pancreatitis, diabetes mellitus, previous gastrectomy and exposure to radiation or chemicals such as chlorinated hydrocarbon based solvents (4-6).

However, despite the numerous studies about pancreas cancer, little is known about this issue. The resections for the cancer of pancreas have entered the therapeutic arsenal for 65 years, but despite the fact that hundreds of articles were written on this subject, there is no unanimity of opinion concerning the efficacy of resection. A number of authors have reported a 5 years survival rate of 30% or even 58%, being supportive of resection (7-9).

In addition, when it comes to the recurrence of the pancreatic cancer, there are also many unknowns regarding the mechanisms and the factors which are implicated. In this way, in this mini-review we want it to make a concise description and analysis for the importance of some molecular factors in recurrent pancreatic cancer, by mainly focusing on CA19-9 and cytokeratins, besides the other clinical factors which are relevant in the diagnostic of the pancreatic cancer recurrence, such as CT, PET, echography or MRI analysis, which of course are not relevant in this context.

FOCUSING ON CA19-9

In this way, CA 19-9 was described for the first time by Koprowski et al. and represents an associated tumoral tissue carbohydrate antigen found on the surface of many types of gastrointestinal tumoral cells. Moreover, Koprowski immunized colorectal cancer cell lines in mice and then isolated the CA 19-9 monoclonal antibodies from the splenocytes of the respective mice. Subsequent studies have shown that the antigen is normally present in salivary mucus and pancreatic exocrine juices, so that, although it originates from colorectal tumoral cell lines, is currently useful in the diagnosis, prognosis, evaluation and monitoring of patients with cancer of the pancreas (10-12).

Also, some researchers have tried to demonstrate the usefulness and efficiency of sequential determination in the dynamics of CA 19-9 in the serum of patients during the postoperative monitoring period. Probably this type studies was first carried out in 1986 at the National Cancer Institute, Bethesda, when the authors notified that patients who experienced a decrease in serum levels to normal values after the operation had a better survival rate than those in which the marker levels maintained at the same increased level. Also they have demonstrated that an increase in the levels of CA 19-9 > 95U/mL or four times its lowest value, immediately post operatory, represents a factor of prediction for the disease progression (13).

In addition, another study has shown that elevated serum level in the post operatory period precedes the occurrence of lesions detectable through CT or clinical examination, with 2 up to 9 months (14).

Also, Montgomery et al. had previously shown that periodic measurements of the CA 19-9 post-operatory level is the best predictor for the survival without recurrence period "disease free survival" and also for the total period of survival. His group also shown that patients in which the level of CA 19-9 returns to normal values for 3 to 6 months, have longer periods of overall survival and survival without relapses than those in which the level is remaining high (15).

Moreover, the same author noticed that the survival rate of the patients in which the post operatory level of CA 19-9 has been less than 180 U/mL at 1 and 3 months, was similar to that of the patients in which the CA 19-9 level returned to normal values in 3 to 6 months (15).

Also, by assessing the usefulness of CA 19-9 in patients with pancreatic cancer versus those with benign lesions, the Safi et al. group found in 92% of patients with pancreatic adenocarcinoma a higher than normal level >37 U/ml, for 775 values > 120 U/ml, with a median value of 528 in patients neoplasm. In contrast, they also found values of 18, 14 and 7 U/ml in patients with chronic pancreatitis, acute or concerned with other benign tumors.

In addition, on another group of patients with different forms of neoplasia: colorectal, gastric, esophageal or extra digestive neoplasia, the CA 19-9 levels for most patients were within normal limits. Moreover, in this series, the sensitivity, specificity and accuracy were 92 %, 85% and 82 % respectively (16).

Moreover, Berger et al. have analyzed retrospectively 129 patients who have had determinations of THAT 19-9 before resection with the visa - mapping between the years 1990 and 2002. They have studied not only the correlation between the level of antigen and recurrence or survival and digestive patients with detectable antigen in serum. The level of the fact that a good preoperative neurological 10-9 has been grouped into 4 categories: undetectable, normal <37, increased 37 to 200 and more than increased > 200 U/ml. Also, the results were contained between 0 and 16.300 U/mL. The median overall survival and survival at 5 years were of 19 months and 11 % respectively (10).

Also, survival was similar between non-secretory and those with normal level, and for both groups the survival was statistically significantly higher than of the other 2 groups with values elevated above normal ($p = 0,003$). The only significant predictive factor for survival, after the univariate and multivariate analysis was the presence of tumor adenopathies ($p = 0.015$ and 0.002) and the level of CA 19-9 ($p = 0.003$). In this way, although the group of patients with normal and undetectable values was submitted with advanced disease, the overall survival was superior (10).

Moreover, there are few studies in the literature about CA 19-9 non-secretory patients. It might be foreseeable that these patients would have a worse prognosis because the level of antigen can not be assessed and, therefore, the prediction for the response to treatment, and for the recurrence and survival is not possible. However, in the study we mentioned before, the non-secretory group had the same prognosis as the one with normal values of the antigen during the preoperative stage. The median survival for these 2 groups of patients was similar: 32 and 35 months respectively. Furthermore, these patients had a significantly better survival than those with higher levels of CA 19-9.

In fact, the patients with undetectable level of antigen have a much higher median value of recurrence-free survival than the other groups: 27 vs. 14, 10 and 10 months respectively, despite the fact that it did not present a clear statistical significance. Also, the second positive predictive factor resulted through uni- and multivariate analysis was the presence of tumoral adenopathies, a fact found in other studies as well. Yet it is difficult and early to draw definitive conclusions because the groups of patients are not numerically statistically sufficient. Also it remains unclear why the CA 19-9 non-secretory patients have better prognosis even if the tumor adenopathies are present. For this reason, the aforementioned authors considered that for resected patients in which the CA 19-9 preoperatory level of is undetectable, the aggressive surgery/therapy must and it is worth being performed (10).

FOCUSING ON CYTOKERATINS

In this way, cytokeratins (CK) are epithelial cellular markers useful in determining the tumoral genesis in multiple neoplasms. The cellular structure of mammals includes 3 different types of protein families: actin-containing microfilaments, microtubules containing tubulin and intermediate filaments. The latter include cytokeratins, desmin, vimentin, neurofilaments and glial fibrillar acidic filaments, representing in fact a family of proteins involved in cell differentiation processes (17-19).

Generally speaking, two cytokeratins have been more intensively studied: CK 7 and CK 20, mainly due to their complementary distribution in normal epithelium and malignant tumor tissue.

In this way, CK 7 is expressed in lung, breast, ovary and endometrium, while CK 20 is found in the gastrointestinal epithelium, urothelium and Merkel cells. Also, it is believed that an alteration of the cellular structure that includes the expression of CK 20 may be due to more aggressive tumor development. Also, a variation of the type of expression of CK 20 in neoplasic lesions has predictive value for the recurrence of the disease. Moreover, the expression of cytokeratins and in particular the CK 20 in neoplasic lesions can be an indicator of poor tumor cell differentiation.

Thus, in a study by Wildi et al., the authors describe the over expression of CK 20 on a significant number of specimens with pancreatic cancer, while its prevalence and significance have not been explored (20). Moreover, the expression of cytokeratins, including CK 20, correlated with the degree of cell differentiation. In this way, a possible explanation for the prognostic significance of CK 20 might be that the specific gene is associated with the neoplasic lesion grading as a marker of differentiation, and CK 20 gene expression is correlated with more aggressive tumor biology.

Also, Winnenthal et al. have investigated the possible correlation between the expression of CK 20 in pancreatic cancer and the infaust prognosis of these patients. In this way, they monitored the R0 operated patients for pancreatic tumors and correlated the presence or absence of CK 20 with survival. The cytokeratins are surface cell markers and therefore may be used in the determination of micrometastases in hematopoietic organs. Also, in particular CK 20 was used to detect the dissemination of neoplastic cells using reverse transcription-polymerase chain reaction (RT-PCR) or immunohistochemistry in bone-marrow

cells, venous blood, or lymph nodes in patients with cancer (21).

Moreover, for colorectal and thyroid neoplasm a correlation between CK 20 positive cells from blood and bone marrow and tumoral stage has been described. In this way, because pancreatic neoplasm has high potential for metastasis, the presence of CK 20 cells in blood has been suggested as a possible marker with high sensitivity in determining the micrometastases. Also, tumoral tissue samples from 63 patients with pancreatic cancer were studied for the presence of CK 20 using the RT-PCR. 34 out of the 63 patients have benefited from R0 resection and were monitored for survival. From these 34 patients, 26 (76%) exhibited positive 20 CK cells and 8 (24%) negative CK20. The average period of monitoring for the entire group was 17 months (limits between 4-36 months) while the monitoring period for 'censored' patients was 23 months (between 10-36 months). Also, in the subgroup of R0 resected patients, 3 (38%) of the 8 patients with negative CK 20 neoplasms and 16 (62%) of the 26 patients with positive CK 20 tumors ($p=0.15$) have died due to the recurrence of the disease. The median survival for patients with positive 20 CK was 13 months (limits, 4-36), the median survival in the R0 and negative CK 20 group was 26 months (limits, 13-35; $p = 0.06$).

In addition, the differentiated survival observed in the patients with negative CK 20 neoplasm cannot be attributed to tumoral stage or tumoral grading variations. Also, the CK 20 expressed by most pancreatic ductal adenocarcinomas seems to be associated with poor prognosis in the R0 resected patients. These data suggest that negative CK 20 pancreatic adenocarcinomas represent a subgroup of patients with a more favorable prognosis.

In this way, the CK 20 expression in resected patients for pancreatic cancer may be important as a prognostic marker that can be easily detected and measured on tumoral tissue. Uncertainties remain, however, regarding a possible association of CK 20 expression with tumoral grading, if CK 20 expression is an independent prognostic marker and if the results after adjuvant therapy differ depending on the CK 20 expression (21).

Also, unlike breast cancer and other neoplasms, pancreatic adenocarcinoma is relatively a homogeneous disease with respect to its infaust prognosis and very few histological variants, which is reflected in the uniform chemotherapeutic standard protocols. While cytokeratins 7, 8, 18 and 19 serve as indicators of normal pancreatic ductal epithelium and of neoplastic cells, CK 20 is absent in the normal fetal and adult pancreas but appears in 30%-60% of pancreatic carcinomas. These observations lead to the assumption that there might be a subset of pancreatic neoplasms that could be associated with this marker.

In addition, the Matros et al. group have investigated the relevance of two cytokeratins, CK 7 and CK 20, in pancreatic cancer and tried to find associations with some clinical features. For determining the moment of CK 20 expression during the pancreatic karyokinesis evolution, the aforementioned authors have evaluated the CK 20 in cancer precursory lesions, called pancreatic intraepithelial neoplasia (PanIN). Thus, they reported for the first time that CK 20 is useful in identifying the subsets of pancreatic cancer with distinctive features. In this way, the classification of human neoplasias into subtypes represents a breakthrough in understanding the

biological behavior of the cancer and the response to conventional or new therapies. Moreover, in some cases, these subclassifications are made in connection to a therapeutic agent, such as the use of Trastuzumab in breast cancer with HER2 amplifications and Gefitinib in small cells lung cancer and EGFR mutations (22-24).

Moreover, the authors found that patients with tumors that have reduced CK 20 expression have a significantly higher survival and this correlation persists even after adjusting other variables that can alter the results. Also, none of the patients with tumors exhibiting moderate or high CK 20 expression survived more than 26 months. This difference between clinical results is somewhat surprising because CK 20 was originally used as origin marker for tumors whose starting point was unknown.

Also, the aforementioned authors have found the CK 20 expression in early lesions (PanIN), prior to carcinoma, which implies that this aberrant pathway is most often acquired early in the tumor progression, even before the development of the invasion. In this way, the expression of CK 20 was present on a wide range of PanIN injuries including PanIN I lesions, which supports the progression hypothesis from low to high-grade PanIN to invasive carcinoma.

Thus, the transition from an epithelial differentiation path with CK 20 present seems to appear very early in the progression of neoplastic lesions and maintains during the development of the invasion. Moreover, recent studies concerning intraductal papillary mucinous neoplasm (IPMN) showed a similar divergence in the differentiation and the progression of non-invasive neoplasms. Also, the IPMN intestinal type is more likely to be positive CK 20 and also more likely to be associated with mucinous adenocarcinoma, when the malignancy is present. In addition, another subdomain in which the epithelial differentiation and cytokeratins profile have been studied is the ductal acinar metaplasia process that occurs in chronic pancreatitis (25).

Also, for the studied group, although CK 7 was highly present in the non-neoplastic lobular tissue, the CK 20 presence was not traced in the non-neoplastic pancreatic ducts. In this way, these findings suggest that the transition to CK 20 positivation appears in PanIN early stages and support the hypothesis that pancreatic ductal epithelial neoplasms may progress onto another distinct carcinogenic path.

In the future, with the development of the researches in the field of pancreatic cancer concerning the diagnosis and management of epithelial noninvasive lesions of high risk, further studies will probably also investigate the role of CK 20 expression in predicting neoplasia development.

Also, despite the recent breakthroughs in understanding the genetic impairments/deterioration in pancreatic cancer, little progress has been made in terms of natural aggressiveness and the reduced survival that characterize this disease. Thus, the general trend is to consider the cancer of the pancreas as a single type of disease, but recent research and findings from other neoplasias have resulted in subclassifications very relevant in terms of the treatment algorithm and etiopathogenic ways.

In this way, as mentioned before, the CK 20 expression indicates reduced survival. Therefore, CK 20 can be used as a new prognostic marker in clinical management of pancreatic cancer and may lead to the discovery of other etiopathogenic ways in managing this devastating disease (18).

Also, the group of Roder et al. (26) has proposed the idea that often the pancreas cancer is being substaged and in many patients, at whom the tumor is considered resectable, the disease is actually systemic from the beginning. In this way, by using immunohistochemistry tests, the aforementioned authors have determined neoplastic pancreatic cells disseminated in bone marrow using monoclonal antibodies (mAbs) CK2, KL1, and A45-B/B3 anti-cytokeratins.

Moreover, prior to operation, bone marrow from the antero-superior iliac crest was sampled from 48 patients and compared to bioptic products from 33 healthy subjects. In this way, 14 (48.4%) of the 31 patients with R0 resections and 10 out of 18 (58.8%) with expanded disease exhibited positive CK cells. Still, there was no significant difference between patients regarding the different stages or the presence of lymph nodes metastases. After a median period of postoperatory monitoring of 22.8 months (3 - 48 months), 6 of 15 (40.0%) patients with R0 resection and tumor cells in the bone marrow exhibited distant metastasis and 7 (46.7%) had local recurrence, as compared with none of the 12 free CK-positive cells patients ($p < 0,05$).

Thus, the univariate analysis revealed that the presence of CK-positive cells has predictive value for reduced survival. Therefore determining the tumor cells from bone marrow of patients with pancreas cancer may help identifying those patients with increased risk of early metastasis. Moreover, this is also important in determining the prognosis and the consequences of the neo-/adjuvant therapy in early pancreatic cancer (26).

Additionally, in the context of the present discussion we could also remember the relevance of the I-st class histocompatibility complexes - related to A chain (MICA), as a relevant aspect for the subject studied here. In this way, Weber et al. (27) reviewed the expression of various major I-st class histocompatibility complexes - related to A chain (MICA), as a transmembranary protein or a soluble protein from the serum of 61 patients with pancreatic cancer and 33 healthy subjects using the ELISA test. Also, MICA relates to a receiver of the NKG2D natural killer cells and is increased in some epithelial cancers: gastric, breast, colon, and melanomas.

In this way, the results indicated that serum MICA in patients with pancreatic cancer were significantly higher than in healthy subjects (1107 pg/mL vs 215 pg/mL, $p < 0,01$). They also showed that the serum level of MICA is lower in T1-T3 tumors (n= 15), when compared to 46 metastatic lesions (437 pg/mL vs. 1325 pg/mL, $p < 0.05$). In addition, MICA serum level in patients with unresectable tumors (n= 37) was significantly higher than in the resected patients (n= 24); (1572 pg/mL vs. 390 pg/mL, $p < 0.05$). Moreover, the survival in resected patients (n= 23) was significantly higher than in the unresected patients (n= 34); (17 months vs. 11 months, $p < 0,05$). Therefore the MICA serum level is significantly increased in patients with pancreatic ductal adenocarcinoma and correlates with tumor size and survival, while also its levels are decreasing after resection and can be used as a prognostic marker (27).

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BREAST INFECTIONS: DIAGNOSIS WITH ULTRASOUND AND MAMMOGRAPHY

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BREAST INFECTIONS: DIAGNOSIS WITH ULTRASOUND AND MAMMOGRAPHY (Abstract): **Aim:** Evaluation of ultrasonographic and mammographic pattern, etiology and risk factors of breast infections. **Material and methods:** Our study included a group of 66 female patients aged 16 to 71 years, examined by ultrasound and mammography in several medical imaging services in Iaşi in the interval 2008-2014; ultrasound was performed in all 66 patients and mammography in 22. **Results:** In our study breast infections occurred mostly during breastfeeding and the most frequent causative agent was *Staphylococcus aureus*; ultrasound established the correct diagnosis in 63 cases and detected one or more of the following aspects in case of breast infections: edema of the fatty tissue, hypoechoic areas in the breast tissue, dilated ducts, or fluid collections. Mammography was not necessary in puerperal mastitis and was performed only in women over 40 years old; in most cases we had encountered a focal asymmetric density which had low specificity for the diagnosis of mastitis or breast abscess. **Conclusions:** Our study proved that ultrasound is a valuable method for the diagnosis of mastitis, especially when an abscess is suspected and established a correct diagnosis in most cases; the abscesses appear as inhomogeneous fluid collections, with poorly defined margins, posterior acoustic enhancement, no Doppler signal inside, sometimes associated with enlarged axillary lymph nodes. Mammography was not helpful for the diagnosis. **Keywords**: BREAST INFECTION, ABSCESS, MASTITIS, ULTRASOUND, *STAPHYLOCOCCUS AUREUS*.

Mastitis and breast abscess appear frequently during breastfeeding (puerperal mastitis) (1); the most frequent causative agents of these infections are *Staphylococcus aureus* (which gives rise very quickly to abscess formation) and *Streptococcus pyogenes* (the manifestation being mastitis, the abscess appearing in the advanced stage) (2, 3). The symptoms of breast infections are: fever, breast pain, swelling breast, palpable breast masses inside the breast. Physical examination can suspect a breast infection, but imaging methods are the ones which confirm the diagnosis (4, 5).

Ultrasound is a valuable method for the diagnosis of mastitis, especially when an

abscess is suspected; ultrasonography can localize, measure and follow-up the abscesses during the treatment (6, 7).

Mammography is not recommended in case of puerperal mastitis. Inflammatory changes increase breast density and can impair the visibility of the lesions.

MATERIAL AND METHODS

This retrospective study included a group of 66 female patients aged 16 to 71 years presenting with one or more symptoms of breast infection and examined by ultrasound and/or mammography in several medical services in Iaşi between 2008 and 2014.

Data were obtained by reviewing the ultrasound and mammography reports and images; the results were processed and interpreted by statistical methods (p value, trend).

RESULTS

All 66 patients were examined by ultrasound and in 20 (30.3%) of them mammography was also performed; 22 (33.3%) abscesses and 44 (66.7%) cases of mastitis, including superficial (30 patients-45.4%) and severe mastitis (14 patients-21.3%) were detected.

We diagnosed 32 (48.4%) breast infections occurring during breastfeeding (15 - 22.7% abscesses and 17 - 25.7% cases of mastitis) and 34 (51.6%) breast infections not related to breastfeeding.

Most cases of severe breast infection (15/66 - 22.7%) occurred during breastfeeding, 5 (7.5%) patients had diabetes mellitus, 2 (3%) of them had no apparent risk factors; 27 (40.9%) women who had superficial mastitis (ultrasound findings: fatty tissue edema and skin thickening) did not have any important risk factors (tab. I).

TABLE I

Risk factors of breast infections

Risk factors	Breastfeeding		Diabetes		No risk factors	
	Abscess	Mastitis	Abscess	Mastitis	Abscess	Mastitis
No. of patients	15	17	5	0	2	27
% of patients	22.7	25.7	7.5	0	3	40.9

In the vast majority of cases breast infections were located on one side: 30 (45.4%) in the left and 35 (53.1%) in the right breast; only one patient (1.5%) had bilateral mastitis.

Breast ultrasound established a correct diagnosis in 63 (95.4%) cases; inflammatory carcinoma was considered in the differential diagnosis in 3 (4.5%) cases.

Mammography was never necessary in puerperal mastitis and was recommended only in women aged over 45-50 years to complete the evaluation of both breasts.

Ultrasonography showed the following:

- in all the cases of superficial mastitis (30 patients-45.4%): fatty tissue edema and thick skin, which correlated with the symptoms were sufficient for the diagnosis; all these patients were treated with antibiotics no case of abscess formation being recorded;

- in case of severe mastitis (fig. 1), one or more of the following: edema of the fatty tissue (37 patients - 56 %), hypoechoic areas in the breast tissue (35 - 53%), dilated retroareolar ducts (34-51.5%), fluid collections (22-33.3%), enlarged axillary nodes (20-30.3%), skin thickness more the 2 mm (19 - 28.7 %), increased vascularisation of the breast tissue (18-27.7 %).

Twenty-two (33.3%) abscesses were detected by ultrasound, which most of the time had a typical pattern: round or oval hypoechoic fluid collections with poorly defined margins, inhomogeneous, with posterior acoustic enhancement, no Doppler signal inside; a fluid-fluid level and internal echoes were identified in one case each; the abscesses were associated with appearances of mastitis in 12 (18.8 %) patients and/or enlarged axillary lymph nodes (12 patients-18.8 %) (fig. 2 a, b).

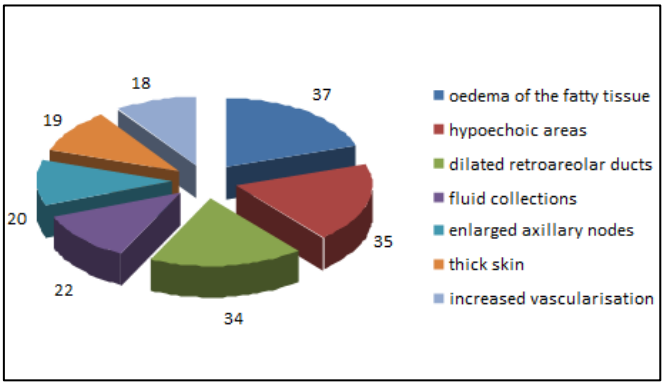
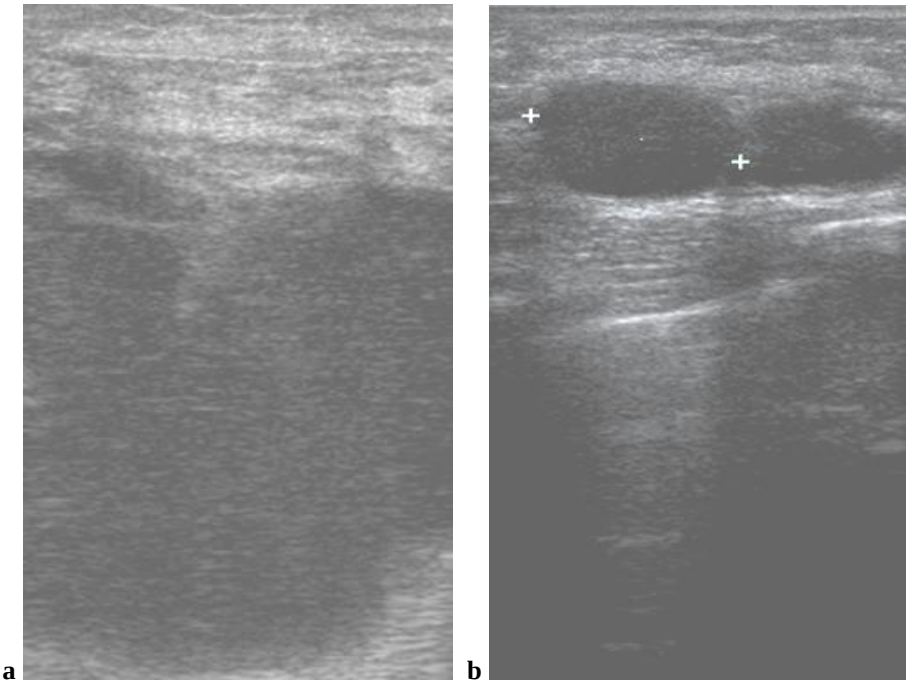


Fig. 1. Ultrasonographic pattern of breast infections



a **b**

Fig. 2 a, b. Breast and axilla ultrasound, B mode: Breast abscess (a), enlarged axillary nodes (b)

Mammography was performed in 22 cases and the findings were: normal in 3

(13.6%) cases, breast edema (increased density of the breast, with effacement of the normal structures) -2 (9%) cases and thick skin (1 case - 4.5%); only in 1 patient (4.5%) with breast abscess mammography discovered a high intensity, homogeneous, oval mass, with poorly defined, irregular margins; in our study in most cases of breast infections (15 cases - 68.1%) mammography showed a focal asymmetric density (fig. 3, 4).

The treatment of breast infections was antibiotic therapy in 48 (72.8%) cases (the patients with mastitis and 4 of the patients with abscesses), antibiotic therapy, incision and drainage in 17 (25.7%) patients with breast abscesses; in one (1.5%) patient after surgery ultrasound guided fine needle aspiration of the remaining collection was also performed.

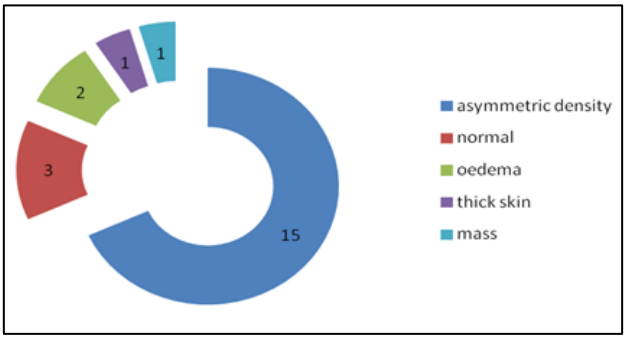


Fig. 3. Mammographic pattern of breast infections

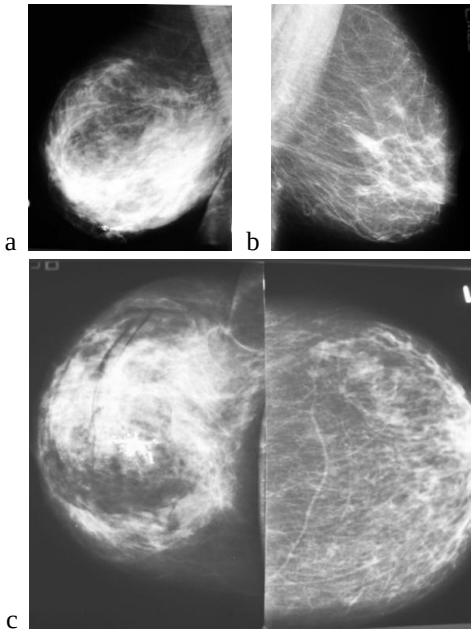


Fig. 4 a, b, c. Bilateral mammography, oblique (a, b) and cranio-caudal (c) views: asymmetric density in the inferior quadrants of the right breast

The causative organisms were studied in 39 (59%) breast infections: 22 (56.4%) abscess cultures and 17 (43.6%) breast milk cultures; the results of the cultures were as follows (tab. II): *Staphylococcus aureus* (33 cultures-84.7%), *Streptococcus pyogenes* (5 cultures-12.8%), *Bacteroides spp.* (1 culture - 2.5%).

TABLE II
The causative organisms of breast infections

Causative organism	No. of patients	% patients
Staphylococcus aureus	33	84.7
Streptococcus pyogenes	5	12.8
Bacteroides spp.	1	2.5

DISCUSSION

In our study, breast infections occurred during breastfeeding (32 - 48.4% patients) and related to diabetes mellitus (5 - 7.5% patients), but 27 (40.9%) women with superficial mastitis and 2 (3%) with breast abscesses had no apparent risk factors for breast infections; less than 50% of breast infections were related to breastfeeding, but this was the most common risk factor in our study. Most infections were caused by *Staphylococcus aureus* (33 infections-84.6 %), accordingly to the literature (8, 9).

Ultrasonography showed edema of the fatty tissue and thick skin in all cases of superficial mastitis, which correlated with the symptoms were sufficient for the diagnosis (10, 11).

In case of an extended breast infection one or more of the following signs of mastitis was present: hypoechoic areas (35 - 53% cases) with increased vascularization (18-27.7% cases) in the breast tissue, dilated retroareolar ducts (34-51.5%), axillary lymphadenopaties (20-30.3%), which along with pain and swelling breast were diagnostic in all cases.

We found at ultrasound 22 (33.3%) abscess, which appeared as hypoechoic collections, with a diameter of 3 to 7 cm; 20 (30.3%) collections were inhomogeneous, with internal echoes and poorly defined, irregular margins; in one case (1.5%) the hypoechoic image was homogeneous and in 1 case (1.5%) had a fluid-fluid level; the lesions were associated with aspects of mastitis in 12 (18.18%) cases and enlarged axillary lymph nodes were present in 12 (18.18%) patients. Only in 3(4.5%) patients the hypoechoic masses appeared to be solid and an inflammatory tumor was suspected (12).

Mammography was performed only in 20 (30.3%) cases and the images were not helpful for the diagnosis; in most cases (15-22.7%) we found a focal asymmetric density with poorly defined, irregular margins which had to be further studied by ultrasound.

CONCLUSIONS

Our study proved that ultrasound is a valuable method for the diagnosis of mastitis, especially when an abscess is suspected and makes a correct diagnosis in most cases. The abscesses appeared as inhomogeneous fluid collections with poorly defined margins, posterior acoustic enhancement, no Doppler signal inside, associated with enlarged axillary lymph nodes. Mammog-

raphy was not helpful for the diagnosis; in most cases we found a focal asymmetric density which has low specificity for the diagnosis of mastitis and breast abscess.

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EPIDEMIOLOGICAL EVALUATION OF THE OUTCOMES AFTER VIDEO-ASSISTED THORACOSCOPIC TALCAGE IN NEOPLASTIC PLEURISY

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EPIDEMIOLOGICAL EVALUATION OF VIDEO-ASSISTED THORACOSCOPY OUTCOMES IN NEOPLASTIC PLEURISY (Abstract). **Aim:** Clinical-epidemiological investigations for further assessing the importance of video-assisted thoracoscopy in the treatment y of patients with neoplastic pleurisy. **Materials and methods**: The researches included a group of 72 patients (31.9% men and 68.1% women aged 31-81 years, mean age ± 60 years) with neoplastic pleurisy who underwent pleural symphysis by video-assisted thoracoscopic talcage.. For statistical-mathematical processing and interpretation the Pearson correlation index with the level of significance at $p = 0.05$ and highly significant at $p < 0.005$ was used. **Results:** Neoplastic pleurisy prevalently affected the age groups 51-80 years (84.9%). Dyspnea was present in all cases, and patient history at the time of admission revealed 14 conditions, of which 25% were lung cancers. Macroscopically nodular and vegetative tumors were found in 66.7% of cases. An amount of 1000 - 2000 ml of pleural fluid was found in 44.5% of the cases and a serocitrin appearance in 50%. In 23.6% of the cases cytology results were positive for malignancy and in 13.8% suspicious. In 65.2% of the cases the pleural fluid was exudative and anatomopathology was suggestive of adenocarcinoma in 34.7% of the cases and breast cancer in 18%. The prevalence of recurrences varied from 1 month to more than 7 months, with 36.4% for 1-2 months. **Conclusions**: The obtained additional data support the important role of pleural symphysis by video-assisted thoracoscopic talcage in the patients with neoplastic pleurisy. **Keywords:** PLEURAL NEOPLASIA: VIDEO-ASSISTED THORACOSCOPY, HISTORY, RECURRENCE, TALCAGE, PLEURAL SYMPHYSIS

In cancer patients, pleural fluid can initially accumulate asymptomatically, possibly associated with that present in the pericardium and peritoneum. (1, 2, 3). In symptomatic pleurisy pleural fluid accumulation occurs during anticancer therapy without requiring local treatment, but in patients in which the neoplasm is not responding to systemic therapy it requires local treatment.

Tumor-induced pleural fluid may or may not contain malignant cells. In 75% of the lung cancer, breast cancer or lymphoma cases the pleural fluid is present.

In symptomatic pleurisy thoracentesis is the first-line procedure and when the fluid returns the drainage tube is necessary for up to two weeks by aspirating 100 ml in 24 hours. Antibiotics and anticancer drugs are introduced according to the established schedule through the drainage tube

(4,5,6,7,8).

Neoplastic pleurisy tends to recur and cause severe inspiratory distress and treatment-refractory state. These symptoms significantly affected the quality of life of patients (8,10).

Therefore, the palliative treatment of neoplastic pleurisy is required in all situations and is consists in the obliteration of the pleural space by video-assisted thoracoscopic talcage (1,12).

MATERIAL AND METHODS

The study included a group of 72 patients (31.9% men and 68.1% women, aged 31-81 years, mean age ± 60 years) with neoplastic pleurisy who underwent video-assisted thoracoscopy completed by talcage.

Information was gathered based on an epidemiological case sheet and data from patients' medical records according to the ethical and legal guidelines.

For statistical-mathematical processing and interpretation the Pearson correlation index with the level of significance at $p = 0.05$ and highly significant at $p < 0.005$ was used.

RESULTS

1. Age-group distribution showed that metastatic neoplastic pleurisy prevalently affected the age groups 51-80 years (84.9% of the total) compared to 12.4% in age-groups 21-50 years, the difference being highly significant ($p < 0.005$) (fig. 1).

2. Admission symptoms. Dyspnea was present in all 72 patients, followed by dry cough, chest pain, chest twinge, productive cough, and fatigue state (fig. 2).

3. Prevalence of prior history recorded on admission included 14 entities with a frequency ranging from 25% for lung cancer to 2.8%, and 22 pathological manifestations recoded in 1 case each (1.4%).

A highly significant prevalence ($p < 0.005$) was found for the first 4 entities: lung cancer, breast cancer, hypertension, and chemotherapy which accounted for 70.8% of the total of 72 patients with metastatic neoplastic pleurisy (tab. I).

One case each (1.4%) of operated brain metastasis, pleurisy, liver metastases, bone metastases, cutaneous squamous cell carcinoma, heart disease, esophageal varices, thrombophlebitis, operated ovarian cyst, chronic heart failure, obstructive arteriopathy, type II diabetes mellitus, lung metastases, operated gastric cancer, leukemia, Hodgkin's lymphoma, mesothelioma, liver cirrhosis, thyroid carcinoma, operated melanoma, non-Hodgkin lymphoma, AIDS were also recorded.

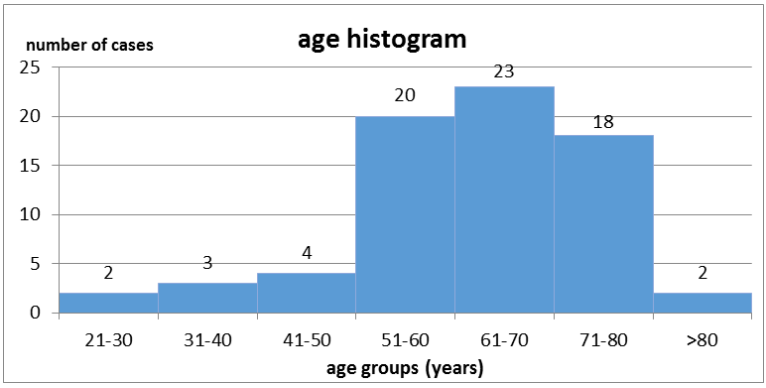

Fig. 1 Age-group distribution of the 72 patients with neoplastic pleurisy

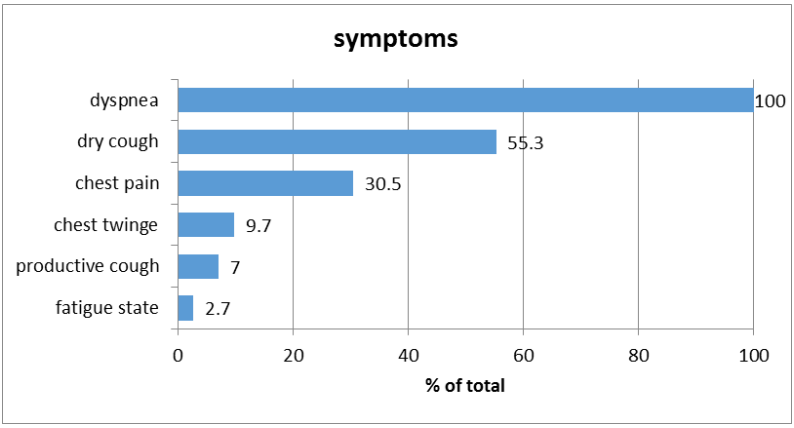

Fig. 2 Admission symptoms in the 72 patients with neoplastic pleurisy

TABLE I

Prevalence of prior history recorded on admission in the 72 patients with metastatic neoplastic pleurisy

Prior history	Nr.	%	Prior history	Nr.	%
Lung cancer	18	25.0	Stroke	2	2.8
Operated breast cancer	16	22.2	Pleural metastases	2	2.8
Arterial hypertension	9	12.5	Radiotherapy	2	2.8
Chemotherapy	8	11.1	Tuberculosis	2	2.8
Operated uterine cancer	5	7.0	Malignant melanom	2	2.8
Operated uterine fibroma	4	5.5	Ischemic cardiopathy	2	2.8
Operated ovarian cancer	3	4.1	Prostate adenoma	2	2.8

4. Detection of metastatic pleural lesions. Macroscopic examination revealed the presence of nodules, vegetative tumors, absence of tumoral features and presence of whitish placards. Nodules and vegetative tumors were present in 66.7% of the cases, the difference between them and the other two remaining findings being highly statistical significant ($p < 0.005$) (Fig. 3).

5. Amount and macroscopic appearance of pleural fluid showed that 1000-2000 ml of pleural fluid was found in 44.5% of cases and a serocitrin appearance in 50.0% (Tab. II).

6. Pleural fluid cytology was positive for malignancy in 23% of cases and suspicious in 13.8%. In a total of 37.4% of the cases cytology suggested a diagnosis of neoplastic pleurisy (fig. 4).

7. Pleural fluid biochemistry. The pleural fluid from neoplastic patients was overwhelmingly of exudative type (65.2%) with a highly significant difference ($p<0.005$) compared to the transudative type (13.8%) (tab. III).

8. The prevalence values of anatomopathological findings pleaded for the diagnosis of malignancy in 65.3% of cases (tab. IV).

9. Frequency of recurrence according

to the interval (months) from surgery showed that recurrences were recorded in 11 (15.3%) of the 72 study cases as follows: after 1 - 2 months = 36.4%; after 4-5 months = 27.2%; ≥ 7 months= 34.4% (tab. V). The 11 (15.3%) cases with recurrence of neoplastic pleurisy were almost equally dispersed in the three time intervals. 1-2 to ≥ 7 months.

10. Length of hospital stays (days) for patients with metastatic pleurisy reflect, at least in part, the costs of care. A hospital stay of 5-10 days was recorded in 73.5% of the 72 study patients (tab. VI).

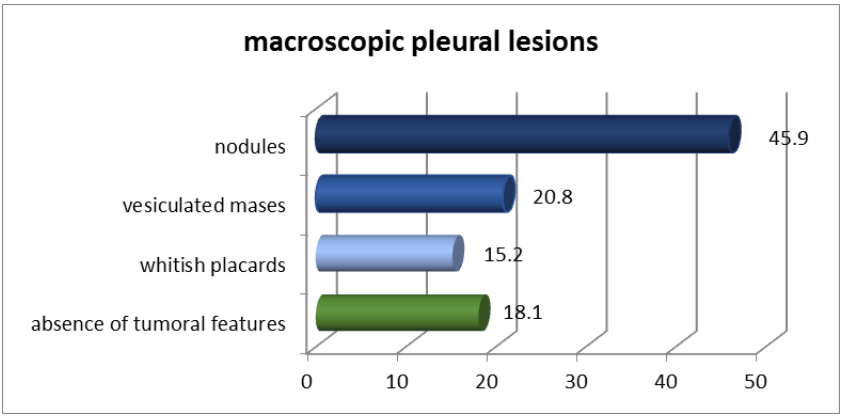

Fig. 3. Macroscopic metastatic pleural lesions in the 72 patients with neoplasic pleurisy

TABLE II

Data on the amount and macroscopic appearance of the pleural fluid

Amount	**No.**	**%**	**Appearance**	**No.**	**%**
< 1000	16	22.2	Serocitrin	36	50.0
1000 – 2000	32	44.5	Serohematic	23	32.0
> 2000	24	33.3	Hemorrhagic	13	18.0
Total	**72**	**100**	**Total**	**72**	**100**

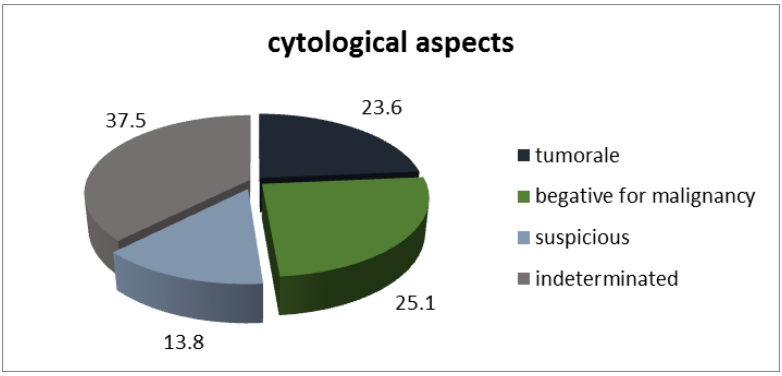

Fig. 4. Pleural fluid cytology in the 72 study patients

TABLE III

Pleural fluid biochemistry

Biochemical aspects	Number of cases	%
Exsudate	47	65.2
Transudate	10	13.8
Indeterminate	15	21.0
Total	**72**	**100**

TABLE IV

Prevalence of anatomopathological findings according to clinical form

Diagnosis	Number of cases	%
1. Pulmonary adenocarcinoma	25	34.7
Breast cancer	13	18.0
Mesothelioma	3	4.2
Uterine body cancer	3	4.2
Malignant melanoma	2	2.8
2. Others		
Inflammatory process	22	30.6
Tuberculosis	3	4.1
Total	**72**	**100.0**

TABLE V

Frequency of recurrence in the cases with metastatic neoplastic pleurisy

Interval from surgery (months)	Number of cases	%
1 -2	4	36.4
4 - 5	3	27.2
≥7	4	36.4
Total	**11**	**100.0**

TABLE VI

Length of hospital stays for patients with metastatic pleurisy

Number of days	Number of cases	%
3 - 4	6	8.3
5 -6	16	22.2
7 – 8	25	34.8
9 -10	12	16.5
11 -12	5	7.0
13 – 14	2	2.8
15 -16	2	2.8
17 – 18	2	2.8
19 -20	2	2.8
Total	**72**	**100.0**

DISCUSSION

In agreement with our findings, other authors (12,13) have highlighted the importance of pleural symphysis by video-assisted thoracoscopic talcage in patients with neoplastic pleurisy (5). Thus, Horvat (11) states that the main indications of video-assisted thoracoscopy are: evaluation of malignant pleural fluid collection, hilar and mediastinal lymphadenopathy, and performing lung biopsies.

If by the previously used procedures in over 40% of the patients with malignant pleurisy the cytological and histopathological results were inconclusive, the diagnostic accuracy of video-assisted thoracoscopy is about 90%. Procedures performed using this technique include pleural and lung biopsy, lysis of adhesions, partial pleural decortication, pleurectomy, pleurodesis and diagnosis of entire pleural cavity.

Homer (12) points out that although used since 1930 - 1940 thoracoscopy is back in current use, with the advent of video system, video-assisted thoracoscopy being a procedure that can be performed without difficulty and risks at an acceptable cost.

Video-assisted thoracoscopy can be performed under local (10 to 15 ml 1% lidocaine) or general anesthesia, and is particularly useful in the diagnosis of pleural disease or for obliterating the pleural space by video-assisted thoracoscopy with talcage.

Other authors (12,13) also insists on the fact that by identifying malignant cells pleural fluid cytology makes an accurate diagnosis of neoplastic pleurisy. More sensitivity may be added to this exam by using monoclonal antibodies, molecular biology techniques and cytogenetics.

CONCLUSIONS

Our clinical-epidemiological research of a representative group of patients provides additional evidence on the importance of video-assisted thoracoscopy in the diagnosis and therapy of neoplastic pleurisy and a better quality of life of these patients.

Of the 72 study patients with metastatic pleurisy included in the study in 56 (77.7%) video-assisted thoracoscopy was followed by aseptic talcage that resulted in stopping the production of pleural fluid.

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NEWS

IS ROBOTIC PARATHYROIDECTOMY A FEASIBLE AND SAFE ALTERNATIVE TO TARGETED OPEN PARATHYROIDECTOMY FOR THE TREATMENT OF PRIMARY HYPERPARATHYROIDISM?

A best evidence topic was written according to a structured protocol. The question addressed was whether robotic parathyroidectomy (RP) is a feasible and safe alternative to targeted open parathyroidectomy for the treatment of primary hyperparathyroidism (pHPT). A total of 36 papers were identified using the reported searches of which 5 represented the best evidence to answer the clinical question. The authors, date, journal, study type, population, main outcome measures and results are tabulated. Targeted parathyroidectomy constitutes the co-gold-standard procedure for pHPT with results equivalent to bilateral cervical exploration. This has led to the proliferation of minimally invasive parathyroidectomy (MIP) techniques for pre-operatively localised adenomas. None has been shown to be overwhelmingly superior. RP constitutes the most recent addition. RP overcomes the limitations of conventional endoscopic surgery and simultaneously avoids a neck scar by concealing it in the axilla or infraclavicular area. The evidence from the present review shows that RP is feasible and leads to a superior cosmetic result compared to targeted open parathyroidectomy (TOP) with an equivalent safety profile. As with every surgical technique, appropriate patient selection is crucial. Long-term data are currently awaited on RP especially in view of its high cost and long operative time compared to TOP and other MIP techniques. Hence, RP offers a viable but costly alternative to other forms of MIP in patients where even the smallest and most cosmetic neck scar is not an option (Garas G, Holsinger FC, Grant DG, Athanasiou T, Arora A, Tolley N. Is robotic parathyroidectomy a feasible and safe alternative to targeted open parathyroidectomy for the treatment of primary hyperparathyroidism? *Int J Surg* 2015 Jan 29;15C:55-60. doi: 10.1016/j.ijsu. 2015.01.019. [Epub ahead of print])

Radu Dănilă

THROMBOTIC EVENTS IN PATIENTS WITH HEPATITIS C VIRUS LIVER CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRALS AND SUSTAINED VIROLOGICAL RESPONSE

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THROMBOTIC EVENTS IN PATIENTS WITH HEPATITIS C VIRUS LIVER CIRRHOSIS TREATED WITH DIRECT ACTING ANTIVIRALS AND SUSTAINED VIROLOGICAL RESPONSE (Abstract): The advent of direct-acting antivirals (DAAs) is a major breakthrough in hepatology representing the therapeutical standard of care in patients with chronic hepatitis C virus infection over the past few years. Despite high rates of sustained virological response (SVR), DAAs therapy doesn't eliminate the risk of thrombotic events. **Aim**: We aimed to assess the incidence and risk factors of thrombotic events in patients with SVR treated with DAAs. **Material and methods:** We retrospectively analyzed a cohort of patients with HCV-related liver cirrhosis treated with paritaprevir/ritonavir, ombitasvir and dasabuvir (PrOD) ± ribavirin and ledipasvir/sofosbuvir (LED/SOF) ± ribavirin for 12/24 weeks, in a tertiary gastroenterology referral center from North-Eastern Romania, between January 1st 2016 and January 1st 2018. All patients with presumption of thrombosis were evaluated by vascular Doppler, abdominal ultrasound and confirmed by CT scan. **Results:** The study included 321 HCV-infected cirrhotic patients treated with PrOD or LED/SOF, with documented SVR, mean age 59,7 ± 8,5 years, predominantly female (59%). Of the total number, 307 (95.63%) received PrOD and 14 (4.36%) patients were treated with LED/SOF. Mean period from SVR and the occurrence of thrombotic events was 250±123 days. Thrombotic complications were reported in **14** (4.36%) patients: 4 (28.57%) with deep vein thrombosis, 4 (28.57%) with portal vein thrombosis (PVT), 6 (42.85%) with malignant PVT. All patients had associated cardiovascular (8- 57.14%) and metabolic comorbidities (6- 42.85%). The main clinical manifestations at diagnosis were: swelling, edema, erythema and lower limb pain in 4 patients, upper digestive hemorrhage in 2 patients, ascitic decompensation in 4 patient and 4 patients were asymptomatic. Biologically there was no significant change in prothrombin serum levels (baseline values in patients treated with PrOD was 11.67 ± 0.91 versus 11.70 ± 0.83 at SVR, p=0.993, respectively 11.5 ± 0.84 sec at baseline versus 11.4 ± 0.68 at SVR, p=0.715 in patients treated with LED/SOF+RBV) and platelet count (126 000 (101 500-162 000) vs. 131 000 (101 000-165 000), p= 0.818 in patients treated with PrOD, respectively 94857.14 ± 32 vs. 92428.57 ± 35, p= 0.853, in patients treated with LED/SOF+RBV). **Conclusions**: We conclude that thrombotic events in patients with HCV-related liver cirrhosis treated with DAAs are not influenced by the variations of coagulation parameters, rather correspond to the hypercoagulability status of the cirrhotic patient. **Keywords**: DIRECT ANTIVIRALS, SUSTAINED VIROLOGIC RESPONSE, THROMBOSIS.

Hepatitis C virus infection (HCV) described by Choo in 1989, represents an important public health problem and according to WHO, there are about 71 million people worldwide with HCV chronic infection (1).

HCV- related liver cirrhosis is a pathology commonly encountered in the current practice, which is associated with increased rates of morbidity and mortality. The complications of liver cirrhosis frequently occur in the evolution of the disease, with a negative impact on the prognosis of patients.

The last years have brought a change regarding the condition of hemostasis in cirrhotic patients. The concept according to which the cirrhotic patient is predisposed to hemorrhagic complications and protected against thrombotic events has been redefined in recent years (2). Studies in this field have shown an equilibrium between the procoagulant and the anticoagulant factors which cause the cirrhotic patient to be in the middle of a very sensitive balance, that can lean the plates towards bleeding or thrombosis (3,4).

The changes that determine a hypercoagulability status in liver cirrhosis are represented by a decrease synthesis of the anticoagulant factors: protein C, protein S and antithrombin III and an increase of von Willebrand factor and VIII factor (5), which may explain the relatively high incidence of thrombotic events among these patients, the most commonly reported being portal thrombosis (PVT) and deep vein thrombosis (DVT) (6).

Direct-acting antivirals (DAAs) have radically changed the management of chronic HCV infection over the past 3 years, with a safety profile and high rates of SVR (>95%). The DAAs mechanism of action directly targets viral protease, polymerase and nonstructural proteins, thus allowing for a high rate of viral clearance without significant side effects (7).

Recent studies have shown the positive impact on liver function, as measured by the CHILD-PUGH and MELD score improvement, as well as the decompensation rate after obtaining sustained virological response with the new oral direct-acting antivirals, but there are no conclusive data regarding the effect on clotting disorders from liver cirrhosis (8,9). In our study, we aimed to assess the incidence and risk factors of thrombotic events in patients with SVR treated with DAAs.

MATERIAL AND METHODS

This is a retrospective study in which we included patients with HCV-related liver cirrhosis which fulfilled the criteria for antiviral therapy, in a tertiary gastroenterology referral center from North-Eastern Romania, between January 1st 2016 and January 1st 2018. The inclusion and exclusion criteria were according to our National Protocol. The regimens used were: Paritaprevir/Ritonavir, Ombitasvir and Dasabuvir (PrOD) ± Ribavirin in patients with HCV-related compensated liver cirrhosis (LC) and Ledipasvir/Sofosbuvir (LED/SOF) + Ribavirin (RBV) for 12 weeks, in patients with decompensated LC. Diagnosis of cirrhosis was established based on clinical features, blood tests and imaging methods.

The study protocol was approved by the Ethical Research Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi and by the Ethical Committee of the "Sf. Spiridon" County Clinical Emergency Hospital. All patients were required to sign an informed consent.

All patients with presumption of thrombosis were evaluated by imaging methods and blood samples. Blood tests were performed at baseline, end of treatment (EOT) and 12/24 weeks after EOT (sustained virological response- SVR12). The imaging tests were: vascular Doppler (SONOTRAX 4, vein ultrasound 4 MHz), abdominal ultrasound (Philips HD 11xe ultrasound system) and computer tomography (Siemens Somatom Emotion 16). Echographically, thrombosis was defined as the presence of a hyperechogenic material within the vascular lumen.

Data collected was statistically analyzed using SPSS 20.0 (Chicago, IL, SUA). All tests were two-tailed with p-value <0.05 was considered statistically significant. The quantitative variables were compared using the t-Student test, and the qualitative parameters were evaluated by the chi-square test.

RESULTS

The study included 321 HCV-infected cirrhotic patients treated with PrOD or LED/SOF, between January 1st, 2016 and January 1st 2018, with documented SVR, mean age 59,7 ± 8,5 years, predominantly female (59%). Of the total number, 307 (95.63%) received PrOD and 14 (4.36%) patients were treated with LED/SOF+RBV. Child-Pugh score at baseline was 7.64 ± 1 in the LED/SOF group versus 5.06 ±0.24 points. Main demographic characteristics are presented in table I.

TABLE I

Demographic characteristics at baseline

Demographic characteristics at baseline	All (n=321)
Females, n(%)	190 (59%)
Age (mean ± SD)	59,7 ± 8,5
Treatment Regimen	
PrOD, n(%)	307 (95.63%)
LED/SOF+RBV, n(%)	14 (4.37%)
CHILD-PUGH Score	
PrOD (mean ± SD)	5.06 ± 0.24
LED/SOF+RBV(mean ± SD)	7.64 ± 1

All patients had SVR documented by undetectable HCV-RNA. In patients treated with PrOD, GGT, AST and ALT had significant decreased values at SVR12 compared to baseline (p<0.001). Also, in the LED/SOF+RBV group, transaminases levels had an improving trend (p<0.001) (tab. 2 and 3).

Mean period from SVR and the occurrence of thrombotic events was 250±123 days. Thrombotic complications were reported in 14 (4.36%) patients: 4 (28.57%) with deep vein thrombosis (DVT), 4 (28.57%) with portal vein thrombosis (PVT), 6 (42.85%) with malignant PVT (fig. 1). All patients had associated cardiovascular (8- 57.14%) and metabolic comorbidities (6- 42.85%). The main clinical manifestations at diagnosis were: swelling, edema, erythema and lower limb pain in 4 patients, upper digestive hemorrhage in 2 patients, ascitic decompensation in 4 patients and 4 patients were asymptomatic (fig. 2).

TABLE II

Dynamic of biochemical parameters in patients treated with PrOD at baseline *vs.* SVR12

Parameters	Baseline	SVR12	P
PLT (/mm^3), median (IQR)	126 000 (101 500-162 000)	131 000(101 000-165 000)	0.818
INR, median (IQR)	1.1 (1.06-1.2)	1.19 (1.12-1.21)	0.002
TP (sec)	11.67 ± 0.91	11.70 ± 0.83	0.993
AST (U/L), median (IQR)	80 (55.5-120)	24 (21-32)	<0.001
ALT (U/L), median (IQR)	85 (62-119.5)	23 (20-33)	<0.001
GGT (U/L), median (IQR)	66 (40-108)	35 (23-55)	<0.001
Bilirubin (mg/dL), median (IQR)	0.93(0.77-1.38)	0.89 (0.61-1.1)	0.038
AFP(ng/mL), median (IQR)	10(5.62-15.6)	10.1(6.3-15.7)	0.871
Creatinine (mg/dL) (mean ± SD)	0.76 ± 0.1	0.83 ± 1.67	0.004
Hb (g/dL)	14.27 ± 1.54	13.48 ± 1.59	0.002
WBC (/mm^3)	5474.17 ± 1616.83	5800.56 ± 1661.76	0.222
Neutrofile (/mm^3)	3124.64 ± 1155.25	3034.15 ± 1035.82	0.624
ALK (U/L)	89.33 ± 29.39	95.88 ± 39.78	0.258
Albumin (g/dL)	4.06 ± 0.43	4.01 ± 0.29	0.557
MELD	7.8 ± 1.79	7.39 ± 1.82	0.192

TABLE III.

Dynamic of biochemical parameters in patients treated with LED/SOF at baseline *vs.* SVR12

Parameters	Baseline	SVR12	P
PLT (mm^3), (mean ± SD)	94857.14 ± 32	92428.57 ± 35	0.853
INR, (mean ± SD)	1.28 ± 0.12	1.20 ± 0.08	0.05
TP (mean ± SD)	11.5 ± 0.84	11.4 ± 0.68	0.715
ALT (U/L), (mean ± SD)	86 ± 49.72	24.07 ± 13.33	< 0.001
AST (U/L), (mean ± SD)	76 ± 32.45	25 ± 18.29	< 0.001
GGT (U/L), (mean ± SD)	43.64 ± 19.66	33.71 ± 15.34	0.148
BT (MG/dL), (mean ± SD)	2.05 ± 0.66	1.50 ± 0.71	0.044
AFP (ng/mL) median (IQR)	9.43 (7.95-17.77)	6 (3.27-9.92)	0.047
Creatinina (mg/dL) (mean ± SD)	0.7 ± 0.22	0.73 ± 0.15	0.626
Hb (g/dL), (mean ± SD)	12.82 ± 1.51	13.17 ± 1.50	0.544
WBC (/mm^3), (mean ± SD)	4471.42 ± 1083.70	4785.71 ± 1377.59	0.508
Neutrofile (/mm^3), (mean ± SD)	2440.28 ± 737	3051.42 ± 1222.57	0.124
ALK (U/L), (mean ± SD)	102.5 ± 32.9	107.42 ± 46.21	0.748
Albumin (g/L), (mean ± SD)	3.30 ± 0.52	3.71 ± 0.39	0.026
MELD (mean ± SD)	12.46 ± 3.07	9.5 ± 3.32	0.024

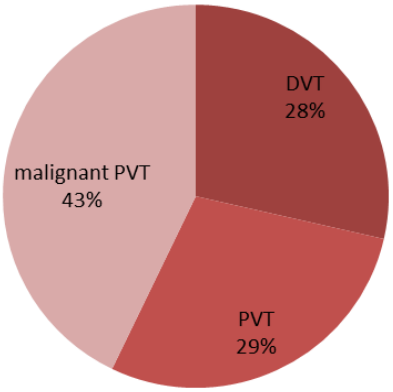

Fig. 1. Thrombotic events after SVR12 PVT= portal vein thrombosis, DVT= deep vein thrombosis, malignant PVT= malignant portal thrombosis

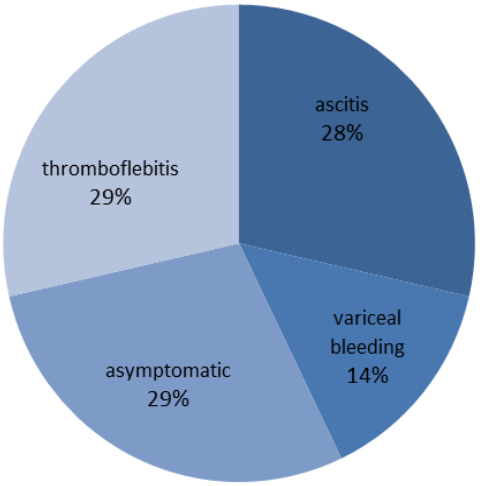

Fig. 2. Clinical manifestations at diagnosis

There was no significant change in prothrombin serum levels in both groups: baseline values in patients treated with PrOD was 11.67 ± 0.91 versus 11.70 ± 0.83 at SVR, p=0.993, respectively 11.5 ± 0.84 sec at baseline versus 11.4 ± 0.68 at SVR, p=0.715 in patients treated with LED/SOF+RBV (fig. 3).

Platelet count had no significant changes at baseline versus SVR in both groups (126 000 (101 500-162 000) vs. 131 000 (101 000-165 000), p= 0.818 in patients treated with PrOD, respectively 94857.14 ± 32 vs. 92428.57 ± 35, p= 0.853, in patients treated with LED/SOF+RBV) (fig. 4).

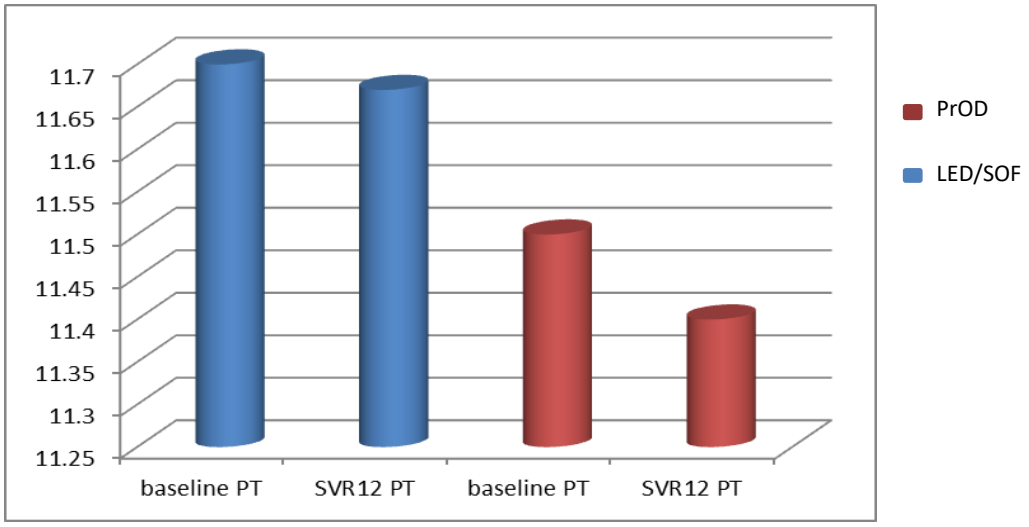

Fig. 3. Prothrombin serum levels at baseline and SVR12

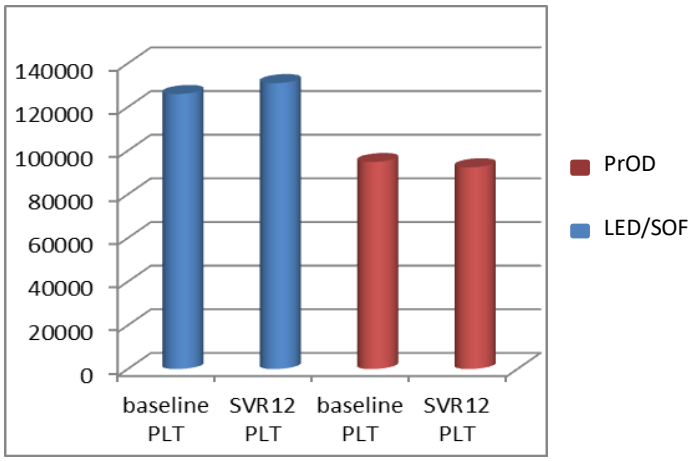

Fig.4. Platelets count at baseline and SVR12

DISCUSSION

The available data from the last few years showed that there is an imbalance concerning the hemostasis status in cirrhotic patients which are nowadays considered to be predisposed to thrombotic events; recent studies have demonstrated a disequilibrium between pro- and anticoagulants factors in cirrhotic patients.

The last few years brought an important change in the therapeutic management of chronic HCV infection. The advent of DAAs represents a breakthrough in hepatology, with a safe profile and high rates of SVR (>97%) (10). Available data shows an improvement in liver function after achieving SVR with DAAs in cirrhotic patients assessed by decreased MELD and CHILD-PUGH scores after 12 weeks from end of treatment EOT (8,9). There are very few studies regarding the coagulation status of cirrhotic patients with SVR treated with DAAs. In our study we aimed to assess the incidence and risk factors of thrombotic events in patients with SVR treated with DAAs.

Most biochemical parameters showed an improvement on SVR12 compared to baseline. Transaminases and GGT had significantly decreased values in both groups of patients, revealing the disappearance of liver injury due to the viral clearance. Also, we observed a decreasing trend for MELD score, showing an improvement in global liver function.

There was a slight improvement of platelet count after SVR in patients treated with PrOD, but not significantly statistic. We didn't find any improvement in platelet count at SVR compared to baseline in patients treated with LED/SOF+RBV, but on the contrary we identified a slight decrease in platelets that can be explained by splenic sequestration and exaggerated destruction, representing a characteristic status in patients with advanced liver disease. Studies that used radiolabeled platelets have shown a low survival to which is added the low level of thrombopoietin, determined by a decreased synthesis in liver cirrhosis (11). Tripodi et al. did not found an appreciable increase in platelet count in their study lot, incriminating the short period of follow-up and the fact that viral eradication and im-

provement of liver function does not change the splenic pool (12). None of the currently used coagulation parameters in cirrhotic patients (PT, INR) had a notifiable statistic change. This finding confirms the idea that the procoagulant status in cirrhotic patients cannot be reflected only by these laboratory parameters.

Our study showed an incidence of 4.36% of thrombotic events in patients with HCV related liver cirrhosis treated with DAAs.

According to recent studies, the incidence of DVT in cirrhotic patients ranges from 0.5% to 8%, depending on the patient's comorbidities (13). We found in our cohort an incidence rate of 1.24% patients with DVT. All patients which developed DVT associated chronic venous insufficiency. None of these patients had previously been treated for prophylactic purposes with anticoagulants or mechanic methods.

PVT represents the most frequent thrombotic event in cirrhotic patients, the most commonly reported etiology being the HCV infection (14). In our study the incidence of non-malignant PVT was 1.24%, like the findings of Luca et al. which showed an incidence of 0.5%-26% in a cohort of cirrhotic patients (15).

Risk factors that were correlated with PVT were the presence of decompensated liver cirrhosis, ascites and age >50 years. If the first two risk factors are the expression of the severity of the liver disease, age above 50 years associates a cumulus of factors such as the presence of comorbidities and the increasing severity grade of the liver disease.

In our study, the incidence of malignant PVT associated with hepatocellular carcinoma occurred in 43% of patients with thrombotic events, these data being consistent with the literature (16, 17).

CONCLUSIONS

Thrombotic events in patients with HCV-related liver cirrhosis treated with DAAs are not influenced by the variations of coagulation parameters, rather correspond to the hypercoagulability status of the cirrhotic patient. The incidence of thrombotic events in cirrhotic was similar to that reported in specialty literature, remaining to be further demonstrated if the mechanism of DAAs can influence the development of thrombotic events in this type of patients.

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NEWS

CDH17 IS A MORE SENSITIVE MARKER FOR DIGESTIVE ADENOCARCINOMA

CDH17 or liver-intestine cadherin, is a cell-adhesion molecule specific to intestinal epithelium, regulated by CDX2 and is involved in the invasion and metastasis process of tumors. A standard panel of CK 7, CK 20, and CDX2 antibodies is usually used in detection of adenocarcinomas of digestive tract. A study compared the usefulness of CDH17 as an immunohistochemical intestinal marker, by evaluating it's sensitivity and specificity in contrast with CK 20 and CDX2. The assessment was done by using immunohistochemistry on formalin-fixed, paraffin-embedded tissue microarrays (TMAs) including normal (n=26) and tumoral tissue (n=884) of colon, stomach and esophagus. CDH17 proved to be a more sensitive and specific marker then CK 20 and CDX2 in diagnose of primary tumors by up to 10% and in metastasis by up to 40%. (Altree-Tacha D, Tyrrell J, Haas T. CDH17 is a more sensitive marker for gastric adenocarcinoma than CK20 and CDX2. *Arch Pathol Lab Med* 2017; 141(1): 144-150).

Victor Ianole

JOD-BASEDOW EFFECT DUE TO PROLONGED USE OF LUGOL SOLUTION-CASE REPORT

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JOD-BASEDOW EFFECT DUE TO PROLONGED USE OF LUGOL SOLUTION-CASE REPORT (Abstract): Graves' disease is the most common form of hyperthyroidism, accounting for 60-80% of all cases of thyrotoxicosis. If left untreated, it may lead to severe thyrotoxicosis with cardiovascular, ocular, psychiatric complication, and in extreme cases thyrotoxic crisis with a high mortality rate. We present the case of a 50-years-old woman diagnosed in another service with Graves' disease and treated for many years with antithyroid drugs (ATDs), admitted to our service for a relapse due to treatment discontinuation. The surgical treatment was planned and the preoperative preparation with Lugol solution was initiated. Due to a misunderstanding, the administration of iodine solution was extended for a period of about 30 days, thus generating the so-called Jod-Basedow effect, with the exacerbation of the manifestations of thyrotoxicosis and risk of thyroid storm. The patient received treatment with high ATDs doses, glucocorticoids, and beta-blockers, resulting in the progressive improvement of symptoms. She was discharged from hospital and given the risk of thyrotoxic crisis the surgery was postponed. After a month, the patient underwent thyroidectomy without preoperative preparation with iodine solution. The operative and postoperative courses were uneventful. **Keywords:** GRAVES' DISEASE, SURGICAL TREATMENT, LUGOL SOLUTION, THYROTOXIC CRISIS

Graves' disease is the most common form of hyperthyroidism, accounting for 60-80% of all cases of thyrotoxicosis. Its prevalence is estimated to be about 0.6% of population, with a female-to-male ratio of 7:8/1. The disease mainly affects young women, aged 30-60 years (1, 2).

It is an autoimmune disorder due to thyroid receptor autoantibodies (TRAb), also named thyroid-stimulated immunoglobulins (TSI), which stimulate growth of the thyroid gland and overproduction of thyroid hormones. The main clinical manifestations are hyperthyroidism, diffuse goiter, ocular signs, and more rarely dermopathy and acropachy (3). If left untreated it may lead to severe thyrotoxicosis with cardiovascular, ocular, psychiatric complication, and in extreme cases thyrotoxic crisis, with a high mortality rate of about 20% even in our days (4).

The optimal therapy for Graves' disease is still a subject of debate. The main treatment options are represented by antithyroid drugs (ATDs) and ablation therapy: radioactive iodine (RAI) or surgery (5). The used ATDs are methimazole, carbimazole, and propylthiouracil. They are acting by blocking thyroid hormone synthesis and may also control hyperthyroidism by immune suppression (6). A major disadvantage of ATDs treatment, which has traditionally been the first-line treatment in Europe, is the high risk of relapse after discontinuation (50-80% of patients) (5, 7). Surgery is an alternative therapeutic option for Graves' disease in many circumstances. Total thyroidectomy has negligible recurrence rates and high cure rates (8, 9, 10). Preoperative preparation with iodine is used by most surgeons in order to prevent thyrotoxic crisis and complications during thyroidectomy (11, 12).

CASE REPORT

We present the case of a 50-years-old woman, living in an urban area, nonsmoker, diagnosed in 2006 in another service with Graves' disease. She was treated with variable doses of methimazole. The patient was admitted to our service in January 2014 for agitation, sinus tachycardia, significant weight loss, and diarrhea. The patient discontinued the treatment with ADTs for 10 months without consulting her physician. Hormone profile revealed severe thyrotoxicosis: TSH < 0.004 μUI/ml, FT4 = 4.88 ng/dl, FT3 = 21.1 pg/ml, positive antithyroid antibodies (AAT) and TRAb. Thyroid ultrasound: increased thyroid volume (40 ml), intense hypoechoic appearance “hell-like" vascularization. ECG: sinus tachycardia. Given the long course of Graves' disease, high thyroid volume and high TRAb levels, surgery was recommended. The preoperative preparation with Lugol solution 20 drops x 3/day for 9 days was initiated. Due to intercurrent respiratory infection surgery was delayed, but without discontinuing the administration of Lugol solution; the patient continued the administration of iodine solution at home for 30 more days. Readmitted for surgical treatment, the patient developed more severe symptoms of thyrotoxicosis (pulse = 140/min, intense sweating, agitation), the surgery being once again delayed, and the patient transferred to the Endocrinology Department. Clinical and laboratory assessments confirmed the diagnosis of Graves' disease; thyroid scan demonstrated the characteristic features of Jod-Basedow effect (large thyroid, highly increased thyroid uptake). Cardiac assessment: hyperkinetic heart, without signs of ventricular dysfunctions (fig. 1, 2).

The patient received treatment with high methimazole doses (Thyrozol 60mg/day), glucocorticoids (Prednisone 30 mg/day) and beta-blockers (Propranolol 40 mg x 2/day). Due to leukopenia (white blood cell count to 2730/μl) with neutropenia (neutrophil count to 920/ μl) methimazole administration was temporary discontinued and continued the administration of corticosteroids with gradual return to normal of leukocyte formula that allowed resumption of high ATDs doses. The clinical signs had progressively improved and the patient was discharged after 14 days. The surgical treatment was once again postponed, given the risk of thyrotoxic crisis. The patient was ambulatory monitored every 2 weeks. In April 2014 thyroidectomy was performed without preoperative preparation with iodine solution. The operative and postoperative courses were

uneventful. The patient developed postoperative hypothyroidism, and currently she is under thyroid replacement therapy. Histopathology confirmed the Graves' disease.

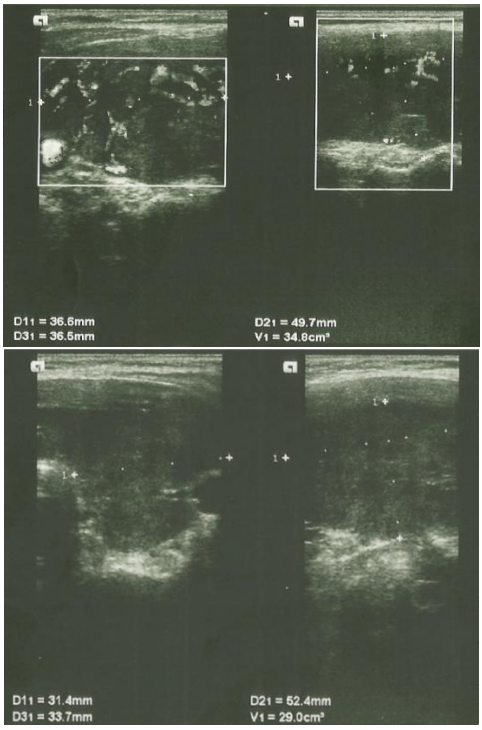

Fig. 1. Thyroid ultrasound showing an increased thyroid volume (63 ml), intense hypoechoic appearance, "hell-like" vascularization

Fig. 2. Thyroid scan: characteristic features of Jod-Basedow effect, large thyroid and highly increased thyroid uptake

DISCUSSION

In patients with Graves' disease treated for many years with ATDs, with an increased number of relapses, large goiter and high TRAb level, surgery may be a safe therapeutic option. Also, surgery provides rapid treatment and permanent cure of hyperthyroidism in most patients (8). Preoperative preparation of patients with hyperthyroidism to achieve euthyroid state is extremely important for avoiding perioperative complications due to severe thyrotoxicosis (13). This can be done using a combination of ATDs, beta-blockers and sometimes glucocorticoids (11). Thyroid storm is an exaggerated state of thyrotoxicosis that occurs in patients who have an inadequately treated thyrotoxicosis and a precipitating event such as thyroid surgery, with a mortality rate that has decreased over time from nearly 100% to approximately 20% in our days (4). Most surgeons administer iodine (as Lugol solution) to provide ≥ 30 mg of iodine/day for 10 days before surgery to decrease thyroid vascularity, the rate of blood flow, and intraoperative blood loss during thyroidectomy (12). These actions are explained by the Wolff-Chaikoff effect of excess iodine, an autoregulatory phenomenon that directly inhibits thyroid hormone synthesis and release. This effect lasts around 10 days, after which it is followed by an ”escape phenomenon”, with resumption of normal organification of iodine and normal thyroid function (14, 15).

In our patient, the preoperative preparation with Lugol solution was extended for a period of about 30 days (due to a misunderstanding), thus providing excessive amounts of iodine to the thyroid gland for a long period of time, and generating the so-called Jod-Basedow effect, with the exacerbation of thyrotoxicosis and risk of thyroid storm.

This condition has a steep onset with fever, marked tachycardia, and arrhythmias, which may be accompanied by pulmonary edema, congestive heart failure, gastrointestinal dysfunction, and central nervous system involvement (16,17). Knowledge of these aspects and careful monitoring of the patient are extremely important because the diagnosis is mostly clinical, the point at which thyrotoxicosis becomes thyroid storm being subjective (17). Even though some authors have proposed a clinical score to confirm the diagnosis, in current practice patients presenting with significant symptoms of thyrotoxicosis should be considered as being at risk for thyroid storm and undergo aggressive treatment, rather than waiting for a specific score threshold (17, 18). In our case, the patient presented an impaired general condition with aggravation of the cardiovascular, digestive and mental manifestations of thyrotoxicosis, thus prompt treatment with high doses of ATDs, beta-blockers, glucocorticoids and sedatives was initiated in an attempt to block the synthesis and peripheral conversion of thyroid hormones, and to control the clinical symptoms.

Besides minor adverse effects such as pruritus, skin rash, arthralgias, and slight elevations in liver enzymes levels, ATDs may rarely cause an aggravation of mild leukopenia that is common in Grave's disease, with risk of agranulocytosis (granulocyte count below 500/mm^3). Agranulocytosis may occur even in patients who previously tolerated ATDs, and multiple exposures may represent a risk factor for developing hematological disturbances (19, 20). This situation was met in our patient, who presented under methimazole a worsening of initial leukopenia, with granulocytopenia and risk of agranulocytosis, which forced us to temporarily discontinue the administration of ATDs, and continue only with glucocorticoids, which gradually normalized the absolute neutrophil count. Normalization of white blood count allowed us to resume the treatment with high doses of methimazole, in order to obtain a more rapid improvement of symptoms, by blocking the synthesis of thyroid hormones.

The clinical symptoms and hormone parameters gradually improved, and the patient was discharged after 14 days of hospital stay.

It is important to know that in thyrotoxicosis ATDs are blocking the thyroid hormone synthesis, not the release of preformed thyroid hormones. Euthyroidism is obtained only when intrathyroidal hormone and iodine stores are depleted, and this process requires up to 6 weeks. Large goiters with abundant deposits of thyroid hormone, especially when iodine excess is present, often show a delayed response to thioanmides (19, 21). In these circumstances, knowing the history of massive thyroid iodine load, confirmed by the increased scintigraphic uptake and the ultrasonographic aspect of the thyroid gland with high volume and intense vascularization, the surgical intervention was postponed again, due to the high risk of thyrotoxic crisis.

CONCLUSIONS

Our case highlights that although preoperative preparation with iodine solution is preferred by most surgeons in order to prevent complications during thyroidectomy, this treatment must be performed in hospital under close supervision by medical personnel, in order to avoid such complications like Jod-Basedow phenomenon and thyroid storm. Thus, surgery remains a safe therapeutic option for Graves' disease in many circumstances.

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PHYSIOPATHOLOGICAL AND THERAPEUTICAL CORRELATIONS IN ALCOHOL DEPENDENCE

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PHYSIOPATHOLOGICAL AND THERAPEUTICAL CORRELATIONS IN ALCOHOL DEPENDENCE (Abstract) No doubt, alcoholism represents nowadays the toxicomany with the highest expansion rate among all population groups, being recognized by the specialists from the medical, social, economic and legal field as a true "toxic pandemy". Researchers consider ethanol, this small but highly aggressive molecule, to have supremacy if we were to consider the number of pages dedicated to it worldwide on daily bases, in the medical or any other specialty literature. Nonetheless, the large volume of data regarding ethanol toxicity does not seen to simplify things, on the contrary it points out new information about the its negative effects on human body. Ethanol represents a toxic that is rapidly and completely absorbed in the intestinal tract being distributed to most tissues and organs; ethanol is recognized as an enzymatic inductor of its own metabolization but also of the metabolization of numerous therapeutic agents. **Keywords:** DEPENDENCE, ALCOHOL, TOXICOMANY, WITHDRAWAL, BEHAVIORAL CHANGES

The biological mechanism of alcoholism are still little known reason why it relies especially on hypotheses concerning, mainly, the disruption of neurotransmission after the action of ethanol on cellular membranes and neurotransmitters. The organism's sensitivity to this action seems to be, to a great extent, determined by genetic factors but, still, behavioral dependence represents the base of alcoholic toxicomany. Its origins indicate an organism predisposed to the consumption of alcohol combined with the availability of alcohol, their good social tolerance and low costs.

Physiopathological factors of physical dependence on alcohol

In general, it is considered to be three key factors involved in the complex phenomenon of the physical dependence on alcohol: intracellular metabolism; biological membranes and neurotransmitters which have constant interactions, especially at the level of neuronal transmission.

The effect of ethanol on intracellular metabolism. The main consequence of the use of alcohol is the considerable increase of the amount of H^+ ions in the cells and the increase of the NADH / NAD relation which regulates the intracellular oxido-reduction potential. The result is the alteration of oxygen consumption and of the activity of mitochondria and of the numerous enzymes that intervene in various metabolic processes. For counteracting these effects, progressive metabolic adjustments

appear: increased degradation of ethanol, enzymatic qualitative and quantitative (metabolic tolerance) adjustments (1).

During withdrawal, the fact that the NAHD / NAD relationship decreases brutally disturbs the cellular functioning, mainly that of the nervous cells, case that might influence the beginning of the withdrawal syndrome. Consequently, it is necessary to increase the basis for the brain protein synthesis during chronic alcoholism.

The effect of ethanol on biological membranes. Biological membranes are functionally active entities due to the molecular intra-membranous components. They regulate transmembranous exchanges and also intervene in certain intracellular metabolisms.

The membranous matrix consists of two layers of phospholipids to which numerous molecules are associated: proteins (hormonal receptors, enzymatic systems as the Na+ -K+ ATP-asis dependent, membranous transport systems) or glycoprotein (with role in cellular recognition, immune response) (1). For this system to work it is necessary:

- The physical condition of the membrane to be normal which is related to the lipids in the membrane and its mobility and it can be assessed by measuring the membrane fluidity;

- The membrane's biochemical condition to be normal, which is the distribution, the spatial structure and the activity of the membrane proteins is satisfactory as well as the lipid-protein interaction.

Modifications of the membrane's physical state

Nowadays it is known that a certain number of substances as detergents and general anaesthetics, including certain alcohols, have physical – chemical effects on the membranes.

Ethanol molecules are liposoluble and they can position themselves between the two layers of membranous phospholipids, conducting to the disorganization of the lipid matrix and the modification of the lipids lateral mobility and increase of membrane fluidity (1).

This effect depends of the dose, the same phenomenon being possible to appear *in vivo* conditions as well, disrupting the fluidity or the optimal micro-viscosity which is essential to the well functioning of the cells. In case of chronic intoxication there is the possibility of cell adjustment which manifests itself as tolerance to alcohol, with insufficiently identified mechanisms (2). Cells seem to be capable to increase the incorporation of saturated fatty acids and to diminish the ratio of unsaturated fatty acids. Another possibility would be the increase in cholesterol content at the level of the membrane, transmethylation, modification of protein synthesis etc. The result consists in lipid rearrangement which strengthens the structure and increases the stiffness of the membrane which diminishes penetration and the fluidizing action of ethanol (just like of all liposoluble molecules, which partly explains the well known resistance of alcoholic patients to general anaesthetics).

During withdrawal, the break of the new biological balance due to the disappearance of the fluidizing effect of ethanol will be responsible for the apparition of the temporary hyper viscosity of the membrane which will disrupt its functioning until the physiological structure is re-established.

Modifications of the membrane's biochemical state

Ethanol is also capable of modifying the

functioning of membrane proteins (enzymes, transport proteins, receptors, surface glycoproteins) but it is important to point out that their answer depends to a great extent of: the proteins involved (for ex. Ca^{2+} dependent ATP-ase seems a lot more sensitive to the action of alcohol than Na^{+} - K^{+} ATP-ase dependent; protein localization, the kinetic features of the receptors seem to vary depending of the cerebral area and the genetic factors which have an important role in adjusting and establishing the sensitivity to effects of ethanol.

Enzymes. Until now there are few studies dealing with the alteration of *biochemical membrane processes* under the action of ethanol. Na^{+} - K^{+} ATP-ase dependent is the most studied enzyme as it intervenes in the active transport of sodium and potassium ions and maintaining their transmembranous gradient (1, 2).

In vitro studies have shown that after the administration of large doses of ethanol this enzyme is inhibited while after the administration of small doses of ethanol it becomes active.

This effect can explain the behavioural modifications induced by ethanol and it can be caused by the disruption of protein microenvironment consequent to phospholipids alteration accompanied by the modification of both spatial configuration and their activity (3).

Calcium, with a key role in membranous stability, in controlling intracellular signals and so in neurotransmission, is also disrupted by ethanol.

In case of chronic intoxication there is an increase of the calcium content in the synaptosomal membranes which seems to be the consequence of long term adjustment to ethanol effects.

Receptors, membrane proteins with particular specificity and affinity features for certain molecules can act at membrane level by coupling with an ionic channel or / and through intracellular syntheses, especially enzymatic, acting on the protein synthesis or on certain metabolisms.

The most famous system is the adenyl cyclase functioning unit – AMP cyclic protein kinase which intervenes mainly in the transmission of catecholaminergic signals. Ethanol disrupts the activity of this complex system either by modifying the physical structure of the surrounding environment of proteins, or by acting at the level of the regulating systems: calcium or/and calmodulin (calcium dependent activation protein). During the beginning of ethanol dependence it seems to be an increase in the sensitivity of the receptors at catecholamines. This can be due to the adjustment of the postsynaptic receptors to the activity of the presynaptic systems and to the effects of ethanol at membranous level (1, 3).

During withdrawal it was experimentally demonstrated the diminished answer of adenyl cyclase to the stimulation with dopamine. This decrease of sensitivity can also be pointed out at adrenergic ß receptors. This effect is inconsistent and depends of the brain area taken into consideration: present in the striatulum, it is absent at mesolimbic level because of the different characteristics receptors have in these two regions (4). The effect disappears if the laboratory animals are given sufficient amount of alcohol, which demonstrates that this system has become dependent on alcohol.

Another factor involved in the development of physical dependence on alcohol is represented by *neurotransmitters*, *acetylcholine* being the most widespread in the

central nervous system, it is heterogeneously distributed and acts on the muscarinic and nicotinic receptors (1, 4).

Ethanol definitely affects the cholinergic transmission; at very high concentrations of alcohol there will be an increased activity of acetylcholine transferase (which intervenes in the synthesis of acetylcholine) while the activity of acetylcholinesterase (degradation) is diminished. The release of acetylcholine is also disrupted (1, 5).

We must underline that that cholinergic system, especially at the level of the striated system is controlled through the D2 receptor of dopamine, the receptors can be affected by alcohol so that the muscalinic type cholinergic receptors undergo certain modifications during alcohol intoxication (2, 5).

The sensitivity of acetylcholine stands for the adapted answer to the effect of ethanol, but it is difficult to establish the eventual role of the cholinergic system, of the parasympathetic one in particular, during the withdrawal syndrome.

Noradrenaline acts through two families of postsynaptic ß receptors (ß1 and ß2) which connect to adenyl cyclase and α, presynaptic (α_1) and postsynaptic (α_2) which are not connected to adenyl cyclase. Presynaptic receptors in association with neuromodulators (dopamine, GABA, endogenous opioids) regulate the synthesis and the release of noradrenalin. The role of this extraordinarily complex noradrenergic system consists in the modulation of the information arriving at the level of the projection systems.

The action of alcohol on noradrenalin and dopamine has been intensely studied but the results are highly contradictory. It appears that the acute administration of ethanol increases the urinary secretion of catecholamine stimulating thus their release at the level of the suprarenal (stress effect). Some experiments demonstrate that *in vitro*, ethanol diminishes synaptic release, reuptake and the renewal process of catecholamines. Some authors have demonstrated the biphasic structure of this effect: increased release and renewal process in a first phase followed by decrease (according to the behavioural effects of ethanol) (2, 4).

The studies conducted during chronic intoxications are more conclusive. They all underline the increasing renewal percent of noradrenalin and dopamine, as well as their synthesis by stimulation of tyrosine hydroxylase. Their release will be just as intense. All these elements certify the increased functional activity of the neurons which can be due to their adjustment to the depressive effects of ethanol.

Serotonin acts through two types of receptors, 5HT1 and 5HT2, with different locations and with different pharmaceutical features. Even if modifications appear, in particular a decrease of brain serotonin that has been invoked in the apparition of tolerance and dependence, it is difficult to point out an effect of alcohol on serotonin, during both acute and chronic phase. Some authors have identified a low level of serotonin during the acute phase and a higher one (due to the adjustment), as a consequence of chronic alcoholism.

There is the possibility that the alteration of serotonin receptors play a role in the appearance of physical dependence. Finally, just like in the case of catecholamine, the disruption of metabolization paths is possible. Thus, the excretion of 5-hydroxytryptophol a compound resulted from the reducing path, increases. Its association with acetilaldehyde will lead,

through various cycles, to the formation of molecules capable of acting at the level of the central nervous system, the β carbolinic acids (3).

β carbolinic acids stimulate the noradrenergic system and they are anxiogenic substances, causing nervousness and favouring the apparition of convulsions, which disappear after the administration of diazepam. The increase of endogenous synthesis of this type of molecules during chronic alcoholism might, consequently, facilitate, if not explain the clinical manifestations recorded during withdrawal.

The gamma-aminobutyric acid (GABA) is widely spread al cerebral level, approximately 30% of brain synapses being GABAergic. Due to its inhibiting feature, it is present mainly in the fast neural circuits, at the level of inhibiting neurons. GABA plays an important role in regulating catecholaminergic systems, inhibiting especially the extrapyramidal dopaminergic and mesolimbic function. Experimentally, an increased level of cerebral GABA diminishes the induced convulsive activities regardless on the induction method.

Various studies dealing with the action of ethanol on the GABA level have contradictory results which seem to be caused by the various protocols used and by the sensitivity differences between the dosing methods (5).

In case of acute intoxication, the GABA level appears to be normal or high, the blocking of Krebs cycle through the excess of H^+ ions having the tendency to increase the level of glutamic acid and GABA, while in case of chronic intoxication, the GABA level is normal or low (4, 5).

During withdrawal, GABA is frequently low. Moreover, alcoholic patients experiencing convulsions seem to have the GABA level in the cephalorachidian liquid smaller if compared to the witnesses. GABA interacts with a complex system of receptors including at least three different sites: GABA receptor, benzodiazepine receptors (one, connected to the chloride channel, is responsible for the anxiolytic and convulsive action of benzodiazepine, the other, regardless of the chloride channel, induces the other features of benzodiazepine, especially the sedative effect) and the receiving site for picrotoxin, the exciting substance that blocks the GABAergic transmission without modifying the attachment of GABA on its receptor.

The inhibition of the GABAergic transmission explains, at least to some extent, the hyper excitability responsible for the apparition of trembling and convulsions during the withdrawal period, the injection of GABA or agonists attenuating or even suppressing these manifestations while its antagonists emphasize them.

Alcohol can, just as much, determine the disruption of benzodiazepine receptors, phenomenon present only for moderate concentrations and it disappears at large amounts of alcohol. In the clinic it can be underlined the potentiating effect of the association of alcohol and benzodiazepine.

In case of chronic alcoholism, the site of picrotoxin can be equally altered by ethanol decreasing the attachment capacity (3).

Endogenous opioids: are substances from the human body whose physiological action is accomplished through various receptors, determining different pharmacological answers. Thus, peripherally, enkephalins inhibit the release of P substance, transmitter of painful signals, and at central level, numerous aminergic structures, especially dopaminergic, are subjected to the control of opioids. At the level of

the hippocampus, enkephalins have an exciting effect: determine the increase of spontaneous release of pyramidal cells and facilitate the release of neurotransmitters inhibiting GABA through the neighbouring inhibiting interneurons.

Ethanol stimulates the release of endogenous opioids, fact witnessed by their plasmatic and intracerebral level. This answer might be on the other hand related to the stress caused effect which induces the suprarenalian and cerebral release of catecholamines and enkephalins and the hypothalamic - hypophysarian secretion of β endorphin and stress hormones, prolactin and especially ACTH. This release might intervene, but only partially, in ethanol dependence.

Much more interesting is the study of the action of ethanol on the functional activity of opioergic systems. It was suggested that the modifications of dopamine metabolism are not due to a direct effect of ethanol but to an action of the enkephalinergic systems. It might be an increase of endogenous enkephalins, at least in some brain regions, but also the action of false neuromediators as tetra isoquinolines on receptors or a modification of the sensitivity of these receptors. It appears that the alteration of the opioidergic systems plays an important role in alcohol tolerance and dependence. In case of chronic administration of alcohol, the adjustment phenomena come into action, interesting the membranes and the secretion of endogenous opioids which will be low (2, 5).

Behavioral dependence. The abusive self administration of a substance relies at the base of the notion of behavioural dependence which can be motivated by a certain emotional condition or motivation; the presence of certain information necessary in triggering the behaviour; the beginning of a learning process; the existence of a voluntary decision to authorize and order the fulfilment of the behaviour.

Two fundamental brain areas intervene in these different phases: the neocortex, the centre of decision and dominating voluntary commands and the paleocortex, which lays at the basis of the state of need and of the learning processes due to the existence of sensorial analysis and memorization systems.

In case of alcohol dependence, the condition is not irreversible as the development of a motivation can allow the reestablishment of the normal control process at the level of the neocortex with the possibility of achieving withdrawal and abstinence.

The consumption of alcohol is induced by external, social-cultural factors and/or for the achievement of pharmacological effects. The perpetuation of this behaviour depends of the continuous presence of external factors, the development of physical dependence and the achievement of pharmacological effects, often being used for overcoming conflict situations which isolated can lead to the development of conjunctural, intermittent alcohol dependence.

CONCLUSIONS

There are numerous neurotoxic substances, alcohol being one of them, capable of determining functional perturbations on the nervous system (functional neurotoxicity) or lesions (structural neurotoxicity).

The word “alcoholism” evokes a particular behavior characterized by the dependence of repeated self-administration of alcohol. This fact requires first of all the existence of an initial impact between a toxic (alcohol) and a predisposed, vulnerable organism. This particular individual

receptivity, possibly genetically determined expresses itself better if the surrounding environment and the social cultural context are favorable.

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NEWS

A NOVEL MOUSE MODEL OF SOFT-TISSUE INFECTION USING BIOLUMINESCENCE IMAGING ALLOWS NONINVASIVE, REAL-TIME MONITORING OF BACTERIAL GROWTH

A recent study conducted by Yoshioka K. and collaborators from Japan successfully established a real-time, in vivo, quantitative mouse model of soft-tissue infection in the superficial gluteus muscle (SGM) using bioluminescence imaging. A bioluminescent strain of MRSA was inoculated into the SGM of BALB/c adult male mice, followed by sequential measurement of bacterial photon intensity and serological and histological analyses of the mice. The mean photon intensity in the mice peaked immediately after inoculation and remained stable until day 28. The serum levels of interleukin-6, interleukin-1 and C-reactive protein at 12 hours after inoculation were significantly higher than those prior to inoculation, and the C-reactive protein remained significantly elevated until day 21. Histological analyses showed marked neutrophil infiltration and abscesses containing necrotic and fibrous tissues in the SGM. With this SGM mouse model, they successfully visualized and quantified stable bacterial growth over an extended period of time with bioluminescence imaging, which allowed them to monitor the process of infection without euthanizing the experimental animals. This model is applicable to in vivo evaluations of the long-term efficacy of novel antibiotics or antibacterial implants. (Yoshioka K, Ishii K, Kuramoto T, Nagai S et al. A Novel Mouse Model of Soft-Tissue Infection Using Bioluminescence Imaging Allows Noninvasive, Real-Time Monitoring of Bacterial Growth. *PLoS One* 2014 Sep 3. PMID: 25184249).

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METABOLIC DISORDERS IN PATIENTS OPERATED FOR PANCREATIC CANCER

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METABOLIC DISORDERS IN PATIENTS OPERATED FOR PANCREATIC CANCER (Abstract): Adenocarcinoma of the pancreas presents a major threat with a 5-years survival rate of 5%. Whipple pancreaticoduodenectomy (PD) is the standard procedure for cephalopancreatic neoplasm. After an extended resection and reconstruction of superior gastrointestinal tract the digestive physiology might be heavily disrupted. A literature review of metabolic alterations of patients who suffered a major pancreatic resection is performed, regarding micronutrients, lipid absorption and pancreatogenic diabetes. Long-term survivors following PD generally have a satisfactory nutritional status although with subclinical iron, vitamin D and selenium deficiency. These patients should be followed-up also regarding these micronutrients and properly dietary supplemented when necessary, also considering the increased life expectancy. Approximately 17-25% of patients will develop insulin-dependent diabetes but pancreatogenic diabetics have elevated levels of serum insulin and minimal or absent response to food intake, as opposed to a type I diabetics, where insulin serum is normal or elevated and there is an exaggerated response to ingestion of sugar. **Keywords**: PANCREATIC CANCER, SURGERY, METABOLIC, NUTRITIONAL, DIABETES

Adenocarcinoma of the pancreas is a major threat to survival, worse than most other forms of gastrointestinal cancer (1). Compared to other gastrointestinal malignancies, exocrine pancreatic cancer is a fairly common malignancy, outnumbered only by colorectal cancer and – slightly – by stomach cancer. Pancreatic ductal adenocarcinoma is the most common malignancy of the pancreas, most frequently arising from the ductal epithelium of the pancreas (2). The age adjusted incidence rates peaks between 60 and 80 years which implies that many of the patients are not fit enough for extensive surgery (3).

Until 1970s, pancreaticoduodenectomy (PD) was a seldom performed procedure, which should be understood against a backdrop of in-hospital mortality of 20-25 percent. However, during the 1980s and 1990s,

experience in performing PD increased steadily and high-volume centers reported low treatment-related morbidity and mortality. One rationale for the increased rate of TP may be an increased diagnosis of diffuse ductal disease in all patients undergoing pancreatic resection. Improved radiographic and endoscopic surveillance during preoperative testing as well as close attention to intra-operative frozen section pathologic analysis of the pancreatic duct margin may contribute to this.

Recently the mortality in high-volume centers is usually close to zero, whereas major morbidity has decreased to 30% or less – even though the procedures have been extended (4). Depending on the stage, the treatment options include chemotherapy, radiation, and surgery. Surgical resection has offered the only hope for cure in the treatment of exocrine pancreatic cancer. Even so, pancreatic cancer still remains a major, unsolved therapeutic challenge and is the most lethal type of gastrointestinal cancer with a 5-year survival rate of 5%. Adjuvant chemotherapy remains to be gemcitabine alone, though fluorouracil offers the same survival and role of radiation remains controversial. Nevertheless, only a few patients survive for at least 5 years after R0 resection and adjuvant therapy (5). Generally, one of the reasons for the bad prognosis of pancreatic cancer is the late diagnosis despite the development of new technologies. This delay in diagnosis is mainly due to the fact that there is no early symptom or sign. Moreover, the initial presenting symptoms of pancreatic carcinoma are generally vague and non-specific.

The development of sophisticated surgical procedures, improved postoperative care, and the capacity for early diagnosis of disease has prolonged life expectancy after pancreatic resection.

Whipple PD is the standard procedure for neoplasm of duodenum, ampulla of Vater, distal coledochus and the head of pancreas. After an extended excision and reconstruction of superior gastrointestinal tract, the digestive physiology might be heavily disrupted. Previous studies have shown that patients might maintain a normal body mass index (BMI) after surgery for pancreatic cancer, although frequently lower than preoperative values (6, 7, 8).

Metabolic disorders consecutive to pancreatic resections have not been widely studied. Compared to healthy individuals, these patients may have measurable reduced concentrations of trace elements and vitamins (selenium, vitamin B12, vitamin D) in whole blood and serum by the time cancer is diagnosed and long before clinically relevant changes in nutritional status are seen (9). Armstrong et al. showed that long-term survivors of duodenopancreatectomy are relatively deficient in several micronutrients compared to non-operated controls taking the same diet. Patients had elevated transferrin and low ferritin levels indicating relative iron deficiency. Patients also showed lower levels of the anti-oxidants -selenium and vitamin E- with 57% of patients with significant selenium deficiencies. Lower levels of vitamin D than controls were found and 30% of patients had a high parathyroid hormone level, suggesting compensatory mechanisms operate to maintain normocalcaemia (10).

The two important antioxidants in the diet, selenium and vitamin E, have an important role in anti-inflammatory reaction and also are protective factors in heart diseases, infectious diseases and in neoplastic processes. After PD 57% of the patients had selenium deficiency which in advanced stages can lead to cardiomyopathy. Although vitamin E level was significantly lower in operated patients, it remained at normal values. It's already

known that the deficit of antioxidant agents contributes to the progression of pathophysiological processes in chronic pancreatitis so we consider that low values of selenium and vitamin E should be avoided in patients operated for pancreatic cancer. Other micronutrients such as copper and zinc which are part of many metalloenzymes and normally play an important role in cell mediated immunity have low values in patients operated for pancreatic cancer. These observations did not have an impact on infection rates so that these changes have no clinical relevance.

In addition to improved endocrine control, exocrine insufficiency may be improved by modern pancreatic enzyme formulations, proton pump inhibitor therapy and the use of the duodenum-preserving pancreatic head resections. In a report from Japan, the pylorus-preserving technique was noted to be associated with significantly improved albumin levels and weight loss over the standard total pancreatectomy (21).

Lipid malabsorption after major pancreatic resections is the result of several mechanisms contributing to early occurrence of steatorrhea compared malabsorption of other nutrients. For various reasons impairment of pancreatic lipase activity cannot be sufficiently compensated by other mechanisms. Digestion of fats in the small intestine is mainly due to the combined effects of lipase and its cofactors, especially bile acids. Intestinal mucosa does not express enzymatic systems that digest triglycerides and lipolytic activity performed by other extrapancreatic sources (salivary and gastric) cannot compensate lipid digestion by pancreatic lipase (11).

The loss of pancreatic parenchyma resulting from pancreatic resection causes an extreme disruption of glucose homeostasis known as pancreatogenic diabetes.

Pancreatogenic diabetes is attracting attention as the primary factor influencing quality of life in patients who have undergone this procedure, but is difficult to predict the effect on carbohydrate metabolism because metabolic changes are from mild to major. The incidence of new-onset diabetes mellitus after pancreatic resection increases as the follow-up period after surgery becomes longer and is related to the progression of underlying disease, the type of surgery, and the extent of resection. In general, postoperative changes are usually moderate to severe and approximately 17-25% of patients will develop insulin-dependent diabetes.

Studies on pancreatic endocrine function after resection are few and controversial. This is probably due to the fact that long-term metabolic consequences may differ even if for the same type of pancreatic resection or surgical indication and depend mainly of the quality of the remaining pancreas tissue (tab. I).

TABLE I

Postoperative consequences after different types of resection and drainage procedures for pancreatic cancer

Distal pancreatectomy	New diabetes	21-40%	Morrow(12)
Cefalic pancreatectomy with presevation of the duodenum	New diabetes	2-15%	Beger(13)
Cephalic pancreatectomy with preservation of the pylorus	New diabetes	12%	Traverso(14)
Whiple duodenopancreatectomy	New diabetes	17%	Schwarz
Total pancreatectomy	Brittle diabetes	100%	Andrén-Sandberg (15)

The pathophysiology of pancreatogenic diabetes is related to pancreatic hormone deficiency and the altered responses of the liver and peripheral organs to lower than normal hormone levels. Experimental results suggest that insulin-independent glucose metabolism after pancreatic resection is done for a large proportion of absorbed glucose. This may explain why patients with pancreatogenic diabetes behave differently than other diabetics sensitive to insulin therapy. These patients with unmanageable diabetes - "brittle diabetics" - may become unpredictable hypoglycemic during treatment with insulin, unrelated to food intake or to physical effort. Diabetes resulting after pancreatic resection differs in several ways from type I (juvenile, or insulin-dependent) and the type II (adult, or non-insulin-dependent).

Hyperglycemia occurs when the amount of insulin produced or administered is insufficient because of unsuppressed hepatic glucose production secondary to a deficiency in pancreatic polypeptide.

In contrast, patients lapse into hypoglycemia when insulin is barely excessive because of enhanced peripheral insulin sensitivity and glucagon deficiency. Nutritional state, pancreatic exocrine function and intestinal function also affect glycémic control. Unlike type I diabetes, patients with pancreatogenic diabetes rare develop ketoacidosis and the degree of hyperglycemia is mild in most cases. Compared with type II diabetes highly resistant to insulin, resected patients are really sensitive to insulin and iatrogenic hypoglycemia episodes are common. These patients have an increased serum level of gluconeogenetic precursors such as lactate and alanine (16). These distinct clinical and metabolic aspects have been attributed to pancreatic glucagon secretion deficits (17).

Pancreatogenic diabetics have elevated levels of serum insulin and minimal or absent response to food intake, as opposed to a type I diabetics, where insulin serum is normal or elevated and there is an exaggerated response to ingestion of sugar (18) (tab. II).

TABLE II

Pancreatogen diabetes, post resection diabetes, compared to classical forms of diabetes

Variables	Type I diabetes	Type II diabetes	Pancreatogenic diabetes
Ketoacidosis	frequent	rare	rare
Hyperglycemia	severe	mild	mild
Hypoglycemia	frequent	rare	frequent
Peripheral insulin sensitivity	normal/elevated	low	elevated
Liver insulin sensitivity	normal	elevated	low
Insulin level	low	elevated	low
Glucagon level	normal/elevated	normal/elevated	low
Pancreatic peptide level	elevated	elevated	low

As pointed out by Frey et al., the incidence of diabetes is directly related to the extension of the resection of pancreatic tissue (19). Patients requiring total or near-total pancreatectomy pay a significant price represented by altered secretion of glycemic regulating hormones. In these patients requiring distal pancreatectomy or only a

limited excision of the pancreas diabetes develops far less frequently than those that require PD or total or near-total pancreatectomy.

It has been noticed an increased sensitivity of extrahepatic tissue to psysiological hyperinsulinemia and increased amounts of insulin binded to receptors from red blood cells in patients with pancreatogenic diabetes. Thus, there is an increase of peripheral insulin receptors in response to insulin deficiency that makes these patients susceptible to hormone replacement.

Patients with PD have a good metabolic status and a significant number of them require insulin replacement therapy, due to failure to control blood sugar with oral antidiabetic agents. Insulin replacement is considered to be the main treatment option for insulin dependent pancreatogenic diabetes. Improvements in glucose monitoring systems, insulin delivery systems and insulin formulations may contribute to superior glycaemic control for these patients. (25)

Surgeons, gastroenterologists and endocrinologists alike may be more comfortable with the management of the apancreatic state that was so feared in the past. Jethwa *et al.* (22) reported that there were no observed mortalities as a result of diabetic complications or metabolic consequences of pancreatic resection during long-term follow-up of 47 patients undergoing TP. They concluded that diabetic control after TP is less difficult and associated with fewer glycaemic complications than generally assumed. Similarly, in the largest single institution review of 124 elective TPs, a group from Germany found TP was performed with the same morbidity rate as partial pancreatectomy, and quality of life was not significantly different in the two groups. (23) This confirmed a previous report from 2005 where quality of life in 34 TP patients was compared with matched type I diabetic controls, and no difference was found. (24)

The significant metabolic derangements of the apancreatic state may not become immediately apparent in the post-operative inpatient recovery phase. Most of these elderly patients do not have baseline endocrine or exocrine insufficiency before surgery and have difficulty adjusting to complex diabetic and nutritional changes seen after TP. Multidisciplinary management is mandatory for improved outcomes and intensive diabetic and nutritional counselling are essential. Providing extended care by discharge to a skilled nursing facility with diabetic education and nutrition capabilities appears appropriate. Additionally, consideration should be given for providing all patients with supplemental enteral feeding for several weeks post-operatively. A strict follow-up protocol should be available for these patients who include close endocrine and nutrition supervision, strict adherence to proton pump inhibitor (PPI) and pancreatic enzyme replacement therapy and adequate hydration and protein intake. These measures may avoid readmission in the majority of the patients, particularly for the diagnosis of dehydration, diabetic complications and failure to thrive (26).

CONCLUSIONS

Mortality and long-term morbidity associated with TP appear to be lessening over the past decade, indicating that the risks of TP appear acceptable compared with the benefits of resection, especially for those patients with premalignant disease. Overall, survival is generally based on the underlying disease process, not a consequence of the surgery.

The goal of the treatment of pancreatic

cancer should be to balance the quantity and quality of life according to individual preferences. Long-term survivors following PD generally have a satisfactory nutritional status although with subclinical iron, vitamin D and selenium deficiency. These patients should be followed-up also regarding these micronutrients and properly dietary supplemented when necessary, also considering the increased life expectancy.

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NEWS

SYNDROME-ASSOCIATED SOFT TISSUE TUMOURS

Soft tissue neoplasms may be associated with a variety of genetic disorders and malformation syndromes, especially when they arise in children, adolescents and early adulthood. While many soft tissue tumours are sporadic and without a known pathogenesis or established risk factors, in recent years there has been an increasing awareness that certain types of benign, intermediate and malignant soft tissue neoplasms may be a manifestation of a genetic or malformation syndrome and that specific syndromes carry with them a higher risk of sarcoma. This is a particular concern for children, adolescents and young adults because of the relatively increased frequency of sarcomas in younger patients. Li–Fraumeni syndrome (including those with germline p53 mutations), hereditary retinoblastoma and neurofibromatosis type are the prototypical syndromes associated with soft tissue tumours, but many other syndromes and genetic conditions have soft tissue tumours as a manifestation, including Gorlin–Goltz syndrome, familial adenomatous polyposis (FAP) and pleuropulmonary blastoma syndrome. Vascular masses, usually malformative in nature, are characteristic of a variety of other disorders, including Maffucci, Sturge–Weber, Klippel–Tr_enaunay–Weber, Proteus and von Hippel–Lindau syndromes. In children, malformation syndromes, congenital anomalies and genetic disorders are among the major risk factors for development of soft tissue sarcoma. In this review, the authors summarize the principal histopathological types of soft tissue tumours which occur in various syndromes, with an emphasis on pathological features, genetic aspects and considerations for the diagnostic pathologist (Coffin C M, Davis J L & Borinstein S C. Syndrome-associated soft tissue tumours. Histopathology 2014, 64, 68–87).

Doina Butcovanu

EFFECTS OF SOME DOPAMINE ANTAGONISTS ON SPATIAL MEMORY PERFORMANCE IN RATS - EXPERIMENTAL RESEARCH

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EFFECTS OF SOME DOPAMINE ANTAGONISTS ON SPATIAL MEMORY PERFORMANCE IN RATS - EXPERIMENTAL RESEARCHES (Abstract)**:** Dopamine is a neurotransmitter with an important role in forming long-lasting memories for some time, especially in episodic memory. Literature data show that dopamine receptor stimulation may be detrimental to spatial working memory functions in lab animals. (R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride derivative - SCH-23390 is a synthetic compound that acts as a selective, high-affinity antagonist of D1 receptors. Experimental studies suggest that SCH 23390 may prevent the spatial working memory disturbances induced by the active substances of marijuana. Melperone is an atypic antipsychotic drug presenting also dopaminergic D2 and 5-HT2A receptor antagonistic activity. This neuroleptic agent is used in the treatment of some types of schizophrenia. **Aim:** Experimental research on the effects of two dopamine receptor antagonists on spatial memory performance in rats. **Material and methods:** The experiment was carried out in white Wistar rats (200-250g), divided into 3 groups of 7 animals each, treated intraperitoneally with the same volume of solution for 14 days, as follows: Group I (Control): saline solution 0.1ml/10g kbw; Group II (coded SCH): SCH-23390 0.3 mg/kbw; Group III (coded MLP): melperone 2 mg/kbw. The dopaminergic agent spatial memory performance was assessed by recording spontaneous alternation behavior in a single session in Y-maze. Each animal was placed at the end of one arm and allowed to move freely through the maze during an 8 min session. Alternation was defined as a consecutive entry in three different arms. The alternation percentage was computed with the following formula: number of alternations divided by total number of arm visits minus 2. Data were presented as +/- standard deviation and significance was tested by SPSS Statistics for Windows version 13.0 and ANOVA method. P-values less than 0.05 were considered statistically significant compared to those in the control group. Experimental researches were carried out in compliance with the regulations of our University Committee for Research and Ethical Issues. **Results:** SCH-23390 (0.3 mg/kbw) and melperone (2 mg/kbw) intraperitoneal injection for 14 days determined a statistically significant ($p<0.05$ and $p<0.01$, respectively) increase in spontaneous alternation rate (compared to controls in Y-maze test). **Conclusions:** Our research revealed that the 14 consecutive days administration of these two dopamine receptor antagonists was associated with the improvement of short-term memory in rats, more intense for SCH-23390 compound. **Keywords:** SCH-23390, MELPERONE, Y-MAZE TEST, MEMORY

Substances mediating dopaminergic transmission have been clinically used in the treatment of different neurological and psychiatric disorders such as Parkinson and Huntington disease, schizophrenia, bipolar disorder, attention deficit hyperactivity disorder. Dopaminergic system, have been also known to play a major role in the emotional response to rewarding, as well to aversive stimuli (1).

SCH-23390 is a R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride derivative acting as a selective, high-affinity antagonist of D1 receptors. Experimental studies have shown that SCH 23390 abolished generalized seizures induced by the chemoconvulsants: pilocarpine and soman (2, 3).

Melperone is a dopaminergic D2 and 5-HT2A receptor antagonist. This butyrophenone derivative structurally related to haloperidol has the ability to produce an antipsychotic effect in man at doses that cause minimal extrapyramidal side effects (4). It is also known to possess anxiolytic properties (5).

Electrophysiological studies showed that this drug determined an increase of dopamine levels in the medial prefrontal cortex and nucleus accumbens and a release of acetycholine in the medial prefrontal cortex. Clinical trial revealed that melperone is used especially in the treatment of neuroleptic-resistant schizophrenia (6, 7).

The supposition that dopaminergic system is involved in modulation of recognition memory is based on different experimental reports indicating the enhancement of memory consolidation by activation of dopaminergic receptor (8).

To directly prove this hypothesis we investigated the effects of two dopamine receptor antagonists, SCH-23390 and melperone, on spatial memory performance in Y-maze, a behavioral test for measuring the eagerness of lab animals to explore new environments.

This standardized test is used to evaluate working memory in rodents by measuring spontaneous exploratory behavior. The substances were administered one hour before the beginning of a behavioral session.

MATERIAL AND METHODS

Male white Wistar rats (150–200g) were used in the study. Animals were housed in plastic cages in an animal room maintained at 23 ± 1°C on a 12-hour dark cycle (light period, 07:00-19:00).

Animals had water *ad libidum* and were maintained at 85% of their free feeding weight by controlling the amount of their single daily meal following the experimental session during 45 min. Before the experiment, rats were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

The animals were distributed into 3 groups of 6 subjects each, and treated intraperitoneally as follows Group: I (Control): saline solution 0.1ml/10 kbw; II (coded SCH): SCH-23390 0.3 mg/kbw; III (coded MLP): melperone 2 mg/kbw.

The used drugs, SCH-23390 and melperone (Sigma-Aldrich Chemical Company), were dissolved with 0.9% saline, prepared immediately before use. All drugs were injected intraperitoneally in a constant volume of 0.1 ml/10 g body weight.

To assess the possible involvement of dopamine agents in the maintenance of spatial cognition, the present study investigated the effects of dopamine receptor antagonists SCH-23390 and melperone on rat memory performance on Y-maze (9). This device consists of 3 identical arms (40

x 9 x 16 cm) situated at 120° from each others, and a central triangular platform. Each arm has walls with specific motifs all over the inner area of the three walls allowing animals to distinguish it from the others.

This experimental model was used to tests if the rat remembered the arm it had just explored and would therefore enter one of the other arms of the maze. Each animal was placed at the end of one arm and allowed to move unreservedly through the maze during an 8 min session. The first 2 minutes were for habituation, and the last 6 minutes for the alternation between arms recorded with photo beam breaks positioned at midpoint of each arm. Repeated activation of the same detector was not considered as an arm-entry until the detector in another arm was activated.

Spontaneous alternation was defined as entry to an arm that was least occupied in recent times (10). Latency to leave the start arm, latency of first arm visit, number of arm visited, alternate arm returns, and the same arm returns were also counted.

A video camera set over the device and connected to a computer in another room was used to allow us to score behavior without distressing the animals. All trials were videotaped and analyzed by an observer unfamiliar with treatment condition. The tasks were performed between 8:00 and 13:00 a.m.

Data for each measure were evaluated separately by analysis of variance for repeated determinations. Results were expressed as arithmetic mean ± SD and represented graphically. All values were processed using SPSS for Windows version 17.0 and ANOVA method. P-value less than 0.05 were considered statistically significant compared to controls.

Experimental protocol was implemented following the recommendations of the "Grigore T. Popa" University Committee for Research and Ethical Issues guidelines for the handling and use of experimental animals, according to the ethical standards of the European Community.

Each animal was used once only and the duration of the experiments was kept as short as possible. For ethical considerations all the animals were sacrificed at the end of the experiment (11).

RESULTS

Spontaneous alternation or free-running in rats refers to their natural tendency to spontaneously choose alternate arms in a Y-maze. Arm exploration was defined as entering at least the first third of an arm. The alternation percentage of saline-treated rats was 61.4 ± 2.28.

Intraperitoneal administration of SCH 22390 resulted in a decreased e number of arm entries (mean ± S.E.M. number of arm entries was 22.67±1.63), but statistically non-significant compared to control animals (mean ± S.E.M. number of arm entries was 28.17±2.14) (tab. I).

The 14-day treatment with SCH-23390 (0.3 mg/kbw) determined a 77.2±1.72 spontaneous alternation rate.

Statistical analysis of the results showed that the alternation rate of SCH-23390-treated rats was statistically ($p<0.05$) higher than that of control rats in Y-maze test, thus suggesting a facilitation of extinction learning (fig. 1).

The administration of melperone (2mg/kbw) resulted in a statistically significant ($p<0.01$) increase of spontaneous alternation rate (86.8±1.16) compared to controls, suggesting effects on spatial memory, especially on short-term memory (tab. II, III).

TABLE I

Descriptive statistics - Spontaneous alternation rate

	N	Mean	Std. Deviation	Std. Error	Min	Max
Controls	6	61.40	2.28	.206	58.40	64.50
SCH-23390	6	77.20	1.72	.118	75.70	80.50
Melperone	6	86.80	1.16	.154	85.70	88.90

* The mean difference is significant at the .05 level.

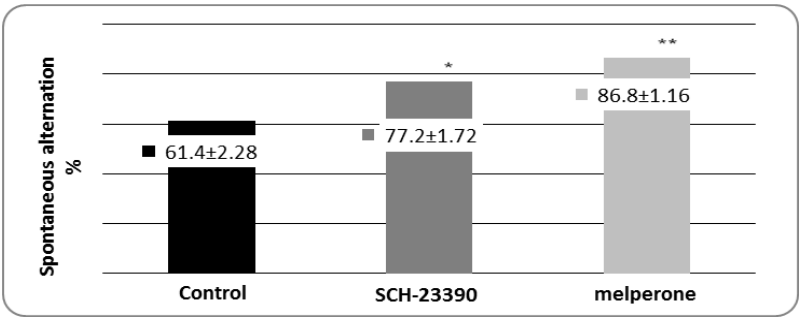

Fig. 1. Effects of SCH-23390 and melperone administration on spontaneous alternation percent/rate in Y-maze. Values were expressed as mean ± S.E.M of spontaneous alternation rate. *p<0.05, **p<0.01 vs control

TABLE II

ANOVA - Spontaneous alternation rate

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	15.048	6	1.112	11.103	.010
Within Groups	10.133	18	.464		
Total	25.181	24			

TABLE III

Multiple comparisons - Dependent variable.
Spontaneous alternation rate. Tukey HSD (J) Substance: Control

(I) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
SCH-23390	.40000	.118	**.046***	.3022	.7084
melperone	.30000	.154	**.007****	.1317	.8112

* The mean difference is significant at the .05 level.

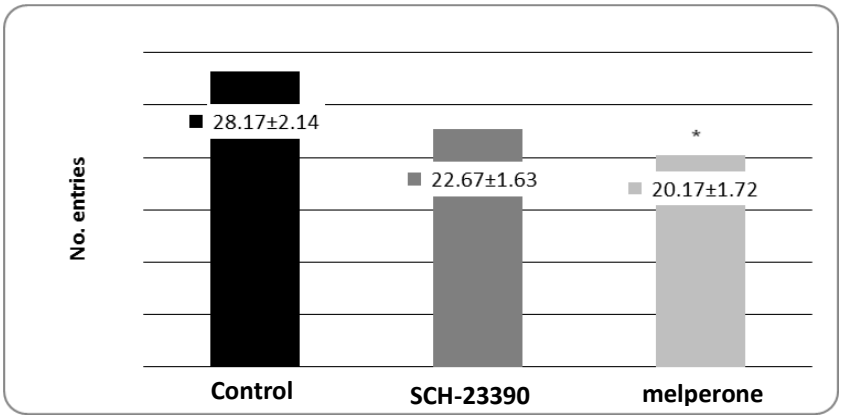

Fig. 2. Effects of SCH-23390 and melperone administration on the number of arm entries in Y-maze test. Values were expressed as mean ± S.E.M. of the number of arm entries. *p<0.05, **p<0.01 vs control

At the same time, only a tendency of reduced exploration capacity after the treatment with SCH 22390 in rats was noticed (tab. IV).

The treatment with melperone determined a statistically significant (p<0.05) diminution of the number of arm entries (mean ± S.E.M. number of arm entries was 20.17±1.72) compared to saline treated rats. These results suggested that melperone generated deficits of performance in Y-maze test (fig. 2) (tab. V, VI).

TABLE IV

Descriptive statistics - Number of arm entries

	N	Mean	Std. Deviation	Std. Error	Min	Max
Control	6	28.17	2.14	.168	26.00	31.00
SCH-23390	6	22.67	1.63	.102	21.00	25.00
melperone	6	20.17	1.72	.117	18.00	23.00

* The mean difference is significant at the .05 level.

TABLE V

ANOVA - Number of arm entries

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.886	6	1.045	10.563	.050
Within Groups	9.791	18	.312		
Total	24.677	24			

TABLE VI

Multiple comparisons - Dependent variable. Number of arm entries. Tukey HSD (J) Substance: Control

(I) Substance	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
SCH-23390	.30000	.102	.062	.2156	.6700
melperone	.50000	.117	**.048***	.1422	.7014

* The mean difference is significant at the .05 level.

This nonspecific depressant behavioral effect observed in Y-maze test could be due to the inhibition of D2 dopaminergic receptor.

The administration of dopaminergic receptor antagonists induced a reduction of the same arm return and alternate arm return number, statistically significant ($p<0.05$) more intense in melperone group compared to controls (tab. VII).

No changes in latency of the first arm visit were found between groups treated with SCH-23390, melperone and saline solution.

TABLE VII

The effect of SCH-23390 and melperone in Y-maze test (same arm return, alternate arm return, latency first arm visit). Values were expressed as mean ± S.E.M. *p<0.05, **p<0.01 vs control.

	Number of same arm return	Number of alternate arm return	Latency first arm visit (seconds)
Control	6.3±1.17	4.2±1.23	22.64±1.73
SCH-23390	4.5±0.73	3.6±0.64	21.17±0.90
melperone	2.4±0.33	1.2±0.17	21.37±1.25

DISCUSSION

The present research established the inhibition of exploratory and locomotors activity of rats induced by consecutive 14 days administration of melperone and only a tendency for reduced exploratory activity following SCH 22390 administrations. This nonspecific depressant behavioral effect could be due to the inhibition of D2 dopaminergic receptor.

Previous reports have described a facilitation of rat spatial working memory determined by D1 receptor antagonist SCH 22390 administrations in the radial arm maze. In this behavioral experimental model SCH-23390 influenced short-time memory, without affecting long-time memory of experimented animals (12).

Different studies using a variety of approaches suggest that dopamine pathway may be a key element in modulating activity in the central areas to facilitate behavioral flexibility (13, 14, 15).

Various researches indicated that dopamine D1 and D2 receptors have been involved in recognition memory processing but their implications in different phases of this cognitive process remains uncertain.

The central dopamine receptors, especially D1 receptors in the prefrontal cortex, may be very important for mediating dopamine effects on cognitive functions (8).

It has been visibly established the involvement of dopamine receptors subtypes in the mediation of cognitive functions and in spontaneous exploratory behavior in rodents (13, 16).

The contribution of different central nervous system areas, particularly hippocampus, in spatial memory and exploration capacity of laboratory animals is well recognized (17).

Experimental studies illustrate that SCH 23390 may prevent the spatial working memory disturbances induced by active substances of marijuana, block of apomorphine-induced stereotypy and methamphetamine-induced lethality in experimental models (18). Also it presented antistereotypic effects, cataleptogenic effect and inhibitory effect on amphetamine circling in different laboratory animal species (3).

Dopaminergic receptors (D1, D2, D3, D4, D5), described in 1979 are G-protein coupled receptors that mediate the activity of the neurotransmitter dopamine, being involved in spontaneous behavior, reward, in pleasure and motivation, blood pressure control and hormonal secretion regulation (19).

D_1 receptors are found at high levels in the typical dopamine rich regions of brain such as the neostriatum, substantia nigra, nucleus accumbens and olfactory tubercle, whereas the distribution of the D_5 receptors is more restricted (20, 21, 22). D_1 receptor mediated important actions of dopamine to control movement, cognitive function and cardiovascular system activity. The functions of D_5 receptors are not very well known until now (23). D_1-like receptors show high affinity for SCH 23390 and SKF 83566 which are selective antagonists for these subtypes and also moderate affinities for typical dopamine agonists such as SKF 82958, SKF 38393, SKF82526 and dihydrexidine (21, 24).

D_2-like receptors show high affinity for selective antagonists such as: butyropenones (e.g. haloperidol) and the substituted benzamides (e.g. sulpiride), phenothiazines and thioxanthines (23). Most antagonists show a stronger affinity for the D_2 receptor (e.g. pimozid) compared with the D_3 and D_4 receptors. The D_2-like subtypes manifest moderate affinities for typical dopamine agonists with the D_3 receptor generally showing higher affinities for agonists than the other subtypes (25).

The D_2 receptor is the principal subtype in the brain and is found at higher levels in typical dopamine rich zones. D_3 and D_4 receptors are found at much lower levels and in a more restricted distribution, especially in limbic areas in the central nervous system (24, 26). The D_2 receptor mediates the effects of dopamine to control movement, some aspects of behavior in the brain and prolactin secretion from the anterior pituitary gland (23).

The functions of the D_3 and D_4 receptors are not clear, although their localization suggests roles in cognitive, affection and behavior function (27).

CONCLUSIONS

The present research indicates that 14 consecutive days administration of D2 dopaminergic receptor antagonist melperone is associated with the enhancement of cognitive functions in rats. This was proved to be more efficient in facilitating extinction learning. Moreover, the experiment revealed the inhibition of exploratory locomotor

activity induced by consecutive 14 days administration of melperone in rats.

The Y-maze test used to assess aspects of cognitive function and learning in rats showed an improvement of spatial memory in the rats exposed to D1 dopamine receptor antagonist SCH 23390 indicated by an increase of spontaneous alternation rate compared to controls, suggesting significant effects on short-term memory.

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NEWS

CANDIDA ALBICANS BIOFILM

Candida albicans is a major human fungal pathogen causing mucosal and deep tissue infections of which the majority is associated with biofilm formation on medical implants. Biofilms have a huge impact on public health, as fungal biofilms are highly resistant against most antimycotics. Animal models of biofilm formation are indispensable for improving our understanding of biofilm development inside the host, their antifungal resistance and their interaction with the host immune defence system. The study's authors have demonstrated for the first time that non-invasive, dynamic imaging and quantification of *in vitro* and *in vivo C. albicans* biofilm formation including morphogenesis from the yeast to hyphae state is feasible by using growth-phase dependent bioluminescent *C. albicans* strains in a subcutaneous catheter model in rodents. They have shown the defect in biofilm formation of a bioluminescent *bcr1* mutant strain. This approach has immediate applications for the screening and validation of antimycotics under *in vivo* conditions, for studying host–biofilm interactions in different transgenic mouse models and for testing the virulence of luminescent *C. albicans* mutants, hereby contributing to a better understanding of the pathogenesis of biofilm-associated yeast infections (Greetje Vande Velde,Sonˇa Kucharíková, Sanne Schrevens,Uwe Himmelreich and Patrick Van Dijck Towards non-invasive monitoring of pathogen–host interactions during *Candida albicans* biofilm formation using *in vivo* bioluminescence. *Cellular Microbiology*, 2014, 16(1): 115–130)

Doina Butcovan

HOMEWORK PARTICULARITIES FOR SMALL SCHOOL CHILDREN

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HOMEWORK PARTICULARITIES FOR SMALL SCHOOL CHILDREN (Abstract): The present study was centered on the particularities of the duration of preparing homework, taking breaks during homework preparation, and the way the breaks should take place for small school children. **Material and methods**: The study has been done on a sample of 235 small school children from Oradea, 114 boys and 121 girls, between the ages 7 and 10 years old, using an anonymous questioner, with 41 items, which investigates the lifestyle of the small school children. **Results and discussion**: The duration of homework preparation it is significantly more reduced for the school children in 1st grade in comparison with the ones in 3rd grade ($p<0.001$); for school children in 2nd grade compared to the ones in 3rd ($p<0.001$) and for school children in 3rd grade compared to the ones in 4th grade. A percentage of 93% of children prepare their homework after lunch. Half of the children from grades I-IV prepare their homework with no break. A very small number of children spend their homework break time in a healthy manner, while the rest prefer to play computer games (46.95%) or to watch television (46.08%). **Conclusions**: More than half of the schoolchildren need 1-2 hours at home to prepare their homework. Most of the school children prepare their homework after lunch, in an optimal interval of time. Half of the questioned children prepare their homework with no break. Those who are taking breaks prefer activities which get the children even more tired, therefore being non-hygienic methods of spending homework breaks. **Keywords:** SMALL SCHOOL CHILDREN, HOME, BREAKS, PREPARATION OF HOMEWORK

While establishing a schedule for the small school child some rules need to be followed: dosing the effort in proportion with possibilities, particularities and health of the growing and maturing organism; alternating harder activities with easier ones; rhythmical alternation of activity with rest, grading the work done (1, 2).

It is appreciated that the 24 hours of a day are split into three groups: professional time, necessary biological time and free time. The professional time encompasses school hours, the time necessary for preparing homework, and the time necessary to go and come back from school. The biological time includes sleep hours, the time necessary for meals and individual hygiene. The remaining difference is the free time (1, 3).

The preparation of homework needs to be done in the interval of maximum output, between 15:00-17:00 o'clock, and not randomly (2, 4, 5). It is recommended to start with the harder subjects. First the written

assignments need to be done, then the reading followed by learning. Switching from one subject to another is done only after completing the homework of the subject first started. Short 10 minute breaks need to be taken in between different subjects, without replacing the school related intellectual activity with similar occupations which strain the nervous system, such as watching television. For the small school children, the homework activity needs to be limited to 1-2 hours daily (6-9).

The present study has centered on the particularities of the duration of preparing homework, taking breaks during homework preparation, and the way the breaks should take place for small school children.

MATERIAL AND METHODS

A study has been done on a population sample of school children from I-IV grade, from an elementary school in Oradea, using an anonymous questioner with 41 items which investigates the lifestyle of small school children.

The group of the small school children studied includes 235 children, out of which 114 boys and 121 girls, with ages between 7-10 years old. There were no significant differences found on the frequency placement by age and sex for small school children, the group being homogenous, $p>0.05$, in terms of distribution of participants by age gender.

Processing and interpretation of data were done using modern methods of advanced medical statistics. The data were electronically filled using Microsoft Excel, 2001 program and interpreted using PASW 18 (*SPSS 18*) 2010. The threshold of statistical significance was established at $p<0.05$, with the exception of the cases where the correction Bonferonni was applied, and the level of threshold of significance is mentioned in the text. The following statistical tests have been applied: chi-squared test, Mann-Whitney test, Kruskal-Wallis test.

RESULTS AND DISCUSSION

1. Homework time (fig. 1)

More than half of the small school children (56.89% in 1st grade, 66.07% in 2nd grade, 62.9% in 3rd grade and 45.76% in 4th grade) need 1-2 hours for homework, according to the hygiene-sanitary norms for this age group of school children. There are some children who need more than 2 hours for homework preparation, 12.5% in 2nd grade, 27.41% in 3rd grade and 49.15% in 4th grade. None of the children needs more than 3 hours for homework preparation, and 39.65% out of the ones in 1st grade need less than one hour for homework.

We found a significant difference in the duration of homework preparation between the grades, $H(3)=65.59$, $p<0.001$. We further investigated this result and the threshold of statistical significance was established at 0.008, according to Bonferroni correction. The duration of homework preparation it is significantly more reduced for the school children in 1st grade in comparison with the ones in 3rd grade, $U=924.5$, $z=-5.23$, $p<0.001$, $\varepsilon = 0.48$, the difference between the two grades being of medium to big size, respectively in comparison with the school children in 4th grade, $U=528.5$, $z=-7.03$, $p<0.001$, $\varepsilon=0.64$, difference between the two grades being of a big size. The duration of homework preparation is significantly more reduced for school children in 2nd grade compared to the ones in 3rd grade, $U=1254$, $z=-3.05$, $p<0.008$, $\varepsilon=0.28$, the difference

between the two grades being of small to medium size, respectively compared to the school children in 4th grade, U=790, z=-5.41, p<0.001, ε=0.50, the difference between the two grades being of big size. The homework preparation time is significantly more reduced for school children in 3rd grade compared to the ones in 4th grade, U=1347, z=-2.82, p<0.008, ε=0.25, the difference between the two grades being of small size. The rest of the differences are of no significance.

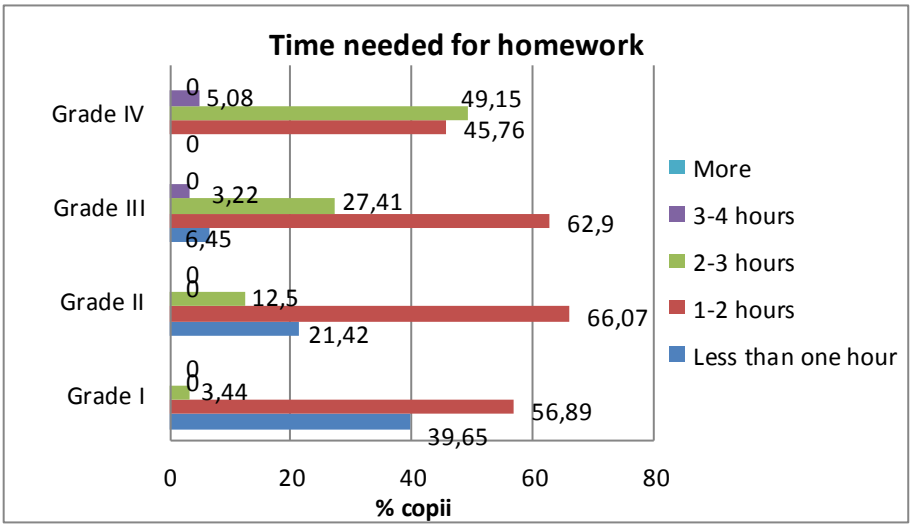

Fig. 1. Homework time for small school children

2. **The time of day when homework are prepared** (fig. 2)

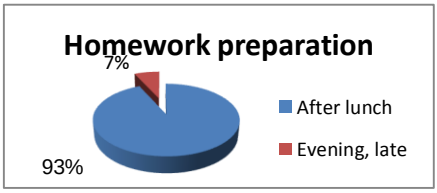

Fig. 2. Small school children homework preparation

The majority of children, a number of 219 (93%) prepare their homework after lunch, but there are 16 children (7%) who delay the preparation of homework for the evening time, entirely contraindicated, since the intellectual output is very low at the end of the day.

3. **The modality of homework preparation and using breaks** (fig. 3,4)

Half of the total of children from grades I-IV prepare their homework with no break, and more specifically 62.06% of 1st graders, 53.57% of the 2nd graders, 51.61% of 3rd graders, and 37.28% of the 4th graders take brakes during homework, 30 children (48.38%) of 3rd graders, 26 children (46.42%) of 2nd graders, and only 22 children (37.93%) of 1st graders.

Nevertheless, the differences in the modality of homework preparation between grades are not significant, p>0.05.

A very small number of children (2 in

2nd grade, 4 in 3rd grade, and 2 in 4th grade) spend their homework break time in a healthy manner, while the rest prefer to play computer games, 46.95% of the children's total, or to watch television, 46.08% of the children's total.

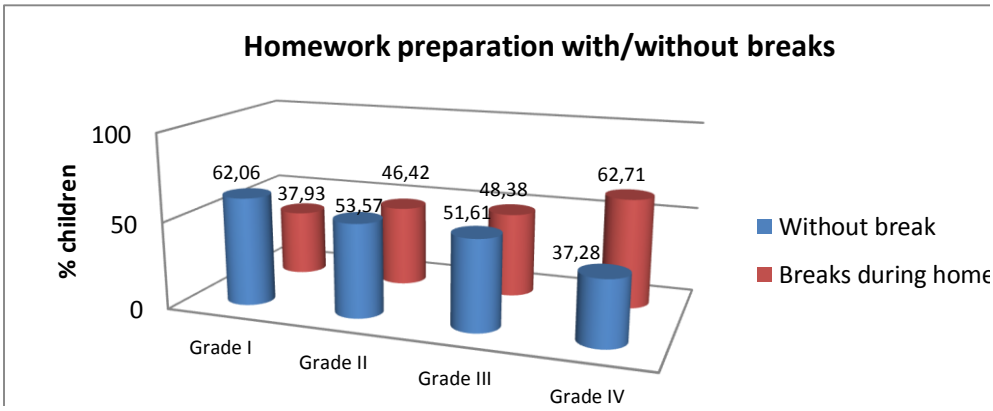

Fig. 3. The modality of homework preparation for small school children

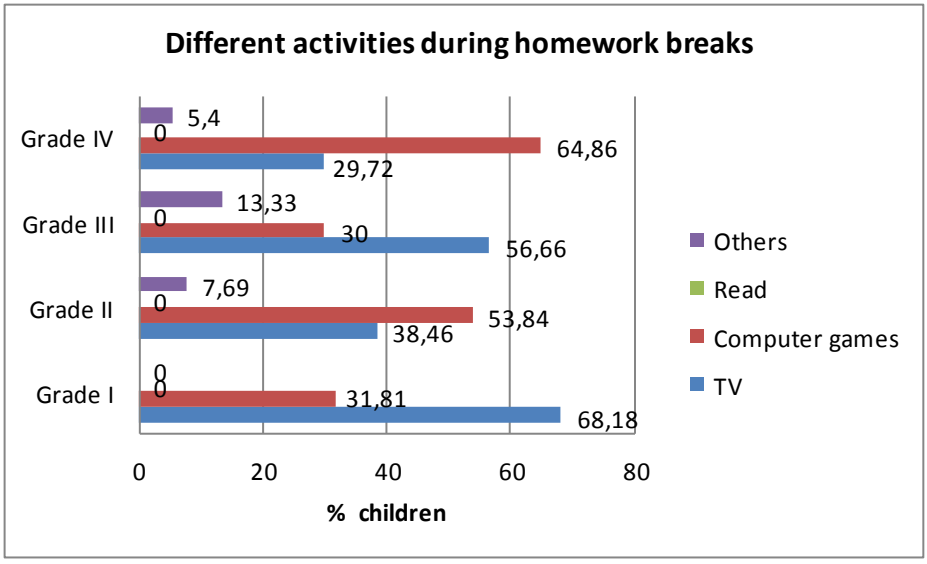

Fig. 4. Small school children use of breaks during homework

Both activities do nothing but get the children even more tired, therefore being non-hygienic methods of spending homework breaks.

CONCLUSIONS

More than half of the studied small school children from Oradea need 1-2 hours to prepare their homework, and are within the hygienic recommendations for this group age. The length of the homework preparation is significantly more reduced at the school children as the grade of study is lower.

A percentage of 93% of the school children prepare their homework after lunch during the optimum interval.

Half of the total school children from grades I-IV prepare their homework without breaks. Out of the ones who take breaks, half prefer to play computer games or to watch television.

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NEWS

ANTIDERMATOPHYTIC ACTIVITY OF *OTACANTHUS AZUREUS* ESSENTIAL OIL

In a study by Houël *et al*., the antidermatophytic activity of *Otacanthus azureus* (Linden) Ronse essential oil (EO) was tested alone and in combination with azole antifungals. The oil was extracted from aerial parts of the plant and its chemical composition was analyzed by gas chromatography/mass spectrometry. In vitro antifungal activity was evaluated by broth microdilution techniques and minimum inhibitory concentrations as low as 4µg ml^{-1} were obtained. The combined effect of *O. azureus* EO with azoles (ketoconazole, fluconazole, itraconazole) was analyzed using chequerboard assays, showing synergism against *Trichophyton mentagrophytes*. Also, cytotoxicity of O. *azureus* essential oil to VERO cells was low. In conclusion, the study highlights that *O. azureus* essential oil represents a promising antifungal agent for treatment of dermatomycoses (Houël E, Rodrigues AM, Jahn-Oyac AD et al. In vitro antidermatophytic activity of Otacanthus azureus (Linden) Ronse essential oil alone and in combination with azoles. *J Appl Microbiol*. 2013. doi: 10.1111/jam.12377).

Teodora Vremera

ETHICAL EVALUATION MODEL FOR TECHNOLOGIES. THE ROLE OF MEDICAL TECHNOLOGY IN THE DEVELOPMENT OF AUTONOMY IN DIABETES PATIENT

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ETHICAL TECHNOLOGY ASSESSMENT MODEL. ROLE OF HYEALTH TECHNOLOGY IN THE DEVELOPMENT OF AUTONOMY IN DIABETIC PATIENT **(**Abstract**):** Romanian Government Decision (GD) No. 8/2012 amending and supplementing GD No. 144/2010 regarding the function and organization structures of the Ministry of Health defines health technology assessment (HTA) as "a systematic and multidisciplinary analysis of the existing and new medical technologies, through which medical, economic, social, ethical and organizational information are synthesized so that medical technologies to be used in a transparent and unbiased manner". We propose an ethical assessment model of technologies used in the care of diabetic patients. The nature of this research was exploratory, giving the novelty of this approach to the clinical and social context of Romania. Material and Methods: The assessment of health technologies used in the care of diabetic patients was based on the following research question: What is the role of health technology in developing autonomy and responsibility in patients suffering from chronic diseases? Individual interviews and focus groups were held from June, 2011 to November, 2012 in Iasi. The criterion for selecting the participants was belonging to the target groups: family doctors or diabetes specialist, patients with type 1 (T1D) and type 2 diabetes (T2D), caregivers and other professionals involved in diabetes patient care. Results and conclusions: The diabetic patient benefits from a specific treatment and has the privilege of self-administering it, his life expectancy and quality of life depending upon the compliance and responsibility he demonstrates. **Keywords:** ETHIC EVALUATION, TECHNOLOGIES, AUTONOMY, DIABETES PATIENTS

Health Technologies (HT) are variously defined. One approach is based on interventions and defines HT as a wide range of products used to diagnose, monitor or treat a disease or medical condition, aimed at improving the quality of healthcare provided to patients with an early diagnosis, less invasive treatment options, and shorter hospital stay and recovery time (1,2). Another definition is focused on structure, HT being understood as "medical services, drugs, medical or surgical instruments and techniques used in health care as well as the organization and support care system provided" (2,3). Health technology assessment (HTA) is necessary for both economic and ethical reasons, HTA meaning "a comprehensive strategic research form (Policy Research) which explores the direct and indirect consequences, intended and unintended, short-and long-term technology applica-

tions, or a systematic evaluation of properties, effects and/or other impacts of technologies in health care" (2,3).

National legal framework regarding health technology assessment

Romanian Government Decision (GD) no. 8/2012 amending and supplementing GD no. 144/2010 regarding the organization and function of the Ministry of Health defines health technology assessment as "the systematic and multidisciplinary analysis of the existing and new medical technologies, through which the medical, economic, social, and ethical information on the use of health technologies are synthesized in a transparent and unbiased and manner " (GD 8/2012). The lawmaker believes that "the purpose of health technology assessment is to provide information support for the formulation of an effective, safe, patient-centred health policy and making decisions that ensure obtaining the greatest possible added value" (GD 8/2012). We draw attention on the approach specific to utilitarian ethics used by the lawmaker in HTA. This assessment is applicable to drugs, medical devices, equipment and advanced medical techniques, health policies, medical services and procedures, organization and management of health systems, including those covered by national health programs (GD 8/2012).

In Romania, the institutional HTA agency is the Ministry of Health which is the competent national authority in this field (GD 8/2012).

The principlism approach of ethical assessment of health technologies

Principlism was first formalized in a document called the Belmont report and the four bioethical principles proposed by Beauchamp and Childress. These four principles in the interpretation of Ion Mureşan (4) are:

1) autonomy: respecting the ability of autonomous individuals to make decisions, giving them the freedom to make choices regarding their life according to their conscience;

2) beneficence: requires weighing the benefits against costs and risks - including the duty of aiding others to promote their interests;

3) nonmaleficence: human subjects should not be harmed, the actions causing more harm than benefits being avoided;

4) justice: consists in a group of norms for fairly distributing benefits, risks and costs - fairly distributed medical goods and services (4,5).

Ion Mureşan shows that the four principles of Beauchamp and Childress are therefore the core of what the authors call "common morality", meaning fundamental moral norms shared by all morally serious persons in all places. Therefore this procedure was called "the four principle bioethical approach" or "principlism" (4).

The assessment proposed in the present research exclusively considers the autonomy principle and the role of health technologies in the development of autonomy and responsibility of chronic patient for his health. For Beauchamp and Childress (5) autonomy is self-empowerment outside the control and intervention of others which generates a meaningful choice. Autonomous, sovereign individuals are free to act according to their own plan (6). The two authors believe that the respect for the autonomy of patients and their healthcare-related choices is one of the four fundamental principles of bioethics (5).

Assessed health technologies

Of the definition of health technology in the Romanian law (GD no. 8/2012 amending and supplementing GD no. 144/2010 regarding the organization and functioning of the Ministry of Health) for the present study we selected the following:

a. drugs: insulin for insulin-dependent diabetic patients and antidiabetics for non-insulin-dependent diabetic patients;

b. medical devices: self-monitoring blood glucose devices, glucometers;

c. equipments and advanced medical techniques: insulin pumps;

d. health policies, medical services and procedures, organization and management of health systems, including those covered by national health programs (GD 8/2012), in this case - the National Diabetes Program.

The primary focus was the ethical parameters in close connection with the related social issues.

MATERIAL AND METHODS

The assumed paradigm of social-constructionist research is that social reality is a product of negotiation between the interpretations of social actors. The research consisted in phenomenological analysis and symbolic interactions based on the study paradigm (2).

The nature of this research was exploratory given the novelty of this approach to the Romanian clinical and social context. The ethical assessment of medical technologies used in the care of diabetes patients relied on the following question: What is the role of medical technologies in developing the autonomy and responsibility of chronic patients? Individual interviews and focus groups were held in Iasi from June, 2011 to November, 2012. The criterion for selecting the participants was that of belonging to the target groups: family doctors or diabetes specialist, patients with type 1 (T1D) and type 2 diabetes (T2D), caregivers and other professionals involved in diabetes patient care (2). Both female and male individuals were selected. Participants were selected with support from Iasi Diabetes Clinic and Family Doctors Association, and invitation directly sent by the organizers to the focus group. Snowball sampling was taken into account: the interviewed suggested interviewing other significant people (2). Interviewed persons were allowed to fully express their ideas without exceeding the thematic focus until they finished what they had to say. Each interview lasted 40-50 minutes, being recorded with the consent of the interviewees, and then transcribed. Research was conducted in compliance with the principles of proper conduct in scientific research (Romanian Law 206/2004), in agreement with national and international research ethics documents.

Validity: Given the generative exploratory nature of this research, sampling had not in view the validity criteria specific to a quantitative study, but the completeness of quantitative information generated by saturating the sample. The sample was considered saturated when no responses appeared significantly different in the new interviewed subjects. The research consisted in a case study conducted in the Iasi County**,** where the only diabetes clinic in Moldova (the Eastern region of Romania) is located (2). Data validity is limited to the specific discursive universe of diabetes specialists, family doctors and diabetes patients in Iasi County, but with at least a nationwide extrapolation potential given the similarities in diabetes clinical practice.

RESULTS

Health behaviour is recognized, articulated and formalized into a major problem for public healthcare programs (2). The new meaning of the term is related to the description of autonomous individuals who can choose whether to act in response to a disease or to maintain health (2). Individual behaviours were analyzed with a less deterministic grid, emphasizing the capacity of individuals to act (7, 8), make choices, and be autonomous in health-related matters (9, 10).

In the last two decades the new approach to health behaviour depicts the patient as a person making autonomous choices about how to act or not to act in relation to his health (2). The approach evolved conceptually from the autonomy seen as central value by Kant, through Mill's interpretation of autonomy as instrumental value to the modern theories on autonomy as authenticity, relational autonomy, informed consent, etc. (6).

A. Insulin and antidiabetic agents

Because chronic diseases require continuing medical care, they may raise the issue of care strategies effectiveness and of the balance doctor/patient involvement in the therapeutic process (12, 13). The participation of professionals and patients in the care process requires the patients to assume responsibility (14, 15), and care providers to provide information and autonomy support, as well as a collaborative process for optimizing long-term outcome (2, 16).

"*First of all, when your glucose level was good all day, do you feel good because you succeeded, you could, you ate, you injected insulin, made exercise, and did everything right? You feel good on the inside, not necessarily ... This and that helped you a lot, I will try to do the same tomorrow, if I was able do it today why shouldn't I do it again tomorrow ? And so, this motivates you the next day to succeed in achieving a good blood sugar level." (Diabetes patient)*

Respondents highlighted the improvements in insulin therapy over the last years.

"During Ceausescu's regime, when injection tools were rudimentary, insulin was injected using glass syringes and Chinese needles that got calcium, tartar and needed to be boiled daily. I had to learn because I was explained that otherwise I could die. (...) The only complaint might be related to a specific punctuality (needed) for insulin injections. Sort of constraint." (Diabetes patient)

Overcoming many drawbacks related to how to self-administer insulin and eliminate its constraints was mentioned as a progress in insulin therapy. In terms of principlism ethics, we are talking about a deeper beneficence of this therapeutic form. In the absence of insulin type 1 diabetics may die relatively shortly, thus therapeutic compliance is mandatory. The degree of inherent discomfort is minimized by eliminating the need for a tools kit. Instead of glass syringes with metal needles that had to be sterilized, disposable needles are used.

"I never perceived it as a constraint, but unlike now, but there was a very large bag of tools; carrying a pot and syringes was not exactly comfortable nor enjoyable and people who didn't have medical information or medical culture looked somewhat strangely at you. It is true that not many people knew that I was a diabetic because the impact on a stranger could get me upset, and spoil my or his mood, too. I often avoided these things." (Diabetes patient)

The ease of today self-treatment and self-monitoring allows the diabetic patient a level of privacy in his social environment.

This approach of individual health behaviours, which emphasizes the social agent skills, has such benefits as reducing costs and decreasing the pressure on the healthcare system, empowering individuals and improving their health, but raises other problems. Increased responsibility for self-care assumed by patients raise specific challenges for those with multiple problems, such as worsening of a problem by treating others (2, 11).

Patients with chronic disease face a range of needs which change their lifestyle and usually lead to behaviours that promote physical and psychological wellbeing, which requires them to modulate their relationship with healthcare providers and to adhere to medical treatment, to monitor their health condition, to make care-related decisions, to manage the impact of the disease on their physical, mental and social functioning (2, 11).

B. Glucometer: self-monitoring and self-management of care

The interviewed physicians emphasized that self-monitoring devices are accessible and provided free of charge.

"You'd rather concentrate on self-monitoring devices, glucometers and test strips, nowadays prescribed free of charge to all insulin-dependent patients, which automatically is a big step forward compared to the years when I started residency, the patients having to make a financial effort. Maybe that is more relevant, I do not know" (Diabetes doctor).

From an ethical standpoint, free of charge self-monitoring devices are an implementation of the principles of beneficence and equity.

"(Regarding) technologies, if you want to know what is really relevant for the diabetic population, in my opinion the daily self-monitoring devices rank first. I think these are the most representative for the ordinary diabetes patient." (Diabetes doctor)

Relevant technologies are viewed in terms of accessibility to patients with median income, in terms of their capability to produce maximum improvement in as many patients. A technology is assessed by doctors according not only to its intrinsic medical value, but from an ethical and social perspective, that is from the perspective of beneficence and utility.

"They should be used with maximum efficiency. (....). Patients go home and eventually the tests expire and they do not use them. A patient came to me with a blood sugar meter and I saw he used it only three times in the last three months. So many test strips payed by the government are wasted; it is a waste of money on people who did not understand well what they got or they go and offer tests to their grandmother, friends, neighbour, priest in his village, and to the teachers and ... who knows who else, or they sell them." (Diabetes doctor)

Doctors discussed how technology is used by patients, insisting on the fact that the importance of this tool in diabetes self-management is not understood. This leads to waste of resources and, in the opinion of respondents, to unfair, unequal allocation.

"I have few diligent patients; they keep a daily record of their blood glucose levels. ... The normal blood glucose level is 100 (mg/dl), but they have 350, 400, 420, day after day, but never once went to ask

the doctor: "Look, my blood glucose is more than 4 times greater". Why do we give these people tests?" (Diabetes doctor).

Physicians underlined the importance of patient responsibility for his health condition focusing on the usefulness of free of charge prescription of blood glucose measuring devices in circumstances of misuse by the patient. They also believe that the use of glucometer, even if daily, is not enough. Knowledge on insulin dose adaptation to blood glucose level is also required; a diabetic patient unable to do that has to ask for medical help.

Also, diabetes patients understand the importance of self-management, taking control of their long term conditions and lifestyle.

"The doctor told me to do this test before I eat certain foods I know to cause an increase in blood glucose level and to see what happens. For example, boiled potatoes, for sure a potato dish will increase my blood sugar no matter how I cook it. She told me to check my blood sugar before I eat potatoes; first eat two potatoes and check for an increase (in blood glucose level), than eat one potato and also check for an increase. Well, I don't have the patience to do that"(Diabetes patient).

One of the interviewed physicians focused on patient opportunity to take control of their care process through frequent blood glucose measurements. This increases the degree of patient autonomy, giving him the chance to make informed decisions, and take responsibility for the decisions he makes.

"It is a minister's order and it says: one hundred tests; if it were my decision if a patient is disciplined, he deserves more tests, maybe 150 or 200; if the patient is not disciplined, I would give him only one box - 50 tests or I would give him nothing at al." (Diabetes doctor).

Inequity occurs between T1 diabetes patients - receiving free of charge blood test for blood glucose, and T2 diabetes patients who do not receive the same kind of gratuity. Inequity is also seen in patients suffering from other diseases not included in national programs.

"There are cases in which T2 diabetes patients who do not take insulin do tests more often than those with T1 diabetes (...) Inequity with other diseases, here there are so many things free of charge and the benefit for some patients is questionable. But heart failure is also a disease, and cirrhosis is a disease and nothing is free of charge in their treatment" (Diabetes doctor.

C. Insulin pump. Medical technology and responsibility for health

Insulin pump is considered cost-prohibitive by interviewed doctors, so they do not consider it relevant for assessing the ethics of technology used in diabetic care.

"If a diabetic comes up with the idea that he wants a pump. Let's see if the pump suits you. Insulin pump is not a device that replaces the pancreas and determines the insulin dose according to blood sugar level. I eat as much as I want and the pump does the job. No. And this device requires sometimes pressing a button, some adjustments. Thus, sometimes it's maybe harder to use a pump than having some already fixed doses of insulin" (Diabetes doctor).

The interviewed physicians considered that the difficulties the diabetes patients are faced with in order to follow an appropriate lifestyle in precarious financial conditions as a main public health problem; in this context of pauperism, technologies, even

the very advanced ones, if available at high costs, are irrelevant for care management and more, they can lead to a number of access-related inequities.

"I do not know if insulin pump is the best example of technology due to the fact that insulin pump is a very expensive device, and automatically prohibitive for most patients" (Diabetes doctor).

D. The National Diabetes Program

Diabetes was identified as the main cause of worsening the problems generated by chronic diseases. WHO statistics allow an estimation of the problem in Romania (2). Thus, in recent years there has been a marked increase in the prevalence of diabetes in Romania, with an almost 5-fold increase compared to the number of cases recorded during the two decades interval 1989 to 2009 (WHO/Europe, European HFA Database, data known to July 2010). Since 2006, Romania has a national diabetes program (Source: Diabetes - The Policy Puzzle, Country overview, Romania, 2008), technically coordinated by the "N.C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Diseases (2). This Institute coordinates the annual monitoring and prevention of diabetes complications, self-monitoring of diabetes patients, access to specific treatments, including insulin pump; the Institute is also the administrator of the National Diabetes Register.

"... let's take a bad example related to technologies: the patient may not realize, as in many other situations, that if something is given free of charge it does not mean that there is no financial effort behind what is offered to him, and I think this a problem with many of our patients. Maybe, sometimes we cannot explain quite clearly, maybe we cannot make ourselves understood, however that stuff costs something, but there is a degree of indifference to what is given free of charge." (Diabetes Doctor)

From an ethical standpoint, the National Diabetes Program is tributary to the utilitarian model of care.

"...creates a new type of addiction, because the diabetic patient becomes addicted to a right he does not use properly, as intended, and cannot be monitored outside medical units. What is he doing, how much is he really doing." (Diabetes doctor)

Respondents pointed out the risk of creating a relation of dependency on healthcare system instead of mobilizing patient's resources to increase self-care management.

National Diabetes Program is considered absolutely necessary for patients, in the absence of free insulin their life being threatened. This Program also compensates patients' socio-economic vulnerabilities.

"Let's hope it will not undergo changes …otherwise… it would be a great misfortune, at least for those (patients) with type 1 diabetes, it will be disastrous if test will not be prescribed free of charge because only few have the financial means to buy their own tests." (Diabetes doctor)

The components of the National Diabetes Program are prevention and control of diabetes, insulin and oral hypoglycaemic agents being provided free of charge. According to the program, family doctors are responsible for the care of noninsulin-dependent diabetics, and diabetes doctors for the insulin-dependent patients. A check-up with the diabetes doctor is required every three months for each diabetes patient, being carried out in the county diabetes centres.

The interviewed doctors emphasized

how important is the patients to be aware of the value of health technology and of the costs of freely provided devices and consumables through the National Diabetes Program.

Beside its medical objective, as underlined by diabetic patients, the National Diabetes Program also has a social dimension.

"At the end of the month, when all my pension money are spent and I have no more tests I go to the inpatient diabetes clinic ... because I have no more money to buy food. Do you believe me? It happened to me last month, and they also did blood glucose tests. I go there to get artificial feeding (intravenous glucose administration) because I have no money for food and if I do not eat I get into ketosis. I can live without food, but last month I lost very much weight, but now I am somewhat better. I have to eat at least once a day, they give me glucose and buffered insulin to avoid other complications, this doesn't seem normal to me." (Diabetes patient)

CONCLUSION

Our findings show that people with chronic illnesses develop autonomy through various relationships in the unique context of their life, social interactions, including mutual reactions of thoughts, feelings, concerns and habits, autonomy being negotiated during daily activities both in professional and family relationships (17).

As shown in the literature, the factors influencing patient autonomy are: health status/associated diseases (co morbidity); treatment; knowledge (about their disease); experience and skills; the personal way of approaching the disease; family patterns; type of relationships with others; history/life experience; social context (2,17). Self-management in diabetes (and other chronic diseases) relies more heavily on medical technology to monitor behaviour and/or biomedical parameters. These technologies are part of the psychosocial aspect of daily life (18).

Our study also shows that the patients suffering from diabetes mellitus have a high degree of autonomy given the type of treatment they receive and the possibility of disease self-management.

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CONTROLLED OVARIAN HYPERSTIMULATION AND IVF PREGNANCY PROGNOSIS

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CONTROLLED OVARIAN HYPERSTIMULATION AND IVF PREGNANCY PROGNOSIS (Abstract): IVF (*in vitro* fertilization) often represents the ultimate step in our attempt to treat infertility. Nowadays this method is more and more used, couple's expectations are higher and higher, so that optimizing the chance that one IVF pregnancy became a healthy newborn child is becoming an important issue. In this study we analyzed the controlled ovarian hyperstimulation impact on IVF pregnancy prognosis. We studied 307 patients who had IVF fresh procedures in "Panait Sîrbu" Clinical Hospital of Obstetrics and Gynecology - Department of Assisted Human Reproduction between 01.01.2008 – 31.12.2010 and obtained pregnancy; pregnancy rate according to our statistics is 34%. For statistic analysis we used the odd Student test. Our data proved better results (defined as a better birth/ abortion ratio) for the patients where recombinant FSH was used towards patients where highly purified urinary gonadotropin or the combination of the two drugs was used. Our statistical data also proved that none of the variables: age, BMI (body mass index), endometrium thickness, number of oocytes, number of embryos or number of days of stimulation is a favorable variable for this better result, but the number of units of gonadotropin is a favorable variable for a better birth/ abortion ratio. We consider that our data gives an interesting perspective upon the connection between controlled ovarian hyperstimulation and pregnancy outcome, especially these days when tendencies are to obtain as many oocytes in one procedure as we can, which often means to use high doses of gonadotropin. **Keywords:** *IN VITRO* FERTILIZATION, CONTROLLED OVARIAN HYPER STIMULATION, PREGNANCY, PROGNOSIS.

Nearly 13-15% of reproductive aged couples are infertile (1). Infertility is a real health problem; it is always associated with emotional stress and affects couple's social life and work. This means a long term impact on society because it affects family stability on one hand, and on the other hand treatment is often expensive and consumptive.

In vitro fertilization (IVF) often represents the ultimate step in our attempt to treat infertility. Nowadays this method is more and more used; couple's expectations are higher and higher, so that optimizing the chance that one IVF pregnancy became a healthy newborn child is becoming an important issue (2, 3). One of the most important adverse outcomes attributed to IVF pregnancy is a high incidence of spontaneous abortion rate: 25 – 35% (4, 5).

In our attempt to understand and minimize the IVF pregnancy spontaneous abortion rate we analyzed the qualitative and quantitative impact of controlled ovarian hyper stimulation during IVF procedures

on pregnancy outcome, defined as the birth/ abortion ratio.

MATERIAL AND METHODS

We studied 307 patients who had IVF fresh procedures in "Panait Sîrbu" Clinical Hospital of Obstetrics and Gynecology, Bucharest - Department of Assisted Human Reproduction between 01.01.2008 – 31.12.2010 and obtained pregnancy; pregnancy rate according to our statistics is 34%. Patients were divided in 3 major groups according to the medication used for controlled ovarian hyperstimulation (recombinant follicle-stimulating hormone (FSH), highly purified urinary gonadotropin and the combination of the two drugs) and we analyzed the birth/ abortion rate for all the 3 groups and the impact on the result obtained of a few variables: age, body mass index (BMI), endometrium thickness, number of oocytes retrieved, number of embryos obtained, number of days of stimulation and number of units of gonadotropin used for ovarian hyperstimulation per day. All patients had normal basal FSH and normal antral follicular count. All patients had long or short protocols for ovarian hyperstimulation. For statistic analysis we used the odd Student Test.

RESULTS

From the 307 patients who obtained pregnancy by IVF fresh procedures, 226 patients gave birth to one or more newborns (73.6%) and 81 patients suffered an abortion in the first or second trimester (26.4%) (fig. 1).

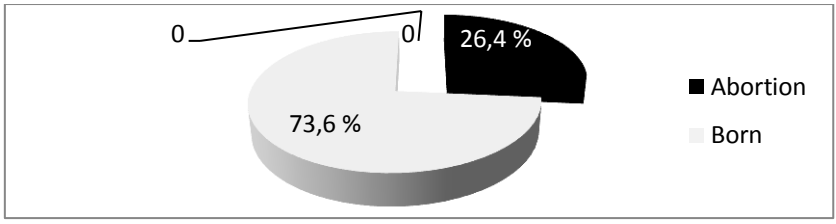

Fig. 1. Pregnancy outcome after IVF fresh procedures

Our first intention was to see if there is any difference between the group of patients whose pregnancies ended with birth and the group of patients whose pregnancies ended with abortion.

Student Test for patients' age, for the patients whose pregnancies ended with birth (group A) towards those whose pregnancies ended with abortion (group B) is presented below (fig. 2.).

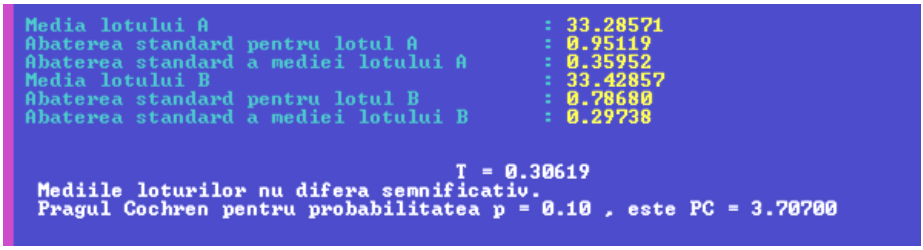

Fig. 2. Student Test for patients' age for patients who obtained pregnancies after IVF fresh procedures.

Average age for the patients who's pregnancies ended with birth (group A = 33.3 years) is not statistically different from average age for the patients who's pregnancies ended with abortion in the first or second trimester (group B = 33.4 years); p > 0.1 and T = 0.31.

Student Test for BMI, for the patients whose pregnancies ended with birth (group A) towards those whose pregnancies ended with abortion (group B) is presented below (fig. 3).

Fig. 3. Student Test for patients' BMI for patients who obtained pregnancies after IVF fresh procedures.

Average BMI for the patients who's pregnancies ended with birth (group A = 22.14 kg/m^2) is not statistically different from average BMI for the patients who's pregnancies ended with abortion in the first or second trimester (group B = 22.7 kg/m^2); p > 0.1 and T = 1.29.

Student Test for endometrium thickness, for the patients who's pregnancies ended with birth (group A) towards those who's pregnancies ended with abortion (group B) is presented below (fig. 4).

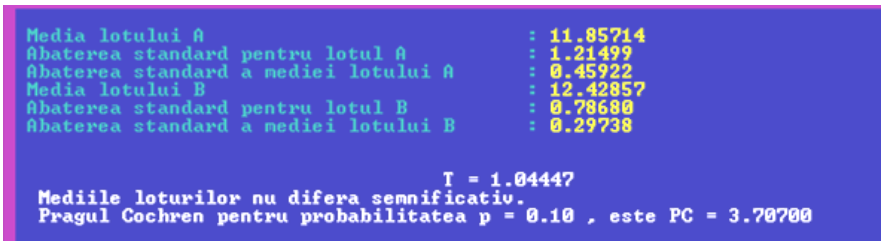

Fig. 4. Student Test for endometrium thickness for patients who obtained pregnancies after IVF fresh procedures.

Average endometrium thickness for the patients who's pregnancies ended with birth (group A = 11.8 mm) is not statistically different from average endometrium thickness for the patients who's pregnancies ended with abortion in the first or second trimester (group B = 12.43); p > 0.1 and T = 1.04.

Student Test for the number of international units (IU) of gonadotropin used per day for ovarian hyperstimulation, for the patients who's pregnancies ended with birth (group A) towards those who's pregnancies ended with abortion (group B) is presented below (fig. 5).

Average number of units of gonadotropin used for ovarian hyperstimulation for the patients who's pregnancies ended with birth (group A = 183.5 IU) is not statistically different from average number of units of gonadotropin used for the patients

who's pregnancies ended with abortion in the first or second trimester (group B = 201.0); p > 0.1 and T = 0.79.

Student Test for the number of days of hyperstimulation for the patients who's pregnancies ended with birth (group A) towards those who's pregnancies ended with abortion (group B) is presented below (fig. 6).

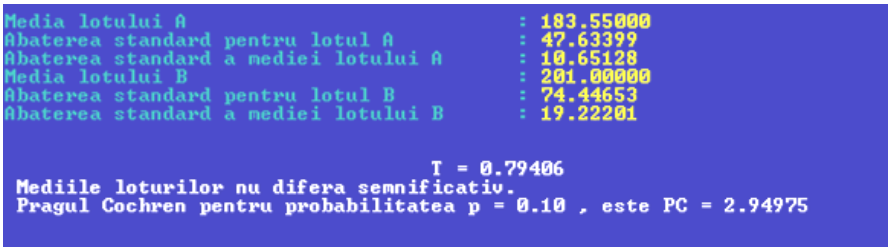

Fig. 5. Student Test for number of units of gonadotropin used for ovarian hyperstimulation for patients who obtained pregnancies after IVF fresh procedures.

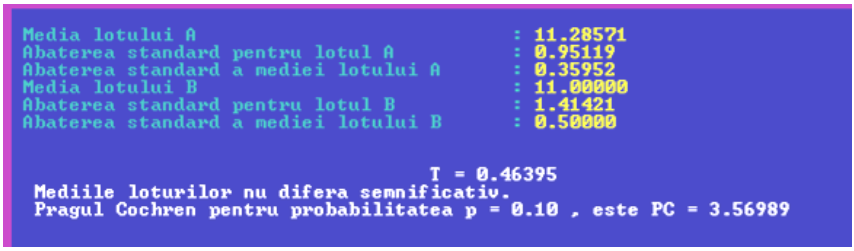

Fig. 6. Student Test for number of days of ovarian hyperstimulation for patients who obtained pregnancies after IVF fresh procedures.

Average number days of ovarian hyperstimulation for the patients who's pregnancies ended with birth (group A = 11.3 days) is not statistically different from average number of days of ovarian hyperstimulation used for the patients who's pregnancies ended with abortion in the first or second trimester (group B = 11.0 days); p > 0.1 and T = 0.46.

Student Test for the number of oocytes obtained from the patients who's pregnancies ended with birth (group A) towards those who's pregnancies ended with abortion (group B) is presented below (fig. 7).

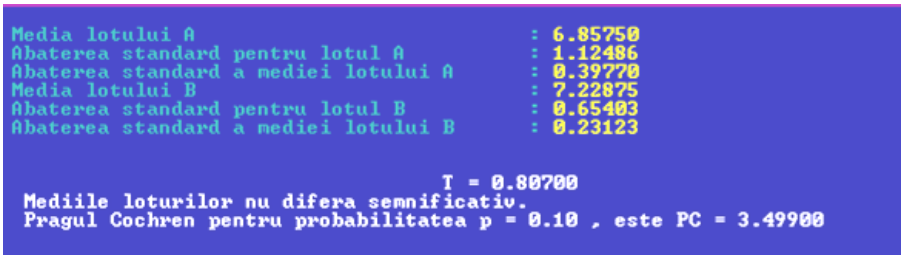

Fig. 7. Student Test for number of oocytes retrieved from patients who obtained pregnancies after IVF fresh procedures.

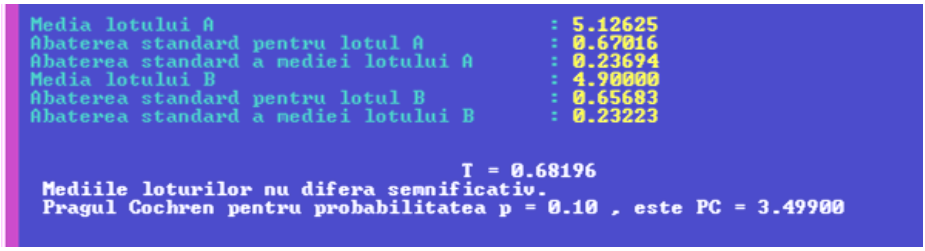

Fig. 8. Student Test for the number of embryos from patients who obtained pregnancies after IVF fresh procedures.

Average number of embryos from the patients whose pregnancies ended with birth (group A = 5.13) is not statistically different from average number of embryos obtained from the patients who's pregnancies ended with abortion in the first or second trimester (group B = 4.9); $p > 0.1$ and $T = 0.68$.

Student Test for the common variables of the two groups (age, BMI, endometrium thickness, number of oocytes retrieved, number of embryos obtained, number of days of stimulation and number of international units of gonadotropin used per day) demonstrated that the average values of these variables are not significantly different.

Our next step was to divide the 307 group of patients according to the medication used for controlled ovarian hyperstimulation: 157 (51.1%) patients received recombinant FSH – group A, 42 (13.7%) patients received recombinant FSH and highly purified human gonadotropin – group B, 106 (34.5%) patients received highly purified human gonadotropin – group C, in 2 (0.7%) patients natural cycle was used – group D and in 1 (0.33%) patient we used a combination of clomiphene, highly purified human gonadotropin and recombinant FSH – E. (fig. 9).

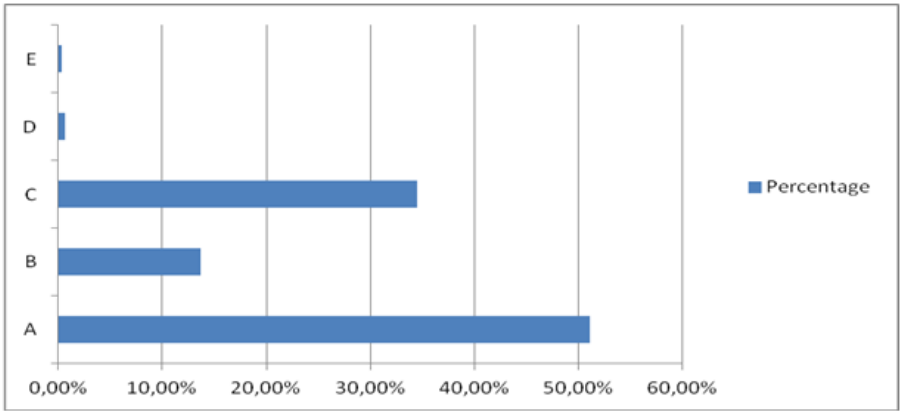

Fig. 9. Patients' distribution according to the medication used for ovarian hyperstimulation

We analyzed only the three groups of patients where the number of patients was good enough for the statistics (A, B and C). We calculated for each group the birth/abortion ratio and we considered the best ratio as the best pregnancy outcome.

For the group of patients who received recombinant FSH for ovarian hyperstimulation the birth/ abortion rate was 3.24 (FSH group). For the group of patients who received highly purified human gonadotropin for ovarian hyperstimulation the birth/ abortion rate was 2.31 (HPHG group) and for the group of patients who received both medications the birth/ abortion rate was 2.73 (FSH + HPHG group). These data point out that the best ratio (3.24) was for the group of patients where recombinant FSH was used, followed by FSH + HPHG group (2.73), the worst result being registered for the HPHG group (2.31) (fig. 10).

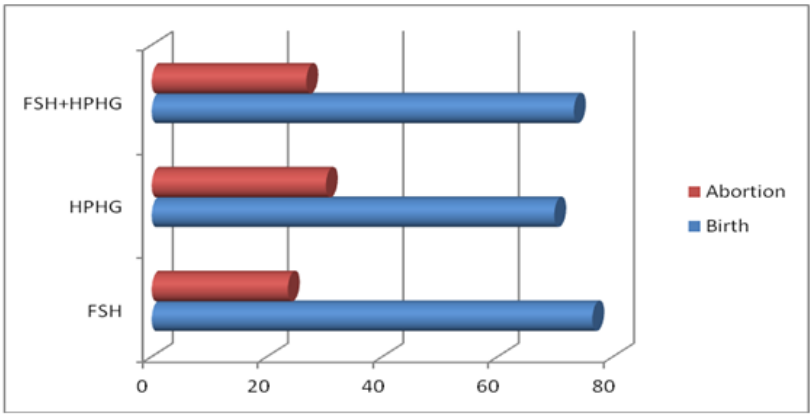

Fig. 10. Percentage distribution of pregnancies (birth and abortion) according to the medication used for ovarian hyperstimulation

The next step was to determine if there is any favorable variable for this result. We used the odd Student Test to calculate the average value for all the variables considered (age, BMI, endometrium thickness, number of oocytes retrieved, number of embryos obtained, number of days of stimulation and number of units of gonadotropin used per day) for all the 3 groups of patients (FSH, HPHG and FSH+HPHG) (tab. I).

TABLE I

Mean variables values according to the medication used for ovarian hyperstimulation

Variable	Unit of measure	FSH	HPHG	FSH + HPHG
Age	Years	33.39	33.78	34.19
BMI	kg/ m^2	22.48	22.72	22.22
Endometrium thickness	mm	11.98	12.01	11.8
Number of oocytes retrieved	-	7.3	6.56	7.05
Number of days of stimulation	-	11.01	11.27	11.8
Number of embryos obtained	-	5.18	4.94	5.18
Number of units of gonadotropin/ day	IU	167.15	215.8	205.66

Student test for the variables: age, BMI, endometrium thickness, number of oocytes retrieved, number of days of stimulation and number of embryos obtained for the

patients in the FSH and HPHG groups indicated that the mean values of these variables are not statistically significantly different.

Student test for the variables: age, BMI, endometrium thickness, number of oocytes retrieved, number of days of stimulation and number of embryos obtained for the patients in the FSH and FSH+HPHG groups indicated that the mean values of these variables are not statistically significantly different.

Student test for the variables: age, BMI, endometrium thickness, number of oocytes retrieved, number of days of stimulation and number of embryos obtained for the patients in the HPHG and FSH+HPHG groups indicated that the mean values of these variables are not statistically significantly different.

Student Test for the number of international units of gonadotropin used for ovarian hyperstimulation for the patients in the FSH group (group A) towards number of international units of gonadotropin used for ovarian hyperstimulation for the patients in the HPHG group (group B) is presented in fig. 11.

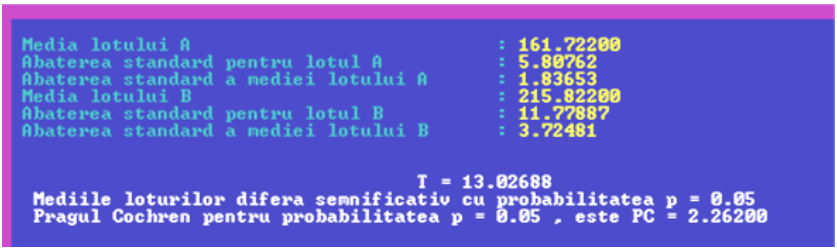

Fig. 11. Student Test for the number of units of gonadotropin used for ovarian hyperstimulation for the patients in the FSH group towards patients in the HPHG group.

Average number of units of gonadotropin used for ovarian hyperstimulation in the FSH group (group A = 161.7 IU) is statistically significantly different from the average number of units of gonadotropin used for the HPHG group (group B = 215.8 IU) for p = 0.05.

Student Test for the number of international units of gonadotropin used for ovarian hyperstimulation for the patients in the FSH group (group A) towards number of international units of gonadotropin used for ovarian hyperstimulation for the patients in the FSH+HPHG group (group B) is presented in figure 12.

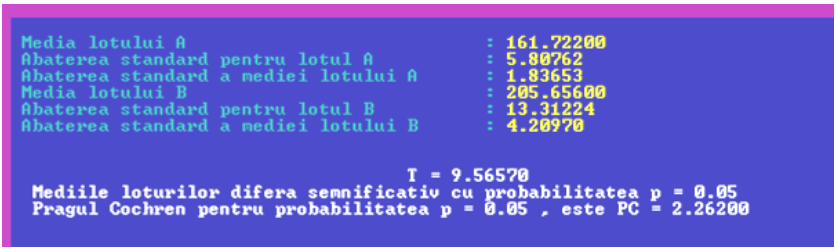

Fig. 12. Student Test for the number of units of gonadotropin used for ovarian hyperstimulation for the patients in the FSH group towards patients in the FSH+HPHG group.

Average number of units of gonadotropin used for ovarian hyperstimulation in the FSH group (group A = 161.7 IU) is statistically significantly different from the average number of units of gonadotropin used for the FSH+ HPHG group (group B = 205.66 IU) for p = 0.05.

Student Test for the number of international units of gonadotropin used for ovarian hyperstimulation for the patients in the HPHG group (group A) towards number of international units of gonadotropin used for ovarian hyperstimulation for the patients in the FSH+HPHG group (group B) is presented in fig. 13.

Average number of units of gonadotropin used for ovarian hyperstimulation in the HPHG group (group A = 216.24 IU) is not statistically significantly different from the average number of units of gonadotropin used for the FSH+ HPHG group (group B = 205.66 IU) for p >0.1.

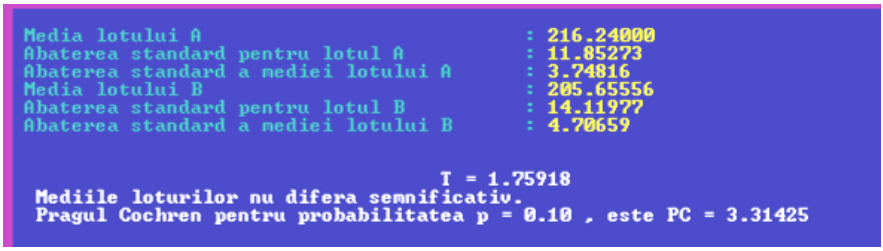

Fig. 13. Student Test for the number of units of gonadotropin used for ovarian hyperstimulation for the patients in the HPHG group towards patients in the FSH+HPHG group**.**

DISCUSSION

The data presented above pointed out that apparently IVF pregnancy prognosis, defined as the best birth/ abortion ratio is not dependent on variables such as : age, BMI, endometrium thickness, number of oocytes retrieved, number of days of stimulation, number of embryos obtained or number of units of gonadotropin used per day for ovarian hyperstimulation.

Still, when we analyzed the best birth/ abortion ratio according to the medication used for ovarian hyperstimulation we could see that the best result was obtained when recombinant FSH was used. Different variables were analyzed and the number of units of gonadotropin used per day for ovarian hyperstimulation was found to be a favorable variable for a better IVF pregnancy outcome. Statistics pointed out that less the number of units of gonadotropin used per day, better the birth/ abortion ratio for the patients where recombinant FSH was used (fig. 14).

Nowadays there are many attempts to identify any parameter that could improve IVF clinical outcome, defined as live birth rate or “baby taken home” rate. Regarding controlled ovarian hyperstimulation recent studies demonstrated the optimal number of oocytes to maximize the live birth rate (6), but few studies focused on the number of units of gonadotropin used per day. One of our previous small studies pointed out the possibility that less number of units of gonadotropin per day could mean better chances for a good pregnancy prognosis (7).

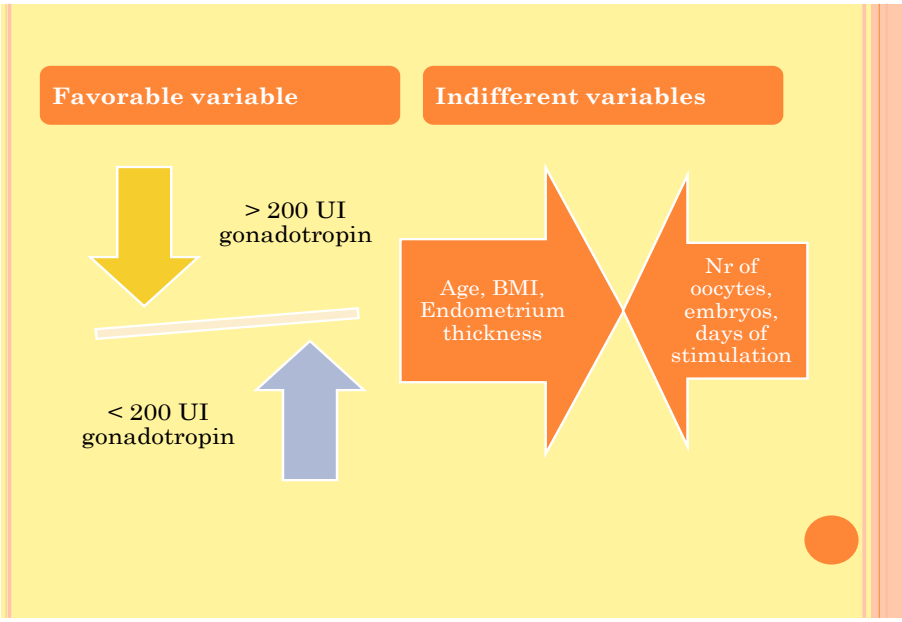

Fig. 14. Favorable variable for the best birth/ abortion rate.

CONCLUSION

The best IVF pregnancy prognosis (the best birth/ abortion ratio) was obtained when recombinant FSH was used. The majority of variables used in this study (age, BMI, endometrium thickness, number of oocytes retrieved, number of embryos obtained, number of days of ovarian hyperstimulation) are not favorable variables for this result, but the number of units of gonadotropin used per day for ovarian hyperstimulation seems to play an important role: less units of gonadotropin for the FSH group seems to be associated in our study group to a better birth/ abortion rate.

We consider that our data gives an interesting perspective upon the connection between controlled ovarian hyperstimulation and pregnancy outcome, especially these days when tendencies are to obtain as many oocytes in one procedure as we can, which often means to use high doses of gonadotropin.

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NEWS

BREAST CANCER RISK AND LIPID METABOLISM GENES

The breast cancer is an important pathology for women. The identification of biomarkers to highlight and differentiate between the estrogen receptor subtypes of breast cancer (sensitive or insensitive to hormones) would enable the adoption of prevention methods specific to each type of breast cancer. According to data published in Cancer Prevention Research, some genes related to lipid metabolism are related also with risk for hormone receptor-positive or hormone receptor-negative breast cancer. Khan and colleagues have analyzed by Illumina expression array and quantitative real-time PCR, the samples obtained by fine needle aspiration on the unaffected breast of women with unilateral breast cancer. The researchers identified 18 genes with high expression levels in samples from estrogen receptor-negative cases. They show that 4 genes (DHRS2, HMGCS2, HPGD and ACSL3) associated with lipid metabolism are a significant overexpression in estrogen receptor-negative cases. Another 2 genes (UGT2B11 and APOD) associated with lipid metabolism have a lower expression in estrogen receptor-positive cases. The results suggest that certain lipid metabolism genes may be potential biomarkers of different subtypes of breast cancer (Wang J, Scholtens D, Holko M, Ivancic D, Lee O, Hu H *et all*. Lipid metabolism genes in contralateral unaffected breast and estrogen receptor status of breast cancer. *Cancer Prev Res*, 2013; 6(4): 321–30).

Cătălina Luncă

RESISTANT HYPERTENSION: THE ROLE OF INTERVENTIONAL THERAPY

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RESISTANT HYPERTENSION: THE ROLE OF INTERVENTIONAL THERAPY (Abstract): Resistant hypertension still represents a major health problem, incompletely resolved by the current therapeutic interventions. Based on the interference of sympathetic over activity in resistant hypertension, novel invasive strategies such as sympathetic renal denervation and carotid baroreceptor stimulation have recently emerged. Despite the promising results and their good tolerability profile, the optimal role of these non-pharmacologic therapies relative to conventional medical regimens, remains unknown. Further investigation is also necessary to establish their real long-term efficacy and safety in clinical practice. **Keywords**: RESISTANT HYPERTENSION, INTERVENTIONAL THERAPY, SYMPATHETIC OVERACTIVITY, RENAL DENERVATION, BARO-REFLEX STIMULATION.

Arterial hypertension is one of the most common worldwide diseases of this century, characterized by an alarming percentage of patients with uncontrolled blood pressure (BP) values. It was estimated that 25% of adult population had arterial hypertension in 2005, and this figure is predicted to rise to 1,5 billion by 2025 (1). There is a linear relationship between BP and cardiovascular risk, accounting for 13.5% premature deaths, 54% of all cases of stroke and 47% of ischemic coronary events in worldwide statistics (2, 3). Despite the availability of various effective antihypertensive agents, target BP is not achieved in a large number of subjects.

In current guidelines, arterial hypertension is defined as resistant to treatment when the concurrent use of at least three antihypertensive drugs (mandatory a diuretic) in appropriate schemes and at maximum doses fails to optimally lower BP (4). The target values are different according to the type of hypertensive patient: < 140/90 mm Hg in general or < 130/80 mm Hg in high risk hypertension (diabetics, patients with chronic renal or ischemic coronary disease etc). The importance of the phenomena is highlighted by the prevalence of 15-20% reported in literature (5). In fact the prevalence of resistant hypertension ranges from 5% to 50% depending on the studied population or hypertension centre. For this reason the exact prevalence is difficult to determine (6-9). The direct consequences of raised BP values, respectively the significant percentage of target organ damage and particular metabolic profile, set patients at high risk for major cardiovascular events, thus making resistant hypertension a clini-

cal and epidemiological priority (10).

In clinical practice, the workup of suspected resistant hypertension involves the following steps (11):

1. to prove failure in controlling BP values irrespective of the type of measurement used: office BP, home BP or 24-hour ambulatory monitoring;

2. to differentiate true resistant hypertension from pseudoresistance caused by BP measurement artifact, white-coat effect, physician inertia, pseudohypertension, poor patient adherence to prescribed therapy because of lack of education/information or treatment regimen (inappropriate/unaffordable combinations, frequent changes in therapeutic scheme, adverse effects, high cost of medication);

3. to establish the potential causes of resistance related to:

a. medication: inadequate diuretic therapy, drug interaction (non-steroidal anti-inflammatory, corticosteroids, anabolic drugs, erythropoietin, oral contraceptives, cyclosporin, tricyclic antidepressants sympathomimetics, cocaine, amphetamines etc), inappropriate combinations/dosing, rebound effect (e.g. for clonidine), adverse effects (reflex vasoconstriction, hydro-saline retention);

b. comorbid conditions: excessive salt intake > 10 g/day, alcohol abuse, smoking>20 cigarettes/day, chronic renal disease, obesity, diabetes mellitus, sleep apnea, elderly, panic attacks, chronic disease causing pain) or ignored secondary causes of hypertension, most common renal parenchymal/renovascular disease, primary aldosteronism.

Along these lines, hypertension can be considered as true resistant and treated according this judgment only after completing the previous analysis. The complex therapeutic schemes and the new classes of antihypertensive drugs are the common option for treatment. However, it is believed that about 10% of patients will continue to be resistant although on 6-7 antihypertensive drugs (12). According to the current recommendations, if arterial hypertension was labeled as true resistant to treatment and subsequent therapy was correctly and completely applied (first and second steps), interventional therapy, which represents the third step, can be considered (13).

PHYSIOPATHOLOGICAL BASIS FOR INTERVENTIONAL THERAPY

The interference of sympathetic over activity, which is shown to be present in most cases of resistant hypertension with no secondary cause, is fundamental for developing the interventional concept (14). Based on the demographic data of Framingham and ALLHAT studies, a particular "phenotype" in resistant hypertension is suggested, in which older age, obesity, aldosterone excess and obstructive sleep apnea are covering most part of patients characteristics. It is well documented that sympathetic nervous system (SNS) activity increases with aging, especially in skeletal muscles (15). Secondly, aldosterone excess, obesity, and obstructive sleep apnea are not only common co morbid conditions in resistant hypertension, but also mechanisms which actively interact. Although not fully elucidated, SNS activation may be a major contributing factor to these interactions.

By using sophisticated techniques for the measurement of sympathetic nerve fibers activity or excess plasma norepinephrine, it was clearly documented that in normotensive obese patients efferent sympathetic fibers to the kidneys and skeletal muscle vasculature are overactive, normal to the skin and hepatomesenteric circulation, and

reduced to the heart. In obese hypertensive patient this kind of heterogeneity is depressed, while the suppression of cardiac efferent sympathetic fibers disappears. It is the visceral adipose tissue distribution that seems to be crucial for SNS activation (16). The discussed mechanisms are extremely complex, including high plasma leptin concentration, hyper-insulinemia, decreased arterial baroreflex sensitivity, but also bidirectional interaction with renin-angiotensin-aldosterone system (RRAS) which promotes inflammation, endothelial dysfunction, arterial stiffness (16-18). The increased variability of BP is the consequence of arterial stiffness, but is also a clinical marker for intensive sympathetic traffic, with negative impact on prognosis (19).

Obstructive sleep apnea, common in obesity, is also independently related to the occurrence of resistant hypertension. Although the mechanisms are elusive, the causal relationship between sleep apnea and SNS overactivation in skeletal muscle is postulated. Sympathetic stimulation during sleep and transient hypoxemia would provide the causal connection. The data are pertinent for hypoxemia, which is clearly involved in tonic chemoreflex activation of sympathetic inflows. The up regulation of endothelin A and angiotensin II receptors in the carotid body by hypoxia supports the role of these molecules as peripheral chemoreceptors and central neuromodulators (20-22).

Aldosterone excess has a reported prevalence of 17-22% in resistant hypertension (23), and the mechanism of SNS activation is becoming increasingly clear. It is experimentally documented that mineralcorticoid receptors in the paraventricular nuclei are closely linked to central sympathetic overactivity (24, 25).

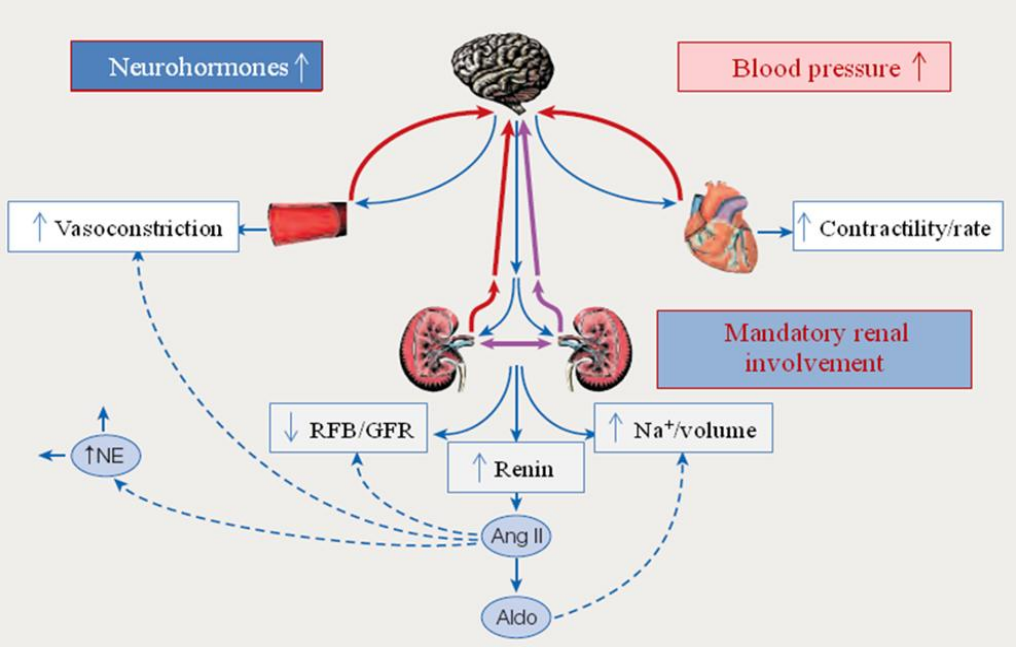

Fig. 1. Effect of SNS on BP regulation (adapted from 12). RBF – renal blood flow, GFR – glomerular filtration rate, NE – norepinephrine, Aldo – aldosterone, Ang II – angiotensin II

The role of renal sympathetic nerves lying in the adventitia of renal arteries is crucial for SNS activation. Efferent sympathetic fibers from the central nervous system activate the RRAS in juxtaglomerular apparatus of the kidneys via $ß_1$-adrenergic receptors, resulting in renin release. There is a concomitant direct stimulation of sodium and fluid reabsorption via α_2-adrenergic receptors, followed by renal vasoconstriction and a consecutive reduction in renal blood flow. Efferent sympathetic activation is linked to the development of insulin resistance, responsible for hyperinsulinemia and metabolic syndrome in over 50% of cases with resistant hypertension. At the same time, afferent sympathetic fibers maintain the central nervous system stimulation with subsequent vasoconstriction, direct structural and functional cardiac effects, hepatic gluconeogenesis (12) (fig. 1).

SYMPATHETIC RENAL DENERVATION

The sympathetic innervations of the kidneys are achieved through a dense network of postganglionic neurons which arise from the dorsal root T_{10}-L_1. The postganglionic nerves run alongside the renal arteries and penetrate the cortical and juxta medullary areas. The activation of these nerves is responsible for deleterious effects on renal hemodynamics. Renal afferent sensory nerves derive from renal pelvis and contain mechanoreceptors which respond to stretch and chemoreceptors which detect renal ischemia, hypoxia and other chemical injuries. From kidneys, the signal travels to the dorsal root ganglia T_6-L_4 and hypothalamus, area responsible for vasopressin and oxytocin release with subsequent modulation of BP level and vascular resistance (26). Electrical stimulation followed by BP elevation, mesenteric ischemia and muscle vasoconstriction is a model for afferent sympathetic fibers action. Conversely, renal afferent sympathetic denervation attenuates the effects, delays or prevents arterial hypertension in several experimental models (13).

In 1930, the overcoming of renal sympathetic stimulation consequences is first reported.

Supradiaphragmatic splanchnicectomy or radical surgical sympathectomy was applied as the last therapeutic options for malignant hypertension, resulting in over 70 mm Hg decrease of BP (12). Despite the spectacular results observed in more than 1000 hypertensive patients, including a parallel improvement of target organ damage, the surgical technique was driven to total obscurity due to severe postoperative complications (27). However, the concept was then developed in experimental studies.

The current therapeutic option is interventional renal denervation, a minimally invasive percutaneous procedure characterized by a fast effect on BP level and absence of significant systemic adverse effects (13, 26, 27). The technique requires renal angiography to assess anatomic eligibility for the procedure and to exclude renal artery stenosis. Denervation is performed using a special flexible catheter (Simplicity TM Catheter System, Ardian/Medtronic Inc.) placed into the distal segment of renal artery, and connected through its proximal end to a radiofrequency generator for ablation (27, 28). Bilateral percutaneous denervation can be accomplished in about 40-60 minutes, after at least six ablations for each renal artery (fig. 2).

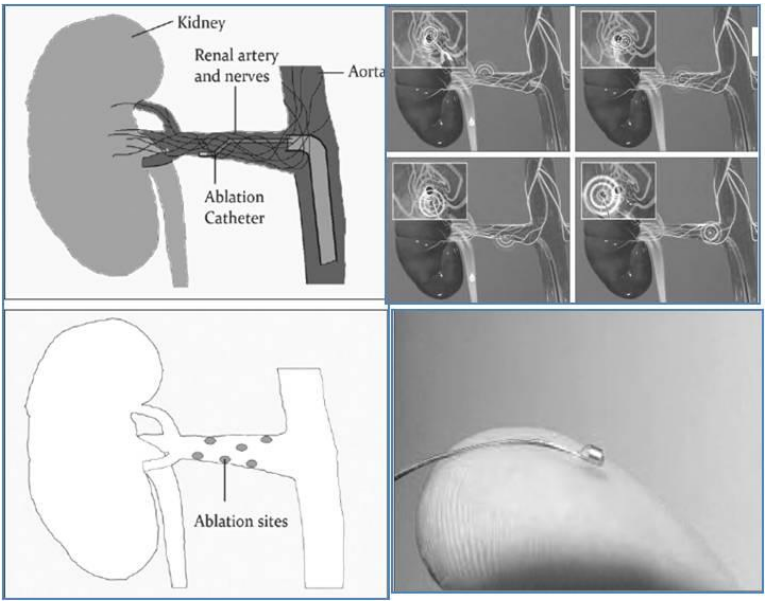

Fig. 2. Technical principle of circumferential sympathetic renal denervation using radioablation (adapted from 12, 26)

The clinical efficacy of the procedure has been proven in Symplicity Hypertension trials (HTN-1 and HTN-2) (29-31). In HTN-1 trial which included 45 patients with resistant hypertension on 5-6 antihypertensive drugs, percutaneous renal denervation induced a progressive and sustained BP decrease. After two years, mean BP was reduced by 32/14 mm Hg. The procedure was considered safe, with no complications in 97% of patients, and stable renal function. HTN-2 trial assessed 106 patients and demonstrated similar rates for BP reduction (by 32/12 mm Hg) at 6 months, with no apparent change in renal function or other significant complications.

For several reasons, the open question remains estimation of long-term procedural efficacy. First of all, renal reinnervation phenomena have been observed in experimental models, and this may attenuate procedural effect (12, 32). Secondly, ambulatory BP monitoring was performed in a limited number of patients in HTN-2 trial, and showed no significant reductions compared to office or home measurements (13). Cardio-circulatory adaptation to exercise is another problem to be evaluated, taking into account the unclear response of these patients and because SNS functional integrity is required. Finally, estimation of the benefit on cardiovascular risk may be necessary, assessing target organ damage outcome, metabolic profile, major cardiovascular events and mortality.

A successful method is subject to compliance with the eligibility criteria. The main criteria for an appropriate application of the method are an accurate practice of the first two steps for analysis and treatment of resistant hypertension, pre-procedural renal artery imaging for assessment of anatomic contraindications (e.g. CT or magnetic resonance renal angi-

ography), and a GFR > 45 ml/min/1.73 m^2. A complete sympathetic denervation is also necessary for the procedural success. From this point of view, any preprocedural efficient marker is currently missing and this could be the answer for some patients which are "nonresponders" or experience an unsatisfactory response. (33). Recently, Steigerwald and coworkers have conducted a morphological assessment of renal arteries after denervation in a porcine model. The authors have proposed optical coherence tomography imaging as a useful method for assessment of postprocedural success and immunostaining technique for neurofilament proteins detection as a relevant surrogate marker for postprocedural nervous viability (34). Finally, it is recommended the method to be performed only in very experienced centers.

A current requirement of experts is comparison of the classic method with different promising experimental procedures. It is about sympathetic denervation performed by local delivery of neurotoxic drugs, cryoablation, ultrasound-induced denervation or using different catheters (Ablation Induced Renal Sympathetic Denervation Trial study) (35). Furthermore, it is currently experimented the effect of local circumferential drugs delivery (guanetidine is the ideal agent) in adventitia of renal artery by using a special balloon catheter (Cricket™ and Bullfrog® Micro-Infusion Catheter), in order to precisely reduce renal sympathetic overactivity without harming SNS in other territories. The authors consider the multiple advantages of this method: removal of adverse drug effects, increase of therapeutic effectiveness, and improvement of patient outcomes and prognosis with concomitant decrease of medical costs (36). The method provides the perspective for treating other different hypertensive patients, including young hypertensive or patient with heart failure and chronic kidney disease (26).

BAROREFLEX STIMULATION

There are many neurohumoral reflexes which regulate the neural feedback mechanism of circulation. The arterial baroreflexes from aortic arch and carotid sinus adventitia play a key role in the short-term control of mean BP level, heart rate and contractility, vasomotor tone. Signals to the nucleus *tractus solitarius* and parasympathetic stimulation are the mechanisms involved. However, the long-term mode of action is less clear. First experimental studies have observed that baroreceptor response gradually diminishes (over minutes to days) in the face of a sustained increase in BP by "blunting" the reflex response. Although a minimal interference in chronic BP modulation has been postulated, it has been recently suggested that baroreceptors may have a substantial role by modulating renal salt excretion and, consequently, systemic fluid balance. Therefore, prolonged baroreflex activation may be responsible for BP sustained fall (36-38). Preliminary experimental studies conducted to reveal how baroreceptors function is restored, showed that carotid sinus stimulation (interpreted by the brain as a rise in BP) leads to BP decrease and reduces plasma NE level (39).

This principle has been applied in clinical practice by using a system that works in a similar fashion to a pacemaker (The Rheos CVRx Baroreflex Hypertension Therapy System). The device consists of a pulse generator which delivers activating energy of 1-7.5 V, connected to two electrodes, one for each carotid sinus, and a program-

mer system. The pulse generator is surgically implanted bellow the right clavicle (40). Recently, in Europe, a second generation miniaturized implantable system has been approved (Barosim neo^{TM} CVRx) (fig. 3).

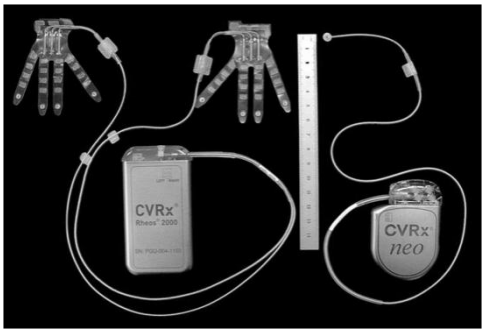

Fig. 3. Implantable baroreflex stimulators for resistant hypertension (adapted from 40)

The first clinical results have been reported by two prospective, phase II studies conducted in Europe (DE-BuT-HT) and USA (US Rheos Feasibility Trial), which documented a mean five-year decrease of BP by 53/30 mm Hg, left ventricular hypertrophy regression, and one-year renal function preservation (41-43). The beneficial results are confirmed by the recent Rheos Pivotal trial report (2011), a double-blind, randomized, prospective, multicenter, phase III clinical study of 265 patients with resistant hypertension. The trial shows a sustained drop in BP after 12 months (88% responders), the safety of method and used device. It appears that at least 6 months are necessary for achieving a sustained effect, and some procedural adverse events can occur. Simultaneously, a multicenter European study conducted by Scheffers and coworkers also demonstrates a more consistent decrease of BP measured by ambulatory monitoring, by 24/13 mm Hg after two-years of treatment, compared to renal denervation (44).

RESPeRATE® system is another type of device approved by FDA as an adjunctive therapy in resistant hypertension. The principle is based on slow and deep breathing ("paced breathing") as a trigger for baroreflex/cardiopulmonary receptors stimulation, increase of central inhibiting rhythms or decrease of chemoreceptors sensitivity (36). The direct consequence is modulation of sympathetic traffic and BP level. The system can be used in daily sessions (45 minutes per week) in order to achieve a decrease in breaths number to < 10/min. The beneficial effect has been documented in seven prospective studies, eight weeks of treatment being necessary for achieving this goal. A larger reduction in BP was observed in older patients and those with higher baseline BPs. This therapy can be combined with other pharmacologic or non-pharmacologic interventions (36).

A growing number of studies also support the significant antihypertensive effect of isometric exercises (e.g. isometric handgrip). The medical literature confirms the benefit on BP decrease after 4-8 weeks, the modulation of muscle sympathetic nerve and autonomic nervous system activity and stimulation of reactive oxygen species being involved (36). Zona PlusTM device has been designed to guide this kind of exercises, recommended to be performed in 12-minute sessions, at least three times weekly. Three randomized studies assessed the effect after at least 4 weeks of treatment and showed a mean BP decrease by 13.6/6.1 mm Hg. It appears that the effect acts in a linear fashion, with no side events (36).

In conclusion, in resistant hypertension, interventional therapy decreases sympathetic stimulation by specific mechanisms depending on each method and involves

direct, beneficial and sustained consequences on BP level, with no notable complications. Current results should be judged with caution taking account of multiple open questions in this early phase. However, the perspective for interventional methods to be used as an effective and well tolerated alternative, and also less long-term expensive for medical system, exists. Systematic and long-term follow-up of a large number of patients with resistant hypertension are the targets for new trials in progress such as Simplicity HTN-3, Global Symplicity Patient Registry or GREAT (German Renal Denervation) in order to settle the current dilemmas (26).

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NEWS

A PROTEIN IS LINK TO SEXUALLY TRANSMITTED INFECTIONS SUSCEPTIBILITY

The sexually transmitted infections (STI) caused especially by chlamydia and herpes simplex virus that affects the young population, predominantly female, are a global health problem. Into a study on mice, Prof. Hertzog and his team have revealed a protein called interferon epsilon (IFN-ε) with a protective effect against the viral and bacterial STI. IFN-ε is an unusual type I IFN because it is not induced by immune cells after contact with a virus or bacteria, by known pattern-recognition receptors pathways. The IFN-ε is a constitutively cytokine produced by epithelial cells of the female reproductive tract and is hormonally regulated, with the variables levels during menstrual cycle but in pregnancy and menopause with his absence. The authors of study finding that the deficit of IFN-ε is associated with increased susceptibility to STI. "Because IFN-ε follows different rules of normal immunomodulatory proteins, it might be an important link to new therapeutic opportunities for STI", noted authors of the study (Fung KY, Mangan NE, Cumming HJ et al. Interferon- Protects the Female Reproductive Tract from Viral and Bacterial Infection. *Science*, 2013; 339: 1088-1092).

Cătălina Luncă

LUNG CANCER – A COMORBIDITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE

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LUNG CANCER – A COMORBIDITY IN CHRONIC OBSTRUCTIVE PULMONARY DISEASE (Abstract): Chronic Obstructive Pulmonary Disease (COPD) is an important public health challenge, representing the fourth leading cause of death in the world. A very important characteristic of COPD is represented by the coexistence of comorbidities. Lung cancer, a major cause of morbidity and mortality throughout the world, is also a frequent comorbidity in COPD. Cigarette smoking represents the best-known risk factor in both COPD and lung cancer, causing common pathogenic mechanisms like oxidative stress and inflammation. However, the etiopathogenic link between COPD and lung cancer implies a common underlying genetic susceptibility, acting in addition to environmental risk factors. These trigger more complex cellular and molecular mechanisms, represented by immune dysfunction, abnormal activation of transcription factors, altered signaling pathways, epithelial to mesenchymal transition. A better understanding of the etiopathogenic link between COPD and lung cancer at the molecular level will allow the discovery of more sensitive diagnostic methods and also new molecular targets for an efficient treatment. **Keywords:** COPD, LUNG CANCER, INFLAMMATION, COMORBIDITY.

Chronic Obstructive Pulmonary Disease (COPD) is a preventable and treatable disease, characterized by persistent airflow limitation, usually progressive and associated with an enhanced chronic inflammatory response in the airways and the lung, to noxious particles and gases (1).

This disease generates an important public health challenge, representing the fourth leading cause of the death in the world, further increases in its prevalence and mortality being predicted in the coming decades (2).

One of the main characteristics of COPD is represented by the coexistence of comorbidities, with a significant impact on disease prognosis. Some comorbidities arise independently of COPD whereas most of these are causally related or share common pathogenic mechanisms.

Lung cancer represents a comorbidity frequently seen in patients with COPD and also a major cause of morbidity and mortality throughout the world. Lung cancer has been found to be the most common cause of death in patients with mild COPD (3).

Clearly, cigarette smoking represents the most common known risk factor for

COPD, and also for lung cancer. However, only a small percentage of smokers go on to develop one of these diseases in their lifetime. This situation is explained by the complex pathogenic pathways involving a common underlying genetic susceptibility generated by multiple polymorphic genes, acting in addition with known shared environmental risk factors.

This article will illustrate some evidences of the epidemiologic, cellular and molecular mechanisms involved in the etiopathogenic link between COPD and lung cancer.

1. Tobacco smoking - a common risk factor in COPD and lung cancer. The results of epidemiological studies have shown a strong association between smoking and both, lung cancer and COPD morbidity and mortality, respectively. The two chronic diseases share tobacco smoking, as an important common risk factor.

A comprehensive report on deaths from tobacco smoking between 1950 and 2000 demonstrates that smoking is associated with 90% of lung cancers, most of these arising from epithelial cells (4).

A meta-analysis of the epidemiological evidences regarding the relation between smoking and COPD, chronic bronchitis and emphysema, published in 2011, has found a elevated relative risk in current smoking (COPD 3.51, chronic bronchitis 3.41, emphysema 4.87) and ex smoking (COPD 2.35, chronic bronchitis 1.63, emphysema 3.52) (5).

2. The link between COPD and lung cancer, independent of tobacco smoking. Beyond the role of smoking as the common most important risk factor in both COPD and lung cancer, there are evidences showing towards the existence of other independent risk factors.

A large observational study confirmed the high incidence density of lung cancer in COPD (16/1000 person-years), by comparing with 1–1.5/1000 person-years in normal smokers. GOLD stages I and II, older age, lower body mass index and lung diffusion capacity of carbon monoxide (DLCO) below 80%, were associated with elevated risk of lung cancer. The incidence of lung cancer over time decreased in a stepwise manner with the increasing of the airflow obstruction severity at baseline, from disease stage GOLD I to IV (6).

DLCO, the most important physiological marker of computed tomography (CT) detected emphysema, independently associated with the diagnosis of lung cancer. CT detected emphysema, has been reported to be an independent risk factor for the development and prognosis of lung cancer (7).

A case–control study investigated COPD prevalence in lung cancer showing that COPD was more than six-fold frequently in the lung cancer group, compared with control group (8).

3. Lung cancer in never smoking COPD. 10% of the men and 20% of the women newly diagnosed with *non small cell lung cancer* (NSCLC), are never smokers. Among these cases, an overwhelming proportion is adenocarcinoma. The increased risk of NSCLC in never smokers has been associated with environmental, genetic, hormonal and viral risk factors (9).

Using the data resulting from Cancer Prevention Study II, a prospective study of cancer mortality conducted by the American Cancer Society, Turner et al. demonstrated elevated lung cancer mortality, associated with the history of emphysema, even among persons who had never been active smokers. Lung cancer mortality was significantly associated with emphysema

(hazard ratio 1.66) and with the combined endpoint of emphysema and chronic bronchitis (hazard ratio 2.44), but no association was observed with chronic bronchitis alone (hazard ratio 0.96). The association between chronic bronchitis and lung cancer was stronger in men (hazard ratio 1.59) than in women (hazard ratio 0.82) (10).

4. Bronchial obstruction, lung emphysema and lung cancer risk. A meta-analysis including population-based studies which examined the relationship between airway obstruction and lung cancer risk showed that reduced forced expiratory volume in one second (FEV1) correlated with the increased risk for lung cancer in the general population. Compared with the highest quintile of FEV1 (>100% of predicted), the lowest quintile of FEV1 (<70% of predicted) was associated with a 2.23 fold increase of the risk for lung cancer in men and a 3.97 fold increase of the risk in women (11).

In addition to airway limitation, the other defining element of COPD, emphysema, represents a strong and statistically significant independent risk factor related to lung cancer (OR, 3.14; 95% CI, 1.91–5.15). This correlation was demonstrated by CT scan assessment of emphysema (12).

A clinical research assessing the prognosis significance of emphysema, associated with lung cancer, demonstrated that the presence of emphysema, with or without airway obstruction, is related with survival, as an independent prognostic factor (HR = 1.49), for patients with NSCLC (13).

5. Stepwise histological progression towards malignancy. Epithelial to Mesenchymal Transition is the process whereby epithelial cells undergo a shift in development to a fibroblastoid or mesenchymal phenotype. Epithelial to mesenchymal transition represents an essential mechanism in normal embryonic development but also in pathologic processes like chronic inflammation and fibrosis (14).

The fragmentation of the reticular basement membrane in the airway of COPD patients appears to be a hallmark of the epithelial mesenchymal transition. A study based on endobronchial biopsies samples demonstrated a significant reticular basement membrane fragmentation and epithelial to mesenchymal transition markers increasing, in current smokers, ex-smokers with COPD and smokers with normal lung function compared with never-smoking subjects (15).

The disruption of intercellular contacts and the enhancement of cell motility, essential features of epithelial to mesenchymal transition, represent alterations suitable for migration, tumor invasion and dissemination. Several interconnected transduction/ signaling pathways have proved to be an important link between epithelial to mesenchymal transition and carcinogenesis (16).

In pathogenesis of lung cancer has been described a stepwise progression from pathological premalignant changes in the bronchial epithelium, towards basal cell hyperplasia, dysplasia and finally, towards squamous cell carcinoma.

The exposure to carcinogens from cigarette smoke damages the epithelium leading to stochastic genetic mutations at widely separated sites. A single mutant epithelial cell clone could expand and extend into widespread areas of the bronchial epithelium (17).

6. Inflammation – the pathogenic link between COPD and lung cancer. The inflammation represents an essential element in generation of the pathogenic link between COPD and the lung cancer, involv-

ing genetic and epigenetic modulations due to chronic tissue injury and abnormal tumor immunity in susceptible hosts. Tobacco smoke represents the main inducer of the inflammatory response in COPD, by providing an exogenous source of oxidative stress in the lung, which overcomes the natural defense mechanism (18).

In COPD, activated macrophages orchestrate the inflammatory process of the disease, releasing reactive oxygen species, extracellular matrix proteins, cytokines and matrix metalloproteinases, essential to the pathogenesis of emphysema and bronchial inflammation. The breakdown of extracellular matrix in parenchymal tissues occurs simultaneously followed by an increase in extracellular matrix production in the adjacent small airways (19, 20).

The macrophages may also be involved in early preinvasive lesions of lung cancer, forming dense clusters into stromal areas surrounding the tumor and producing focal breakdown of the epithelial basement membrane. In the areas of invasion where tumor-associated macrophages act, these promote cancer cell motility and metastasis. In the avascular and perinecrotic areas surrounding the tumor, hypoxic macrophages stimulate angiogenesis (21).

The increased inflammation has been demonstrated to associate a high risk for malignancy. Baseline C-reactive protein levels above 3 mg/dl seem to be a biomarker of increased likelihood of developing lung cancer (22).

Inflammatory mediators from COPD may induce proneoplastic mechanisms: production of immunosuppressive factors, cellular mutations and resistance to apoptosis, angiogenesis, invasion and metastasis. Inflammatory cells such as macrophages and T lymphocytes can communicate with the neoplastic cells through a reciprocal and self-perpetuating interaction, resulting in increased growth and resistance to immune destruction (23).

The inflammatory milieu is an important factor for tumor growth regulation. Proinflammatory cytokines, such as IL-1β, promote malignancy through immune suppression generated by induction of myeloid suppressor cells, counteracting immune surveillance and allowing the proliferation of malignant cells (24).

7. Prostaglandins in COPD and Lung Cancer. The enzyme cyclooxygenase-2 (COX-2) and its product, prostaglandin E2 (PGE 2) play a critical role in the regulation of inflammatory responses, in respiratory diseases. COX-2 gene expression and the enzyme activity are increased in the peripheral lung of COPD patients (25).

In lung cancer cyclooxygenases mediate production of prostaglandins from arachidonic acid, promoting tumor growth, angiogenesis and metastasis.

The role of COX-2 in lung cancer development was assessed by genotyping lung cancer and control subjects, for polymorphisms of the inflammation-related genes prostaglandin synthase-2/cyclooxygenase-2 (COX2/PTGS2). The results showed that carriers of the C allele of a polymorphism in the 3'-UTR region of COX2 had a significantly increased risk of lung cancer, with odds ratios of 4.28 for homozygotes and 2.12 for heterozygotes (26).

Data obtained in earlier studies, immunehistochemically staining the COX-2 protein in lung cancer, showed positivity in 90% of adenocarcinomas and in all squamous cell carcinomas (27).

The daily treatment with aspirin as COX-2 inhibitor has been found to decrease the risk of lung cancer, confined to

adenocarcinomas (28).

8. COPD and lung cancer – common intra/inter-cellular signaling pathways. The complex pathogenic relationship between COPD and lung cancer is based on inflammation mediators and some specific cell signaling pathways.

The transcription factor NF- kB promotes chronic inflammation, playing a significant role in the pathogenesis of COPD by releasing of inflammatory mediators. The inflammation is perpetuated through increased transcription of target genes encoding chemokines, cytokines and adhesion molecules and promoting cell survival (29).

NF-kB provides a critical link between inflammation and carcinogenesis, up-regulating the expression of tumor promoting cytokines, such as interleukin-6 (IL-6) or tumor necrosis factor (TNF-α), and survival genes, such as Bcl-XL. NF-kB activation is present in certain solid tumors, but no oncogenic mutations responsible for its activation in carcinomas have been identified (30). The researches demonstrated the role of NF-kB-dependent inflammatory response in inducing lower airways lung cancer in cigarette smoke exposed animals (31). NF-κB activity correlates with proneoplastic events like proliferation, apoptosis, angiogenesis and may determine chemo-radioresistance in cancer treatment (32).

- Phosphoinositol 3-kinase (PI3K) pathway is involved in many cellular functions, including cell growth, motility, proliferation, and survival. PI3K activated by cell-surface receptors, initiates intracellular signaling cascades, generating the lipid secondary messenger phosphatidylinositol-3,4,5-triphosphate (33). There is an increased expression of the PI3Kδ isoform in macrophages, in the peripheral lungs of patients with COPD, compared with healthy smokers and nonsmokers (34).

In smokers, PI3K pathway activation in bronchial dysplastic epithelium represents an early event in lung tumorigenesis. The PI3K activity remains elevated in lung cancer, independent of smoking status. The treatment with myo-Inositol, a precursor of phosphatidylinositol, may determine the regression of airway dysplasia by inhibition of PI3K, associated with increased expression of genes that are repressed on PI3K activation (35).

Signal Transducer and Activator of Transcription (STAT3) is a molecule with an important role in regulating cytokine-dependent tumor-promoting inflammation and transducing signals from various oncogenic pathways. STAT3 promotes pro-oncogenic inflammatory pathways, including nuclear factor NF-kB and IL-6 (36).

IL-6 is one of the main causative agents of emphysema, through inducing apoptosis in the lung (37). IL-6 and STAT3 are co-expressed in alveolar type II epithelial cells in the lung. The increased IL-6 levels in exhaled breath condensate reflect airway inflammation in patients with COPD (38). STAT3 signaling plays an important role in initiation and progression of pulmonary adenocarcinoma. The averages of STAT3 up-regulated gene expression levels are 2-fold to 8-fold higher in human adenocarcinomas, in comparison with normal subjects (39).

STAT3 also regulates the expression of MUC1, a gene which mediates tumorigenesis, lung cancer cell survival and metastasis (40).

Epidermal growth factor receptor (EGFR) overexpression is one of the earliest abnormalities in the bronchial epithelium of high-risk smokers. It is present at the stage

of basal cell hyperplasia and persists through squamous metaplasia, dysplasia, and carcinoma in situ. EGFR overexpression is found in all squamous carcinomas and in 65% of adenocarcinomas and may link airway inflammation from COPD to lung carcinogenesis (41).

CONCLUSIONS

Lung cancer represents an important comorbidity in COPD, dramatically increasing the mortality of this disease.

Cigarette smoking is the most important risk factor, underlying the pathogenic link of the two diseases, triggering common pathogenic mechanisms involving oxidative stress, chronic inflammation, immune dysfunction, abnormal activation of transcription factors, altered signaling pathways, epithelial to mesenchymal transition.

A greater understanding of the molecular and cellular pathobiology and common signaling pathways involved in COPD and lung cancer will allow the discovery of more sensitive diagnostic methods and also new molecular targets for an efficient treatment.

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NEWS

RESIN-COMPOSITE CYTOTOXICITY VARIES WITH SHADE AND IRRADIANCE

The study was aimed at investigating the cytotoxicity of different composites as a function of composite shade and the light curing unit employed. Non-polymerized and polymerized samples of the composites Grandio® (VOCO,Cuxhaven), Solitaire® (Heraeus Kulzer, Hanau) and Filtek Z 250® (3M/Espe, Seefeld) in two markedly differing shades (A2, C2) were prepared. Polymerization was performed with two light curing units: Heliolux II (Ivoclar/Vivadent, Ellwangen) and Swiss Master Light (EMS, Nyon, Switzerland). To obtain composite extracts, the samples were immersed in cell culture medium (DMEM – Dulbecco‘s Modified Eagle Medium), which was replaced daily up to the 7th day of the experiment, and then on the 14th, 21st and 28th day. After incubation of human gingival fibroblasts with the extracts obtained, cytotoxicity was determined using the MTT test. With the non-polymerized samples, essentially no influence of the composite shades investigated on fibroblasts viability was detected, with the exception of the Solitaire material, where a higher cytotoxicity of the shade C2 in the non-polymerized state was found at the end of the observation period. After polymerization of the different composites, the cytotoxic reaction observed for the extracts of shade C2 was stronger than that observed for A2. After polymerization with the Heliolux II, the extracts of composites Grandio and Solitaire C2 were significantly more toxic than those of the A2 shade ($p < 0.01$). Polymerization with the Swiss Master Light reduces this cytotoxic effect. The extracts of the Grandio composite showed the least cytotoxic effect throughout the observation period, irrespective of the curing unit used. For the extracts of the Z250 specimens, the cytotoxicity observed was generally higher. These results show that the shade of the composite has an influence on its cytotoxicity and that this cytotoxicity is also influenced by the light curing unit used. It was observed that composites of the darker shade (C2) had a higher cytotoxicity, which varied with the light curing unit employed (Bernd W. Siguscha, Torsten Pflauma, Andrea Völpela, Kerstin Gretscha, Sandra Hoya, David C. Watts, Klaus D. Jandt. Resin-composite cytotoxicity varies with shade and irradiance. *Dental Materials* 2012; 28: 312-319).

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SOCIO-DEMOGRAPHIC AND LIFESTYLE CHARACTERISTICS ASSOCIATED WITH THE RISK FOR OBSTRUCTIVE SLEEP APNEA SYNDROME IN A RURAL POPULATION

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SOCIO-DEMOGRAPHIC AND LIFESTYLE CHARACTERISTICS ASSOCIATED WITH THE RISK FOR OBSTRUCTIVE SLEEP APNEA SYNDROME IN A RURAL POPULATION (Abstract): **Aim:** To evaluate socio-demographic and lifestyle characteristics in relationship to the risk for obstructive sleep apnea syndrome (OSAS). **Material and methods:** The study was carried out on 254 subjects**.** Socio-demographic data (age, gender, occupation, education level, and economic status), smoking status, alcohol consumption, and hours/day spent watching TV were recorded. For physical activity assessment the International Physical Activity Questionnaire and for identifying the risk for OSAS Berlin Questionnaire were used. **Results:** Of the 254 subjects, 29.4% of men and 36.8% of women were at high risk for OSAS (p=0.22). Age was higher in the group at high risk for OSAS (p=0.002) and in women (p<0.001). The highest percentage (47.5%) of participants at high risk for OSAS was found among retired people, whereas farmers generated the lowest percentage (18.6 %) (p<0.001). 57.1% of retired women were at high risk for OSAS as compared to 5.6% of the farmers (p<0.001). Education level and monthly income did not influence the risk of OSAS (p=0.172, p=0.113, respectively). No differences in the risk of OSAS were found in relation with smoking (p=0.887), alcohol consumption (p=0.688), and sedentary lifestyle. We found that individuals at low risk for OSAS performed more physical activity at work (p=0.053) and significantly more vigorous physical activity (p=0.036). **Conclusions:** In the study rural population, age, occupation and physical activity are related to the risk for OSAS. **Key words:** OBSTRUCTIVE SLEEP APNEA SYNDROME, SOCIO-DEMOGRAPHY, LIFESTYLE, PHYSICAL ACTIVITY.

Obstructive sleep apnea syndrome (OSAS) is an increasingly prevalent sleep disorder characterized by repetitive episodes of upper airway obstructions leading to sleep fragmentation and excessive daytime sleepiness (1). One of the first studies to evaluate the prevalence of OSAS estimated it to be about 4% of men and 2% of women (2). Later on, the same author found that a very high proportion of the general adult population is undiagnosed (3) and a decade later, he estimated that the prevalence of OSAS in adults 30 to 69 years of age is approximately 17% (4).

Evidence from several studies reveal that undiagnosed and untreated OSAS is independently related to increased mortality rates (5), traffic accidents (6), diminished quality of life (7), and work disability (8). Strategies to increase the diagnostic rates and the detection of risk factors for OSAS need to be developed. Excess weight represents the strongest risk factor for OSAS (4) but other risk factors have been cited, including age, gender, family history, smoking, alcohol, and menopause. The social and cardiovascular consequences of OSAS seem most pronounced among patients of low socioeconomic status (9).

Among potentially modifiable risk factors, the ones related to lifestyle, namely excess weight, alcohol, smoking, could represent targets for health policies. Even though data suggest that obstructive sleep apnea is associated with these factors, the only evidence-based intervention strategy is weight loss (10), hence the usefulness of weight control and increased physical activity, as a preventive measure or even as a treatment for OSAS. The aim of this study was to evaluate the socio-demographic factors including age, gender, occupation, education level, economic status, and some elements of lifestyle (smoking, alcohol consumption, sedentary lifestyle and physical activity) in relation to the risk for OSAS in a rural population.

MATERIAL AND METHODS

We conducted a cross-sectional study on 254 subjects in Deleni, the largest rural community in Iași County. The subjects were randomly selected from a database of 3248 individuals created during the 2007-2008 National Health Programs, the study sample being representative for this population (11). All participants signed a written informed consent before enrolling, and the study had the approval of the University Ethics Committee.

The following data were recorded during an interview: socio-demographic data (age, gender, occupation, education level, and monthly income/family member), smoking status, and alcohol consumption - each type of alcohol being assessed separately. According to smoking habit subjects were divided into 3 groups: non-smokers, former smokers (over 6 months abstinence) and current smokers. Alcohol consumption of more than 3 servings/day for men and more than 2 servings/day for women was considered to be excessive (1 serving = 50 ml strong alcoholic drink =150 ml wine =350 ml beer).

The assessment of physical activity was done by using the International Physical Activity Questionnaire (IPAQ) – long form (12), which measures the intensity and duration of physical activity performed during the last 7 days, in 4 domains: at work, during transportation, household activity (domestic), and during leisure time, or exercise. The results were expressed in two different ways: as continuous variables, in MET-minutes/week (total and for each activity), and category variables, establishing if the subjects were sedentary, moderately active or active. Sedentary lifestyle was evaluated using two separate tools: number of minutes/day spent sitting, regardless of the level of physical activity (item included in IPAQ), and number of hours/day spent watching TV.

The risk for OSAS was evaluated using Berlin questionnaire (13), which consists of 10 items organized in 3 categories, referring to snoring and witnessed apneas, daytime sleepiness, and the presence of either hypertension or a body mass index ≥30

kg/m^2. The risk for OSAS is high if two or more categories are positive and, by opposite, the risk is low if less than two categories are positive.

The statistical package SPSS version 17.0 for Windows (Chicago, IL, USA) was used for data analysis. The significance of differences between continuous variables was assessed using the *t* Student, One-way ANOVA, or Kruskal-Wallis tests, and for categorical variables Pearson Chi-Square test. A *p* value <0.05 was considered to be statistically significant.

RESULTS

Descriptive statistics of the studied population

Of the 254 subjects included in our study, 102 were males (40.2%), and 152 females (59.8%). The mean age was 54.75±16.9 years, with no statistically significant differences between men and women (p=0.824). Regarding occupation, almost half of the subjects were retired (48%), the remainder being farmers (16.9%), employees (14.6%), and housewives or unemployed (18.4%). As to education level, approximately 60% of the subjects were with 8 or less years of education, and only 17.3% with over 10 years of education. The per capita monthly income was in the majority of our subjects (72.4%) of ≤400 Lei/month; 53.9% with a per capita monthly income of 100-400 Lei, and 18.5% <100 Lei/family member. Only 6.7% of the study subjects reported excessive alcohol consumption. When assessing smoking status, the results showed that 14.6% of the subjects were current smokers and 7.1% former smokers.

Relationship between socio-demographic factors and risk for OSAS

No gender differences in the percentage of subjects at high risk for OSAS were found (χ^2=1.505, p=0.22) – 29.4% of men and 36.8% of women being at high risk for OSAS. In the group of subjects at high risk for OSAS, age was significantly higher than in those at low risk (p=0.002), namely 59.24±13.32 yrs, as compared to 52.45±17.14 yrs.

This difference was noticed only for women (p<0.001) – 61.23±12.19 years, as compared to 50.68±16.47 years (tab. I). Differences were observed in the percentage of participants at high risk for OSAS in relation to occupation (χ^2=20.782, p<0.001), the highest percentage (47.5%) being registered among retired persons, while the lowest percentage among farmers (18.6 %). When analyzing by gender, a statistically significant difference was found only for women, (χ^2=25.861, p<0.001), 57.1% of retired women being at high risk for OSAS, as compared to 5.6% of the farmers. Education level had no influence on the risk of OSAS (χ^2=4.992, p=0.172), neither in men (χ^2=1.034, p=0.793), nor in women (χ^2=5.676, p=0.129), the same being also true for the monthly income (χ^2=5.978, p=0.113).

Relationship between lifestyle characteristics and risk for OSAS

Smokers had no higher risk for OSAS than non-smokers (χ^2=0.24, p=0.887). No differences were observed regarding alcohol consumption between the two groups, at high risk and low risk for OSAS, respectively. Only in women, the results showed that those at high risk for OSAS drank less alcohol (0.2038±0.358 servings/day as compared to 0.3423±0.636 servings/day, p=0.046) (tab. I). We found that individuals at low risk for OSAS performed more physical activity at work than those at high risk - 1898.61±3843.744 METS/week compared

to 1106.72±3161.272 METS/week, differences with a trend towards statistical significance (p=0.053) - and significantly more vigorous physical activity (p=0.036), meaning 1037.61 ±2399.858 METS/week compared to 589.83±1964.142 METS/week. Men at low risk for OSAS do more physical activity by walking than those at high risk (p=0.016) - 1859.71±1884.661 METS/week vs. 845.43±1134.006 METS/week (tab. I).

TABLE I

Significant value of mean differences in relation with the risk for OSAS

	Total	**Men**	**Women**
Age	0.002	0.847	<0.001
Alcohol	0.903	0.433	0.046
Total MET Work	0.053	0.220	0.539
Total MET Transport	0.208	0.191	0.614
Total MET Domestic	0.308	0.650	0.179
Total MET Leisure	0.645	0.409	0.609
Total MET Walk	0.082	0.016	0.721
Total MET Moderate	0.426	0.911	0.261
Total MET Vigorous	0.036	0.341	0.420
TOTAL MET	0.924	0.408	0.431
Average Sitting	0.562	0.719	0.238
Total Sitting	0.933	0.354	0.267

DISCUSSION

The majority of population-based studies that have estimated gender specific prevalence reported a 2- to 3-fold greater risk of OSAS in men compared to women (14). Some researchers showed that gender differences lie in the anatomic structure of the upper airway, and pattern of fat deposition (15). Also, the role of sex hormones in OSAS pathogenesis and the increasing prevalence of OSAS in postmenopausal in women, incriminating a protecting role of estrogen hormones (16), have been proposed to explain the higher male risk for OSAS. Our results are in disagreement with the current literature findings: a high risk for OSAS was found in a higher percentage of women, but without statistical significance. A possible explanation could be that in this population, as previously shown, all OSAS symptoms were more frequently reported by women (17), whereas most studies reveal that women underreport specific symptoms (18) or present with complaints suggestive of other disorders than OSAS (19).

Several studies have found OSAS to be highly prevalent in people older than 60 years. In our study, age was significantly higher in subjects at high risk for OSAS, but the statistical significance was noticed only for women. Similar to our findings, observational studies that have used polysomnographic recordings as a diagnostic method showed that age was higher in women with OSAS (19).

Excessive daytime sleepiness is a noticeable problem because it affects the ability to work at best performances and in safety. Assessment of the risk factors and

symptoms of OSAS is therefore important to prevent productivity loss and work-related accidents (20). In the Wisconsin Sleep Cohort Study, OSAS severity was significantly, but weakly related to diminished psychomotor efficiency (21). With respect to occupation, our results showed statistically significant differences in the percentage of subjects at high risk for OSAS, the highest percentage of being recorded among retired persons, and the lowest among farmers. This association was investigated in several studies, showing that individuals without occupation have a higher frequency of OSAS (18).

The study conducted by Tarasiuk et al. revealed that low socioeconomic status was found to be a novel independent risk factor for cardiovascular disease among adult OSAS patients requiring treatment (22). According to our results, education level and economic status did not influence the risk for OSAS, although there is evidence that daytime sleepiness is associated with a lower education level (20). In another study, socioeconomic status and occupation had a minor effect on the likelihood that the population was hospitalized for OSAS (23).

In our study, no differences were recorded regarding alcohol consumption and smoking status in subjects at high and low risk for OSAS. Only in women, we found that those at high risk for OSAS consumed less alcohol, but the analysis was not adjusted for obesity or postmenopausal status. Alcohol ingestion has been demonstrated to increase the number of apneal episodes (24).

Smoking represents one of the strongest risk factors for cardiovascular disease and although the association with OSAS is relatively weak, it may add to the cardiovascular risk associated with OSAS (25). Wetter et al. (26) found that current smokers were three times more likely to have OSAS than never-smokers. However, former smokers were not more likely to have OSAS than never-smokers.

Physical inactivity is a risk factor for obesity, which is known to be the most important risk factor for OSAS. It has been previously demonstrated that, in our study population, the high risk for OSAS is associated with obesity, both in men and women (27). An analysis from the Wisconsin Sleep Cohort Study (28) examined this association and estimated that, in persons with mild OSAS, a 10% weight gain increased 6-fold the odds of developing moderate or worse OSA. The results of our study showed that individuals at low risk for OSAS performed significantly more vigorous physical activity. Our results are in agreement with those of the Sleep Heart Health Study which showed that at least 3 h/week of vigorous physical activity reduced the odds of sleep disordered breathing (29).

CONCLUSIONS

The results of our study show that of all assessed socio-demographical characteristics only age and occupation were associated with a high risk for OSAS. Women at high risk for OSAS were older, the highest percentage being represented by retired women.

Of the studies lifestyle features, only physical activity showed significant influence on the risk for OSAS - individuals at low risk for OSAS performed more physical activity at work and significantly more vigorous physical activity. Men at low risk for OSAS performed more physical activity by walking than those at high risk for OSAS.

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NEWS

THE PLASMA LEVELS OF CAFFEINE AND THE COGNITIVE STATUS

The recent researches suggest that the consumption of caffeine and coffee may be protective against the development of Alzheimer's disease. A new case-control study evaluated the degree to which initial plasma caffeine and cytokine levels would predict changes in cognitive status. Two separate cohorts were cognitively assessed and a blood sample was taken for determination initial level caffeine/ cytokine. Over the next 2 to 4 years, researchers annually reassessed the cognition of the participants (normal, mild cognitive impairment (MCI), dementia) and the level caffeine/ cytokine. Plasma caffeine levels at study onset were lower (-51%) in MCI subjects who later progressed to dementia compared to levels in stable MCI subjects. The study showed that a 1200-ng/mL plasma caffeine level appears to be an important threshold for avoided progression MCI to dementia. Patients with MCI may be able to avoid developing dementia by drinking 5 cups coffee daily or amount 500 mg of caffeine. The research also suggests that 3 cytokine (granulocyte colony-stimulating factors (G-CSF), interleukin-10 (IL-10), and interleukin-6 (IL-6)) could be signal for impending conversion to dementia among those with MCI. The MCI subjects who later progressed to dementia, but not stable MCI subjects with high plasma caffeine levels, had reduced plasma levels of G-CSF, IL-10, and IL-6. The scientists concluded that results provided the first direct evidence that caffeine or coffee intake is associated with a reduced risk for dementia or delayed onset of dementia, particularly for those who already have MCI (Chuanhai Cao, David A. Loewenstein, Xiaoyang Lin et a . High Blood Caffeine Levels in MCI Linked to Lack of Progression to Dementia. *J Alzheimer Dis*. 2012;30:559-572).

Cătălina Luncă

EMERGENCE OF A NEW GROUP CTX-M ENZYME IN ROMANIA AND RISK FACTORS FOR EXTENDED SPECTRUM BETA-LACTAMASES PRODUCING *E. COLI* INFECTIONS

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EMERGENCE OF A NEW GROUP CTX-M ENZYME IN ROMANIA AND RISK FACTORS FOR EXTENDED SPECTRUM BETA-LACTAMASES PRODUCING *E. COLI* INFECTIONS (Abstract): Antibiotic resistance rates in *E. coli* are rapidly rising, with worrisome aspects especially regarding community - acquired resistance to third- and fourth-generation cephalosporins and fluoroquinolones. The objectives of this prospective cohort study was to determine the resistance profile of *E. coli* for two categories of patients (<49 years and ≥ 50 years), risk factors for ESBL positivity and to investigate the molecular epidemiology of ESBL type CTX-M enzymes. A total of 885 strains of *E. coli* were isolated in the Infectious Diseases Hospital laboratory between June 2008 and June 2011 and *E. coli* resistance due to ESBL production was noted in 17% of cases. We found that previous therapy with cephalosporins, hospitalization and urinary catheter were risk factors for ESBL positivity. We noted significant differences concerning resistance rate between patients under 49 years and aged more than 50 years for ciprofloxacin (19% and 38%, respectively, p=0,0001), for gentamicin (15% and 23%, p=0,008), ceftazidime (15% and 24%, p=0,001) and ESBL positivity (14% and 20%, p=0.009). This study highlights the predominance of CTX-M producing strains (92.5% of ESBLs-positive *E. coli* harboured *bla* CTX-M genes); CTX-M-15 producing isolates were the most common, accounting for 96 % of isolates. Only 4% were belonging to CTX-M group-9, an emerging ESBL group which is newly described in Romania. **Key words**: *ESCHERICHIA COLI*, RESISTANCE, CTX-M ENZYMES

A problem of global magnitude is represented by the dissemination of Gram-negative bacteria genes which codify extended-spectrum β-lactamase (ESBLs), enzymes of particular clinical and epidemiological interest that can rapidly hydrolyse, or confer resistance to oxyiminocephalosporins (extended spectrum cephalosporins and monobactams) but not carbapenems.

The emergence of extended-spectrum β-lactamases (ESBL)-producing strains mainly with CTX-M-type enzymes in the community, is an alarming phenomenon that could have major implications for antimicrobial therapy (1, 2, 3).

A lot of reports confirmed that CTX-M (frequently CTX-M-15) are associated with an important number of mobile resistance genes and that these genes have the ability to disseminate between different strains of

Escherichia coli (4)**.**

In our region of North-Eastern Romania there were no studies to evaluate risk factors such as previous treatment with cephalosporins or fluoroquinolones, presence of urinary catheters, hospitalizations during the previous 6 months, and age (>50 years) for developing an infection with ESBL-producing *E.coli* strain.

Therefore, the aim of our study was to asses all these risk factors and also**,** to investigate the molecular epidemiology of ESBL enzymes**.**

MATERIAL AND METHODS

Design of the study

The study was performed at a University Hospital of Infectious Diseases of Iasi, Romania. A total of 885 *E. coli* strains were isolated from different infections (predominantly from urine) during June 2008 and September 2010. Antimicrobial susceptibility testing was performed according to the CLSI criteria by disk diffusion method and using ATB G- strips (bioMerieux, France). ESBL production was confirmed by CLSI disc diffusion and Etest (5). Molecular characterization was done using polymerase chain reaction (Palm Cycler Corbett Research) and sequencing of *bla* CTX-M (Beckman Coulter CEQ8000) (6).

A t-test, Fisher's exact test or Pearson chi-square test was applied where appropriate, and a p-value of < 0.05 was considered to be significant.

RESULTS

The overall resistance rate for *E. coli* to cefotaxime, ceftazidime, ceftriaxone, amoxicillin - clavulanate, ciprofloxacine, gentamicin and amikacin was 34%, 27%, 43%, 36%, 30% , 27%, and 14%, respectively.

E. coli resistance due to ESBL production was identified in 17% of cases (157 strains).

Use of fluoroquinolones in the preceding 6 months were noted in 9% of the ESBL-positive patients (14 cases) and in 3.2% in the non - ESBL group (23 cases) (p=0.001). Previous treatment with a third - generation cephalosporin was described in 6.4% of the ESBL-positive patients (10 cases) and in 1.1% in the non - ESBL group (8 cases)(p=0.0001) (tab. I).

42% of patients with an ESBL – positive *E. coli* infection (47 patients) and 15% of patients from ESBL-negative group (97 patients) were hospitalized in the previous 6 months (p=0.001). Statistical analysis demonstrated that previous therapy with cephalosporin; hospitalization and urinary catheter were risk factors for ESBL positivity.

TABLE I

Risk factors for ESBL – producing *E. coli* infections

	ESBL negative	ESBL positive	p
Previous fluoroquinolone treatment	23 (3.2%)	14 (9%)	0.001
Previous third generation cephalosporins treatment	8 (1.1%)	10 (6.4%)	0.0001
Urinary catheter	19 (2.6%)	16 (10.2)	0.0001
Previous hospitalization	109 (15%)	62 (40%)	0.001

The rates of resistance for two cathegories of patients (<49 years and ≥ 50 years) are shown in Table II.

Among ESBL negative *E. coli*, resistance rate to cefotaxime or ceftazidime was up to 9%.

TABLE II
Rates of resistance according to age*

	< 49 years (n=381)(%)	≥ 50 years (n=504) (%)	p-Value
Ampicillin	256/371 (69) *	260/486 (74)	0.2
Ceftazidim	53/346 (15)	112/464 (24)	0.001
Cefotaxime	56/310 (18)	124/437 (24)	0.001
Amoxicillin-clavulanic acid	64/368 (17)	110/439 (22)	0.15
Piperacillin-tazobactam	2/121 (1.7)	4/149 (2.5)	0.49
Ciprofloxacin	71/372 (19)	188/494 (38)	0.0001
Gentamicin	50/332 (15)	106/455 (23)	0.008
Amikacin	3/80 (4)	11/220 (9)	0.2
ESBL - positive	53/381 (14)	104/504 (20)	0.009

*Data are present as no. of strains/total of tested strains (%)

We noted significant differences concerning resistance rate between patients under 49 years and aged more than 50 years for ciprofloxacin (19% and 38%, respectively, p=0,0001), for gentamicin (15% and 23%, p=0,008), ceftazidime (15% and 24%, p=0,001) and ESBL positivity (14% and 20%, p=0.009).

Molecular analysis by PCR performed in 157 ESBL positive strains revealed that 145 of ESBL-positive isolates (92,4%) harboured *bla* CTX-M gene and 12 (7,6%) were negative for this gene (non-CTX-M ESBLs). Analysis with specific primers showed that 139 (96%) of CTX-M belonged to group-1 (sequenced as type CTX-M-15) and in 6 cases (4%) CTX-M belonged to group-9. No producers of other group CTX-M enzymes were detected.

DISCUSSION

ESBL – positivity in our study was found to be higher than the rate reported by EARSS and exceeded the rate comunicated in a recent study in Turkey (11%) (7, 8). *E.coli* resistance to fluoroquinolones of more than 25% was reported in Italy (38%), Cyprus (45%), Turkey (52%) and Hungary (26%) (8).

Several recent reviews have detailed how CTX-M beta-lactamases have become dominant among extended spectrum beta-lactamases producing *E. coli* in many European countries, but data from Romania are very limited. In our study CTX-M group-1 producing isolates was the most common accounting for 96 % of isolates. Only 4% were belonging to CTX-M group-9 extended spectrum beta-lactamases.

Similar with molecular findings from our study, an absolute predominance of CTX-M group-1 was reported in Italy and in the London area (9, 10). CTX-M group-9 is the predominant group in Spain (2).

The emerging strains of *E. coli* producing CTX-M group-9, for the first time being recently described in Romania only in one strain by Tuchilus *et al* (11), may reflect introduction via foreign travel, or by food imported from other countries where the genotypes belonging to this group are very frequent (12). In our study sequencing 200 pairs of bases belonging to CTX-M-9 revealed a high degree of similarity with the fragment of CTX-M-113 described in a Shigella strain in China (13), but, subsequently, further investigations are necessary for a complete description of the gene.

Our results are in accordance with previous data reporting that the major risk factors for ESBL type resistance is antibiotic use in the preceding 6 months but we have also

demonstrated the implication of urinary catheterization, previous hospitalization and age more than 50 years as risk factors (7).

A notable limitation to our study is the limited epidemiological data about travel history and food or water exposure especially in those patients with group-9 CTX-M enzymes.

CONCLUSIONS

Our data contributed to the epidemiological and molecular investigations of antibiotics resistance of *E. coli* in Romania, with a great clinical impact concerning optimization of first intention therapy.

This study highlights the predominance of ESBL-type CTX-M producing strains, reveals that CTX-M-15 is preponderant and shows that CTX-M group-9 has emerged in Romania being described only in the North-Eastern region.

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Conflict of interest: nothing to declare.

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DIFFICULTIES IN DIAGNOSIS AND TREATMENT OF CHALAZIA IN CHILDREN. CASE REPORT

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DIFFICULTIES IN DIAGNOSIS AND TREATMENT OF CHALAZIA IN CHILDREN. CASE REPORT (Abstract). Chalazion is a granulomatous inflammation caused by blocking the Meibomian glands, which affect the eyelids, predominantly the upper ones. The purpose of this article is to highlight the clinical and histopathological aspects of chalazia at children. This paper is a retrospective study of a child, 3-year-old, who presents with multiple upper and lower eyelid chalazia in both eyes, mechanical ptosis, blepharospasm and ocular pain. The history of the disease begins 2 months ago with the initial RE (right eye), for which it received general antibiotic and local treatment for 10 days with unfavorable evolution. The child was subsequently examined by another physician recommending topical treatment with Sinerdol (Rifampicin) and Dexamethasone, and empirical treatment consisting of applying a wooden spoon to injuries and hot compresses. Inappropriate treatment has led to an unfavorable evolution, and its continuing may lead to several local complications. After attempting to identify other etiologies, surgical excision was performed with slow favorable evolution. Postoperatively, general and local antibiotic and anti-inflammatory treatment with slow development was recommended. Appropriate treatment initiated from onset would have limited the extension of the chalazia; with the possibility of avoiding the surgical risk involved in general anesthesia. **Conclusions:** Ophthalmologists should consider acne rosacea as a potential diagnostic for any child who has any combination of Meibomian disease, chronic blepharitis, recurrent chalazion and chronic symptoms of photophobia, eye irritation and redness that does not respond to routine medical treatment. **Keywords:** CHALAZION, CORTICOSTEROID, ACNE ROSACEA, QUALITY OF LIFE.

Chalazion is a focal inflammation of the eyelids resulting from a obstruction of the Meibomian glands (an internal posterior hordeolum). Meibomian glands are sebaceous glands, located in the tarsal region of the upper and lower eyelids. Obstruction of the opening of the gland at the level of the palpebral margin may lead to accumulation of the tarsal and neighboring soft tissue collection, accompanied by an inflammatory reaction with pain and skin erythema. The clinic is a benign, well defined, palpable, congestive nodule that can grow. It is often confused with a palpebral carcinoma. Affection is often associated with reddish acne and chronic posterior blepharitis (1, 2). It is considered the most common benign eyelid tumor. The exact role of *Staph-*

ylococcus aureus in chalazion is not fully understood (1, 2, 3, 4). It is often confused with a palpebral carcinoma (1, 5, 6). Excision of a specimen and hematoxylin staining, histopathological examination may reveal characteristic changes (1, 5, 6). Affection is often associated with reddish acne and chronic posterior blepharitis. It tends to begin in childhood as a facial skin eruptive in response to stress.

Diagnosis is difficult to put on, it is good to consider a child with meibomitis, chronic blepharitis and recurrent chalazion. Due to diagnostic confusion and therapeutic behavior it may vary. Studies often show that multiple pseudo-tumoral formations are highlighted, recurring questions as to whether they are associated with systemic ailments and require investigation of the case in identifying a possible collateral cause (2-8). Higher location may cause a mechanical ptosis to induce a decrease in visual acuity. In the infant, any untreated form of palpebral formation accompanied by mechanical ptosis may lead to amblyopia, with long-term impairment of visual acuity and quality of vision and life will be modified (9). Also, the therapeutic behavior can vary. Initial treatment in acute inflammation is the application of warm compresses and palpebral hygiene, the antibiotic associated with local anti-inflammatory is useful when secondary infection occurs but does not completely solve the disease.

Occasionally, in chronic inflammation, the incision is the best solution, with removal of the inflammatory collection and excision of the inflammatory tissue adjacent to the collection (1, 2, 10). Multiple studies compared conventional treatment vs intralesional injections of corticosteroid, the results being similar in some studies, others inclining the balance in favor of injections that would be considered more effective and safer. They recommend corticosteroid injection in children and patients with local anesthetic allergy, and surgery in large, multiple and recurrent chalazia (10, 11, 12). Other studies suggest that the association of the incision of the formation with the local corticosteroid injection provides the resolution of the inflammation in 95% of cases (1, 10). An appropriate initial treatment could prevent the spread of inflammation in both eyelids and the appearance of local complications.

CASE REPORT

We will present a case of a 3-year-old patient who presents with multiple upper and lower eyelid chalazia, mechanical ptosis, blepharospasm and eye pain (fig. 1), for both eyes (BE). Diagnosed two months ago at RE (right eye) with upper eyelid chalazion for which she followed general treatment with oxacillin and topically with chloramphenicol, a 10-day period with unfavorable evolution.

Fig. 1. Biomicroscopy of anterior pole (multiple inflammatory formations at upper and lower eyelid)

He was subsequently consulted by another physician recommending topical treatment with Sinerdol (Rifampicin) and Dexamethasone, and empirical treatment consisting of applying a wooden spoon to injuries and hot compresses. Inappropriate treatment has led to an unfavorable evolution, and its continuing may lead to several local complications. From her personal history we mention atopic dermatitis and needed to be mentioned that her mother shows reddish acne. The specialized exam revealed: VABE = 1fcnc (visual acuity, both eyes), at the anterior pole focal inflammatory formations, mobile on the underlying, confluent planes, painful located at the upper and lower eyelid, mechanical pythosis, the orifices of prominent Meibomiam glands, congestive conjunctiva. Eye fundus evaluation did not reveal pathological changes.

Laboratory analyzes did not reveal any changes. After the diagnosis was made, the multiple chalazia excision was performed under general anesthesia and the following postoperative treatment was recommended: general (Azithromycin 150 mg / 12 hrs., Dexamethasone 2 mg / 12 hrs.), topic (Tobradex ointment, artificial tears) and eyelashes-palpebral massage with Betadina 5%, Blefagel, with favorable post-operative evolution. From the extracted collection is sent for histopathological and bacteriological examination (fig.2, 3), which reveals a lymphogranulomatosis inflammation and does not isolate the microbial flora.

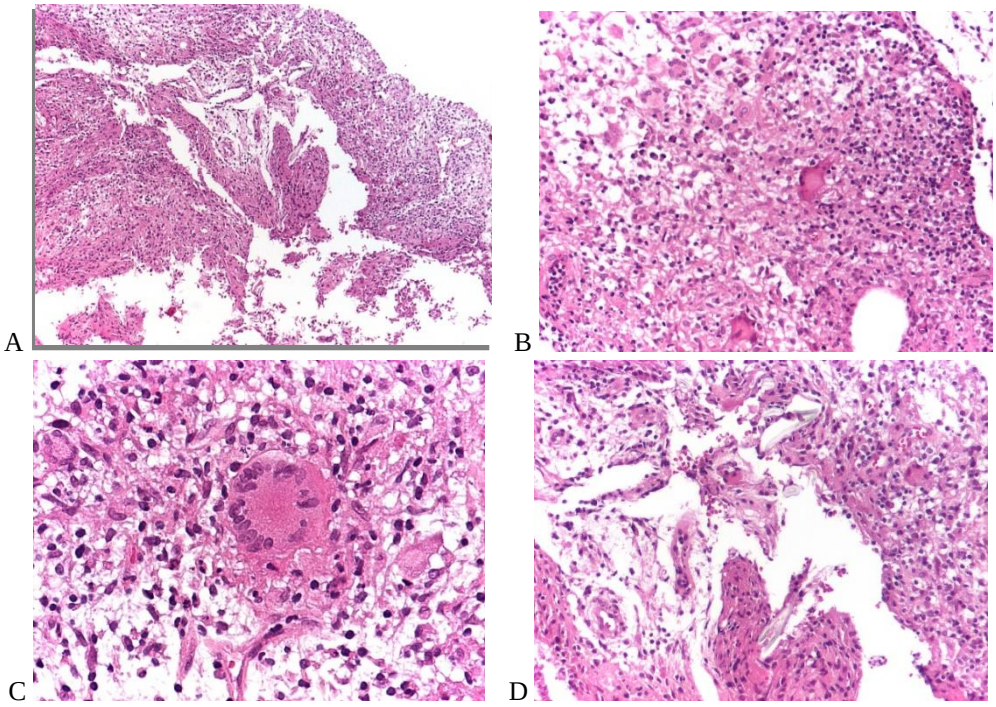

Fig. 2. Histopathological examination (lymph granulomatous inflammation)
A. Aggregate, polymorphic inflammatory granulation tissue, HE, x 4. B. Multinucleated giant cells with rare epitheliomas and polymorphic inflammation, HE, x 10
C. Multinucleated giant cells, HE, x 20 D. giant multinuclear cells on hair, HE, x 10.

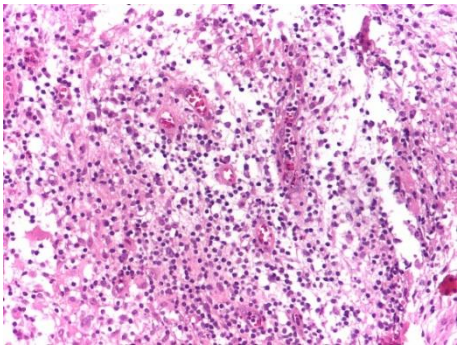

Fig. 3. Histopathological examination, granulation tissue, detail, HE, x 10

At the 1, 2-week control, was observed the absence of pseudo tumoral formations, the reduction of the congestion of the palpebral, only a slight thickening of the palpebral margin (fig.4). Prognosis is favorable, but it requires rigorous hygiene and long-term follow-up.

Fig. 4. Clinical appearance 2 weeks post-operatively

DISCUSSION

Chalazion is a chronic inflammatory granuloma of the Meibomian gland. The major effects are cosmetic deformation with variable discomfort. Microscopy reveals lipogranulomatosis reaction caused by released fat globules, histiocytes surrounded by multinucleated giant cells of epithelial and interstitial neutrophils, lymphocytes, and plasma cells (3, 4, 5, 6). Clinically presence of a pseudo tumoral form at the palpebral level must exclude inflammation of the meibomian glands, idiopathic facial granuloma, palpebral carcinoma and hordeleum etc. It is often associated with rosacea acne, especially in the child, but the diagnosis is difficult to put. Studies show that 58% of patients with rosacea have eye-eye symptoms. Rosacea tends to start in childhood as a common rash, often in response to stress. A diagnosis beyond this initial stage of rosacea is unusual in the pediatric population. If a child is identified with an intermediate stage of rosacea, consisting of papules and pustules, an eye examination should be performed to exclude ocular manifestations (4, 5, 6).

Ophthalmologists should consider acne rosacea as a potential diagnostic for any child who has any combination of meibomian disease, chronic blepharitis, chalazion recurrent and chronic symptoms of photophobia, eye irritation and redness that does not respond to routine medical treatment. Centikaya *et al.* maintain that patients respond well to generalized treatment with erythromycin or doxycycline (13). Another diagnosis we should think of when experiencing recurrent, multiple salicylates ,is hyperimmunoglobulin E syndrome (Job's syndrome). It is a rare form of immunodeficiency characterized by skin, pulmonary, and ocular palpebral abscesses (multiple, giant chalazia). Characteristic is the presence of an increased level of immunoglobulin E (3). It is rarely found in avitaminosis cases or may be induced by a viral infection (14,15). Excluding reddish acne in our case is quite difficult. We must also keep in mind the mother's heredal collateral history, as well as the good response to the general specific antibiotics that the patient received.

Sebaceous carcinoma is notorious for masquerading clinically as other benign

lesions such as chalazion (16, 17). In one study, it was the most commonly missed malignancy (1.1%) among 1,060 cases diagnosed clinically as chalazion (18). Other studies have found the rate of misdiagnosis of sebaceous carcinoma as chalazion to be 25.6% and 20% (19)

Therapeutic conduct should be appropriate to the case and should also consider the causes of recurrent palpebral inflammation. Studies recommend both local excisional therapy and intralesional anti-inflammatory injection, the results being similar (10, 11, 12). In our case, the appropriate initial treatment would have led to a limitation of the extent of inflammation and implicitly avoidance of local complications due to lack of treatment or inadequate treatment. Fortunately, the postoperative evolution was favorable, and the prognosis is good, but requires rigorous local hygiene and adequate follow-up. The case was difficult to manage on the one hand because of the severity of injuries due to an inadequate treatment, the content of granular tablets Sinerdol has no ophthalmic use (chemical burns), on the other hand empirically treatment applied could cause further complications (thermal burns).

CONCLUSIONS

Ophthalmologists should consider acne rosacea as a potential diagnostic for any child who has any combination of Meibomian disease, chronic blepharitis, recurrent chalazion and chronic symptoms of photophobia, eye irritation and redness that does not respond to routine medical treatment. Appropriate treatment initiated from onset would have limited the extension of the chalazia; with the possibility of avoiding the surgical treatment and the risk involved in general anesthesia. Reddish acne does not have standardized diagnostic criteria for the child, which is why the patient should be followed in the long run by a multidisciplinary team of ophthalmologist, dermatologist and pediatrician.

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NEWS

TRANSABDOMINAL CERCLAGE CAN IMPROVE THE PROGNOSIS OF PREMATUR DELIVERY?

Transabdominal cerclage (TAC) tends to be the best form of treatment aiming to improve the obstetric prognosis for patients with cervical incompetence as a result of a large conization, but also for patients who face cervical cerclage after radical trachelectomy. A retrospective review of 11 cases in which TAC was performed showed that five patients with an ultra-short cervix continued their pregnancies until the time for the scheduled caesarean section. A Teflon thread is inserted into the avascular space between the uterine vessels and uterine muscle, and it's tied firmly at the front side of the uterus. That can be kept for 2 years utmost, but if no abnormal findings are detected at the time of cesarean section, this term could most likely be extended. Complications result from the use of non-absorbable thread. In order to avoid two additional laparotomies, a laparoscopic surgery might be an alternative (Ishioka S, Kim M, Mizugaki Y, Kon S, Isoyama K, Mizuuchi M, Morishita M, Baba T, Sekiya T, Saito T. Transabdominal cerclage (TAC) for patients with ultra-short uterine cervix after uterine cervix surgery and its impact on pregnancy. *J Obstet Gynaecol Res*. 2017, doi: 10.1111/jog.13487).

Alina Mădălina Luca

NON-INVASIVE EVALUATION OF LIVER FIBROSIS IN CHRONIC HEPATITIS C

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NON-INVASIVE EVALUATION OF LIVER FIBROSIS IN CHRONIC HEPATITIS C (Abstract): Chronic hepatitis C (CHC) is a major public health concern, with around 180 million individuals affected worldwide. Liver fibrosis and its end-point cirrhosis are the main causes of morbidity and mortality in patients with CHC. Liver biopsy (LB) has traditionally been considered the „gold standard" for pre-treatment evaluation of liver fibrossis in patients with CHC. However, LB is an invasive procedure with several shortcomings (intra- and interobserver variability, sampling errors, expensive) and the risk of rare but potentially life-threatening complications (biliary peritonitis, hemo-peritoneum, and death in 1/10,000). The aforementioned shortcomings of LB have led to development of several non-invasive methods for the assessment of liver fibrosis in CHC. Among the non-invasive methods, Fibrotest and Fibroscan are the most widely used in our country and offer a viable alternative to LB for pre-treatment assessment of liver fibrosis in patients with CHC. This review aims to discuss the advantages and usefulness of non-invasive methods of liver fibrosis in CHC. **Key words:** CHRONIC HEPATITIS C, LIVER FIBROSIS, LIVER BIOPSY, FIBROTEST, FIBROSCAN.

Chronic hepatitis C (CHC) is a major public health problem and a leading cause of cirrhosis, end stage liver disease and hepatocellular carcinoma (1). An estimated 180 millions people are affected worldwide, and the health burden associated with CHC is expected to increase during the next 20 years (2). Liver fibrosis and its end-point cirrhosis are the main causes of morbidity and mortality in patients with CHC and therefore, the assessment of fibrosis is an important issue in the management of these patients (3). Liver biopsy (LB) is still regarded as the „gold standard" for evaluating the liver fibrosis in spite of its several shortcomings: 1) *inter-observer and intra-observer variability of histopathologic interpretation*: there are significant differences between pathologist's interpretation of the biopsies (4); 2) *sampling errors:* inadequate biopsy specimens size will make histopathological examination irrelevant and miss the diagnosis of cirrhosis in more than 25% of patients (5); 3) *complications* may be minor (anxiety, pain, transient hypotension) or major, including bleeding (intra-hepatic, intra-peritoneal), bile-peritonitis, puncture of pleura, colon,

and kidney, with the mortality rate ranging from 0.01% to 0.1% (6); 4) *other limitations:* high costs, variable accessibility, low acceptance by patients and, a method which provides only a static measure of fibrosis and therefore not suitable for repeated evaluation. The above mentioned shortcomings of LB have led to development of non-invasive methods for the assessment of liver fibrosis in CHC; these methods range from serum marker assays to advanced imaging techniques. This review aims to discuss the advantages and usefulness of non-invasive methods of liver fibrosis in CHC.

SERUM MARKERS OF LIVER FIBROSIS

The serum markers of liver fibrosis are commonly divided in two groups:1) *direct serum markers* which are directly linked to the modification in extra-cellular matrix turnover produced by hepatic stellate cells during the process of fibrogenesis in the liver; 2) *indirect serum markers* which reflect alterations in hepatic function.

The "direct markers" include glycoproteins (hyaluronate, laminin, YKL-40), collagen family (procollagen III, type IV collagen), collagenases and their inhibitors (matrix metalloproteases, tissue inhibitory metalloprotease-1). The "indirect markers" are biochemical parameters determined in routine blood tests (7). Several combinations of "direct" and "indirect" serum markers have been proposed and validated in CHC (table 1).

TABLE I

Combinations of serum markers for non-invasive diagnosis of liver fibrosis

Marker	Description
APRI	AST to platelets ratio index
Fibrotest	Age, gender, alpha-2-macroglobulin, γGT, haptoglobin, apolipoprotein A1, total bilirubin
Lok index	AST/ALT ratio, platelets, INR
Forns index	Age, BMI, γGT, cholesterol
Fibroindex	AST, platelets, gamma-globulins
Fib-4	Age, AST, ALT, platelets
Hepascore	Bilirubin, γGT, hyaluronic acid, alpha-2-macroglobulin, age, sex
Fibrometer	Age, AST, platelets, hyaluronan, INR, alpha-2-macroglobulin, urea
Fibrospect	Alpha-2-macroglobulin, hyaluronan, TIMP1
ELF*	Age, hyaluronic acid, type III procollagen, TIMP1
FIP*	Age, past alcohol intake, cholesterol, AST, HOMA-IR
Virahep-C	Age, AST, platelets, alkaline phosphatase
GUCI*	AST, INR, platelets

* ELF: European liver fibrosis group; FIP: Fibrosis Probability Index;
GUCI: Göteborg University Cirrhosis Index

Fibrotest and APRI are the most widely used before making a decision on antiviral therapy in patients with CHC. APRI (AST/platelets ratio) is a simple, chip, and readily available in the clinical practice, with an accuracy between 60% and 82% for significant fibrosis (8). Fibrotest is a patented test, with the accuracy reported ranges from 70-85% (8). To improve the performance of Fibrotest for the assessment of fibrosis in CHC, its combination with Fibroscan has been suggested. With such combination one study reported areas under the receiver curve (ROC) for 0.88 $\geq$ F2 (stage in the Metavir scoring system), 0.95 for $\geq$ F3 and 0.95 for F4 (9).

There are some limitations of serum markers; none is liver specific, and have poor performance in diagnosis of intermediate stages of liver fibrosis. However, it is important to note that the performance of each non-invasive marker is evaluated against LB which is an imperfect "gold standard", and the apparent failure of non-invasive markers to make accurate distinction between different stages of intermediate fibrosis could be the consequence of misclassification from LB (10). Poynard et al (11) have assessed the accuracy of Fibrotest, Fibroscan and biopsy using methods without gold standard in patients with CHC and found that both Fibrotest and Fibroscan to be accurate for the diagnosis of advanced fibrosis and cirrhosis in such patients.

IMAGING TECHNIQUES

Transient elastography **(**Fibroscan®, Echosens, Paris, France) measures the liver stiffness in a volume that is at least 100 times greater than a standard LB sample and therefore, may be more representative of the entire liver. Fibroscan is composed of an ultrasound transducer probe mounted on the axis of a vibrator; vibration is transmitted to induce an elastic shear wave that propagates through the liver. Pulse-echo ultrasound acquisition is used to measure the velocity of the shear wave, which is directly related to the liver stiffness: the stiffer the liver, the faster the shear wave propagates. Results are expressed in kilo-Pascals (kPa), and the values range from 2.5 to 75 kPa, with normal values less than 5.5 kPa. According to several studies, a cutoff value of 7.2 to 8.7 kPa defines significant fibrosis, and cirrhosis is diagnosed by cutoff value of 12.5 to 14.5 kPa (12). Several studies have confirmed that Fibroscan can accurately assess liver fibrosis in patients with CHC (13); it is an excellent method for the diagnosis of cirrhosis (F4) (14), and this is the reason that Fibroscan is gaining popularity worldwide to confirm or to rule out cirrhosis in patients with CHC.

Fibroscan has several advantages: it is painless, quick (less than 5 minutes), with results immediately available, inexpensive, and easy to be performed in the outpatient clinic and at the bedside. Fibroscan measurement failure is due to obesity, narrowed intercostal space, and limited operator experience.

Acoustic Radiation Force Impulse (ARFI) imaging combines conventional ultrasonography of the liver with evaluation of local liver stiffness. Results are expressed in meter/sec and the method allows operator to avoid large blood vessels or ribs. Recent studies have shown excellent diagnostic accuracy to identify significant fibrosis and cirrhosis and in some of them ARFI measurements were highly correlated with those of Fibroscan (15).

Magnetic resonance elastography (16) directly visualizes and measures acoustic shear wave's propagation through the liver;

the advantages of this method include the possibility to be used in patients with obesity or ascites. The main shortcoming of MR-elastography is its high cost which precludes its use in clinical practice for hepatic fibrosis screening and therefore, it remains as a research tool.

CONCLUSIONS

Unfortunately, so far no single ideal method (high sensitivity and specificity in identifying different stages of fibrosis, safe, with no risk for the patient, inexpensive, readily available, reproducible) exists for liver fibrosis. Therefore, we should choose the method with enough diagnostic accuracy and no risk for the patient with CHC. It is obvious that LB has several shortcomings, and that non-invasive methods offer viable alternatives to be used for staging of fibrosis in patients with CHC who are candidates for antiviral therapy. The rate of adoption of non-invasive tests by hepatologists differs from country to country. In France, a survey of 546 hepatologists revealed that 81% of them used the non-invasive methods (17). Our team has been the first in Romania which tried and succeeded to break the monopoly that LB has maintained on the evaluation of liver fibrosis in patients with CHC. Thus, Fibroscan and Fibrotest are used as first-line staging of fibrosis before making a decision on antiviral therapy. Once direct-acting antiviral therapy becomes available in many countries, and the allocation system will be based on „medical need" (therapy for the sickest patients), non-invasive assessment tests with highest accuracy for severe fibrosis/cirrhosis (i.e., Fibroscan) will play an important role. Therefore, it is surprising that many experts in the field of hepatology, still recommend LB for evaluation of fibrosis in patients with CHC. We believe that the time has arrived for moving forward to non-invasivity, no risk for patients, and high diagnosis accuracy in evaluating advanced stages of hepatic fibrosis in patients with CHC candidates for antiviral therapy.

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HIPEROSTOZA CERVICALĂ ANTERIOARĂ – O CAUZA RARĂ DE INTUBAȚIE DIFICILĂ ÎN URGENȚĂ

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ANTERIOR CERVICAL SPINE HYPEROSTOSIS - A RARE CAUSE OF DIFFICULT INTUBATION ÎN EMERGENCY (Abstract): DISH (Diffuse idiopathic skeletal hyperostosis) of cervical spine is a rare condition which causes dysphagia in 23% of cases and occasionally dyspnea. The authors report the case of a 74 years old male, known with progressive dysphagia and recurrent episodes of dysphonia and dyspnea, who suffered a sudden episode of respiratory distress that need finaly tracheotomy after ineffective attempts of orotracheal intubation. **Key words** OLLA, OROTRACHEAL INTUBATION, ENTEZOPATHY

DISH (hiperostoza scheletică difuză idiopatică), este o afecțiune descrisă de Forestier şi Rotes Querol în 1950, caracterizată prin rigidizarea tendoanelor şi ligamentelor prin calcifiere şi osificare difuză la nivel spinal şi extraspinal (umăr, cot, mână, şold, genunchi, plantă) (1, 2).

Scheletul axial este frecvent implicat, osificarea ligamentului cervical anterior este frecventă şi discontinuă, în special localizată la nivelul segmentului toraco-lombar. Localizarea cervicala este rară (3, 4).

PREZENTARE DE CAZ

Pacientul D. R., în vârsta de 74 de ani, cunoscut cu disfagie progresivă de 2 ani, disfonie şi dispnee intermitentă (aflat în investigații pentru o eventuală patologie malignă farino-laringiană), se prezintă la camera de gardă cu un episod brusc de insuficiență respiratorie acută. Întrucât tentativele de intubație orotraheală au eşuat, s-a solicitat serviciul O.R.L. şi s-a efectuat traheotomia de urgență.

Din antecedentele patologice s-a reținut diabetul zaharat (DZ) tip 2, aflat sub tratament, iar analizele de laborator au evidențiat o trombocitopenie severă (12000 trombocite/mm^3, la internare, apoi 20000 după 3 zile de administrare de dexametazonă), care nu s-a corectat pe parcursul internării.

Examenul clinic a evidențiat bombarea peretelui posterior al oro şi hipofaringelui, dar laringele nu s-a putut examina indirect inițial (laringoscopie indirectă); ulterior, fibroscopia a arătat un edem important al vestibulului laringian probabil datorat tentativelor de intubație, fără afectarea corzilor vocale, cu moderată stază salivară în sinusurile piriforme (excluzându-se astfel patologia tumorală faringiană şi laringiană).

Radiologia a tranşat diagnosticul, con-

form criteriilor lui Resnick (6): 1) punți osoase antero-laterale la cel puțin 4 corpuri vertebrale, 2) neafectarea aparentă a spațiilor intervertebrale, 3) absența ankilozei apofizare şi a sclerozei sacroiliace.

Examenul imagistic (RMN) a evidențiat hiperostoză voluminoasă, compresivă la nivelul C2-C6 având aspectul unei formațiuni cu aspect fuziform pe secțiunile sagitale, situată anterior de ligamentul vertebral ventral şi posterior de muşchiul lung al gâtului (pe secțiunile axiale) pe care îl deplasează lateral, cu efect de masă la nivelul hipofaringelui şi laringelui, în hipersemnal T2 şi izosemnal T1 fat sat după administrarea substanței de contrast (gadolinium) cu corpii vertebrali adiacenți (fig. 1, 2).

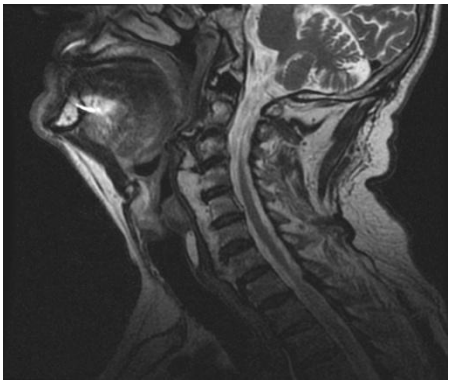

Fig. 1. Secțiune sagitală –IRM

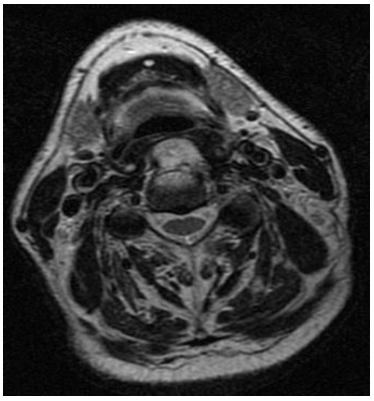

Fig. 2. Secțiune axială - IRM

Datorită trombocitopeniei, pacientul a fost ulterior transferat într-un serviciu de hematologie pentru investigații şi tratament de specialitate, iar riscul de insuficiență respiratorie fiind important, pacientul a rămas cu canula traheală; tarele sale (DZ şi trombocitopenia) nu au permis o abordare chirurgicală a hiperostozei cervicale.

DISCUȚII

DISH este o spondiloartropatie cunoscută de asemenea sub numele de boala Forestier, hiperostoza ankilozantă senilă (5).

Este o boală neinflamatorie manifestată în principal prin osificarea difuză şi calcifierea tendoanelor, ligamentelor, fasciilor atât a scheletului axial cât şi articulațiilor periferice, ceea ce i-a determinat pe cercetători să utilizeze termenul DISH (2,3).

Această boală are o predilecție pentru bărbați (65%) şi este frecventă la pacienții cu vârsta peste 50 ani, cu o prevalență de aproximativ 15-20% din populația generală în vârstă (6,7). Prevalența DISH în populația generală variază foarte mult în funcție de locația de studiu. A fost citată o rată de prevalență de până la 27,3% la bărbați şi 12,8% la femei (8). Frecvența internațională arată că aproximativ 0,16% din caucazieni suferă de această boală (9). Un alt studiu de prevalență DISH a populației de negri din Africa de Sud cu vârsta de peste 40 de ani a raportat ca prevalenta DISH a fost de 3,9% (3,8% bărbați şi 4,2% femei). Prevalența a crescut odată cu vârsta. Prevalența DZ la pacienții cu DISH a fost de 52,4% (10). Coloana vertebrală toracică este afectată frecvent (95%), urmată de afectarea coloanei lombare şi cervicale (4,11). DISH determina osificarea ligamentului longitudinal anterior (OLLA), fiind raportat la 25% bărbați şi 15% femei, din populația generală cu vârsta peste 50 ani (7).

În populația din Asia, în special în rândul populației japoneze, osificarea ligamentului longitudinal posterior (OLLP) are prevalența de 1,9-4,3% (12). OLLP şi DISH apar frecvent împreună, 50% din cazurile de DISH fiind raportate în asociere cu un anumit grad de OLLP (13).

Etiologia DISH este încă un mister, fiind sugerate mai multe teorii, motiv pentru care este greu de a identifica factorii de risc. Lista cu posibilii factori de risc include medicamente specifice - retinoizi, isotretinoid (Accutane-R), similare cu vitamina A, ce pot creşte riscul de DISH, dar încă nu este clar dacă un aport ridicat de vitamina A creşte riscul de DISH (14,15). Un alt studiu făcut pe animale a arătat că nivelurile plasmatice mari de vitamina A cresc riscul de DISH. Pe model uman astfel de studii nu au fost găsite în literatura de specialitate (17). Un articol de revizuire recenta a bolii Forestier realizat de Sarzi-Puttini şi Atzeni arată o prevalență mai mare a DISH in cazul pacienților cu obezitate şi hiperuricemie (18) dar indicele de masa corporala luat singur nu este considerat factor de risc (16,18). Vezyroglou a arătat că pacienții cu DZ, boală coronariană, hipertensiune arterială, hiperuricemie (gută), dislipidemie sunt mai susceptibili de a dezvolta DISH (16).

Manifestările clinice sunt variabile. Unii subiecți sunt complet asimptomatici, în timp ce alții se plâng de cervicalgii, rigiditate şi simptome secundare compresiunii excrescentelor osoase asupra structurilor vecine. Compresiunea faringo-esofagiană şi traheala se manifestă prin disfagie, dispnee, stridor, pneumonie de aspirație, mielopatie, apnee in somn (19, 20). Incidenta disfagiei raportată la pacienți cu DISH este cuprinsă în intervalul 0,2-28%. Dispneea este mai comună (21). Disfagia la pacienți cu DISH cu localizare cervicală apare ca urmare a compresiunii de diferite grade la nivelul esofagului, afectarea mobilității epiglotice şi a funcției sfincteriene a laringelui, distorsiuni ale cartilajelor laringiene (21). DISH predispune la complicații cum ar fi leziuni iatrogene ale mucoasei laringiene şi esofagiene în timpul procedurilor endoscopice şi intubare orotraheală (19).

Pentru confirmarea diagnosticului de DISH de obicei este suficientă radiografia convențională, radiografia cu bariu. Videofluoroscopia dinamică ar trebui să fie rezervată pentru a demonstra relația exactă a modificărilor la nivelul coloanei cervicale cu funcția de deglutiției la acei pacienți care se confruntă cu disfagia (22). Radiografia cu bariu şi videofluoroscopia va confirma comprimarea esofagiana şi obstrucția în ceea ce priveşte prezența osteofitelor mari anterior. Endoscopia la aceşti pacienți prezintă un risc de perforare, dar este necesară pentru a exclude alte cauze intrinseci ale disfagiei cum ar fi stricturi esofagiene, esofagite, tulburări de motilitate, tumori, candidoze (23).

Tomografia computerizată (T.C.) şi investigațiile prin rezonanță magnetică nucleară (R.M.N.) la nivelul coloanei cervicale pot fi folosite pentru o mai buna detectare a osteofitelor (mărime, formă), în raport cu faringele, laringele, esofagul, sau a complicațiilor DISH (stenoza canalului vertebral, mielomalacia) precum şi prezența OLLP asociat în 50% din cazuri cu DISH (24).

În prezent diagnosticul de DISH este bazat pe criterii de clasificare, care necesită prezența de osteofite cu dispoziție anterolaterală la nivelul coloanei vertebrale. Criteriile de clasificare stabilite de Resnick şi Niwayama implică cel puțin 4 vertebre învecinate, conservarea spațiului de disc

intervertebral, păstrarea integrității discului, absența ostesclerozei marginale, lipsa eroziunilor şi sclerozei în articulația sacroiliacă (25). Utsinger a redus pragul de implicare a coloanei vertebrale la 3 corpuri vertebrale învecinate, dar a adăugat prezența entezopatiei periferice pentru diagnosticul de DISH (4). Julkunen a sugerat că implicarea a 2 corpuri vertebrale în cel puțin 2 locuri ale coloanei vertebrale sa fie caracteristice pentru DISH (26). Modificările coloanei în segmentul cervical, în special C3-C4, afectează funcțiile laringelui inclusiv cea respiratorie (22), în timp ce dezvoltarea hiperostozei în segmentele C5-C7, poate produce spasmul sfincterului esofagian superior şi compresiune esofagiană (23,27).

CONCLUZII

DISH este o afecțiune comună trecută cu vederea la persoanele vârstnice. Etiologia DISH nu a fost definită dar există asociații cu obezitatea, diabetul, hipercolesterolemia şi guta. DISH cu afectarea coloanei cervicale este o cauza mai puțin frecventă de disfagie însă poate fi trecută cu vederea, deoarece disfagia este des întâlnita la persoanele vârstnice. Diagnosticul de DISH se stabileşte de obicei în faza ireversibilă, pe deplin dezvoltată a osteofitelor, când apar complicațiile şi riscul de perforare în timpul endoscopiei şi a intubației orotraheală. Intervenția chirurgicală (osteofitectomie) poate fi luată în considerare, dar datorită asocierii factorilor de risc (vârsta, diabetul, HTA) se preferă tratamentul conservator, care include medicamente antiinflamatorii nesteroidiene, corticosteroizi, relaxante musculare şi modificarea dietei.

Cunoaşterea DISH cu localizare cervicală înainte de intubația orotraheală poate indica dificultăți ce pot impune măsuri aşazise invazive de restabilire a funcției respiratorii. Deşi rar, DISH cu afectare cervicală trebuie suspicionat la orice pacient cu disfagie progresivă şi dispnee, disfonie intermitentă şi/sau sindrom de apnee în somn, iar consultul interdisciplinar (O.R.L.) şi investigațiile radiologice trebuie efectuate pentru determinarea diagnosticului şi a eventualității unei intubații dificile.

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EVALUĂRI DESCRIPTIVE PRIVIND CARACTERISTICILE UNUI LOT DE PACIENŢI CU POLIARTRITĂ REUMATOIDĂ, INTERNAŢI ÎN VEDEREA INSTITUIRII TRATAMENTULUI RECUPERATOR, ÎNTR-O CLINICĂ DE SPECIALITATE, ÎN PERIOADA 2008-2010

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DESCRIPTIVE ASSESSMENTS OF A SERIES OF RHEUMATOID ARTHRITIS PATIENTS ADMITTED TO REHABILITATION TREATMENT BETWEEN 2008 AND 2010 (Abstract): **Aim:** To assess some demographic features in a series of 741 rheumatoid arthritis patients admitted to the Clinic Hospital of Rehabilitation at Iasi in different stages of disease in order to select the most adequate rehabilitation teratment. **Material and methods**: Data were collected from patients' records, hospital statistical reports, and then processed and interpreted using EPI/INFO and MS Excel. **Results**. Sex distribution revealed that the disease was more common in females (male/female ratio 0.2). The prevalence was higher after the age of 40, from 15.4% to 33.8% between 51 and 60, and 40.2% after 61. The most important comorbidities were related to osteoporosis (48.9%), ischemic cardiopathy and essential arterial hypertension (40.1%), impaired glucose tolerance and diabetes mellitus (36.9%), viral hepatitis B and C (24.02% of all cases). **Conclusions**. In selecting the most adequate rehabilitation treatment (drug therapy, kinetotherapy, and physical therapy) patient's age, and his various associated diseases have to be considered. **Key words:** RHEUMATOID ARTHRITIS, PREVALENCE, COMORBIDITIES, REHABILITATION

Poliartrita reumatoidă este o boală rară la bărbaţi sub 30 de ani, dar incidenţa ei creşte şi atinge vârful după 60 de ani. La femei, prevalenţa bolii creşte constant începând cu jumătatea celei de a 3-a decade atingând un nivel constant şi ridicat între 45 şi 75 de ani. Uneori debutează postpartum, iar relaţia cu traumatisme fizice sau stress psihologic este uneori posibilă (1, 2, 3).

Dacă prognosticul vital este rar compromis după o perioadă obişnuit de lungă de boală, cel funcţional devine, cu fiecare zi, tot mai precar, iar cheltuielile financiare ocupă un loc important, atât în bugetul familial, cât şi în cel social (4, 5). În acest sens, sunt de menţionat tratamentul îndelungat, cu un cost ridicat, precum şi numărul de zile de spitalizare, incapacitatea de muncă. La toate acestea se adaugă costul investigaţiilor paraclinice, precum şi tratamentul recuperator, în vederea ameliorării inserţiei sociale (6, 7, 8). Concomitent se

are în vedere ameliorarea vieţii cotidiene şi realizarea unei inserţii socio-economice optime cu efecte favorabile pentru bolnav, familie şi societate (9, 10, 11, 12).

Studiul de faţă are ca scop evaluarea unor caracteristici demografice ale lotului de pacienţi cu poliartrită reumatoidă, unele aspecte ale factorilor de risc implicaţi în etiologia bolii, ale investigaţiilor paraclinice, în legătură cu instituirea şi eficienţa exerciţiului fizic la aceştia, ca metodă terapeutică.

MATERIAL ŞI METODE

Studiul clinic de tip descriptiv a fost efectuat pe un lot de 741 pacienţi diagnosticaţi cu poliartrită reumatoidă, aflaţi în variate stadii de evoluţie şi care au fost internaţi şi asistaţi terapeutic şi evolutiv în Spitalul Clinic de Recuperare, Iaşi, în perioada 2008-2010. Acest lot a cuprins şi reinternările, deci, studiul se referă la prevalenţa bolii.

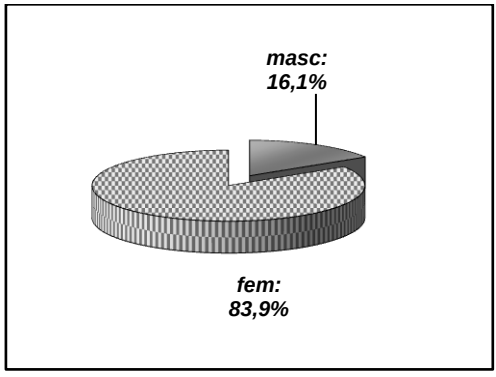
Culegerea informaţiilor s-a realizat prin evaluarea foilor de observaţie ale pacienţilor, precum şi a raportărilor statistice ale Spitalului Clinic de Recuperare, Iaşi. Pentru prelucrarea şi interpretarea datelor acumulate s-au folosit programele computerizate MS Excel, EPI/INFO, iar metodele biostatistice au inclus calcularea prevalenţei globale, specifice, a probabilităţii, regresiei liniare şi tendinţei.

REZULTATE ŞI DISCUŢII

1. Structura lotului

Repartiţia pe sexe a evidenţiat predominanţa femeilor, atât pentru fiecare an, cât şi cumulativ pentru cei 3 ani luaţi în studiu, cu un raport masculin / feminin de 0,2. Această valoare obţinută de noi este în concordanţă cu rezultatele altor studii privind predominanţa poliartritei reumatoide la sexul feminin, cu o diferenţă puternic semnificativă ($p<0,001$). *Repartiţia pe medii de reşedinţă a pacienţilor* a fost în favoarea celui urban, însă cu diferenţe statistic nesemnificative, în toţi anii luaţi în studiu ($p>0,05$). Această constatare se regăseşte în datele din literatura de specialitate, publicate în străinătate, cât şi în România (fig. 1).

Explicaţia ar consta nu în faptul că, în mediul rural, ar exista un cumul de factori de protecţie şi deci o lipsă a factorilor de risc pentru poliartrita reumatoidă, cât, mai degrabă, o deficienţă a adresabilităţii către medic a pacienţilor din rural, comparativ cu cei din mediul urban, aceştia din urmă având şi un acces mai facil la cabinete, spitale şi investigaţii de specialitate.



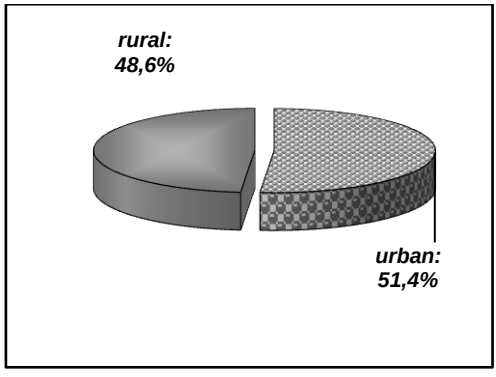


Fig. 1. Repartiţia pe sexe şi medii de reşedinţă a pacienţilor studiaţi

Repartiţia pe grupe de vârstă. Poliartrita reumatoidă a fost înregistrată la toate grupele de vârstă: 2,4% din cazuri au fost semnalate până la vârsta de 30 de ani; 8,2% pentru 31-40, remarcându-se o creştere a prevalenţei cazurilor după vârsta de 41 ani, de 15,4% până la 50, 33,8% între 51 şi 60 şi 40,2% cazuri cu vârsta peste 61 ani. Date similare au fost menţionate şi în alte cercetări (2, 3, 6), de unde rezultă necesitatea preocupării pentru depistarea precoce a poliartritei reumatoide, la toate vârstele, cu deosebire pentru femei şi grupele de vârstă de peste 40 de ani (fig. 2).

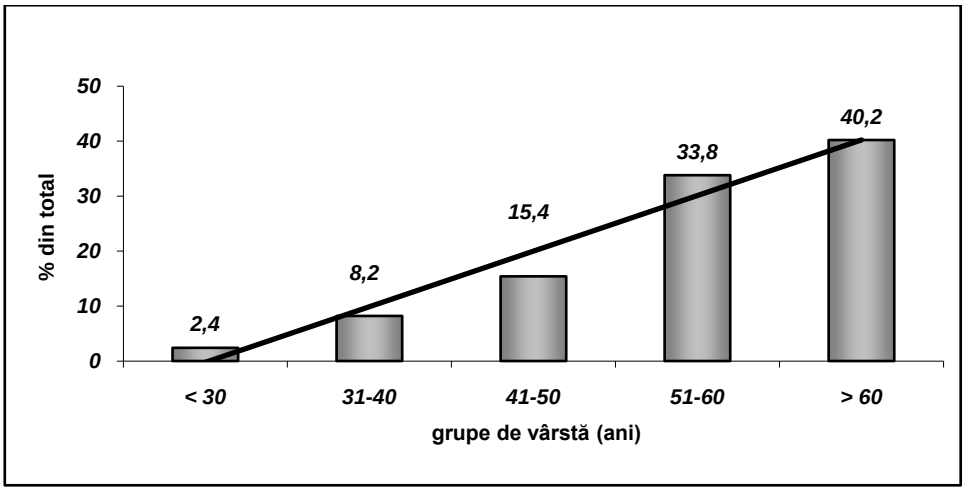


Fig. 2. Repartiţia pe grupe de vârstă

Calcularea regresiei liniare arată faptul că prevalenţa cazurilor de poliartrită reumatoidă are o prevalenţă cu tendinţă crescătoare spre grupele de vârstă de peste 40 ani. Această constatare am întâlnit-o, de asemenea, în studiile altor autori publicate în literatură (5, 8, 9).

O altă modalitate de a descrie lotul de studiu este distribuţia pacienţilor cu poliartrită reumatoidă pe *categorii ocupaţionale* active sau pasive. Se observă faptul că 298 (40,2%, adică aproape jumătate din lotul studiat) dintre bolnavi aparţin grupei de vârstă de 61 ani şi peste, persoane cu o activitate fizică mai redusă, posibil, din cauza pensionării, dar şi a prezenţei comorbidităţilor. Acest fapt poate avea influenţă asupra terapiei, în special kinetoterapiei şi recuperării.

2. Consideraţii asupra diagnosticului şi comorbidităţilor

Structura lotului studiat în relaţie cu diagnosticul. Din cei 741 pacienţi luaţi în studiu, în perioada 2008-2010, 635 (85,7%) au prezentat diagnosticul de „*Alte artrite reumatoide seropozitive cu localizări multiple*" (codul OMS: M05.80), iar 106 (14,3%) dintre ei au primit diagnosticul de „*Artrită reumatoidă seronegativă, cu localizări multiple*" (cod OMS: M06.00). Astfel, am subîmpărţit lotul de studiu în două grupuri, denumindu-le conform codurilor OMS: grupul „M05.80" şi grupul „M06.00", pe care le-am analizat, luând în consideraţie variabilele statistice sex, medii de reşedinţă şi grupe de vârstă.

Din analiza de statistică descriptivă, se constată că prevalenţa specifică atinge cea mai mare valoare pentru grupele de vârstă

de peste 60 ani, cu predominanţa sexului feminin, iar distribuţia pe medii de reşedinţă (urban/rural) nu a prezentat diferenţe statistic semnificative (tab. I, II).

TABELUL I

Distribuţia pe grupe de vârstă, sexe şi medii de reşedinţă a cazurilor cu diagnosticul de „*Alte artrite reumatoide seropozitive cu localizări multiple*”

Grupa de vârstă (ani)																			
< 30				**31-40**				**41-50**				**51-60**				**> 60**			
Fem		*Masc*		*Fem*		*Masc*		*Fem*		*Masc*		*Fem*		*Masc*		*Fem*		*Masc*	
R	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*
3	9	3	1	24	22	5	2	28	47	14	5	72	116	14	14	86	93	45	42
12		*4*		*36*		*7*		*75*		*19*		*188*		*28*		*179*		*87*	
Total 16				***43***				***94***				***216***				***266***			
Total general = 635 cazuri																			

TABELUL II

Distribuţia pe grupe de vârstă, sexe şi medii de reşedinţă a cazurilor cu diagnosticul de „*Artrită reumatoidă seronegativă, cu localizări multiple*”

Grupa de vârstă (ani)																			
< 30				**31-40**				**41-50**				**51-60**				**> 60**			
Fem		*Masc*		*Fem*		*Masc*		*Fem*		*Masc*		*Fem*		*Masc*		*Fem*		*Masc*	
R	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*	*R*	*U*
0	0	2	1	4	1	2	0	5	10	1	2	7	22	4	1	25	12	3	4
0		*3*		*5*		*2*		*15*		*3*		*29*		*5*		*37*		*7*	
Total = 3				***7***				***18***				***34***				***44***			
Total general = 106 cazuri																			

Se observă că, în ambele grupuri de diagnostic, se păstrează ponderea grupelor de vârstă, cu diferenţe statistic nesemnificative. Date descriptive similare au fost publicate şi de alţi autori, în concluziile studiilor referitoare la poliartrita reumatoidă (1, 2, 3, 7, 9). Aceste constatări sunt utile în alegerea terapiei recuperatorii şi a exerciţiilor fizice, la pacienţii cu poliartrită reumatoidă, asistaţi în clinica noastră.

*Observaţii privind distribuţia comorbidităţilor***.** În perioada luată în studiu, cele mai importante patologii asociate la cazurile cu poliartrită reumatoidă au interesat sistemul osteoarticular (osteoporoză în 48,9% din total cazuri), circulator şi cardiac (cardiopatie ischemică şi hipertensiune arterială esenţială – 40,1%), pancreasul (scăderea toleranţei la glucoză şi diabet zaharat – 36,9%), ficatul (hepatită virală B şi C – 24,02%) atât separat, cât şi în contextul patogenezei autoimune a bolii (fig. 3). Datele obţinute, referitoare la patologiile asociate întâlnite la pacienţii cu poliartrită reumatoidă, sunt în acord cu cele concluzionate de alţi cercetători (1, 2, 5, 6).

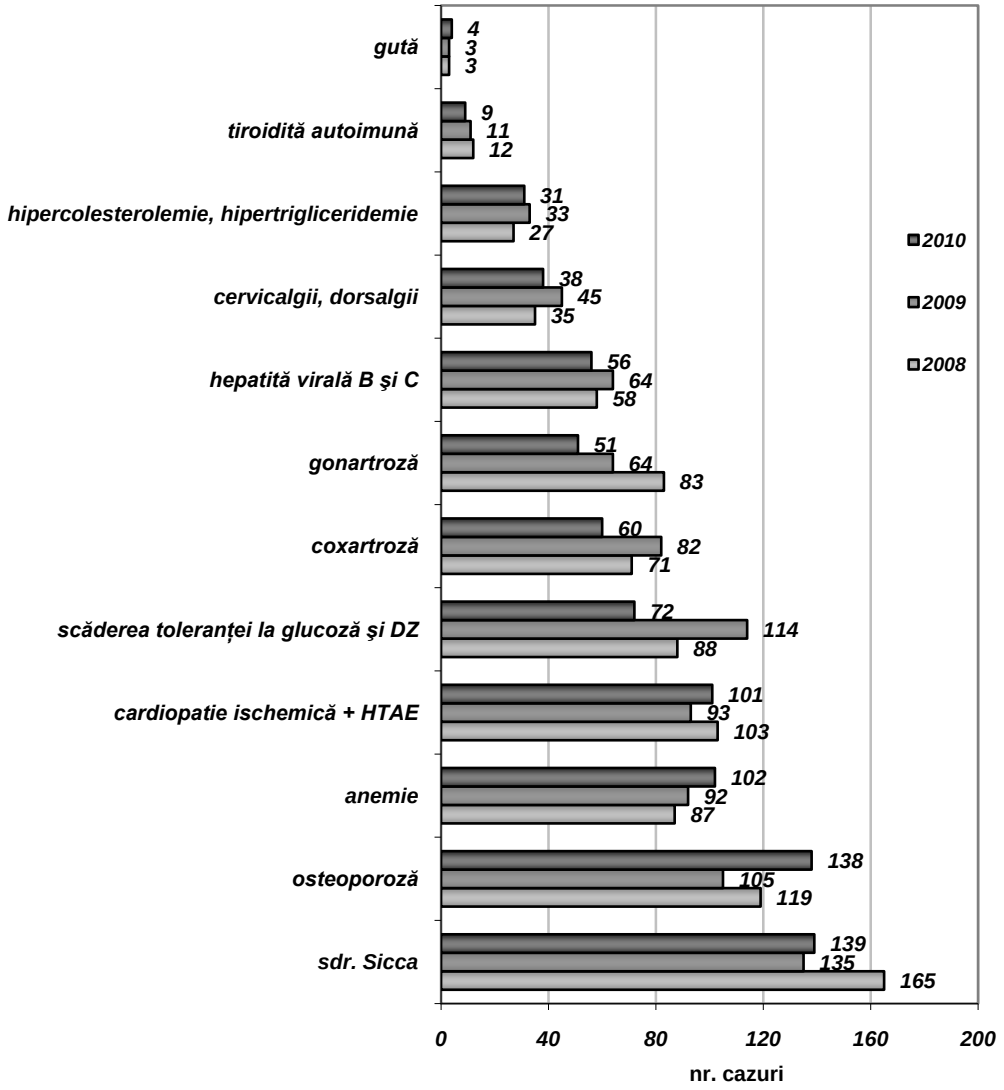


Fig. 3. Distribuţia pe comorbidităţi a cazurilor de poliartrită reumatoidă

3. Unele investigaţii de laborator efectuate la pacienţii luaţi în studiu

Bolnavii cu poliartrită reumatoidă au fost investigaţi în relaţie cu funcţia hepatică (ALT, AST, GGT), renală (acid uric, creatinină, urocultură), glicemia, semnele de anemie, semnele de inflamaţie (ASLO, CRP, VSH), prezenţa diferitelor tipuri de anticorpi (Ac anti HBc, anti Hbe, anti VHC) sau antigene virale (Ag HBs), crioglobulinele şi prezenţa factorului reumatoid (prin metoda Latex şi Waaler-Rose). Luând în discuţie doar prezenţa factorului reumatoid, am constatat că acesta a fost prezent în diferite titruri la 393 pacienţi (55,9%) şi negativ la 310 bolnavi investigaţi (44,1%). Prezenţa

factorului reumatoid a fost documentată la mai multe valori, cele mai frecvent întâlnite fiind titrul de 64, în 48 cazuri (51,6%) şi de 128, la 46 pacienţi (49,5%) (fig. 4).

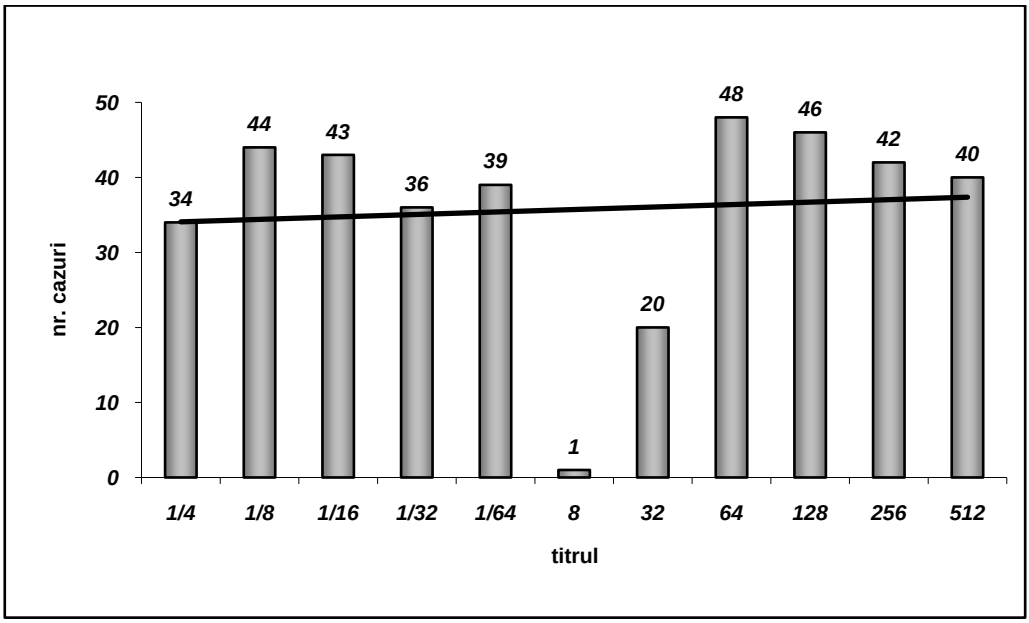


Fig. 4. Factorul reumatoid la pacienţii cu poliartrită reumatoidă (Latex)

CONCLUZII

Dificultăţile în realizarea diagnosticului precoce, care ar favoriza obţinerea unor rezultate terapeutice optime şi consecutiv, reducerea impactului, uneori dezastruos, asupra bolnavului, familiei şi societăţii, situează poliartrita reumatoidă între patologiile cu severe implicaţii sociale şi economice.

Aproape jumătate dintre pacienţii cu poliartrită reumatoidă, incluşi în lotul de studiu (40,2%) a aparţinut grupei de vârstă de 61 ani şi peste, persoane cu o activitate fizică mai redusă, posibil, din cauza pensionării, dar şi a prezenţei comorbidităţilor. Acest fapt poate avea influenţă asupra terapiei, în special asupra kinetoterapiei, exerciţiului fizic şi recuperării pacienţilor.

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NOUTĂȚI

PACIENŢII PEDIATRICI CU DISFUNCȚII ALE TRACTULUI URINAR INFERIOR – CALITATEA VIEȚII LOR ŞI A CELOR CE ÎI ÎNGRIJESC

Interesul privind calitatea vieţii (QoL) a crescut iar aceasta este un instrument util pentru evaluarea şi compararea furnizării de ingrijiri medicale şi impactul pe intervenţiile asupra stării de sănătate. Percepţia QoL diferă de la un individ la altul. Caracterizarea QoL este deosebit de dificilă în grupa de vârstă pediatrică, unde fiecare stadiu de dezvoltare prezintă necesităţi diferite. Prevalenţa disfuncţiilor congenitale ale tractului urinar inferior este mare şi îngrijirea acestora modifică rutina zilnică a pacientului şi a familiei sale. Într-un studiu transversal, autorii au evaluat QoL a 28 copii şi adolescenţi cu malformaţii urinare şi a celor ce îi îngrijesc folosind un chestionar (Autoquestionnaire Qualité de Vie Enfant Imagé-AUQUEI) respectiv forma scurtă 36 (Short –Form 36 – SF 36) şi au comparat rezultatele cu un grup –control de 38 copii sănătoşi de vârstă comparbilă/persoane ce îi îngrijesc. Patru întrebări au fost adăugate la chestionarul pacienţilor pentru a evalua problemele legate de îngrijirea lor urologică. Rezultatele au arătat un scor total mic AUQUEI în grupul pacienţilor ($p<0,213$, test Fisher), care au de asemenea probleme în ceea ce rpiveşte aspectele sociale, cum ar fi să se afle în clasă, manifestau trăiri negative în relaţie cu pierderile urinare diurne dar păreau să se adapteze bine la cateterizarea intemitentă. Tendinţa pentru un scor nefavorabil QoL a fost înregistrat şi pentru persoanele de îngrijire din grupul pacienţilor, în legatură cu durerea şi limitarea domeniilor fizice. (Lopey M, Farraro A, Filho UD, Kuczinski E, Koch V, Quality of life of pediatric patients with lower urinary tract dysfunction and their caregivers. *Pediatr Nephrol* 2011; 4(26), 571-577).

Mihaela Munteanu

SISTEME DE CLASIFICARE ŞI STADIALIZARE PENTRU COLANGIO-CARCINOMUL HILAR (TUMORA KLATSKIN): APLICAȚIE CLINICĂ ŞI RELEVANȚĂ PRACTICĂ

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CLASSIFICATION AND STAGING SYSTEMS FOR HILAR CHOLANGIO-CARCINOMA (KLATSKIN TUMORS): CLINICAL APPLICATION AND PRACTICAL RELEVANCE (Abstract): Hilar cholangiocarcinomas or Klatskin tumors have been classified in 1975 by French surgeons Henri Bismuth and Marvin B. Corlette and this remains largely used in clinical practice. The authors present the TNM classification and the changes introduced by the sixth and seventh edition of Union for International Cancer Control regarding the tumors of the proximal bile duct and describe Blumgart classification for tumors of this site. The usefulness of these systems is assessed considering the last six years experience of the service**. Key words**: HILAR CHOLANGIOCARCINOMA, KLATSKIN TUMOR, TNM CLASSIFICATION, STAGING

Colangiocarcinoamele sunt tumori dezvoltate din epiteliul ductal biliar. Ele pot apărea în orice segment al ductelor intrahepatice sau extrahepatice, de la ficat pînă la ampula Vater.

Cele care interesează confluența biliară sau colangiocarcinoamele hilare (CCH) au fost descrise de Gerald Klatskin în 1965 (1) primind ulterior acest eponim. De interes este faptul că trei cazuri de o astfel de tumoră fuseseră descrise 8 ani mai devreme de către William A. Altemeier de la University of Cincinnati (2), fapt menționat de către Klatskin în lucrarea sa, ceea ce a îndreptățit pe unii autori să denumească aceste tumori Altemeier- Klatskin (3).

MATERIAL SI METODĂ

În perioada 2005-2010 au fost internați în Clinica a III-a Chirurgicală Iaşi 43 de pacienți cu diagnosticul de tumoră Klatskin. Au fost studiate foile de observație clinică, din care au fost extrase detaliile referitoare la intervențiile chirurgicale efectuate cu intenție curativă şi rezultatele examinărilor anatomo-patologice, urmărindu-se evidențierea concordanțelor clasificărilor şi stadializărilor uzuale.

REZULTATE

Intervenție chirurgicală cu intenție curativă s-a efectuat la 3 pacienți, restul intervențiilor fiind paliative sau laparotomii exploratorii.

Primul pacient, de 72 de ani, cu tumoră la nivelul convergenței canalelor hepatice, a beneficiat de rezecția căii biliare extrahepatice cu anastomoză bi-hepatico jejunală

pe ansă „în Y”. Examenul anatomo-patologic extemporaneu a confirmat tranşele de secţiune negative ale căilor biliare în segmentele proximal şi distal, iar cel definitiv a evidenţiat doi limfoganglioni regionali cu metastaze de colangiocarcinom.

La al doilea pacient, de 49 de ani, cu tumoră interesând convergenţa canalelor hepatice, cu extensie la nivelul canalului hepatic stâng s-a realizat hepatectomie stângă (segmentele II, III şi IV) şi rezecţia segmentului I, în bloc cu calea biliară principală (secţionată distal în segmentul retroduodenal), calea biliară restantă de la nivelul hemificatului drept situându-se pe canalele sectoriale anterior şi posterior; continuitatea biliodigestivă s-a realizat, după completarea limfadenectomiei regionale, pe o ansă jejunală „în Y” à la Roux suturata la teaca pediculilor glissonieni ai sectoarelor drepte. Examenul anatomo-patologic a evidenţiat absenţa metastazelor ganglionare.

Celui de-al treilea pacient, în vârstă de 63 de ani, cu tumoră Klatskin având extindere tot pe canalul hepatic stâng, i s-a efectuat rezecţia căii biliare principale în bloc cu hemificatul stâng (segmentele II, III şi IV), anastomoză colangio-jejunală la nivelul tranşei de rezecţie a canalului hepatic drept; ganglionii regionali nu au fost găsiţi invadaţi.

DISCUŢII

Cronologic, *prima clasificare* a CCH a fost cea propusă în 1975 de către Henri Bismuth şi Marvin B. Corlette (4), larg folosită şi astăzi, în variantă modificată, fiindcă articolul original cuprindea numai primele trei tipuri (tab. I).

Ea grupează tumorile pe baza extensiei lor longitudinale în sistemul ductal hepatic, fiind o clasificare anatomică (fig.1), care poate ghida terapia (rezecţională sau paliativă) însă nu poate preciza care dintre pacienţi sunt candidaţi chirurgicali şi nici nu furnizează informaţii privind prognosticul sau corelaţii cu rata de rezecabilitate (5).

TABELUL I

Clasificarea Bismuth-Corlette

Tipul I: tumora invadează confluenţa biliară dar canalele drept şi stâng comunică
Tipul II: tumora invadează confluenţa biliară iar canalele drept şi stâng nu comunică
Tipul III: tumora invadează confluenţa biliară şi are extensie pe canalele hepatice IIIa: extensie pe canalul hepatic drept IIIb: extensie pe canalul hepatic stâng
Tipul IV: tumora este multicentrică sau invadează canalele hepatice drept şi stâng

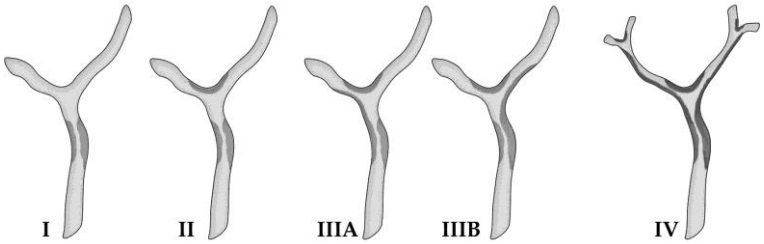

Fig. 1. Reprezentarea schematică a clasificării Bismuth- Corlette

Cel mai răspândit şi folosit sistem de clasificare a tumorilor solide este bazat pe aprecierea extinderii tumorale (T), a interesării ganglionilor (nodulilor) limfatici regionali (N) şi a prezenţei metastazelor la distanţă (M), cunoscut sub denumirea abre-

viată TNM, care permite planificarea tratamentului şi estimarea prognosticului. Compararea diagnosticelor şi terapiilor este posibilă dacă extensia neoplazică anatomică este definită conform standardelor general acceptate (6).

În ianuarie 2003 Union for International Cancer Control (UICC) a introdus în uz a şasea ediție a clasificării TNM a tumorilor maligne (7). Aceasta a introdus *modificări majore* în clasificarea cancerelor căii biliare extrahepatice, renunțându-se la diferențierea între invazia *presupuselor* diferitelor straturi parietale ale căii biliare principale precum „țesutul conjunctiv subepitelial (T1a)", „stratul fibromuscular (T1b)" sau „țesutul conjunctiv perifibromuscular (T2)" din ediția a cincea (8).

Astfel, tumora T1 este limitată la calea biliară, iar dacă invadează dincolo de calea biliară este clasificată T2. În cazul unei boli mai extinse, cu invazia ficatului, vezi-culei biliare, pancreasului şi/ sau tributarelor unilaterale ale venei porte sau arterei hepatice, tumora este stadializată T3 şi T4 atunci când invadează oricare din următoarele: vena portă sau tributarele bilateral, artera hepatică comună sau alte structuri adiacente

O altă modificare majoră a constat în contopirea categoriilor N1 şi N2 în N1, metastaze în ganglionii regionali.

Noutățile ultimei ediții (9) intrată în uz în ianuarie 2010 sunt reprezentate de clasificarea cancerelor căii biliare extrahepatice în proximale (cuprinzând canalele hepatice drept, stâng şi cel comun) şi distale (de la abuşarea cisticului în canalul hepatic comun), cu diferențe în definirea categoriilor TNM şi a stadializării; de asemenea, dispariția categoriei Mx din toate stadializările tumorilor solide.

Definițiile categoriilor T, N şi M pentru CCH ale ediției a şaptea se regăsesc în tabelul II iar stadializarea în tabelul III.

TABELUL II

Parametrii TNM ai clasificării UICC, ediția a 7-a, pentru cancerele căilor biliare extrahepatice proximale

T- tumora primară	
T1	Tumoră limitată la calea biliară
T2a	Tumora invadează dincolo de peretele căii biliare
T2b	Tumora invadează parenchimul hepatic adiacent
T3	Tumora invadează tributare unilaterale ale venei porte hepatice sau arterei hepatice
T4	Tumora invadează trunchiul venei porte sau tributarele bilateral şi/sau artera hepatică comună
N - ganglioni limfatici regionali (GL)	
N0	Fără metastaze în GL regionali
N1	Metastaze în GL regionali
M- metastaze la distanță	
Mx	*CATEGORIE ELIMINATĂ DIN TOATE STADIALIZĂRILE TNM!!!*
M0	Fără metastaze la distanță
M1	Metastaze la distanță

TABELUL III

Stadializarea cancerelor căilor biliare extrahepatice proximale

Stadiul I	T1	N0	M0
Stadiul II	T2a-b	N0	M0
Stadiul IIIA	T3	N0	M0
Stadiul IIIB	T1-3	N1	M0
Stadiul IVA	T4	Orice N	M0
Stadiul IVB	Orice T	Orice N	M1

Al treilea sistem de clasificare a CCH a fost propus de grupul condus de Leslie H. Blumgart de la Memorial Sloan-Kettering Cancer Center (MSKCC) din New York, acesta fiind o modificare a parametrului T al sistemului American Joint Comission on Cancer (AJCC), care ia în considerare trei factori legaţi de extensia tumorală, identificaţi de imagistica preoperatorie (10,11,12). Aceşti factori sunt: situarea şi extinderea invaziei biliare (conform clasificării Bismuth-Corlette), prezenţa sau absenţa invaziei portale şi prezenţa sau absenţa atrofiei hepatice lobare. Studiul publicat în 1998 cuprindea patru categorii T, cel din 2001 numai trei, prin comasarea a două categorii din propunerea iniţială (11,12) (tab. IV).

Scopul acestor modificări este categorizarea acelor pacienţi care sunt candidaţi pentru explorare chirurgicală cu intenţie rezecţională curativă ca şi furnizarea de informaţii anatomice privind extensia tumorală locală . Această clasificare nu a fost însă adoptată şi de alţi autori.

Este de amintit că în 1985 Gazzaniga (13) a publicat propria procedură de stadializare, care ţine seama şi de extinderea extrabiliară a tumorilor, în primul rând posibila interesare vasculară, arterială sau portală, care s-au regăsit ulterior în clasificările UICC. Mai mult, în această clasificare, îmbunătăţită ulterior (14) fiecare stadiu tumoral este corelat cu potenţialul terapeutic – tratament chirurgical curativ în stadiile 1- 3 sau paliativ în stadiul 4. Această clasificare nu a avut, însă, impactul şi notorietatea celorlalte trei menţionate.

TABELUL IV

Definirea parametrului T în clasificarea Blumgart

Stadiu	Criterii
T1	Tumora invadează confluenţa biliară+/- extensie unilaterală la ramuri biliare de al doilea ordin
T2	Tumora invadează confluenţa biliară+/- extensie unilaterală la ramuri biliare de al doilea ordin **şi** invazie venoasă portală ipsilaterală +/- atrofie hepatică lobară *ipsilaterală*
T3	Tumora invadează confluenţa biliară + extensie bilaterală la ramurile biliare de al doilea ordin ; **sau** extensie unilaterală la ramuri biliare de al doilea ordin cu invazie venoasă portală contralaterală; **sau** extensie unilaterală la ramuri biliare de al doilea ordin atrofie hepatică lobară contralaterală; **sau** invazie venoasă portală bilaterală sau a trunchiului principal

Majoritatea covârşitoare a pacienţilor cu stricturi hilare şi icter au tumoră malignă (15). Diferenţierea benign - malign a stricturilor hilare este dificilă şi reprezintă o problemă majoră în managementul acestor leziuni, 10-15% din pacienţi fiind de aşteptat să aibă alt diagnostic decât CC (16).

Diagnosticul diferenţial al leziunilor stenotice de la nivelul hilului hepatic include-colangiocarcinomul, metastazele ganglionare limfatice, colangiocarcinomul intrahepatic cu extensie hilară, limfomul Hodgkin şi non-Hodgkin, respectiv coledoco-litiaza, sindromul Mirizzi, granuloamele, fibroza benignă, tuberculoza, sechelele traumatice sau postoperatorii, fibroza retroperitoneală (15,17,18).

Pacienţii din studiul nostru au fost încadraţi postoperator (Bismuth-Corlette şi TNM) după cum urmează: primul pacient - tip II Bismuth-Corlette şi stadiul IIIB (T1N1M0); al doilea pacient - tip IIIb Bismuth-Corlette şi stadiul IIIa (T3N0); al treilea pacient- tip IIIb Bismuth-Corlette şi stadiul II (T2N0).

Comparativ, clasificarea Bismuth-Corlette preoperatorie, făcută pe baza explorărilor imagistice (colangio-RMN şi computer-tomografie) a clasificat pacienţii în mod diferit. Astfel, la primul pacient tumora a fost supraevaluată imagistic (tip IIIb) datorită dificultăţilor de a obţine detalii la o rezoluţie mai mare de achiziţie şi prelucrare a imaginilor . La doilea pacient, de asemenea suprastadializat (tip IV), tumora de confluent biliar superior a fost etichetată ca având şi invazie arterială şi de perete venos portal, condiţie infirmată la disecţia perivasculară care a făcut posibilă izolarea tumorii de elementele vasculare la nivelul plăcii hilare. La cel de-al treilea caz examinarea tomografică a descris o tumoră de dimensiuni mai mici faţă de cele reale (tip II, substadializare), extensia tumorii nefiind precizată corect datorită caracterului infiltrativ.

Se remarcă neconcordanţele dintre încadrarea cazurilor în tipuri Bismuth-Corlette pre- şi postoperator, ceea ce confirmă limitele acestei clasificări. Simplitatea şi claritatea o fac însă larg şi cvasi-general folosită pentru caracterizarea CCH.

Pe de altă parte, stadializarea TNM fiind stabilită postoperator nu poate fi utilizată în conturarea strategiei terapeutice şi prognosticului în faza iniţială. În schimb, fiind standardizată şi efectuată în toate cazurile constituie baza comună de comparare a oricărei serii raportate în literatură.

În practica serviciului nostru nu a fost utilizată stadializarea propusă de Blumgart şi colectivul de la MSKCC New-York.

Studiul, chiar realizat pe un număr extrem de mic de pacienţi (atât datorită rarităţii acestui tip de tumoră cât şi numărului şi mai mic de pacienţi rezecabili) a permis ajungerea la concluzii similare celor din literatură.

CONCLUZII

Clasificarea Bismuth-Corlette a colangiocarcinoamelor hilare (tumorile Klatskin) este limitată doar la descrierea extensiei longitudinale a tumorii şi este larg folosită în practica clinică. Clasificarea TNM reprezintă standardul internaţional şi cunoaşte modificări continue de la o ediţie la alta, dar nu s-a dovedit a avea valoare prognostică preoperatorie, iar clasificarea Blumgart are calităţi privind prognosticul dar nu este utilizată decât de centrul în care a fost propusă.

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ANAL AND PERIANAL DISORDERS MANAGEMENT

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ANAL AND PERIANAL DISORDERS MANAGEMENT(Abstract): Perianal disorders are the main cause of addressability to the family doctor and to the gastroenterologist. The study is conducted on the background of the increasing incidence of anal pathology in relation with the decrease in the age of appearance. **Aim**: to propose a protocol for management of patients with perianal disease. **Material and methods:** The study included a group of 2,000 patients, who were clinically and imagistic investigated. **Results**: The statistics show a much higher incidence of perianal pathology and an increasing prevalence of it in younger age. **Conclusions**: we propose a management plan for these patients that facilitates early diagnosis and determines the specific treatment required. The impact of this study is both medical and socio-economic as it shortens patient suffering and the time required for active social reintegration. **Keywords**: PERIANAL DISORDERS, HEMORRHOIDS, MANAGEMENT, ANAL DISEASES.

Perianal disorders are the main cause of addressability to the family doctor and to the gastroenterologist. The wide range of pathologies, from benign (hemorrhoids, anal fissures, fecal incontinence, fistulas and perianal abscesses, thrombosis) to malignant (rectal carcinoma), involves a clinical examination and a complete and correct anamnesis (1)

Anal fissure is a linear or oval ulcer in the anal canal below the dentate line extending to the anus. The most frequent location is at the posterior median line, being explained by several theories with anatomical substrate: the elliptical shape of the external anal sphincter, which leads to low resistance of anal canal and relative ischemia of the posterior commissure, which compromises the blood circulation and increases anal sphincter tonus (2).

Hemorrhoids are the increasing and/or distal movement of protrusions of the anal mucosa formed by smooth muscle and blood vessels, along with connective tissue, termed "cushions". Hemorrhoids usually consists of three cushions in the left lateral, right anterior and right posterior positions (2).

These anatomical structures are in the submucosal layer of fibrovascular tissue and due to lack of smooth muscle wall are considered as sinusoidal structures. Hemorrhoids are suspended in the anal canal due to Treitz's muscle, thus explaining the increase in the incidence of pathology with aging, because of the loss of the muscular tonus. When they increase their size become pathological, because of the venous stasis. This process can be accelerated by other pathologies such as high blood pres-

sure, portal hypertension, and abdominal tumors.

Anal cryptitis and papillitis are inflammatory proctologic illnesses with increased incidence. Papillitis is the inflammation of the anal papillae at the distal end of Morgagni's rectal columns. Cryptitis is the inflammation of an anal crypt and corresponding anal glands immediately above the dentate line. Initially, the inflammation occurs at the crypt of Morgagni, and then extends to the anal papilla. Inflammation of the papilla due to the cryptitis and its volumetric growth increase the likelihood that fecal material remains captive in the crypt and initiates inflammation, and if it forms the abscess, anal fissure and perianal fistula may occur.

Cryptoglandular fistula is the most common cause of perianal infection and affects especially males. It represents the infection of the Hernmann and Desfosses glands from the intersphincteric space and their opening to the crypts of Morgagni (1, 2).

Malignant pathology of the perianal region includes rectal cancer as a subtype of colorectal cancer (CCR) and anal cancer. In colorectal cancer, neovascularization is a critical event during tumor genesis, with an early peak in malignant process (3-8). Rectal cancer has a worse prognosis because it is characterized by an increased rate of local recurrence and a higher metastasis presence at the moment of diagnosis (9).

MATERIAL AND METHODS

This retrospective study was performed in a group of 2,000 patients, investigated at Medical ON Group, Iasi, between 12.04.2015-14.05.2018, with ages between 20-80 years old, of which 63% were men and 37% women.

Patients presented with various perianal pathologies: hemorrhoids, perianal abscesses fistula, condylomatosis, various anal and rectal tumors and anal polyps. Patients' anamnesis was performed, following the clinical manifestations, methods of investigation and the therapeutic methods used in the past, followed by the clinical examination of the perianal region and the anal canal. This was done by rectal digital examination, video-anuscopy, and in some cases by rectoscopy to confirm or refute the presence of a colorectal tumor (up to 25 cm) or ulcerative colitis.

A total of 2,050 minimally invasive interventions were performed, such as: rubber band ligation, infrared coagulation and resection with radiofrequency cautery. Using statistical methods, we calculated the prevalence of perianal affections according to the associated risk factors.

RESULTS

We calculated the proportion of each disease in total perianal pathology by reporting the total number of patients with certain pathology in total patients with perianal pathology (tab. I).

TABLE I

The distinctive variation of pathologies in total perianal pathologies

	Hemorrhoid	Perianal abscesses	Perianal fistula	Anal polyps	Anal papilloma	Condylomatosis	Squamous-cellular cancer	Rectal cancer
2015	89.90%	2.57%	1.28%	3.85%	0.51%	0.25%	0.25%	1.28%
2016	84.50%	6.34%	2.11%	4.22%	0.21%	0.63%	0.42%	1.47%
2017	87.64%	7.17%	1.18%	1.66%	0.16%	0.66%	0.50%	1.00%
2018	89.42%	3.15%	4.26%	1.66%	0.37%	0.37%	0.18%	0.55%

The environment from which the patient originates is also important. The emergence of an increasing number of sedentary persons in cities, correlated with the reduced addressability to doctors of the patients in the rural areas leads to significant statistical differences between the two situations (fig. 1).

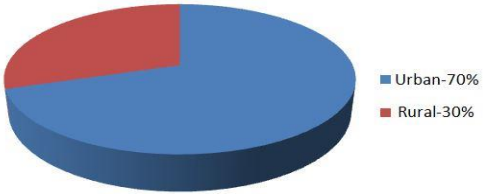

Fig. 1. Distribution of patients according to their origin

The distribution of patients by age and gender shows an increased prevalence of male perianal pathology and draws an alarm signal related to the early onset of age. The most affected age group oscillates between 30-60 years old, with an apex of 40-50 years (72%), as demonstrated in the literature, with respect to the group of 20-30 years, where the incidence of pathologies is low (2 %). We observed that at the age at which the incidence of perianal pathology is increased (40-50 years old) men are 1.6 times more affected than women in the study group (fig. 2).

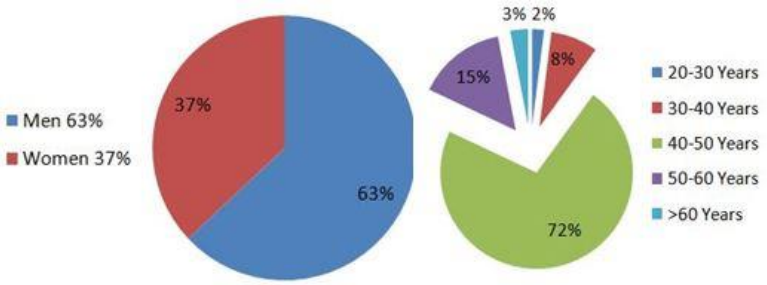

Fig. 2. Distribution of patients by age and gender

We have structured the types of studied pathologies as a percentage of the perianal disease and we observed that prevalence of hemorrhoidal disease is 88% with a total of 1,757 cases since 2,000 and the low prevalence of benign (70 cases) and malignant (28 cases) tumors between 12.04.2015-14.05.2018. In the case of patients with hemorrhoids, the anuscopy revealed a small number of anatomical atypia, 10% presenting turgescent vascular bundle outside the three major bundles. As symptoms, lower digestive hemorrhage and proctalgia are the most frequent.

From the analysis of the living and working conditions of the study group, we obtained data about the occupation and the time spent in the seating position (fig. 3). It was obvious that in the study group, patients with more than 5 hours per day in seating position had an increased risk for perianal diseases.

There is an increased prevalence of hemorrhoidal disease compared to other pathologies, most of the cases being diagnosed in 2015-2016, most of them being men, from urban areas, aged 40-50 years, the most common, in over 50% being hemorrhoidal disease, perianal abscess, perianal fistula and anorectal polyp and the physical activity rate being slightly low, 55% of them spending 5-10 hours in the sitting position.

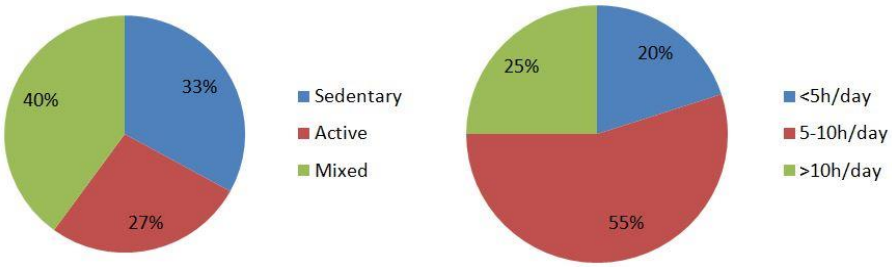

Fig. 3. Distribution of patients according to type of job and time spent sitting

DISCUSSION

Specific prevalence on pathologies over the period 2015-2018 according to the formula Prevalence of period = total number of cases of illness / number of examined persons X 100, were 50% for hemorrhoidal pathology, 4% for perianal abscess, 1.8% for perianal fistula, 2.16% for perianal fissure, 2.16% for anorectal polyps, 0.24% for anal papilloma, 0.4% for condylomatosis, 0.28% for squamous cell cancer and 0.84% for rectal cancer.

Particular attention should be paid to patients with chronic cardiovascular pathology, pacemaker carriers. At the same time, we noticed a special category of patients - vocal artists and those using wind instruments. In their case, the intraabdominal pressure raised during artistic act by contraction of the abdominal anterolateral muscles, correlated with a high vocal tract, can cause the hemorrhoidal disease in time (10-12).

Following anamnesis, the dominant symptom was bleeding with red blood, small but repetitive abundance, conditions that led patients to see their doctor. Of these, 100 patients experienced pain, especially patients who had complicated hemorrhoids with thrombosis. The patient's reported swelling was 70% due to the hemorrhoid prolapse of Stage III-IV. Serum mucosal leakage was also reported in 35% triggering anal pruritus. The beginning of symptoms was acute, first-degree hemorrhoids patients presenting to their physician immediately, and those with a chronic evolution either did not take self-medication or used various unguents (13-17).

The minimally invasive surgical interventions in the ambulatory (18-23) were grouped as follows: 71 patients under IRC (infrared coagulation), 1,686 for RBL (rubber band ligation), 100 incisions and drainage, 17 fistulectomies, 28 fistulotomies, 19 excisions, 2 curettage and 5 excisions and curettages.

Following the study, we have proposed a protocol for ambulatory patient management:

- **Patient's history:** to insist on the symptoms, disease evolution, the debut, the medical history, the exposure to risk factors: sedentary, sitting position - for the benign pathologies and abuse of alcohol, smoking, HIV virus, sexually unprotected anal sex - for malignant pathology or condylomatosis.
- **Clinical examination**: inspection of the anal and perianal region may reveal external fistulae that secretes pus, the presence of lesions produced by laceration in the case of acute anal fissure, or the presence of the tegument impression at the distal end of the chronic fissure. In case of prolapsed internal hemorrhoids, the anal

mucosa appears together with the turgescent venous formation at the level of the anal ring and in the case of the superficial perianal abscess it can be observed an area showing signs of inflammation. Papilloma and prolapsed anal polyps are a perianal skin color formation at the anal orifice. Condylomatosis is white formation, in the anal or perianal orifice or perianal skin. In the case of malignant pathology, these formations may be elastic, mobile in the case of polyps or may be friable, stenotic, and harsh.

- **Imagistic evaluation**: video scanning, eventually fistulography and exploration of the fistula with a buttoned probe and in the case of suspicion of rectal cancer, anal cancer or acute and chronic complicated perianal suppuration, it is recommended to perform a CT, MRI or PET-CT.
- **Preoperative evaluation** with the anamnesis orientated to possible allergic reactions, the medication taken by the patient, the anesthetic-surgical history, possible postinterventional complications, stopping or passing the antiaggregant / anticoagulant therapy on fractional low molecular weight heparin.
- **Treatment** of hemorrhoidal disease is performed according to their degree and complications: in grade I - prophylactic treatment, local and general administrated drugs, minimally invasive - infrared coagulation; in grade II-III - prophylactic, local and general medical treatment, minimally invasive - infrared coagulation corroborated with Rubber Band Ligation; in grade IV - prophylactic, local and general medical treatment, minimally invasive - infrared coagulation, in conjunction with Rubber Band Ligation and classic surgical treatment in case of relapses. In: perianal abscess - incision, evacuation and drainage; perianal fistula - fistulotomy, fistulectomy; anorectal polyps, anal papillae - radiofrequency excision; acuminate condylomas - excision and curettage with radiofrequency (24, 25).

CONCLUSIONS

Under modern society, most patients do not have the time to hospitalize, this problem being solved by minimally invasive ambulatory treatment due to by lack of hospitalization, anesthesia, complications, minimal risks with efficiency, speed and safety, and reducing recovery days. In the last years there has been an increase in the incidence of hemorrhoidal disease (especially) among the young population (20-30 years), with direct socio-economic implications. This is due to the emphasis on sedentary life, especially in the urban areas, but also on labor migration to new areas such as IT.

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FACTORS AFFECTING EARLY MORBIDITY AND MORTALITY IN NON-SMALL CELL LUNG CANCER SURGERY, THE EXPERIENCE OF SURGICAL CLINIC NO. 1, TG. MUREŞ

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FACTORS AFFECTING EARLY MORBIDITY AND MORTALITY IN NON-SMALL CELL LUNG CANCER SURGERY, THE EXPERIENCE OF SURGICAL CLINIC NO. 1, TG. MUREŞ (Abstract): **Introduction.** The incidence of lung cancer has increased alarmingly. Lung cancer represents the first cause of death in men. Thoracic surgery is engraved with increased morbidity and mortality. Therefore, a rigorous selection of patients undergoing such surgery is imposed. In order to establish the correct therapeutic attitude,paraclinical explorations are of the ulmost importance. **Material and methods:** We conducted a retrospective observational study over a period of six years. We used the casuistry of Surgical Clinic no. 1, Mureş County Emergency Hospital. We studied the observation sheets of all the patients admitted in the Surgical Clinic no. 1 over a period of six years (1th of January 2005 to the 31th of December 2010). We studied 197 patients admitted to surgery in our clinic for lung cancer. **Results**: In the group studied, the majority of patients were in the 5th and 6th decade of life. The average age was 59.48 years. In our study group, most patients were in an advanced stage of the disease, probably due to the late stage diagnosis of lung cancer in general. The majority of the patients were in the stage IIB and IIIA of disease. The overall postoperative morbidity rate was 21.82% (43 cases), In the study group we registered 8 deaths (4.06%) – it should be noted that we considered in-hospital mortality within the first 30 days postoperatively. **Conclusions:** The main risk factors for postoperative complications are: late age, male gender, pneumonectomy, low FEV_1 value. Certain risk factors are predictive of postoperative mortality. The most important of them are: late age, male gender, type of surgery, FEV value, associated diseases (especially cardiovascular and diabetes). **Key words**: LUNG, CANCER, MORBIDITY, MORTALITY, FISTULA

In the past decades the incidence of lung cancer has increased alarmingly. It represents the principal cause of death in both men and women in the United States of America (1).

In our country, the incidence of lung cancer has increased dramatically in the last 10 years, becoming the most frequent type of cancer in men and the third one in women (after breast cancer and cervical cancer). In 1990, the incidence ratio men/women was 9 to 1, now it has reached 2 to 1.

The purpose of the present study is to evaluate the most important factors that af-

fects early morbidity and mortality of patients with lung cancerthat were admitted and operated in the Surgical Clinic nr. 1 of the Mureş County Emergency Hospital, for over a period of six years, between the 1th of January 2005 and the 31th of December 2010.

MATERIALS AND METHODS

We conducted a retrospective observational study over a period of six years. We used the casuistry of Surgical Clinic nr. 1, Mureş County Emergency Hospital. We studied the observation sheets of all the patients admitted in the Surgical Clinic nr.1 over a period of six years (1th of January 2005 to the 31th of December 2010). We studied 197 patients admitted to surgery in our clinic for lung cancer.

The 197 patients in the study were divided into two groups :

- Group A : patients with malignant lung tumors that underwent typical pulmonaryresections (lobectomy, bilobectomy or pneumonectomy).
- Group B : patients with malignant lung tumors that underwent atypical pulmonary resections (wedge resections, exploratory thoracotomy with biopsy).

A standard set of preoperative investigations were performed on all the patients, necessary for both diagnostic purposes and for the type of surgery. Thus, the following tests were carried out : blood tests (blood count, biochemistry and coagulation), chest radiography in two incidents, chest CT, ventilatory function tests, ECG, echocardiography.

Staging was preformed postoperatively, using the TNM system (pTNM).

RESULTS

We have entered into the study 197 patients admitted with malignant lung tumors in the Surgical Clinic Nr. 1 in the period 1th of January 2005 to the 31th of December 2010. The two groups used were :

- Group A : 106 patients (53.8%) that underwent typical resections (lobectomy, bilobectomy, pneumonectomy)
- Group B : 91 patients (46.2%) that underwent wedged resections and exploratory thoracotomy

In terms of distribution per year of patients taken into the study, we obtained a relatively uniform distribution of cases. By studying the observation sheets of patient's and Surgical Clinic nr.1's casuistry, we obtained the following distribution of cases by year :

Of the 197 patients studied, 41 were women (20.82% of the cases) and 156 were men (79.18% of the cases).

Most patients were in the 5th and the 6th decade of life. The average age of patients in the study was 59.48 years. The average age in the group of men was 57.07 years, and in the group of women was 60.12 years. The P value was calculated to be 0.107 so there is no statistically significant diffrence between the age of patients.

In the group studied the vast majority of patients were smokers – 160 patients (81.21%) – the role of smoking as a risk factor in the etiology of lung cancer is well known. In terms of the correlation between smoking and the sex of the patients, 35 women (85.36%) and 125 men (80.12%) were smokers. From a statistical viewpoint, the p value was calculated to be 0.502 so we can not say that there is a statistical association between the two values.

Given the fact that lung cancer generally affects advanced ages, in the groups studied 75 patients (38.07%) had comorbidities. They were represented by : cardiovascular disease : hypertension (28 cases – 14.21%), ischemic heart disease (32 cases – 16.24%), arrhythmia (6 cases – 3.04%), chronic PAD (3 cases – 1.52%), MI scars (6 – cases 3.04%), stroke sequelae(4 cases – 2.03%). We also had patients with respiratory disease : COPD (8 cases – 4.06%), chronic bronchitis (4 cases

TABLE I
Patients / year

Year	Nr. of cases
2005	38 patients
2006	31 patients
2007	30 patients
2008	36 patients
2009	26 patients
2010	36 patients

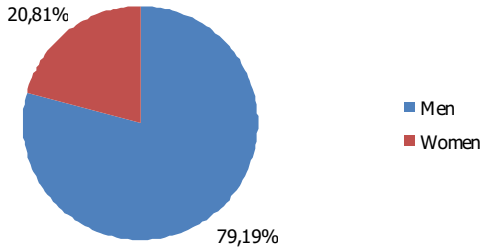

Fig. 1. Distribution of patients according to sex

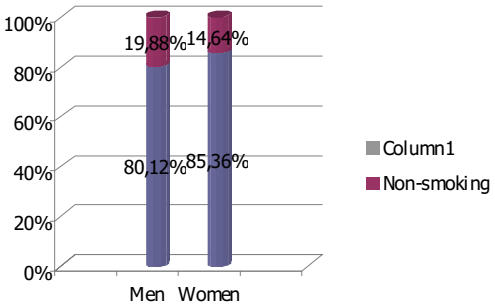

Fig. 2. The incidence of smoking by gender

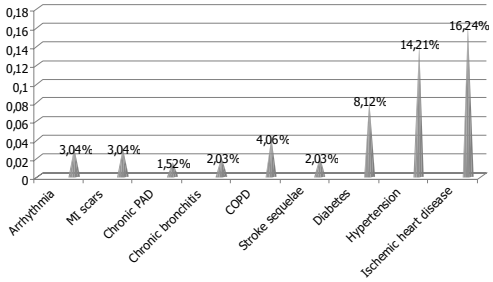

Fig. 3. Associated disorders

– 2.03%). The incidence of diabetes mellitus was 8.12% (16 cases). We also recorded diseases with a lower rate of incidence: GI tract disorders (4 cases – 2.03%), kidney disorders (4 cases – 2.03%), orthopedic disorders (1 case – 0.5%).

By analyzing the FEV parameter (FEV_1), we observed that most patients (53.29% - 105 patients) had a FEV over 60% of the ideal value and 46.71% (92 patients) had a FEV_1 value below 60% of the ideal value. The FEV_1 ideal value is calculated based on age, sex and weight. We used as the standard the value of 60% since the literature considers that patients with a FEV_1 below 60% have a higher risk of pulmonary excision surgery. In terms of distribution according to the two groups studied we observed that:

- in group A (typical resections): 89 patients (83.96%) had a FEV_1 over 60%, and 17 patients (16.04%) had a FEV_1 below 60%
- in group B (wedged resections): 75 patients (82.41%) had a FEV_1 below 60%, and 16 patients (17.59%) had a FEV_1 over 60%

We studied the two groups of patients depending on the type of surgery. In group A we had 106 patients that underwent typical resections: 13 bilobectomy (12.27%), 52 lobectomies (49.06%), 41 pneumonectomies (38.67%). In group B we had 91 patients that underwent atypical resections: 54 wedged resections (59.34%) and 37 exploratory thoracotomies with biopsy (40.66%).

In our study group, most patients were in an advanced stage of the disease, probably due to the late stage diagnosis of lung cancer in general. The distribution of these patients based on disease stage is represented on figure nr. 7. In the analysis of the casuistry based on disease stage we excluded the 15 patients with lung metastases.

In order to study the incidence of bronchial stump fistula we divided the patients that presented this complication into two lots: those who developed the fistula early and those who developed it late. We divided

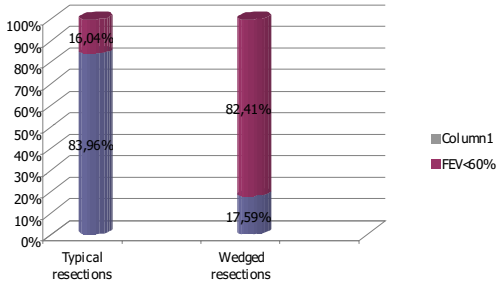

Fig. 4. Groups distribution according to FEV

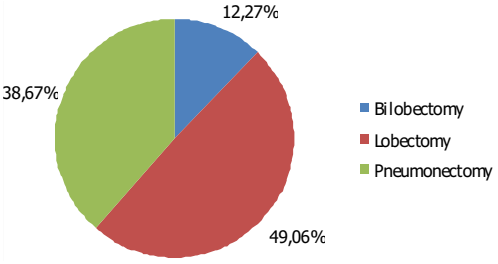

Fig. 5. Distribution of cases that underwent typical resection

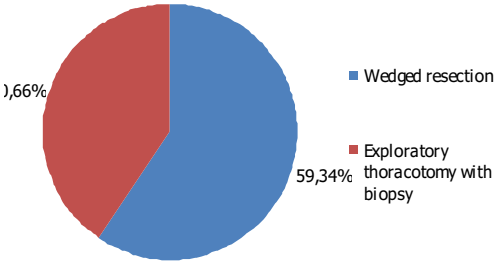

Fig. 6. Distribution of cases that underwent atypical resection

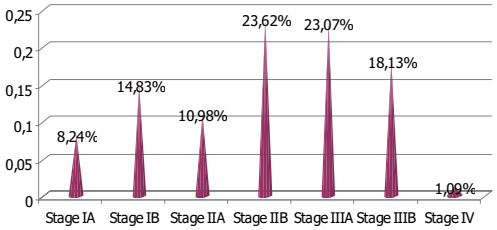

Fig. 7. Distribution of cases according to disease stage

these patients based on the etiological causes that lead to early bronchial stump fistula hence they are different from the ones that are responsible for the apparition of late bronchial stump fistula. The incidence of early bronchial stump fistula was 1.88% (2 cases, 1 right pneumonectomy and 1 left pneumonectomy) while the incidence of late bronchial stump fistula was 4.71% (5 cases : 1 lobectomy, 1 bilobectomy, 1 left pneumonectomy and 2 right pneumonectomies). In all the patients that presented this complication we preferred a conservative approach : passive pleural drainage, pleural cavity lavage with antiseptic solutions on the drain (Betadine), antibiotic treatment according to the sensibility tests from the secretions on the drain and symptomatic treatment.

To analyze the postoperative complications that occurred in our study group we split the complications into 2 lots : minor postoperative complications (non – life – threatening) and major postoperative complications (life threatening). We also took into account the sex of the patients that developed postoperative complications, the type of surgery performed on these patients. We excluded patients with bronchial stump fistulas as we studied them separately. The overall postoperative morbidity rate was 21.82% (43 cases), there were 13 patients (6.59%) who had postoperative complications, both minor and major. The results were as follows :

- Minor postoperative complications : 37 cases (18.78)
- Major postoperative complications : 19 cases (9.64%)

We divided the group of patients who experienced postoperative complications by gender. We observed that 88.37% of them were men (38 cases), compared with 11.63% (5 cases) – women. Thus there is a direct corelation between patients gender and the occurence of postoperative complications, male gender representing an independent risk factor.

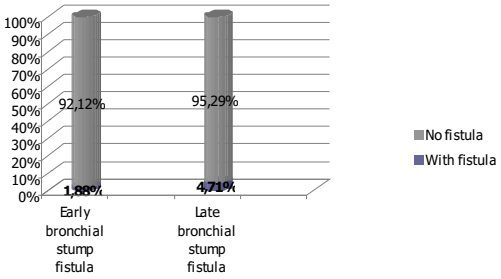

Fig. 8. The incidence of bronchial stump fistulas

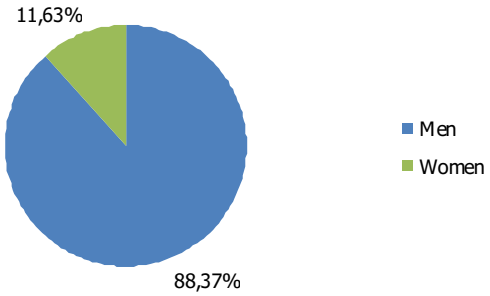

Fig. 9. The correlation between patient's sex and the occurrence of postoperative complications

TABLE II
The incidence of minor postoperative complications

Complications	Nr. of cases	Percentage
Atelectasis	3	1.52%
Bronchial obstruction	3	1.52%
Recurrent laryngeal nerve paresis	2	1.02%
Minor air loses	8	4.06%
Suppuration of the wound	2	1.02%
Arrhythmias	19	9.64%
Total:	37	18.78%

We also took into account the type of surgery performed in patients who had postoperative complications. The distribution was as follows: 3 cases –thoracotomy (6.97%), 16 cases –lobectomies (37.20%), 2 cases – bilobectomy (4.65%), 15 cases – pneumonectomy (34.88%) and 7 cases with wedge resections (16.27%). A total of 33 patients (76.74%) underwent typical pulmonary resections while atypical resections were performed on only 10 patients (23.26%). We thus obtained a direct correlation between the type of surgery and postoperative complications.

We studied the postoperative morbidity rate for each type of surgery and we concluded: 8.10% of patients that underwent exploratory thoracotomy, 12.96% of patients that underwent wedge resections, 30.76% of patients that underwent lobectomies, 15.38% of patients that underwent bilobectomies had postoperative complications. The highest morbidity rate we found was on patients that underwent pneumonectomies (36.58%).

In the study group we registered 8 deaths (4.06%) – it should be noted that we considered in-hospital mortality within the first 30 days postoperatively. The main causes of death were respiratory and cardiovascular failure but we also had one case of acute renal failure.

The main causes of death are shown in the table below:

An independent predictive factor in postoperative mortality and morbidity is the preoperative value of FEV_1. We studied

TABLE III
The incidence of major postoperative complications

Complications	Nr. Of cases	Percentage
Stroke	2	1.02%
Pulmonary embolism	3	1.52%
Acute pulmonary edema	1	0.51%
Hemothorax	6	3.05%
MI	1	0.51%
Respiratory failure	5	2.54%
Acute renal failure	1	0.51%
Total	19	9.64%

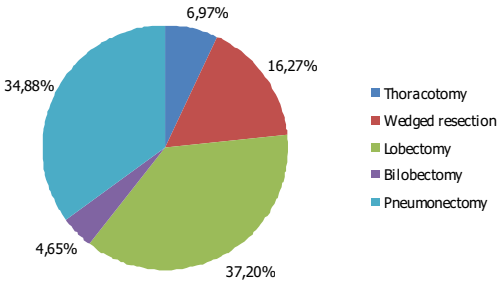

Fig. 10. Distribution of postoperative morbidity according to the type of intervention applied

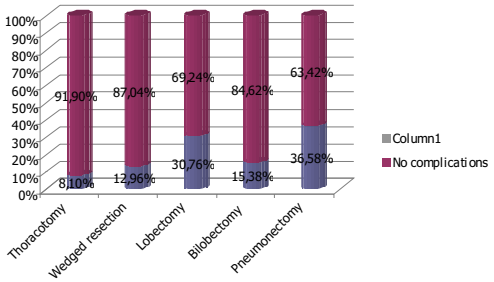

Fig. 11. The incidence of postoperative complications according to type of surgery

the FEV_1 value in patients deceased within 30 days postoperatively. We used as a standard the FEV_1 value of 60% of the ideal value. The FEV_1 ideal value is calculated based on age, sex and weight. Of the 8 patients who died, 5 (62,5%) had a FEV_1 on addmission over 60%, 3 patients (37,5%) had a FEV_1 under 60%. On the other hand, 4,76% of patients with FEV_1 over 60% died and and 3,26% of the patients with FEV_1 below 60% died. We did not obtained a statisticaly significant corelation between patient's death and the value of FEV_1 determined preoperative ($p=0.5943$).

We also did a review of the age of patients who died in the first 30 days postoperatively. In the group of the patients that died, the average age was 58.87 years, lower than the overall average of the lot which was 59.48 years. Of the patients who died, 6 were males (75%) and 2 were women (25%).

We also studied the correlation between overall mortality and type of surgery. Depending on the type of surgery, we recorded the highest mortality rates for patients who underwent lobectomy (3 cases – 1.52%); pneumonectomy (3 cases – 1.52%) and we recorded 3 deaths in patients who underwent exploratory thoracotomy (2 cases – 1.02%).

We studied separately the mortality rates for each type of surgery. The highest mortality was in patients who underwent pneumonectomy (3 cases – 7.31%).The mortality rate in patients who underwent lobec-

TABLE IV
The main causes of death

Cause of death	Nr. of cases	Percentage
Stroke	2	1.02%
Pulmonary embolism	2	1.02%
MI	1	0.50%
Respiratory failure	2	1.02%
Acute renal failure	1	0.50%
Total	8	4.06%

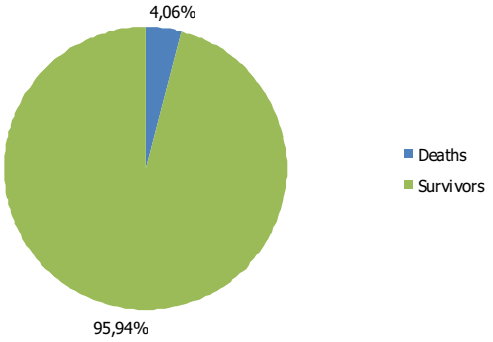

Fig. 12. Overall mortality rate

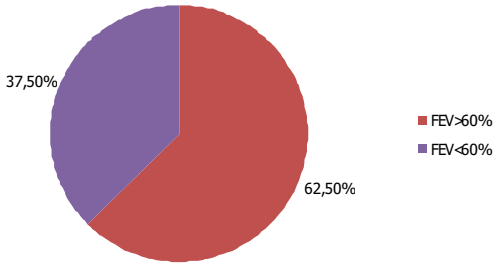

Fig. 13. Correlation between mortality and FEV's value

tomy was 5.76% (3 cases) and patients who underwent exploratory thoracotomy had a mortality rate of 5.40% (2 cases). In patients deceased following pneumonectomies, out of the 3 patients, 2 had a right pneumonectomy and 1 had a left pneumonectomy. There were no deaths in patients who underwent wedged resections or bilobectomy.

The analysis of deceased patients, in terms of associated diseases, revealed that the majority of patients had cardiovascular disease, diabetes or renal disease (2 cases of chronic renal failure). These patients usually had more than one associated diseases. We did not obtain a statistically significant relationship between mortality and associated diseases ($p = 0.2051$).

DISCUSSION

Thoracic surgery is engraved with increased morbidity and mortality. Therefore, a rigorous selection of patients undergoing such surgery is imposed. In order to establish the correct therapeutic attitude, paraclinical explorations are of the ulmost importance. The investigations of lung cancer patients serve a double purpose: both for diagnosis and for determining the surgical indication.

Laboratory examinations performed for the diagnosis of lung tumors used in our study were: chest X-ray, thoracic and abdominal CT scans, bronchoscopy. The remaining laboratory examinations were performed to determine the possibility and desirability of a surgical intervention.

ECG and echocardiography were used to diagnose possible cardiac co-morbidity. Echocardiography can detect neoplastic changes in the cavities of the heart and the major vessels of the heart which is a contradiction to surgery (T4 stage cancer). Chest CT can

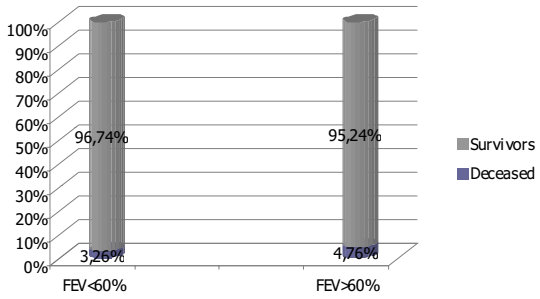

Fig. 14. Mortality rates according to FEV's value

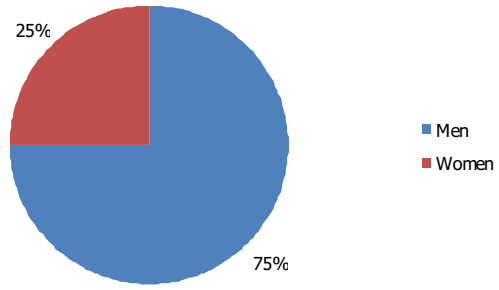

Fig. 15. Gender distribution of patients who died

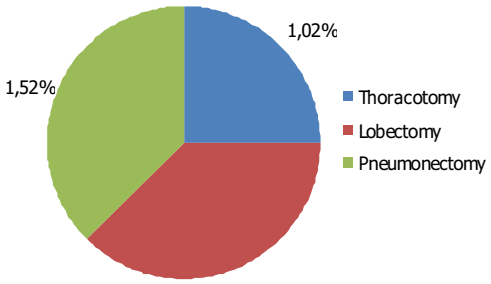

Fig. 16. Mortality rate by type of surgery

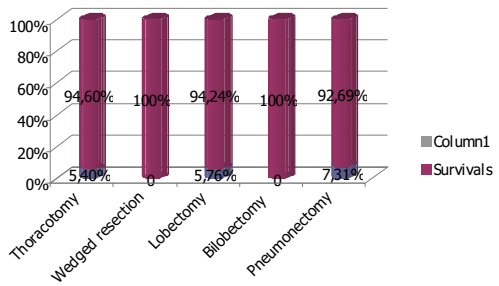

Fig. 17. Mortality rate by type of surgery

detect the invasion of major vessels, esophagus, distant metastases – also a contradiction to surgery (stage IV disease). PET-CT was only done in cases barely within the limit of surgery for a more accurate preoperative staging (1).

A central role in determining the surgical indication is occupied by ventilatory function tests. Olsen (1994) considers that operability of a patient can be established based on ventilatory function tests. For a patient to withstand lung surgery the FEV_1 must be over 60% of the ideal value and the FEV / VC ratio over 50% of the ideal.The ideal value is calculated based on age, sex, weight (2).

The majority of patients who underwent typical resection had a FEV_1 value of over 60% while the patients that underwent atypical resection had a FEV_1 value of below 60%. This difference is due to a rigorous selection of the patients that will undergo typical resection. On the other hand there were patients, who were in an operable state, but due to the low values of the ventilatory function tests, surgery with curative intent was not feasible, thus we opted for a wedged resection or an exploratory thoracotomy with biopsy. The ultimate goal of typical resections (lobectomy, bilobectomy and pneumonectomy) is practicing pulmonary typical resection combined with mediastinal lymphadenectomy.

There are 17 patients in our study who had a FEV_1 value of below 60% and yet typical resections were performed. 16 of these patients underwent lobectomy and 1 patient underwent a left pneumonectomy. For those 16 patients, surgery was performed due to their good clinical condition and the neoplastic disease was in it's infancy. The one patient with FEV_1 below 60% who underwent a pneumonectomy was out of necessity.

One of the most frequent complications after typical resections is the bronchial

TABLE V
Diseases associated to deaths

Associated diseases	Nr. of cases	Percentage
Stroke sequelae	3	37.5%
Diabetes	3	37.5%
Ischemic heart disease	3	37.5%
Hypertension	1	12.5%
COPD	1	12.5%
MI scars	2	25%
Chronic renal failure	2	25%

stump fistula. Bronchial stump fistula greatly increased the morbidity and mortality of these patients.

Regarding the postoperative complications, most of them occurred in males (88.37%) compared with just 11.63% in women. We place this discrepancy between sexes on the associations with cardiovascular disease. The higher incidence of atherosclerotic disease is well documented to be higher in males compared to women, especially in smokers.

76.74% of the patients who underwent typical resection had postoperative complications, while only 23.26% of the patients who underwent atypical resections had postoperative complications. This statistically significant association between the type of surgery and the postoperative morbidity is consistent with the literature data. Also, the highest postoperative morbidity rate was in patients that underwent pneumonectomy (36.58%), data are consistent with the literature.

The majority of postoperative complications, both major and minor were complications of the cardiovascular system and the respiratory system. These complications are to be expected in lung cancer surgery; the heavy smoker has a specific pathology which includes cardiovascular disease.

Morandi (1997) published a study on the occurrence of major postoperative complications after typical pulmonary resections, the incidence of these complications in their study was 9.64% (3). In the group we studied the incidence of these complications was 9.64%, slightly lower, probably because we studied patients with both typical resections, atypical resections and exploratory thoracotomy.

The average hospitalization duration is smaller for patient that underwent atypical pulmonary resection in comparison with patients that underwent typical pulmonary resection. These results are normal, atypical pulmonary resections are interventions with reduced morbidity and mortality compared with typical pulmonary resections; also the postoperative respiratory adaptation in much faster after an atypical resection. We obtained a lower mean duration of hospitalization when comparing bilobectomies with lobectomies because of the small number of bilobectomies (13 cases) compared to the lobectomies (52 cases). Morbidity was higher when lobectomies were practiced.

Achieving average length of hospitalization so high (16.84 days) shows that lung cancer surgeries are interventions with increased morbidity, increased financial costs to the health system and a longer term reintegration of these patients.

Pulmonary excision surgery is major surgery requiring a rigorous selection of patients (4,5).

A particularly important role in select-

ing patients is an accurate assessment of the ventilatory function tests. Not only the preoperative value is important as is predicting the postoperative value depending on the type of surgery performed. The FEV is regarded as the standard predictive risk factor for postoperative mortality in the literature (6,7).

FEV's importance as a risk factor for typical pulmonary resections manifests itself especially when pneumonectomies are performed. Understandably so, because these types of surgery, remove a far wider surface involved in haematosis.

In our study we did not obtain a statistically significant correlation between mortality and the value of preoperative FEV. A percentage of 4.76% patients with FEV over 60% died and 3.26% of patients with FEV below 60% died ; this diffrence is probably due to the fact that major surgery (typical pulmonary resection) was not performed on these patients.

Strand (2007), believes that another predictive factor in postoperative mortality is the patients age and type of surgery. The higher the age, the higher the postoperative mortality is, both because of the disease itself and because of co-morbidities. Typical pulmonary resections are associated with higher mortality and morbidity than atypical resections, especially right pneumonectomy (8).

In our study group, mean age of patients who died was 58.87 years, lower than the overall group which was 59.48 years. Our data are in contradiction with those published by Strand in 2007, probably due to the difference between the groups studied, associated pathology and different stages of the disease.

Instead we got a direct correlation between the sex of the patients and postoperative mortality, 75% of them were men, while only 25% were women.

We found a direct correlation in our study group between the type of surgery and the rate of mortality. We recorded the highest mortality rate in patients who underwent pneumonectomy (7.31%), followed by a 5.76% mortality rate in patients who underwent lobectomy.This is consistent with data published in the literature, various authors published a mortality rate after pneumonectomy ranging between 3.5% and 11.2% (9, 10,11).

Reviewing the literature, we found that typical pulmonary resections mortality varies between 2% and 12% . These relatively high differences are due especially to the size difference in the studied groups in each paper published. We obtained a mortality rate of 4.06% which is included in those obtained in literature.

We tried achieving a correlation between the rate of mortality and associated diseases ; the majority of patients had cardiovascular diseases and diabetes, but we could not obtain a statistically significant correlation between the two parameters.

Romano, in an article published in 1992, adds to the predictive factors with regard to postoperative mortality diabetes, ischemic heart disease and COPD (12). This is also true for our study.

CONCLUSIONS

Pulmonary excision interventions remain interventions with high mortality and morbidity. Pulmonary excision surgery is major surgery, so it requires a rigorous selection of patients that are undergoing such interventions. The vast majority of patients with lung cancer have numerous co-morbidities in addition to the respiratory disease. The main risk factors for postoperative complications are : late age, male gender, pneumonectomy, low FEV_1 value. Certain risk factors are predictive of postoperative mortality. The most important of them are : late age, male gender, type of surgery, FEV value, associated diseases (especially cardiovascular and diabetes).

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ENDOSCOPIC VACUUM-ASSISTED CLOSURE (E-VAC) IN ACUTE MEDIASTINITIS FROM ANASTOMOTIC LEAKAGE AFTER INTRATHORACIC ESOPHAGEAL JEJUNAL ANASTOMOSIS

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ENDOSCOPIC VACUUM-ASSISTED CLOSURE (E-VAC) IN ACUTE MEDIASTINITIS FROM ANASTOMOTIC LEACKAGE AFTER INTRATHORACIC ESOPHAGEAL JEJUNAL ANASTOMOSIS (Abstract): Our aim was to assess the role of negative pressure therapy in the treatment of anastomotic leakage, after intrathoracic esophageal jejunal anastomosis by the evaluation of the time required for healing and the functional outcomes of patients. We present the case of a 73 years old male patient admitted in our clinic with esophageal perforation that appeared possible after a left thoracotomy necessary for an intrathoracic esophageal jejunal Roux-en-Y procedure, after a total gastrectomy for gastric neoplasm. There are groups that propose different approaches such as surgery variations or combinations of the surgery with or without adjuvant treatments, as the use of a hyperbaric chamber or vacuum devices such as Endoscopic Vacuum-Assisted Closure (E-VAC). Using a modified double-lumen naso-gastric probe adapted to the negative pressure unit and using the same principle as in the negative pressure treatment of soft tissue wounds we have achieved positive results with successful cure of esophageal perforation. The toraco-abdominal computed tomography done with contrast substance confirmed the esophageal perforation. The patient had a full recovery after 19 days of hospitalization with a positive check-up at one and two months after leaving our clinic. After the basic wound treatment, auxiliary methods such as negative pressure contributed to healing. Endoscopic vacuum treatment for parietal lesions of the esophageal wall is a great procedure in the management of this pathology with a high mortality and the surgeons and gastroenterologist must have considered as a treatment option in this kind of lessons. The prospective and comparative studies are necessary for the evaluation of this new and minim invasive procedure. **Keywords**: ENDOSCOPIC VACUUM-ASSISTED CLOSURE (E-VAC), ANASTOMOTIC LEAKAGE, INTRATHORACIC, ESOPHAGEAL JEJUNAL ANASTOMOSIS.

INTRODUCTION

Acute mediastinitis caused by bacterial infection due to esophageal perforation or sternotomy is a devastating disease which is frequently unresponsive to best medical therapeutic efforts, but if is diagnosed in its incipient state, and a well-planned treatment is instituted may favorably alter the prognosis (1).

Inflammation of the mediastinum may have several causes, among which are median sternotomy and esophageal perforation caused by Boerhaave's syndrome, dilatations or foreign bodies, as well as head and neck suppurations such as peritonsillar abscesses, deep cervical abscesses. Other less common causes include pleural empyema, osteomyelitis of the vertebrae and ribs, retroperitoneal abscesses and sub phrenic abscesses (2).

Although long recognized as a complication of certain infectious diseases, most cases of mediastinitis are associated with cardiac surgery (>300,000 cases per year in the United States). This complication affects approximately 1-2% of these patients (3).

The perforation of the esophagus is usually iatrogenic, from endoscopic procedures such as esophageal dilatation for strictures and for achalasia. It can also result from surgical interventions in close proximity to the esophagus. In about 15% of the cases, the rupture of the esophagus can be produce spontaneous without preexisting pathology. This is mostly related with intra-abdominal pressure from intense vomiting or severe retching, a clinic-pathologic entity originally called Boerhaave's syndrome. The same underlying mechanisms is involved in esophageal ruptures related to weight lifting, parturition, status epilepticus, defecation or to use of the Heimlich maneuver. Rare causes of perforation include external air-blast trauma and blunt trauma (4).

Another risk of esophageal perforation are deep neck infections of the oropharyngeal region. Delay in the diagnosis and treatment of deep neck infections may result in grave consequences. Once patients with this infection is complicated by descending necrotizing mediastinitis, one of the most disastrous complications, the mortality rate may be as high as 60%.

Conventional treatment of esophageal perforation is surgical. However, more recently, endoscopic treatment has become the treatment of choice given its less invasive nature compared to surgical treatment. This includes endoscopic clip administration, endoscopic stent placement, endoscopic suturing, endoscopic vacuum therapy and tissue sealants which are all administered via the endoluminal route. Whilst small defects (< 2 cm) may be closed with endoscopic clips, larger defects require stent placement or suturing. Removable esophageal stent is an effective method of treatment in cases with esophageal perforation as they allow minimal invasive repair of perforation and rapid nutrition. Endoscopic suturing can be used both to fix the stent to prevent migration and to primarily close the perforation. If perforation is associated with a mediastinal collection, drainage is mandatory, and this procedure can be performed by computed tomography guided percutaneous drainage, thoracoscopy or endoscopic vacuum therapy. In some cases, a combination of these minimally invasive methods is required. Since endoscopic methods provide better quality of life and outcomes and shorten length of hospitalization, such methods have become the treatment of choice for esophageal perforation (5).

Most surgeons believe that early diagnosis and invasive treatment are mandatory to effectively control the acute mediastinitis. Surgeons have not reached a consensus on the standard treatment protocol for such patients due to diversity in the causes and locations of infection. Some cases need surgical intervention, some kind of drainage or debridement. But the optimal technique of drainage and the most suitable surgical approach remain debatable (6).

Endoscopic vacuum-assisted closure (E-VAC) therapy is a new method for repairing defects of the upper gastro-intestinal system with different etiologies. This method has high success rates. E-VAC therapy consists of placing a sponge either within the lumen or within an abscess cavity connected with a nasogastric tube to a negative pressure system, thus decreasing bacterial contamination and edema and promoting granulation tissue proliferation, thereby gradually decreasing the cavity size until complete closure (7).

This method was developed in the early 1990s and is now standard of care for chronic surface wounds, ulcers, and burns. Adapting vacuum sponge therapy for use intraluminal for perforations of the esophagus was first reported in 2008 (8).

Required materials are a double-lumen naso-gastric probe, vacuum S size kit, surgical instruments for small surgery, and a vacuum pump.

Device preparation requires information about the size of the defect to be explored and also provides data for endoscopic guidance in preparing contact material (foam kit) and sizing the naso-gastric tube dimensions that will be mounted on the sponge. Obtaining the necessary data for the intervention is done by performing a CT scan (fig. 1).

The intervention involves a mixed endoscopic-surgeon-anesthetist team and the therapeutic gesture will take place in the surgery room, patient requiring general anesthesia with oro-tracheal intubation.

Fig. 1. The endoluminal vacuum kit.

Preparing the sponge according to the size of the cavity will be made respecting all three dimensions measured by the CT scan previously performed. The naso-gastric probe is adjusted according to the length of the sponge. The holes in the naso-gastric probe should not exceed the proximal and distal edge of the future sponge sleeve.

Perforated sponge is drilled by following an imaginary line through its center along its entire length, representing the future path through which the naso-gastric probe will be passed.

After preparing all this upper gastrointestinal endoscopy is performed with viewing the cavity of the wound, inspection and lavage.

The naso-gastric probe is passed through the nasal orifice and exteriorized through mouth, when the previously made sponge is attached with its firm anchorage. The endoscopist takes and drives the device created to the defect by placing it inside the cavity to be drained actively. The aspect of the assembly is visualized by the retraction of the endoscope and also under visual control is initiated the aspiration with negative pressure.

The endoscope is withdrawn, and the naso-gastric probe firmly fixed to the nose wing.

CASE REPORT

We present the case of a 73 years old male patient, which was admitted in our clinic with severe pain in the superior abdominal quadrant, lack of appetite, and loss of weight (about 20 kilograms in 10 months), eructation. From the personal pathological antecedents, we remember chronic hepatitis with C virus, chronic cardiac failure and mitral insufficiency. From the patient statement we understood that he was admitted in the Gastroenterological Institute with these symptoms, where the final diagnose was gastric neoplasia. We performed on the 2nd of November 2017 an exploratory laparotomy with adhesiolysis, total gastrectomy, DII lymphadenectomy and an intrathoracic esophageal jejunal anastomosis a la Roux-en-Y, pleural and multiple peritoneal and mediastinal drainage. In the first day after intervention the patient presented an anastomotic leakage on the esophageal jejunal anastomosis. A reintervention was performed with the suture of the fistula a mediastinal drainage. On the 10th day postreintervention the radiologic examination with opac index revealed a fistula tract on the left postero-lateral side of the esophageal jejunal anastomosis, fistula confirmed by the endoscopic and computed tomography examination.

We decided the minim invasive treatment - Endoscopic Vacuum-Assisted Closure (E-VAC) therapy on 17th of November 2017 and also, we performed a jejunostomy tube feeding, followed by the closure of the anastomosis defect thru inserting the E-VAC kit by superior endoscopy (fig. 2).

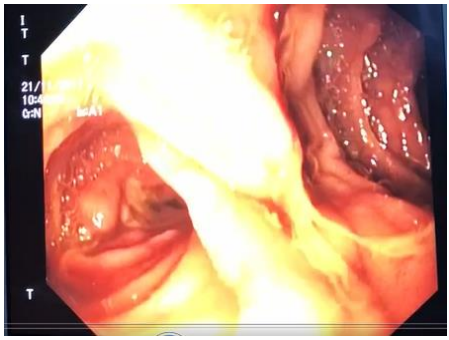

Fig. 2. Endoscopic initial aspect

We prepared the endoluminal vacuum kit (fig. 3) as we described before, with the

placement of the kit in the interior of the perforation following the description of the CT examination.

We set the device at a 165-mmHg pressure. The patient was motorized in the ICU Clinic for his biological status, enteral and parenteral feeding, and the good function of the vacuum kit (the washing of the kit to avoid the clotted of the secretions in the tube system).

We performed the change of the vacuum kit in the first state at 4 days after installing it, after that at 5 days under endoscopic control, with the checkup of the cavity that was smaller every time and the general status of the patient was better.

On post-operative day 19 the endoluminal vacuum kit was suppressed under endoscopic control, with complete healing (fig.3) which is also supported by the appearance of the superior digestive examination with opac index (fig. 4).

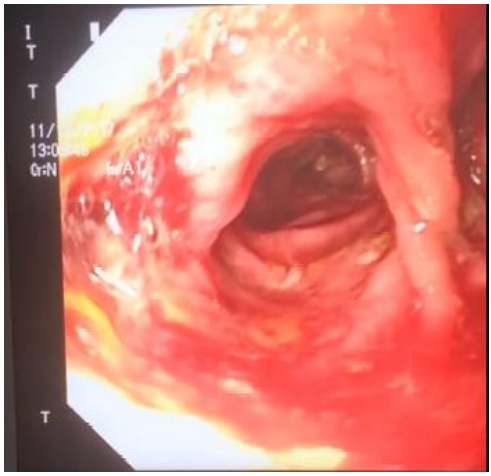

Fig. 3. Endoscopic control aspect

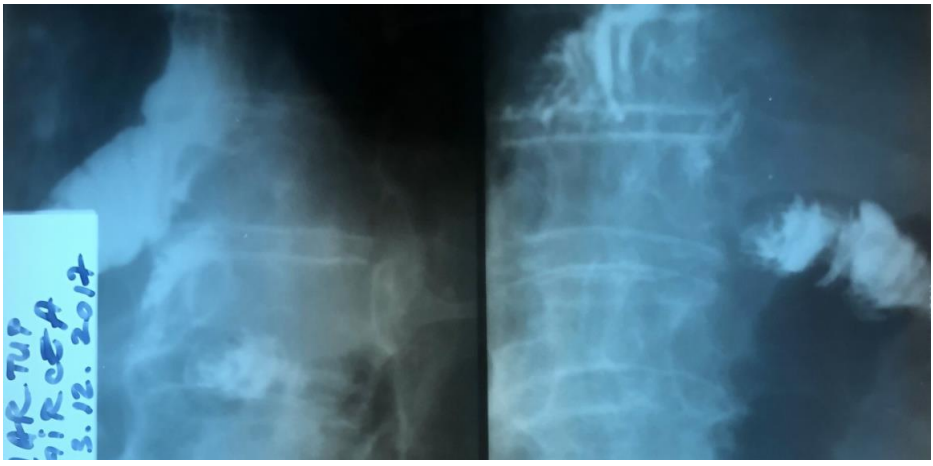

Fig. 4. Radiologic control aspect

DISCUSSION

Esophageal perforation is a rare and potentially life-threatening condition. Early clinical suspicion and imaging are important for case management to achieve a good outcome. However, recent studies continue to report high morbidity and mortality greater than 20% from esophageal perforation. At least half of the perforations are iatrogenic, mostly related to endoscopic instrumentation used in the upper gastrointestinal tract, while about a third are spontaneous perforations. Surgical treatment remains an important option for many patients, but a nonoperative approach, with or without use of an endoscopic stent or

placement of internal or external drains, should be considered when the clinical situation allows for a less invasive approach.

The rarity of this emergency makes it difficult for a physician to obtain extensive individual clinical experience; it is also challenging to obtain firm scientific evidence that informs patient management and clinical decision-making. Improved attention to nonspecific symptoms and signs and early diagnosis based on imaging may translate into better outcomes for this group of patients, many of whom are elderly with significant comorbidity (4). In a study performed on 17 patients with esophageal perforation the success rate of E-VAC to seal all esophageal perforations was 88% (8).

Endoscopic vacuum assisted closure is a new technique for the drainage of mediastinal collections. In acute esophageal perforation, appropriate drainage of the extra luminal contamination is necessary for a successful treatment modality.

Drainage procedure may be performed by open or minimally invasive surgery or interventional radiology. In addition to that, recently, E-VAC sponge system started to be used for the treatment of perforation as it was used to close soft tissue defects. While this approach is mainly used for chronic fistulas, it was also thought to be used for acute esophageal perforation (9). A study performed one 52 cases, 39 cases had anastomotic leakage, 13 cases had esophageal perforation, and 94% success rate was reported (10). Brangewitz *et al*. compared 39 patients who were treated using SEMP or SEPS with 32 patients who were treated with E-VAC for the treatment of intrathoracic esophageal leakage. This could be also correlated in some small degree with the latest interest of our group in a multidisciplinary approach on laparoscopic surgery and metabolic dysfunctions associated deficits (11-15), especially considering that esophageal perforation can be indeed associated with a multitude of other manifestations (16).

In a multi-variable analysis, successful wound closure was found to be independently associated with E-VAC treatment. The closure rate of the leakage in E-VAC group (84.4%) was found to be significantly higher compared to SEMS / SEPS group (53.8%) (17).

A study performed to evaluate the more effective therapy for the postsurgical gastro esophageal leakage by a head-to-head comparison of endoscopic vacuum therapy and endoscopic stent implantation with self-expandable metal stent, conclusion that vacuum is more effective and has fewer adverse effects than stent therapy as a treatment for postsurgical gastroesophageal leakage.

They believe that the advantages of vacuum therapy include a shorter median closure time, shorter hospital stay, and lower complications compared with stent therapy, which make it a potential alternative option for treating postsurgical gastroesophageal leakage (18).

CONCLUSIONS

Endoscopic vacuum treatment (EVT) for parietal lesions of the esophageal wall is a great procedure in the management of this pathology with a high mortality and the surgeons and gastroenterologist must have considered as a treatment option in this kind of lesions.

The prospective and comparative studies are necessary for the evaluation of this new and minim invasive procedure.

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ACTUALITĂŢI ÎN TRATAMENTUL MEDICAL AL ENDOMETRIOZEI

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MEDICAL TREATMENT OF ENDOMETRIOSIS. AN UPDATE (Abstract): Endometriosis is a major health problem, because of difficult diagnosis and limited therapies available. The surgical approach is done by very specialised surgeons, doing classical or laparoscopic ablative interventions. This article is an up-to date evaluation of medical treatment, reviewing the hormonal drugs (contraceptives, progestatives, GnRH analogues) and non-hormonal ones (anti-aromatase, anti-progestative, anti-tissular growth factors). The conclusion is that the medical treatment should be used in well selected cases, and adapted regimens, chossing among continuous contraceptives, intrauterine devices with levonorgestrel, or aromatase inhibitors. The main objective in this cases is rarely the complete cure, but more frquently optimal timing before surgery. **Key words**: ENDOMETRIOSIS, CONTRACEPTIVES, PROGESTATIVES, GNRH ANALOGUES

Endometrioza este definită ca implantarea de ţesut cu epiteliu glandular de tip endometrial şi celule stromale în afara cavităţii. Această localizare priveşte ovarele, peritoneul pelvin, şi trompele. În 75% din cazuri endometrioza externă afectează componente ale aparatului genital (uter, trompe, ovare, ligamentele largi, rotunde şi utero-sacrate). Restul de 25% din cazuri se referă la implicarea unor regiuni extragenitale intra şi extraperitoneale.

Această afecţiune reprezintă **o problemă majoră de sănătate** ea afectând circa 10-15% din femeile de vârstă reproductivă prin diversele sale manifestări (1). După Pauerstein, în fiecare an în SUA 4 femei din 1000 cu vârste de 15-64 ani sunt spitalizate pentru endometrioză.

CONDUITA CURATIVĂ MEDICAMENTOASĂ

Tratamentul endometriozei este în esenţă unul chirurgical, legat şi de diagnosticul afecţiunii, accesibil prin metoda endoscopică. Scopul acestui articol este de a revedea, pe baza dovezilor ştiinţifice existente, tratamentul medical.

Terapii neconvenţionale

O serie de abordări non-chirurgicale au fost enunţate în timp, având însă beneficii reduse sau chiar îndoielnice:

- Sarcina poate fi considerată ca un tratament hormonal natural, care ar determina clivajul mucoasei endometriale, inclusiv din focarele endometriozice
- aderenţele şi obstrucţiile endometriozice ale trompei pot fi desfăcute prin instilaţii utero-tubare, histerosalpingografie sau histeroscopie.
- Radioterapia- în scop de castrare- dar cu risc gebnetic, carcinogenic şi aderenţial semnificativ
- acupunctura, în şedinţe de 30 min, în puncte energetice specifice
- modificări ale regimului de viaţă: reducerea consumului de cafea, băuturi car-

bogazoase, ciocolată, ceai natural, zaharuri rafinate. Creşterea aportului de vegetale şi fructe, efortul fizic, înotul-ar fi factori protectivi.

- Radioterapia – în scop de castrare- dar cu risc genetic, carcinogenic şi aderenţial semnificativ
- Un studiu randomizat cu control (2) a arătat diminuarea dismenoreei şi hemoragiei, inclusiv în endometrioză de administrarea de vitamina E
- Electrostimularea, fizioterapia asociate cu acupunctura ar ameliora simptomele (mai ales durerea)- recom ESHRE guidelines 2008 categoria C (3)

Tratamente hormonale

Au fost propuse mai multe preparate cu componenţă sau efect hormonal :

- contraceptivele orale
- progesteronul, în formă clasică sau ca DIU
- antiprogestative
- modulatori ai receptorilor la progesteron
- danazol, antindrogenice
- analogi şi antagonişti de GnRH
- antiaromatazice
- agonişti de receptori beta de estradiol

a) Progestativele

Sunt printre cele mai frecvent utilizate medicamente în endometrioză.

Mecanismul de acţiune ar putea fi :

- decidualizarea endometrului, cu atrofie asociată
- efect anti ovulator dacă sunt administraţi în mod continuu, sau minim 20 zile/28, cu doze antigonadotrope
- contrabalansarea efectului promotor al factorilor de creştere determinat de estrogeni
- posibilă acţiune de inhibare a metalproteinazei matriciale endometriale, cu rol însemnat în timpul ciclului menstrual.

1) Acetat de medroxiprogesteron injectabil : meta-analiza Cochrane făcută de Prentice (4), arată, în puţinele studii cu control realizate, că există o eficienţă semnificativă pe durerile pelvine, lombare si legate de defecaţii ; efectele asupra infertilităţii par mai puţin importante.

2) Linestrenolul. Intr-un studiu complex făcut de Donnez (5) în care a utilizat linestrenol comparat cu alte regimuri (gestinone, danayol, buserelina spray sau implant) în endometrioze stadiu 3-4 postchirurgie, eficienţa a fost redusă, cu 80% leziuni încă active la 6 luni. Un studiu asemănător al lui Regidor (6) a arătat că tratamentul de primă intenţie rămâne cel cu antagonişti de GnRH, iar linestrenolul rămâne ca a doua opţiune, mai ales la tratamentele de lungă durată.

3) Didrogesteron (Duphaston). In aceeaşi meta-analiză Cochrane este citat un studiu în care utilizarea în faza luteală, după picul de LH, pe o durată lungă, a arătat un efect asupra durerii mai important doar la doze de 60 mg/zi, cu beneficii dipărute la un an de urmărire.

4) Acetatul de nomegestrol (Lutenyl) a fost analizat într-un studiu efectuat de Dorangeon (7) pe un număr mic de cazuri, iar rezultatele nu au părut semnificative statistic.

5) Acetatul de ciproteron (Androcur). Fedele a analizat într-un studiu mai vechi (8) comparativ un regim cu ciproteron acetat cu etinil estradiol (Diane 35 TM) faţă de danazol 600 mg/zi, 6 luni. Eficienţa asupra durerii a părut asemănătoare pentru cele două grupuri.

6) Utilizarea DIU cu levonorgestrel a făcut obiectul unui studiu al lui Vercellini (9), care a analizat efectul după tratament chirurgical asupra dismenoreei pe un lot cu DIU şi un lot cu placebo. La un an, dismenoreea apăruse într-un număr semnificativ mai mic la lotul cu DIU, iar autorii au concluzionat că inserţia imediat postchirurgicală a evitat recidiva dureroasă în o treime din cazuri. Un alt studiu (10) comparativ între eficienţa dispozitivului faţă de analogi de GnRH sugerează eficienţă asemănătoare asupra durerii, mai ales pentru stadiile mai

avansate de endometrioză. Aceleşi concluzii, de eficienţă pe durere în formele uşoare sau moderate, sunt citate de alţi autori. (11)

7) Modulatorii de receptori la progesteron (anti-progestative) se referă în principal la RU 486 (Mifegyne TM) şi Danazol. Dintre studiile existente asupra utilizării RU 486 în endometrioză, cel al lui Kettel (12) a arătat, pe un lot mic de 9 paciente, ameliorarea durerii pelvine, şi regresia focarelor în 55% din cazuri. Efectele secundare)menstre neregulate, sindrom de citoliză, hiperplaziile endometriale) limitează doza la 5 mg/zi, în timp ce autorii susţin că o doză eficace ar fi de peste 50 mg/zi.

b) Combinaţiile estro-progestative

Pornind de la constatarea că sarcina reduce simptomele şi limitează extensia endometriozei, a fost normal ca printre tratamentele hormonale încercate în endometrioză să se includă şi terapia estro-progestativă, datorită statusului pseudo-gestaţional indus de aceasta.

Mecanismul ar fi aşadar decidualizarea cu creşterea endometrului iniţial, iar ulterior o atrofie. Este vorba aşadar de activarea mecanismelor apoptozei pe care Meresman a demonstrat-o la o lună de la debutul tratamentului cu anticoncepţionale (13).

Literatura în acest domeniu nu este însă foarte vastă, şi meta-analiza efectuată de Moore şi colab pentru Cochrane database a găsit un singur studiu prospectiv randomizat controlat (14). Acest studiu a analizat tratamentul cu contraceptiv minidozat (20 gamma etinil estradiol) timp de 6 luni, comparat cu agonişti de GnRH, şi a constatat eficienţe asemănătoare asupra durerii şi dispareuniei, dar simptomatologia recidiva la 6 luni după întrerupere.

O variantă de terapie propusă mai recent este terapia cu contraceptive în regim continuu. Mahutte arată în studiul său o rată de satisfacţie a pacientelor sub tratament de 60-70%, asemănătoare cu alţi autori (15). Efectele secundare citate sunt prezente în 10% din cazuri: metroragii, senzaţie de balonare, greaţă, creşteri ponderale şi cefalee.

Recomandarea Colegiului American de Obstetrică Ginecologie (ACOG) în 2000 este că, faţă de abstinenţa terapeutică, tratamentul cu contraceptive este mai eficient în formele uşoare şi medii de endometrioză, fără a diferenţia între tipul ciclic şi cel continuu de administrare. In condiţiile lipsei de efect la 3 luni, recomandarea precizează necesitatea schimbării medicaţiei (16).

Agoniştii de GnRH

Efectul administrării agoniştilor de GnRH se exercită pe receptorul hipofizar pentru GnRH- cu iniţială creştere a eliberării de gonadotrofine, urmată apoi de o inhibiţie prelungită a secreţiei FSH şi LH, ca rezultat al desensibilizării celulelor gonadotrope la GnRH. Se vorbeşte de o castrare medicamentoasă reversibilă. Există diverse preparate disponibile, administrate pe cale subcutanata, intramusculara sau nazală: decapeptyl (Triptorelina), enantone (Leuprorelina), synarel (Nafarelina). Tratamentul uzual în endometrioză durează 6-12 luni, cu adăugarea din a 4a lună de "add-back therapy", reprezentat de estradiol şi progestativ.

Efectul agoniştilor a fost evaluat în numeroase studii, atât comparativ între ele, cât şi contra placebo, a danazolului etc.

Asupra infertilităţii, AFSSAPS citează 6 studii (17), în care tratamentul a durat 12--18 luni, fără diferenţe semnificative asupra numărului de sarcini obţinute faţă de pacientele cu abstinenţă terapeutică. Ca tratament preoperator, la czurile cu endometrioză cunoscută de stadiu 3-4, Audebert a arătat că scorul AFS s-a ameliorat semnificativ după 6 luni de tratament (18). Utilizarea postoperatorie pentru prevenirea recidivei a fost analizată de Busacca, într-un studiu randomizat cu control, la care concluzia a arătat că nu există un beneficiu clar al tratamentului postoperator cu leuproreline in administrare lunară (19).

Efectele adverse ale tratamentului cu

analogi de GnRH sunt :

- reacţii alergice, în general locale
- efecte de tip hipoestrogenie (bufee de căldură, cefalee, modificări de libido, dispareunie, instabilitate emoţională, astenie), chiar dacă necesită tratamente adjuvante, nu reprezintă motive de întrerupere a terapiei
- greaţă, vărsături, HTA, creştere ponderală, febră, anomalii de câmp vizual – rareori semnalate
- osteoporoza- pierderea de masă osoasă la 6 luni de tratament este de 10-25%, cu recuperare incompletă la oprirea terapiei

Rolul agoniştilor de GnRH în tratamentul infertilităţii şi pregătirea pentru FIV este disputat :

- Unii autori susţin o creştere de 4 ori a ratei sarcinilor obţinute prin FIV: (20, 21)
- Concluzii sunt discutabile din punct de vedere a metodologiei studiilor incluse în metaanaliză, a tipului de protocol asociat, a criteriilor de includere în studiu, a rezervei ovariene a pacientelor etc. (22)
- Alte studii susţin că endometrioza nu influenţează semnificativ succesul tehnicilor FIV (23)

Inhibitorii de aromatază

Celulele stromale endometriozice au o expresie excesivă a aromatazei P450, care converteşte sterozii C19 în estrogeni. Există autori care susţin că această anomalie se însoţeşte de un deficit de 17 steroid dehidrogenază, care inactivează estradiolul astfel rezultat, ceea ce favorizează suplimentar apariţia de implante endometriozice (24,25).

Preparatele de a treia generaţie a inhibitorilor de aromatază (anastrozol, letrozol) au o toleranţă bună. Anastrozolul (Arimidex) utilizat în cancerele de sân, a fost propus în studiul endometriozei de Ailawadji, timp de 6 luni, la paciente cu forme rezistente la tratamentul medico chirurgical. Scorul algic şi AFS a diminuat semnificativ la cele 10 paciente urmărite, dar bineînţeles că acest studiu trebuie extins în durată şi număr de paciente (26).

Antiinflamatoare nesteroidiene

Aceste medicamente sunt prescrise frecvent în dismenoree, iar cum multe cazuri sunt legate de endometrioză, a fost normal să fie evaluate în tratamentul acestei afecţiuni- în special al durerii.

Dintre preparatele recomandate de studiul AFSSAPS (17) citǎm :

- derivaţii de acid aril-carboxilic : acid tiaprofenic (Surgam TM), Ibuprofene (Nurofen TM), Ketoprofen - cu cea mai mare eficienţă
- Diclofenac, care a ameliorat semnificativ în studii randomizate simptomele dureroase
- acid mefenamic (Ponstyl TM, Vidan TM) este de asemenea eficient pentru dismenoree

Concluzia articolului menţionat este că antiinflamatoarele nesteroidiene trebuie recomandate de primă intenţie în tratamentul dismenoreei şi durerii abdomino-pelvine din endometrioză.

Danazolul

Derivat de 17 etinil-testosteron, danazolul are efect antigonadotrop la nivel hipotalamo-hipofizar ; de asemenea interferă cu mecanisme enzimatice ale steroidogeneză, crescând nivelul de testosteron plasmatic.

Efectul asupra durerii a fost demonstrat faţă de placebo, analogi de GnRH sau acetat de medroxiprogesteron. Sapte studii din literatură permit concluzia unei eficienţe asemănătoare cu analogii de GnRH, superioară celorlaltor terapii. Doza recomandată este 600-800 mg/zi, în medie 6 luni.

In privinţa numărului şi volumelor implantelor endometriozice, corelaţia între durerea ameliorată şi danazol nu a fost regăsită de unii autori. Deşi există o ameliorare a scorului AFS, eficienţa comparată cu analogii de GnRH este uşor mai mică.

Fertilitatea în urma tratamentului cu danazol nu a prezentat ameliorări semnificativ

diferite faţă de analogii de GnRH, coagularea chirurgicală, sau abstinenţa terapeutică.

Efectele secundare la dozele uzuale sunt semnificative. Este vorba mai ales de efecte androgenice : creştere ponderală, acnee, hirsutism, iritabilitate- în 12-28% cazuri, ca şi modificări ale metabolismului lipidic.

Alte direcţii de cercetare :

- Medicamente angiostatice, şi împotriva VEGF au arătat rezultate interesante experimentale, dar nu au fost studii umane (27).
- Unii autori susţin utilizarea modulatori selectivi de receptori progesteronici (28) – fără studii semnificative statistic : asoprisnil, CDB 4124 (proellex, progenta), PGL 4001 (ulipristal).

CONCLUZII

Tratamentul medical este încă subiect de cercetare şi nu există studii largi şi dovezi ştiinţifice care să confirme o terapie de elecţie. Nici terapia post-operatorie nu are un standard precis, iar situaţiile dificile (chist ovarian endometriozic bilateral la paciente cu infertilitate, endometrioza profundă extinsă la organe învecinate) nu au tratament de pregătire a intervenţiei chirurgicale cu scop curativ.

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DYNAMICS OF CLINICAL AND BIOLOGICAL ELEMENTS IN MECHANICAL BOWEL OBSTRUCTIONS – EXPERIMENTAL STUDY ON LABORATORY ANIMALS

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DYNAMICS OF CLINICAL AND BIOLOGICAL ELEMENTS IN MECHANICAL BOWEL OBSTRUCTIONS–EXPERIMENTAL STUDY ON ANIMALS (Abstract): **Introduction:** the bowel obstruction represents a syndrome with significant clinical and biological modifications. **Material and methods:** we performed an experimental study on Wistar rats in which we induced a mechanical bowel obstruction. One group of subjects was used as control, on another group we ligated the sigmoid colon with surgical thread and on the last group we ligated a small bowel loop. **Aim:** our objectives were to analyze, in different time moments (after 2 days and 4 days, respectively, from the moment of obstruction), the clinical modifications (temperature, diuresis, neurological symptoms, body weight, abdominal circumference) or the biological imbalances (by monitoring the blood count, serum glucose, urea, creatinine levels). **Results:** the initial protocol could not be fully complied with, due to the premature deaths of a few subjects with bowel obstruction in the jejunum. From the point of view of the clinical features, we noted the occurrence of dizziness, reduction of the appetite and changes in diuresis in a number of subjects. We observed variations of abdominal circumference, temperature curve and body weight of the rats. We also determined modifications of biological parameters such as hemoglobin values, serum platelet count, glycemic values, serum urea and creatinine. **Conclusions:** the bowel obstruction leads to the installation of major clinical and biological imbalances, with the occurrence of major systemic disorders, finally leading to death in the absence of a quick, adequate treatment. **Keywords:** RAT, BOWEL, OBSTRUCTION, EXPERIMENT.

The bowel obstruction is a syndrome that occurs in approximately 20-30% of cases of acute abdomen encountered in surgical departments (1). Regarding me-

chanical bowel obstructions, they are characterized by important changes in the morphology of the digestive tract adjacent to the obstructive process, as well as alteration of the clinical parameters (2-13) and the installation of major biological imbalances (14-17).

Since patients usually do not present at the hospital when clinical manifestations occur, as well as the fact that the obstruction (especially in the case of tumors, adhesions or complicated abdominal wall defects) occurs progressively, it is difficult to establish a period of time between the installation of the pathological process and the determination of specific parameters.

We have thus outlined a study in which, using experimental animals, we can induce a mechanical bowel obstruction at different levels of the digestive tract, and afterwards, at well-established moments, to observe the changes that will occur, both clinically and biologically.

MATERIAL AND METHODS

The study was conducted within the "Cantacuzino" National Institute of Medico-Military Development Research, Băneasa Department, Preclinical Testing Unit, during a period of 5 days. The conditions for GLP (Good Laboratory Practice), in accordance to the OECD (the Organization for Economic Co-operation and Development) standards were respected, as well as the current Romanian laws and the internal protocols, with the authorization of the Veterinary Authority and Ethics Committee of the institute.

As laboratory animal we used the rat, Wistar strain, weighing between 150-200 g (grams) and between 6-8 weeks of age at the start of the study (fig. 1). We included in the study a number of 26 rats, both females and males, juveniles-adults, healthy, that had not been used in previous tests and whose source was represented by the Băneasa animal breeding department of the "Cantacuzino" Institute. Animal acclimatization was 8 days long and was performed under the same conditions as the study. The animals were housed in separate cages, made of polycarbonate, identified by a label indicating all the individual characteristics of the subject and the experiment, respectively, according to the internal protocols (fig. 2).

Fig.1-2. The Wistar rat used in the experiment and the individual cages

The ambient temperature was 18-24°C (Celsius), with a relative humidity of 30-70%, providing a 12-hour light/dark cycle. The animals were fed with standardized granulated fodder and drinking water, *ad libitum*, with no known contaminants that could have interfered with the results of the experiment.

The animals were divided into 3 groups, A, B, and C, each of them having 2 subgroups - A_1 and A_2, B_1 and B_2, and respectively C_1 and C_2, depending on the gender of the animal (tab. I). Group A was the control group consisting of 6 rats, group B was composed of 10 rats to which we induced a mechanical bowel obstruction in the large bowel, and group C was also composed of 10 rats to which we induced a mechanical bowel obstruction in the small bowel.

TABLE I
Distribution in the groups of the experiment

Group	Sub-group	Subjects' gender	Number of subjects /sub-group
A	A_1	Females	3
	A_2	Males	3
B	B_1	Females	5
	B_2	Males	5
C	C_1	Females	5
	C_2	Males	5

Ten hours (h) before the surgical procedure was performed, the animals were placed on an alimentary diet. General anesthesia of the animals was performed with a mixture of Ketamine (0.35 mg (milligrams)/kg (kilogram) of body weight) + Acepromazine (0.30 mg/kg of body weight), administered intramuscularly. Just before the procedure, performed at a moment we named T_0, a number of clinical parameters such as body weight, temperature (determined intrarectally), abdominal circumference (measured over the umbilicus), as well as laboratory assays such as blood count and serum creatinine, urea and glucose, were collected from each subject.

Blood sampling was made from the retroorbitary venous plexus under general anesthesia. Hematological determinations were made from blood sampled on EDTA (ethylene diamine tetra acetic acid) and the biochemical determinations were made from plasma obtained by centrifugation for 2 minutes at 4°C at 12,000 rotations per minute from blood sampled on heparin lithium.

At the T_0 moment, after collecting the above-mentioned parameters, the subjects in the control group (group A) were euthanized with an anesthetic overdose, each animal undergoing a complete necrotic examination.

For all the 10 subjects in the B group, a median incision was performed in the lower half of the abdominal wall. The muscular-aponeurotic layer was cut, entering the peritoneal cavity. The sigmoid loop was identified, placing a stenotic ligature with a 2.0 polyglycolic acid thread in the middle of the loop, without the involvement of the mezosigmoid, thus producing a mechanical bowel obstruction (fig. 3). Subsequently, the closure of the abdominal wall in a total layer was performed, using a 0 polyglycolic acid thread in surjet manner. The wound was toileted, and the subjects were placed in their respective cages.

In the case of the 10 subjects in group C, the surgical procedure was similar to the subjects in group B, with the difference that the incision was performed in the upper half of the abdominal wall and the ste-

notic thread was placed on a small bowel loop, without the involvement of the mesentery, at approximatively the junction of the upper third and lower two thirds of the jejuno-ileonum (fig. 4).

Following these procedures, each animal in groups B and C was closely monitored during the anesthesia recovery. In the next 3 days, each animal received anti-inflammatory (Ketofen, 3 mg/kg of body weight) and antibiotic (Enrodexil, 5 mg/kg of body weight) treatment, both administered intramuscularly, for the prophylaxis of postoperative infections and for the pain relief.

Fig. 3. Stenotic threads placed on the sigmoid colon

Fig. 4. Stenotic threads placed on the jejuno-ileonum

The initial study plans involved daily monitoring of the study subjects, recording the temperature, body weight, diuresis, as well as local or general clinical manifestations – loss of appetite or neurological disorders (agitation or dizziness).

Two days after the bowel obstruction was induced, at a moment we noted with T_2, it was desired to collect the biological parameters from the remaining subjects and measure their abdominal circumference, choosing randomly immediately afterwards a number of 5 subjects from each group, B and C, to be euthanized.

After another 2 days (4 days from the initial procedure, moment defined as T_4), it was hoped that the remaining 5 rats from each group would be subjected to similar procedures, with the dynamic analysis of the mentioned parameters, followed by the euthanasia of these last subjects, as well.

RESULTS

Compared to the initially established protocol, the viability for the subjects in group B was 100%, while for the subjects in group C it was 60%, given that 4 rats out of 10 from this group died before the scheduled time (2 rats at 48 hours, a rat at 53 hours and another one at approximately 61 hours from the onset of the experiment). So, concerning the subjects in group C, we had to overtake the initial plan and determine the parameters of the last 3 subjects from this group at 72 hours from the beginning of the experiment, in a moment we recorded with T_3, due to the alteration of their general condition and the estimation that they would not have survived until T_4 (tab. II).

In terms of clinical parameters, modified diuresis was found in 60% of subjects in both groups B and C, some of the rats

showing dizziness and reduction of the appetite. However, it needs to be mentioned that a quantifiable measurement of diuresis or feces weight could not be performed, as in the case of the blood pressure, which could not be determined.

TABLE II

Survival rate of the subjects in the experimental study

	Number of living animals at a given time (before the procedure from the respective day)				
Sub-group	**T_0 (onset of the obstruction)**	**T_1 (T_0 + 24h)**	**T_2 (T_0 + 48h)**	**T_3 (T_0 + 72h)**	**T_4 (T_0 + 96h)**
A_1	3	-	-	-	-
A_2	3	-	-	-	-
B_1	5	5	5	2	2
B_2	5	5	5	3	3
C_1	5	5	4	2	-
C_2	5	5	4	1	-

There were no problems with the abdominal wounds, no suppurations, seromas and we had no cases of evisceration.

The weight of the subjects, measured in grams, daily, is listed in the table below (table III) and represented in the following figure (fig. 5). For a better optimization, it was noted the average value of the subjects in each sub-group that remained alive at the time of the determination of the parameters, this principle being respected for the other numeric parameters that we will expose subsequently. Also, it must be noted that there were cases in which a sub-group was made up of a single subject at a given time.

Below, we present the temperature values of the studied sub-groups, mentioning that at the moment T_0 these values were measured under general anesthesia, as well as the fact that the normal temperature of the rat is between 35.9°C-37.5$^{0°}$C (tab. IV and fig. 6).

The values of the abdominal circumference of the subjects, measured in cm (centimeters), over the umbilicus, are shown below (tab. V and fig. 7).

We also represented graphically the dynamics of the average values of the hemoglobin (fig. 8,9), white blood cells count (fig. 10, 11) and the number of platelets (fig. 12, 13) in both groups B and C.

TABLE III

Variation of the average weight (in grams) of the subjects

Subgroup	T_0	T_1	T_2	T_3	T_4
A_1	182.33	-	-	-	-
A_2	189.66	-	-	-	-
B_1	189.00	196.46	183.54	178.35	180.18
B_2	186.00	198.45	185.82	189.32	183.11
C_1	186.20	184.58	181.69	162.98	-
C_2	177.80	176.43	171.69	174.12	-

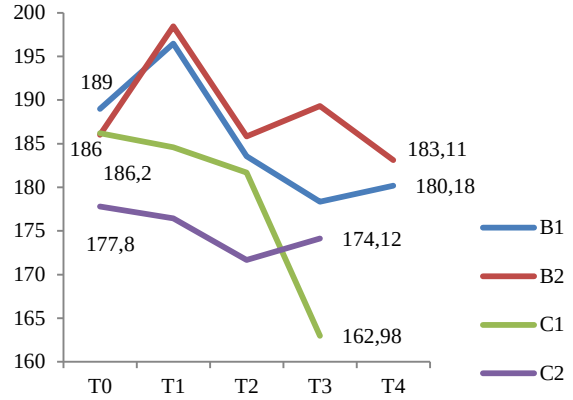

Fig. 5. Variation of the average weight (in grams) of the subjects (for simplification are noted only the initial and the final values)

TABLE IV

Variation of the average temperature (in Celsius degrees) of the subjects

Subgroup	T_0	T_1	T_2	T_3	T_4
A_1	35.50	-	-	-	-
A_2	35.56	-	-	-	-
B_1	36.72	37.68	37.64	37.15	36.90
B_2	35.84	37.68	37.52	36.93	36.20
C_1	37. 22	37.48	37.00	36.10	-
C_2	36.04	37.00	35.67	36.00	-

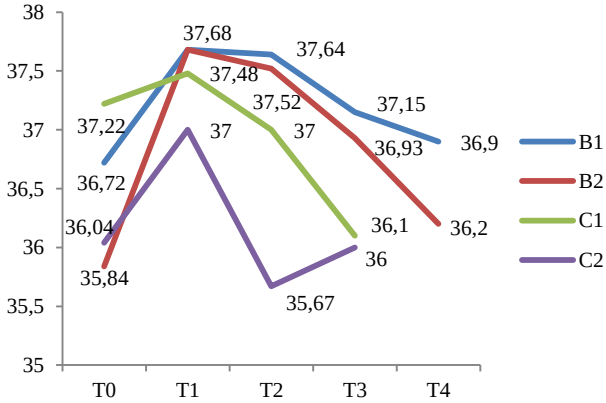

Fig. 6. Variation of the average temperature (in Celsius degrees) of the subjects

TABLE V

Average abdominal circumference (in cm) from the studied sub-groups

Subgroup	T_0	T_1	T_2	T_3	T_4
A_1	14.96	-	-	-	-
A_2	15.13	-	-	-	-
B_1	15.42	-	16.25	-	17.00
B_2	14.74	-	16.15	-	16.50
C_1	15.34	-	17.40	17.00	-
C_2	15.26	-	16.80	16.60	-

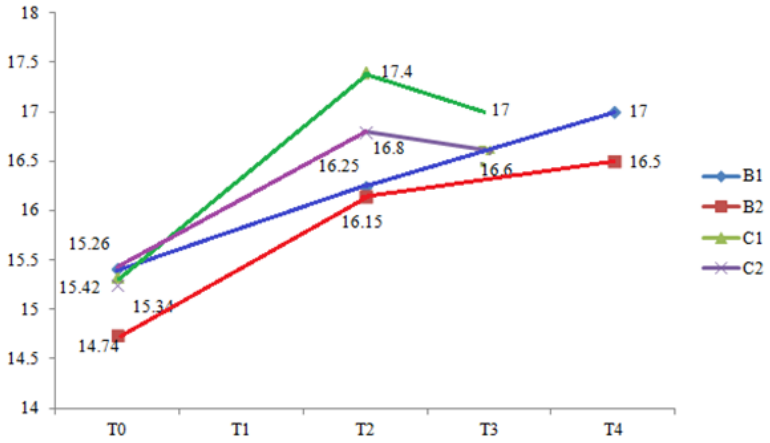

Fig. 7. Average abdominal circumference (in cm) from the studied sub-groups

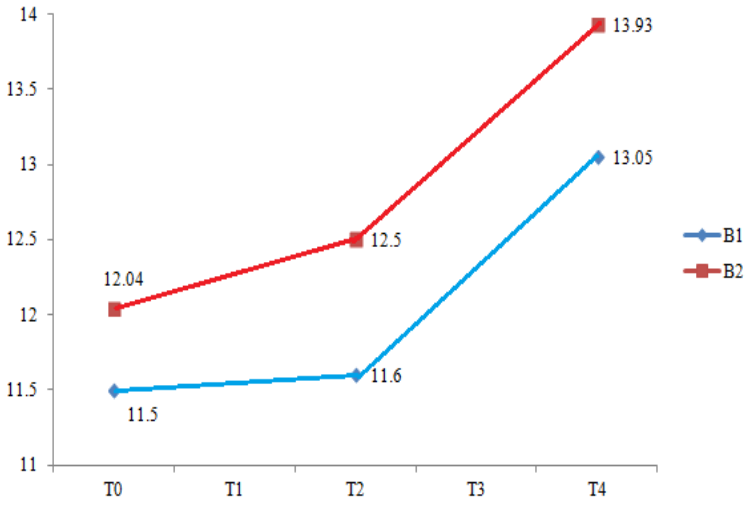

Fig. 8. Dynamics of the average values of hemoglobin (g/dL(deciliter)) in subjects from group B (unit reference values between 13.3-16.7 g/dL)

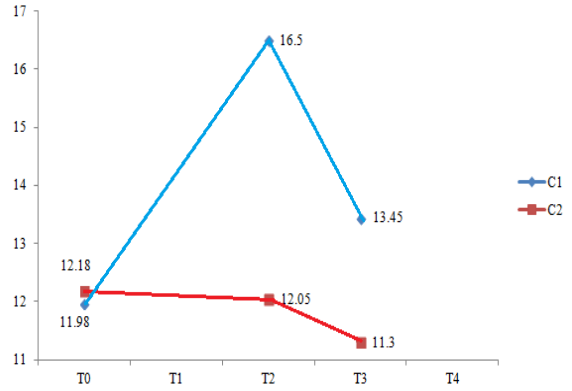

Fig. 9. Dynamics of the average values of hemoglobin (g/dL(deciliter)) in subjects from group C (unit reference values between 13.3-16.7 g/dL)

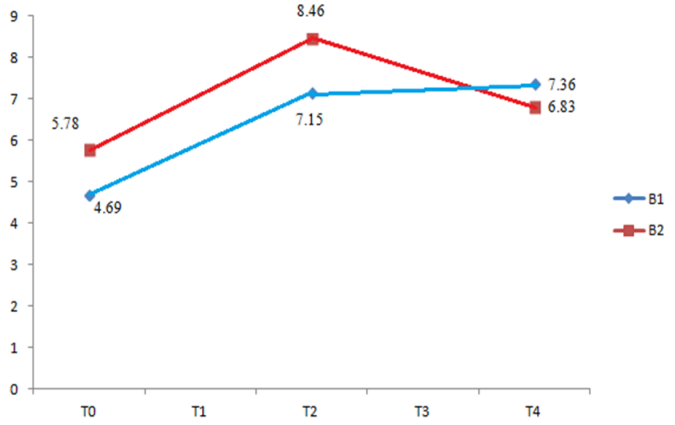

Fig. 10. Dynamics of the average values of white blood cells count (x 10^9/L (liter)) in subjects from group B (unit reference values between 3.27-11.14 x 10^9/L)

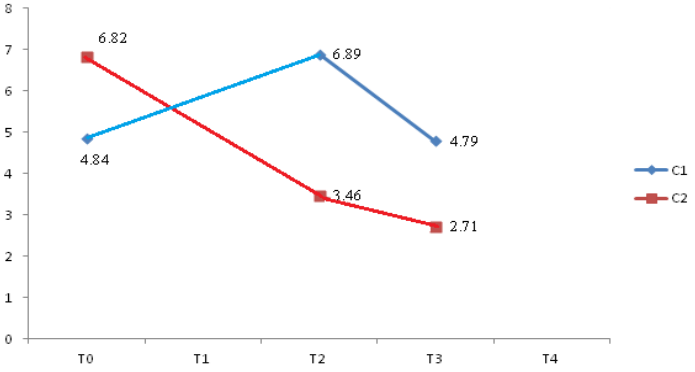

Fig. 11. Dynamics of the average values of white blood cells count (x 10^9/L (liter)) in subjects from group C (unit reference values between 3.27-11.14 x 10^9/L)

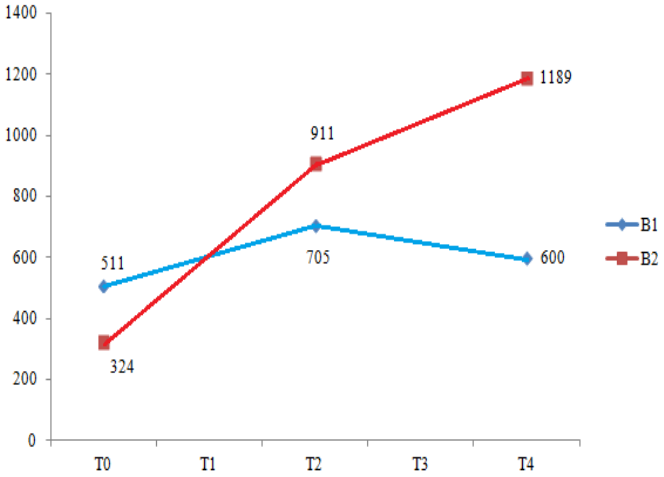

Fig. 12. Dynamics of the average values of the number of platelets (K (1000)/µL (microliter)) in subjects from group B
(unit reference values between 300-700 K/µL)

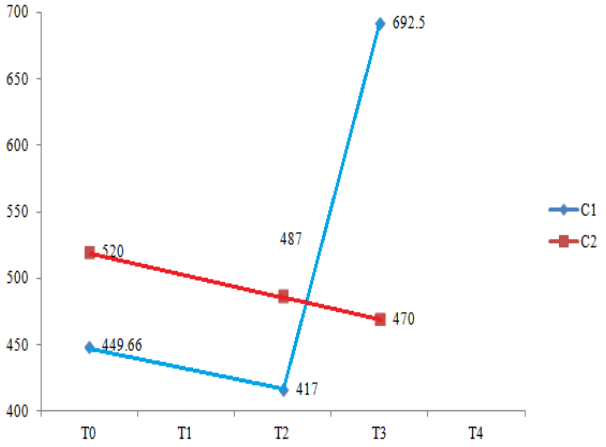

Fig. 13. Dynamics of the average values of the number of platelets (K (1000)/µL (microliter)) in subjects from group C
(unit reference values between 300-700 K/µL)

We also found a difference between the aspect of the blood plasma of the subjects in the B group, compared with that of subjects in group C, 48 hours after the induction of the bowel obstruction (fig. 14).

Regarding the biochemical parameters (blood glucose, urea, creatinine), we obtained the following values (tab. VI-IX), represented in the graphs below (fig. 15-20).

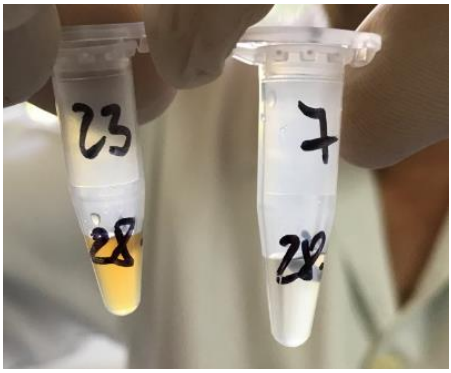

Fig. 14. The plasma aspect at the T_2 moment of a subject in group B (right, marked with 7) and a subject from group C (left, marked with 23)

TABLE VI

Average values of the biochemical parameters of the subjects in groups A, B, C, at the moments T_0, T_2, T_3 and respectively T_4

Parameter	Sub-groups						Unit reference values
	A1	A2	B1	B2	C1	C2	
Glucose - mmol (millimoles)/L	8.22	8.68	8.43	8.07	7.8	7.45	2.78-7.50
Creatinine - µmol (micromoles)/L	29	19.66	15.6	12	16.2	11.2	4.0-57
Urea - mg/dL	19.33	18.33	18.2	19.4	18.8	17.2	15-21

TABLE VII

Average values of the biochemical parameters of the subjects in groups B and C at the moments T_0, T_2, T_3 and respectively T_4

Parameter	Sub-groups				Unit reference values
	B_1	B_2	C_1	C_2	
Glucose - mmol/L	28.33	19.50	17.39	17.24	2.78-7.50
Creatinine - µmol/L	24.66	32.50	39	25	4.0-57
Urea - mg/dL	27.33	37.50	31	28	15-21

TABLE VIII

Average values of the biochemical parameters of the subjects in group C at the moments T_0, T_2, T_3 and respectively T_4

Parameter	Sub-groups		Unit reference values
	C_1	C_2	
Glucose - mmol/L	17.89	21.12	2.78-7.50
Creatinine - µmol/L	32.50	34	4.0-57
Urea - mg/dL	33.50	33	15-21

TABLE IX

Average values of the biochemical parameters of the subjects in group B at the moments T_0, T_2, T_3 and respectively T_4

Parameter	Sub-groups		Unit reference values
	B_1	B_2	
Glucose - mmol/L	34.5	20.33	2.78-7.50
Creatinine - µmol/L	26	23	4.0-57
Urea - mg/dL	40	33	15-21

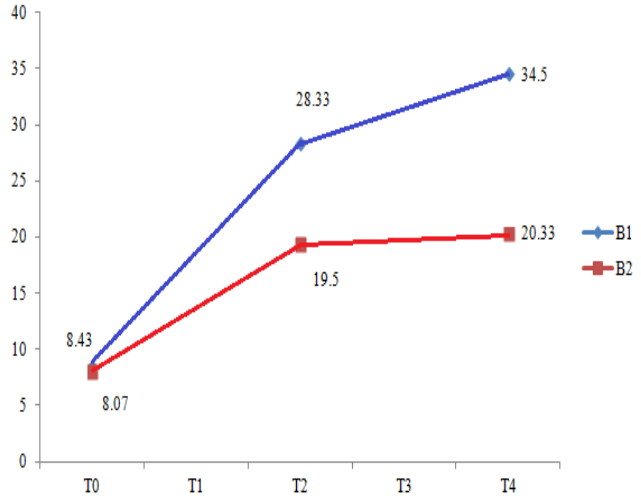

Fig. 15. Dynamics of the average values of blood glucose (mmol/L) in subjects from group B

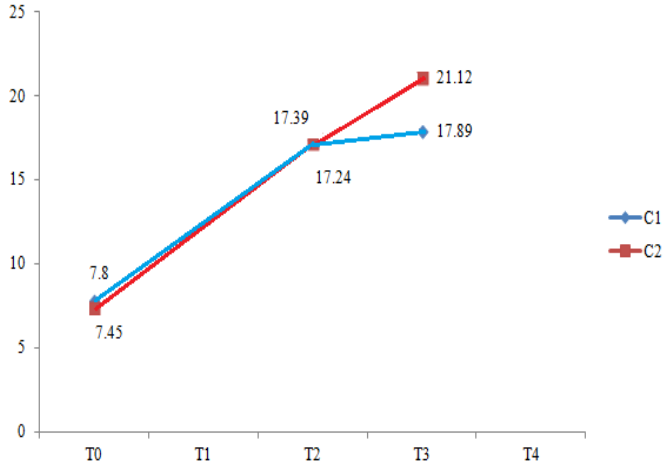

Fig. 16. Dynamics of the average values of blood glucose (mmol/L) in subjects from group C

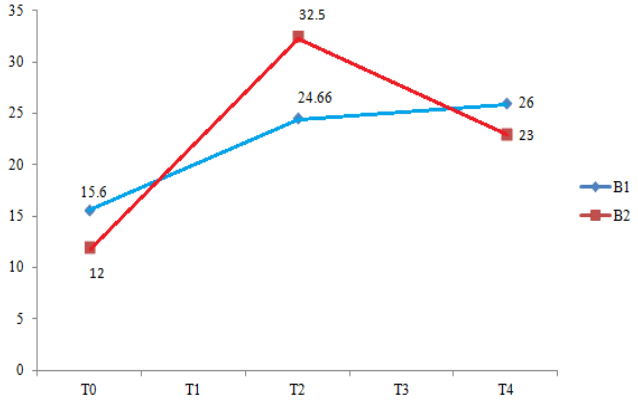

Fig. 17. Dynamics of the average values of serum creatinine (µmol/L) in subjects from group B

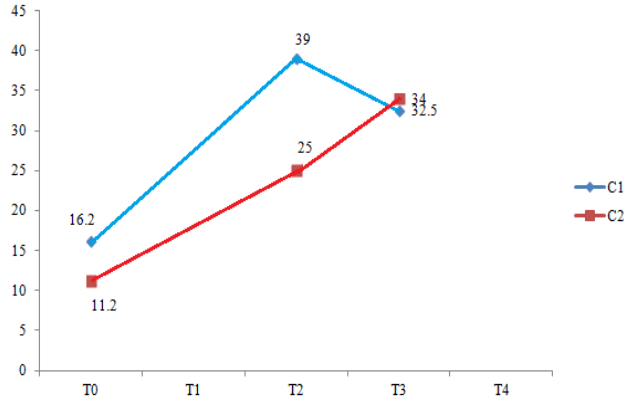

Fig. 18. Dynamics of the average values of serum creatinine (µmol/L) in subjects from group C

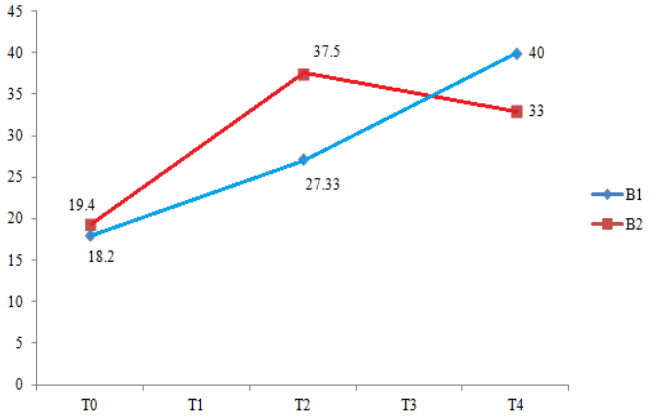

Fig. 19. Dynamics of the average values of serum urea (mg/dL) in subjects from group B

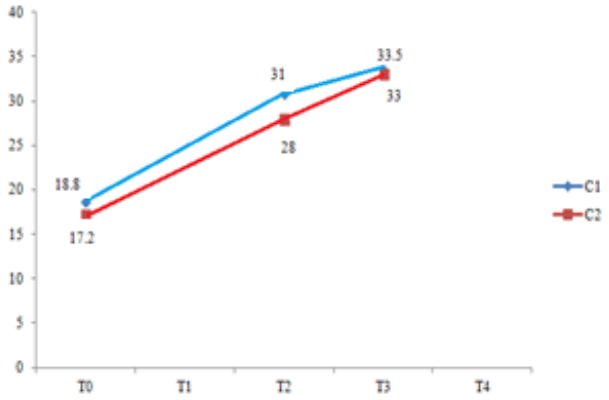

Fig. 20. Dynamics of the average values of serum urea (mg/dL) in subjects from group C

DISCUSSION

Clinical positive diagnosis of bowel obstruction is based on the specific symptoms (abdominal pain, abdominal distension, vomiting and stoppage of the intestinal transit), on the objective local examination of the patient as well on a series of extra-abdominal general signs (1), the latter being represented by cardiovascular features (tachycardia, hypotension), oliguria, fever or dehydration (13, 15).

In the bowel obstruction syndrome, a number of biological parameters such as metabolic acidosis, hexosaminidase, inorganic phosphate, serum amylase, intestinal fatty acid binding protein (I-FABP) or serum D-lactate were incriminated, their growth having been associated with an already established intestinal ischemia (18, 19). In the absence of these parameters, the usual biologic assays can highlight a number of changes in serum biochemistry, serum ionogram and blood count.

In terms of biochemistry, elevated glycemic values, as well as an alteration of renal parameters, with high levels of serum urea and creatinine, are often found, the renal failure worsening in the final stages of the bowel obstruction.

Hydro electrolytic imbalances are due to losses of sodium, chlorine and potassium ions. The kidneys try to compensate the hyponatremia by exchanging sodium ions with the potassium ions, amplifying the initial hypopotassemia. Subsequently, through hyper catabolism and tissue destruction, the hyperpotassemia occurs. As for the acid-base balance, by alkaline losses, usually a mixed acidosis is found.

In the case of the blood count, leukocytosis and thrombocytosis can be frequently found, while low hemoglobin may suggest a bleeding tumor in the digestive tract. Increased hemoglobin levels can occur in the case of hemoconcentration due to dehydration (14-17).

In the medical literature, a series of experimental studies have been published, that have analyzed changes in blood circulation, intraluminal pressures, bacterial flora multiplication, electrophysiological mechanisms or various imaging aspects using complete or incomplete intestinal obstruction models on different animals such as rats, dogs, cats, rabbits or pigs (20-34).

Regarding the subjects in our study, the protocol we imagined implied complete obstruction at various levels of the digestive tract (jejunum and sigmoid colon, respectively), monitoring dynamically a series of biological and clinical parameters, during several days.

However, this initial protocol could not be fully complied with, due to the premature deaths of a few subjects with bowel obstruction in the jejunum. Thus, after 3 days from the start of the experiment, we had to determine the parameters of the remaining living subjects in the respective group earlier, due to the severe alteration of their general condition and the improbability of their survival for another 24 hours.

From the point of view of the clinical features, we noted the occurrence of dizziness, reduction of the appetite and changes in diuresis in 60% of the subjects who were induced a bowel obstruction. It needs to be mentioned that due to some structural features of the diaphragm and the transmission of the nervous signals from the central level, the rat does not vomit (35).

The body weight of the rats determined dynamically revealed a decrease in values from the start of the experiment, this result being explained by the lack of food intake and dehydration of the subjects.

Temperature curve values showed an increase in the measured intrarectal temperature 24 hours after the obstruction, most likely in the context of the invasive surgical procedure. Subsequently, this parameter was normalized, the mechanism of the obstruction being a non-ischemic one, strictly to the digestive lumen, without the interest in the intestinal vascular supply and the subsequent possible onset of parietal intestinal necrosis.

The abdominal circumference also showed an increase of its values, because of the digestive tract distension located upstream of the obstacle.

Regarding the biological parameters, there was a slight increase in hemoglobin values as a result of dehydration, while the white blood cell count remained within normal limits. This latter parameter can suggest a lower rat systemic response to the obstructive process, taking also into account the effect of the anti-inflammatory treatment that was administered perioperatively in order to alleviate pain symptoms. The serum platelet count, in most subjects, showed an increase in the dynamic values, similar to the glycemic values, the latter most likely being explained by the unloading of the stress hormones into the blood stream. In addition, elevated levels of serum urea and creatinine suggested the occurrence of the renal failure with the progression of the obstructive syndrome.

CONCLUSIONS

The present experiment succeeded in inducing a mechanical bowel obstruction at different levels of the rat digestive tract (jejunum, respectively sigmoid colon), but the initial protocol, which involved the measurement of a series of clinical and biological parameters at well-established moments, required changes during the study due to premature deaths among subjects with jejunal topography, which suggests the higher severity of upper digestive obstruction.

From the clinical point of view, a number of changes were observed in the group of subjects, such as abdominal circumference increase, body weight decrease, dizzi-

ness, appetite decrease, ascension of the temperature curve in the first postoperative day, but there were also a number of parameters who were difficult to quantify (precise measurement of the diuresis volume, amount of feces or the blood pressure).

The biological features were represented by the increase of the serum values of hemoglobin, platelet count, glycemia and renal parameters.

Between the bowel obstruction in rats and humans there are a number of similarities regarding clinical (increased abdominal circumference) or biological parameters (increase of glycemic values, thrombocytosis, renal failure), but we have also encountered a number of animal-specific features in our study, such as the absence of vomiting or systemic leukocytosis.

The bowel obstruction leads to the installation of major clinical and biological imbalances, with the occurrence of major systemic disorders, finally leading to death in the absence of a quick, adequate treatment.

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NUTRIŢIA ÎN COLESTAZĂ ŞI IMPACTUL PE TERMEN LUNG DUPĂ TRANSPLANT

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NUTRITION IN CHOLESTASIS AND LONG-TERM IMPACT ON TRASNPLANT (Abstract): **Aim**: Plurietiological hepatic cholestasis involves an increased risk of protein--caloric malnutrition and specifical nutritional deficiencies. Biological investigations to determine deficiencies in fat-soluble vitamins are essential for specific nutritional therapy . Although malnutrition is not an absolute contraindication for liver transplantation, its gravity has complex consequences in relation to this intervention. **Material and method**: The authors present a study conducted over a period of 5 years following the analysis of 293 children diagnosed with intra-or extrahepatic abnormalities that caused varying degrees of cholestasis. **Results**: In the study group, the percentage of infants with cholestasis was 45.39% (133 cases) and among them 62.12% had malnutrition (82 children). Clinical evaluation of fat-soluble vitamins deficiency, in particular, but also of the soluble and minerals was performed in all patients. Liver transplantation was successfully performed in three cases. **Conclusions**: Most important factors affecting growth after transplantation were age at the time of the liver transplant and primary diagnosis that required transplant. The role of nutritional support prior to liver transplantation is of great importance. Quality nutritional support change impact that malnutrition has on survival after liver transplantation. **Key words**: CHOLESTASIS, LIVER TRANSPLANT

Colestaza hepatică plurietiologică implică un risc crescut de malnutriţie protein--calorică asociind şi deficienţe nutriţionale specifice. Statusul nutriţional trebuie atent monitorizat în dinamică. Criteriile antropometrice convenţionale se pot utiliza cu mare dificultate şi nici nu au semnificaţia obişnuită în stadiul final al bolilor hepatice din cauza ascitei şi a edemelor, necesitând interpretări nuanţate (1,2).

Greutatea corporală este un parametru variabil în cadrul complicaţiilor bolii, iar creşterea ei poate chiar semnifica agravarea prin ascită refractară, edeme, hepatosplenomegalie, neavînd relevanţă specifică pentru malnutriţie. Talia poate aprecia gradul malnutriţiei cronice, fiind mai corect asociată perturbării nutriţionale. Dimensiunile pliurilor şi circumferinţa medie a braţului se modifică precoce în comparaţie cu talia, dar sunt influenţate de retenţia de lichide în zonele declive. Fluidele în exces se acumulează decliv şi de aceea trebuie notate dimensiunie pliurilor în 1 superioară a corpului (3).

Investigaţiile biologice pentru determinarea deficienţelor în vitamine liposolubile sunt esenţiale în monitorizarea biochimică în scopul terapiei nutriţionale nuanţate. Mecanismul major implicat în apariţia malnutriţiei îl reprezintă diminuarea concentraţiei de acizi biliari secundară scăderii fluxului biliar. Colestaza determină reducerea semnificativă a absorbţiei lipidelor şi a trigliceridelor cu lant lung, care la rândul lor determină reducerea balanţei energetice şi malabsorbţia vitaminelor liposolubile (4).

În stadiile finale ale bolilor hepatice cro-

nice malnutriţia este o regulă. Deşi malnutriţia nu reprezintă o contraindicaţie absolută a transplantului hepatic, gravitatea ei are consecinţe complexe în relaţie cu această intervenţie în evoluţia imediată postoperatorie, complicaţiile precoce, statusul vital pe termen lung, calitatea vieţii.

Există numeroase riscuri pe termen lung pentru deteriorare nutriţională şi metabolică la copiii cu transplant hepatic. Principiul de bază în tratamentul deficitului nutriţional din cadrul colestazei este de a corectă statusul nutriţional astfel încât să se poată preveni deficienţele nutriţionale (5).

Instalarea progresivă a malnutriţiei creşte riscul de morbiditate şi mortalitate, explicat de faptul că sindroamele de deficienţă sunt refractare chiar şi la măsuri terapeutice de mare complexitate. Ameliorarea statusului nutriţional este o verigă importantă pre şi posttransplant hepatic. Terapia nutriţională trebuie aplicată indiferent de dificultăţile şi reversibilitatea unor manifestări.

MATERIAL ŞI METODĂ

Studiul efectuat a fost atât retrospectiv (în perioada 01 ianuarie 2005- 01 noiembrie 2006), cât şi prospectiv (01 noiembrie 2006--31 septembrie 2009), analizând copiii internaţi în această perioadă în Clinica a II-a Pediatrie a Spitalului Clinic de Copii „Sf. Maria" Iaşi pentru diverse afecţiuni posibil generatoare de colestază intra- sau extrahepatică.

Criteriile de includere în studiu a pacienţilor au fost :

- Vârsta cuprinsă între 0-18 ani
- Confirmarea colestazei
- Confirmarea patologiei generatoare de colestaza prin mijloace biologice si imagistice, analizand ulterior rezultatele pe categorii etiologice conform unei clasificari prestabilite.

Astfel au fost evaluaţi 293 copii diagnosticaţi cu anomalii intra- sau extrahepatice ce au determinat diverse grade de colestază. Lotul de studiu a fost subdivizat în două mari grupe :

I. Situaţiile clinice ce au determinat *obstrucţie extrahepatică* au reprezentat 10,23% din cazuri fiind reprezentate astfel :

- Litiaza biliară - 18 cazuri (60%)
- Atrezie căi biliare extrahepatice - 8 cazuri (26,6%)
- Chist de coledoc - 4 cazuri (13,4%).

II. Patologia intrahepatică a fost întâlnită cu o frecvenţă mai mare (89,77%) decât cea extrahepatică şi a fost grupată astfel :

- Cauze infecţioase - citomegalovirus (87 cazuri), toxoplasma (32 cazuri), cauze infecţioase-toxice cu endotoxemie-sepsis, infecţie urinară cu *Escherichia coli* (8 cazuri).
- Cauze metabolice- fibroza chistică (46 cazuri), hipotiroidie (14 cazuri), boala Wilson (12 cazuri) , glicogenoza tip I (3 cazuri), boala Gaucher (2 cazuri).
- Hepatita neonatală idiopatică - 26 cazuri.
- Displazia arterio-hepatică (sindromul Alagille) - 9 cazuri.
- Cauze anatomice (chist hepatic) - 8 cazuri.

REZULTATE ŞI DISCUŢII

Relaţia între boala hepatică şi gravitatea malnutriţiei se corelează în special cu vârsta mică. Peste 60% dintre sugarii cu boli hepatice colestatice au malnutriţie după studiile lui Sokol şi Stall (6). În lotul studiat, procentul sugarilor cu colestază a fost de 45,39% (133 cazuri) iar dintre aceştia, au prezentat malnutriţie 62,12% (82 copii) (fig. 1).

Evaluarea clinică a deficitului vitaminelor liposolubile, în special, dar şi a celor hidrosolubile şi a mineralelor s-a efectuat la toţi pacienţii şi s-au obţinut următoarele rezultate :

- Deficitul de vitamina A – 84% dintre copiii cu colestază prezentau pielea uscată.
- Deficitul de vitamina D - a fost monitorizat prin urmărirea semnelor clinice dar şi paraclinice (biologice şi radiologice) de rahitism, prezent în special la sugari

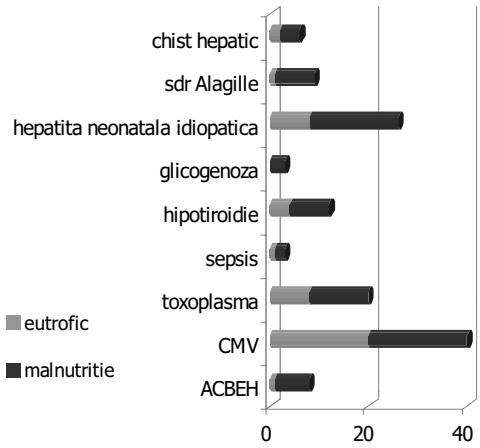

Fig. 1. Repartiţia cazurilor de malnutriţie în lotul sugarilor cu colestază

(66%), evidenţierea osteopeniei la densitometria osoasă (38%), hipotonia musculară (9%), fracturi pe os patologic (3 cazuri) - fig 2.

- Deficitul de vitamina E- a fost mai rar întâlnit în lotul studiat (3%) iar determinarea serică a alfa-tocoferolului nu a putut fi realizată.
- Deficitul de vitamina K - nu a avut un răsunet major în tabloul clinic al copiilor cu colestază însă a fost demonstrată biologic prin alungirea timpului Quick şi a timpului de protrombină în 71% din cazuri.
- diverse grade de anemie au fost întâlnite la majoritatea copiilor cu colestază (85%), aceasta datorându-se mai multor factori secundari malabsorbţiei induse de colestază (deficit de acid folic, vitamine B_1, B_6, B_{12}, fier)

În ceea ce priveşte terapia nutriţională a acestor copii scopul este de a asigura un aport caloric de 130% din aportul caloric zilnic recomandat de vârstă. Dacă acest scop nu este atins prin hrănire pe cale orală, hrănirea pe sondă nasogastrică ar trebui luată în considerare, furnizând o cantitate suficientă pentru a ajunge la 150 Kcal / kg / zi (4). Aceste măsuri ar trebui să fie luate imediat ce se observă o întârziere în creşterea ponderală pentru a putea preveni instalarea malnutriţiei.

Fig. 2. BR, atrezie de căi biliare extrahepatice

Astfel, copiii cu malnutriţie din lotul studiat au beneficiat de o dietă individualizată, administrată pe cale preponderent enterală, încercându-se suplimentarea variatelor deficienţe, urmărind câteva principii :

- menţinerea unui aport caloric şi proteic crescut (2,5 - 3 g/kg corp/zi)
- conţinut ridicat de calorii : 0.8 - 1 Kcal / ml (Dextrine maltoză, 60% din aportul energetic)/ caloriile necesare depăşesc 130 – 150% din raţia recomandată pentru greutatea totală, ajungînd la 150 – 200 cal/kg /zi
- dietă îmbogăţită cu aminoacizi cu lanţ ramificat (0,25 g/kg/zi)
- glucide -15 – 18 g/kg/zi , administrat sub formă de polimeri de glucoză
- lipide 8 – 9 g/kg/zi (15% trigliceride cu lanţ mediu)
- creşterea conţinutului de vitamine liposolubile
- acizii grasi esenţiali
- conţinut scăzut de sodiu, cupru şi conţinut ridicat de zinc întrucât enzimele esenţiale în funcţionalitatea hepatică necesită cofactori de activare reprezentaţi de oligoelemente
- raportul calciu / fosfor = 2

În ceea ce priveşte terapia deficitelor în vitamine liposolubile şi minerale, dozele au fost stabilite după următoarele formule :

- Vitamina K : - 1 mg/kg, maximum 10 mg, intra-muscular, o dată la 1-2 săptămâni
- Vitamina E : - 10 mg/kg, IM, la fiecare 2 săptămâni/ 25 UI/kg, oral
- Vitamina A : - 25000 - 50000 UI, IM, o dată pe lună
- Vitamina D - 30000 - 60000 UI, IM, o dată pe lună
- Calciu : - 50 mg/kg/zi, oral
- P : 25 mg/kg/zi, oral
- Zinc : - 1 mg/kg/zi, (zinc sulfat), oral

În multe cazuri cu boală hepatică terminală la copil, numai transplantul hepatic reprezintă o modalitate efectivă de tratament. În funcţie de starea pacientului, pediatrul trebuie să decidă dacă este momentul optim pentru transplant hepatic astfel încât statusul nutriţional să nu fie un impediment pentru realizarea transplantului. Obiectivul major este ca pacientul să fie preluat de echipa de transplant hepatic într-o stare nutriţională care să permită avansarea unui prognostic evolutiv bun posttransplant (7,8).

În lotul studiat, transplantul hepatic s-a efectuat cu succes în trei cazuri – doi băieţi cu sindrom Alagille şi o fetiţă cu atrezie de căi biliare extrahepatice. Vârsta efectuării intervenţiei chirurgicale a fost în medie de 2 ani în toate cele trei situaţii. Statusul nutriţional în cazul fetiţei cu atrezie de căi biliare extrahepatice a fost normal atât precât şi posttransplant. În situaţia copiilor cu sindrom Alagille, aceştia prezentau o malnutriţie protein calorică gradul I în momentul efectuării intervenţiei chirurgicale, menţinând şi în prezent, (la 3, respectiv 4 ani posttransplant) hipotrofie staturoponderală.

Conform rapoartelor anuale prezentate de SPLIT (studii despre transplantul hepatic la copil), aproximativ 20% din beneficiarii unui transplant hepatic menţin un retard al creşterii după transplant. Se consideră că o creştere nesatisfăcătoare pretransplant nu poate fi corectată complet deşi un procentaj mare de copii recuperează deficitul staturo-ponderal (9,10).

S-a observat că în situaţia celor doi copii cu sindrom Alagille ce au beneficiat de transplant hepatic, retardul ponderal s-a accentuat iniţial, în primele 6 luni posttransplant, pentru ca ulterior, curbele ponderală şi staturală să evolueze ascendent ; în ambele situaţii, curba staturală se menţine în jurul valorii de -2DS, în timp ce curbele ponderale se situează în jurul valorilor de -1 DS. Pentru prevenţia şi terapia încetinirii creşterii, copiii pot beneficia de administrarea hormonului de creştere recombinant.

Potrivit aceluiaşi raport, factorii cei mai importanţi care afectează creşterea dupaă transplant sunt vârsta în momentul efectuării transplantului (pacienţii cu vârsta sub 2 ani au avut cea mai mare creştere), scorul Z la transplant, şi diagnosticul principal ce a impus efectuarea transplantului (pacienţi cu atrezie biliară par să aibă cele mai bune recuperări ale creşterii). Astfel, studiul de faţă, deşi pe un număr mic de pacienţi, susţine ideile mai sus menţionate (11,12,13).

CONCLUZII

Malnutriţia protein-calorică în patologia hepatică reprezintă un aspect dramatic care poate fi prevenit printr-un program individualizat fiecărui caz.

Suportul nutriţional scade morbiditatea prin infecţii (în special datorate deteriorării imune din malnutriţie şi agravate de imunosupresia posttransplant), asigură necesarul metabolic disponibil funcţiilor de sinteză, stocaj şi detoxifiere a ficatului grefat, scade mult rata complicaţiilor chirurgicale, asigură creşterea lineară, dezvoltarea mentală şi previne osteopatia metabolică.

Rolul suportului nutriţional premergător transplantului hepatic este de mare importanţă. Asistenţa nutriţională de calitate modifică impactul pe care malnutriţia îl are asupra supravieţuirii după transplant hepatic. Creşterea normală posttransplant este

un indiciu al funcţionalităţii grefei, dar un discret deficit în talie se menţine câţiva ani în orice condiţii.

Evaluarea necesită, de asemenea, măsuri speciale iar corecţia trebuie adaptată fiecărui caz, metode clasice de aport caloric fiind contraindicate în cazurile cu hipertensiune portală.

Evaluarea nutriţională permanentă, terapia nutriţională eficientă şi reală necesită o pregătire de specialitate, suport material şi logistic, o bună colaborare interdisciplinară, echipe de nutriţionişti care să colaboreze permanent cu echipa de transplant.

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EPIDEMIOLOGICAL CONSIDERATIONS ON POLYTRAUMA IN CHILDREN AND ADOLESCENTS

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EPIDEMIOLOGICAL CONSIDERATIONS ON POLYTRAUMA IN CHILDREN AND ADOLESCENTS (Abstract): Polytrauma is a major public health problem and a leading cause of death and disability for children at any age. **The study aim** was to identify the factors which influence the lesion trauma model and can be a starting point in setting the strategy for approaching violent accidents in children and young people. **Material and methods**: The descriptive study was carried out on a group of 1532 patients with polytrauma, admitted in the Emergency Department (ED) of the "Sf. Ioan" Children's Emergency Clinical Hospital, Galati, Romania, during 2012 - 2016. The data were analyzed using the *SPSS V.24* software. **Results**: Road traffic accidents encountered the highest incidence (54.9% - 841 cases). The mean age was 9.4 ± 4.32 SD. An increased frequency of urban cases (53.46%) – and males (67.49%) were highlighted. The comparative analysis of the monthly incidence showed a significantly higher incidence during August (13.3%). The distribution of cases by days of week noted an increased incidence in Sunday (21.1%). **Conclusions**: Characteristics of the case that may be admitted more frequently in our ED were those of a 9-year-old child, most likely male, from rural area, with polytrauma due to a road traffic accident that occurred more frequently in summer at the weekend. Our study could be useful for a better medical and prevention management of polytrauma at pediatric age. **Keywords**: EMERGENCY DEPARTMENT, POLYTRAUMA, EPIDEMIOLOGY, CHILDREN.

Polytrauma continues to be a main cause of death and disability in all age groups, especially in young people. The first assessment and management of seriously injured patients is a difficult task and requires a rapid and systematic approach. This systematic approach can be used to increase the speed and accuracy of the evaluation process, but a good clinical assessment is also required. The term of polytrauma defines a severe acute condition that occurs following an accident due to a physical or a chemical aggression, characterized by the presence of multiple lesions affecting body organs or systems. Polytrauma due to road traffic accidents is the main cause of mortality in people aged between 1-40 years. Deaths due to polytrauma in children aged of 1-15 years exceed all other causes counted together, and they are only the tip of the iceberg because for 1 deceased child, 2 children remain with disabilities, 34 children require long-term hospitalization, and 30 children are check out in emergency rooms and outpatient units (1, 2).

Approximately 1.35 million people die

each year due to road traffic accidents. The 2030 Agenda for Sustainable Development set an ambitious target of halving the overall number of deaths and injuries caused by road traffic accidents by 2020. Road traffic hazards are the main cause of death for 5-29-year-old children and adolescents. Data for Romania show that in 2016 there were 1913 road traffic accidents, 73% in men and 27% in women, with a fatality rate estimated by the WHO at 10.3% of 100,000 inhabitants (3).

A recent report (4) from 34 countries showed that more than 8,100 children died in road traffic accidents in the last decade in the European Union. In 2016, a number of 630 children died in a traffic accident. In the EU, 46% of all road traffic deaths occur on rural roads, 46% on urban roads and 7% on highways. In Romania, about 74% of all road traffic deaths occur on urban roads, as compared with 67% in Croatia or 64% in Serbia. Although the incidence of polytrauma in children has decreased in EU22, by 4.6% annually, from 2006, in Romania, the number of children injured in road traffic accidents increased by almost 2% each year.

The aim of the study was to identify the factors which influence the lesion trauma model and can be a starting point in setting the strategy for approaching violent accidents in children and young people.

MATERIAL AND METHODS

The present descriptive study was carried out on a group of 1532 patients with polytrauma, admitted in the Emergency Department (ED) of the "Sf. Ioan" Children's Emergency Clinical Hospital, Galati, Romania. The study was performed retrospectively between 2012 and 2016.

Statistical analysis

The data were analyzed using the *SPSS V.24* software (IBM Statistical Package for Social Sciences, Chicago, Illinois). Student t test, Pearson-χ^2, Fisher's test, Levene's test, ANOVA test, and Pearson linear regression were used. The descriptive statistical indicators (mean, standard deviation, standard error, minimum, maximum, and quartile intervals) were calculated for continuous variables. In the descriptive statistics we calculated the values of the localization indicators (mean, median, mode values), spreading (dispersion, standard deviation, amplitude), we determined, where appropriate, the 95% confidence interval for the study of proportions. In the statistical analysis we applied tests to determine the regression model or Chi square test (χ^2) to compare the proportions. The calculation of the intervals of variation of the statistical indicators as well as the statistical hypothesis testing was performed with the 95% confidence interval and a significance threshold of p-value = 0.05.

RESULTS

Patients with polytrauma of various etiologies were evaluated and stabilized in the ED, followed by specific treatments by admission or transfer of patients to other units. The casuistry analysis (n = 1532) showed an increase in the frequency of cases from 272 patients recorded in 2012 to 396 cases registered in 2016 (fig. 1).

It is noteworthy that among polytrauma cases admitted in the ED, road traffic accidents encountered the highest incidence (54.9% - 841 cases). A high frequency has also polytrauma caused by falls from height (23% - 353 cases). A frequency of less than 10% was found for polytrauma caused by hetero aggressions (8.6%), bicycle / scooter accidents (6.1%), accidents caused by carts (4.6%) (tab. I).

The age of polytrauma cases was between 0 and 18 years, according to the specialty of the hospital, with a maximum of accidents recorded in 9-year-old patients (fig. 2). The mean age was 9.4 ± 4.32 SD. Quartile intervals indicated that 50% of cases were less than 10 years of age, and 25% under the age of 6 years. The value of quartile 3 indicated that 25% of cases were aged of 13-18 years.

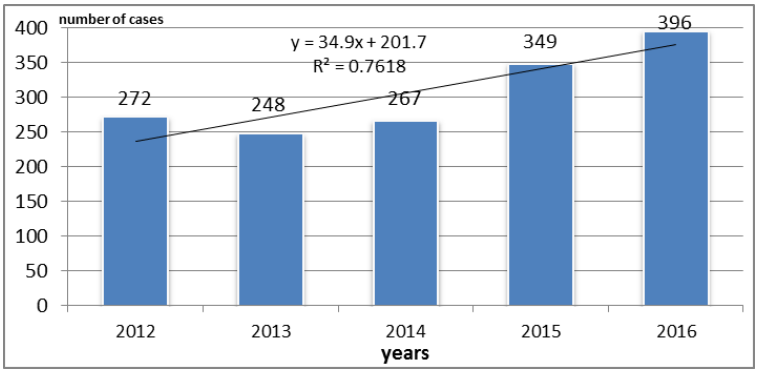

Fig. 1. Frequency of polytrauma cases by year of study

TABLE I

Polytrauma etiologies

Etiology	2012	2013	2014	2015	2016	Total
Silverman's syndrome	1	1	2	2	2	**8**
Accidents caused by sleigh	1	2	1	1	1	**6**
Domestic animals hit	2	1	2	3	1	**9**
Work accidents	3	4	5	4	4	**20**
Cart accidents	8	11	16	17	19	**71**
Bicycle / scooter accidents	18	21	15	19	20	**93**
Hetero aggressions	35	22	19	26	29	**131**
Fallen from height	71	62	58	79	83	**353**
Road traffic accidents	133	124	149	198	237	**841**
Total of cases	**272**	**248**	**267**	**349**	**396**	**1532**

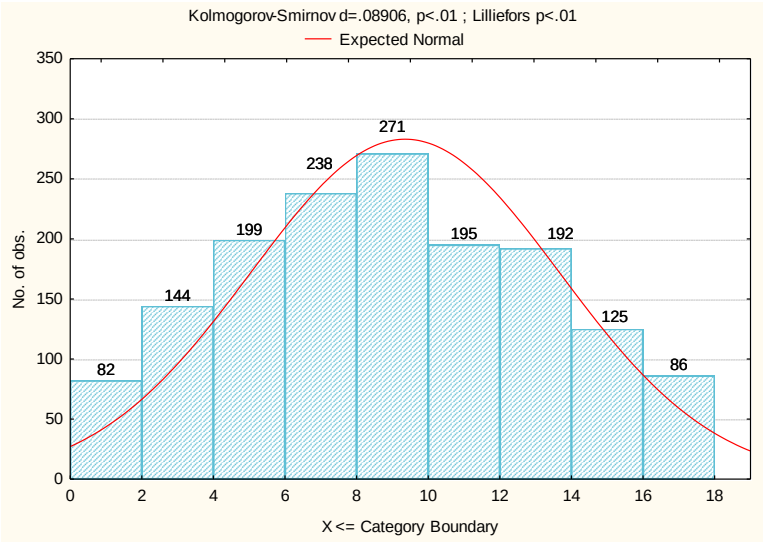

Fig. 2. Age histogram

The analysis of demographic characteristics regarding *residence area* revealed an increased frequency of urban cases 53.46% (p = 0.046) compared to those from the rural area (46.54%).

The distribution of cases by gender indicated that 1034 were males (67.49%) and 498 females (32.51%), with a statistical significance (p = 0.0027, 95% CI) of polytrauma among male patients (tab. II, III).

TABLE II

Demographical characteristics

Demographical characteristics		n	%	p (Chi-square test –95%CI)
Gender	Male	1034	67.49%	0.00274*
	Female	498	32.51%	
Residence area	Urban	819	53.46%	0.0463
	Rural	713	46.54%	
Total of cases		**1532**		
(*) p <0.05 – significant differences				

TABLE III

Statistical indicators: age in relation with patients' gender

Gender	Age average	Mean		Std. Dev.	Std. Err.	Min	Max	Q25	Median	Q75
		-95%	+95%							
Male	9.43	9.16	9.69	4.31	0.13	0.29	18.00	6.00	10.00	13.00
Female	9.20	8.82	9.59	4.34	0.19	0.36	18.00	6.00	8.00	13.00
Levene test: F=2.4702, p=0.1162; ANOVA: F(1,1530) = 0.8911, p = 0.3453										

The seasonal evolution of cases revealed significant differences in the frequency of according to the calendar months (fig. 3). The comparative analysis showed a significantly higher incidence during the summer months (July - 10.9%, August - 13.3%). A small incidence was found for December (5.4%) and January (4.4%). The incidence was significantly influenced by the calendar seasons (χ^2 = 21.56, p = 0.002, 95% CI), being increased in the summer season (34% of total cases).

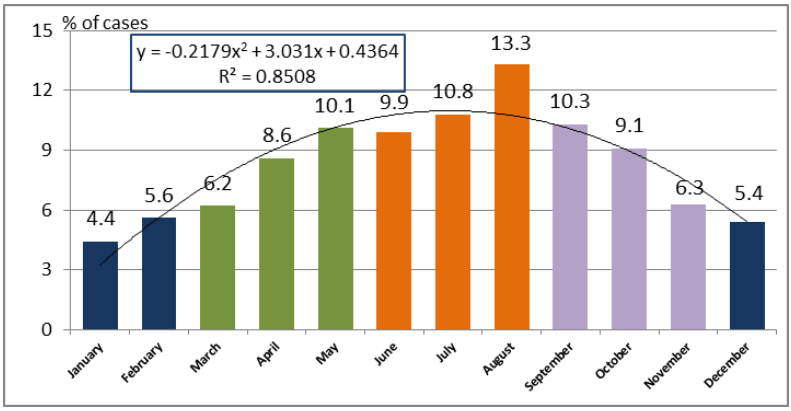

Fig. 3. Monthly incidence of polytrauma cases

The distribution of cases by days of week noted an increased incidence in weekend days, Friday (14.6%), Saturday (19.7%), and Sunday (21.1%). The smallest incidence of polytrauma cases was found on Wednesday (10.2%), but close values were encountered for Monday (11.9%), Tuesday (11.2%), and Thursday (11.2%). The association of the polytrauma cases frequency with the weekdays was statistically significant (χ^2=17.53, p=0.0396, 95% CI) (tab. V, fig. 4).

TABLE V

Distribution of polytrauma cases by days of week

Days of week	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
n	183	172	156	171	224	302	324
%	11.9%	11.2%	10.2%	11.2%	14.6%	19.7%	21.1%
χ^2=17.53, p=0.0396; Chi-square test (McNemar Chi-square)							

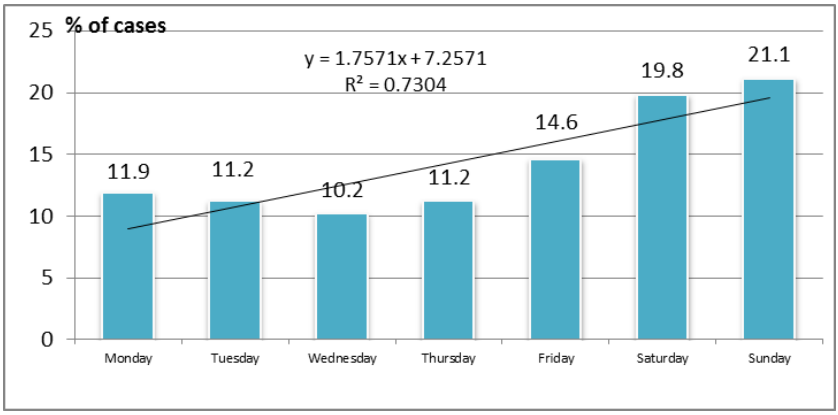

Fig. 4. Polytrauma cases incidence in relation with days of week

DISCUSSION

According to annual WHO reports, approximately 200,000 deaths among children were registered due to road traffic accidents. Trauma due to road traffic accidents, by their very high incidence, represents a major public health problem worldwide. Due to their age-specific peculiarities, children are a population at high risk for trauma, in general, due to their capability of attention, perception, and discernment involved in producing events with vital risk (5, 6).

There are differences in the frequency of gender-related polytrauma starting from the first year of life. Frequency of polytrauma is similar up to 5 months of life, with differences occurring when the child begins to walk. The difference is progressively accentuating with a maximum in adolescents, the risk of death caused by polytrauma being higher in boys. The gender differences in incidence are attributed to behavior, risk exposure by games or sports activities (7, 8).

Urban and rural areas have notable differences, and serious road traffic accidents involve mainly children from rural areas who are supposed to be less supervised, spending most of time outdoor, thus being more exposed to serious injuries. The case severity is also due to the relatively long

distance to a specialized medical service (9, 10, 11).

The variations during the study interval showed a minimum in 2012 and a maximum of cases in 2016, in line with national statistical reports, but also from Galați County. The continuous increases in the number of motor vehicles and driving license holders result in an increase in the number of road traffic accidents with polytrauma, deaths and disabilities (12).

Studies conducted in Europe and North America highlighted the fact that starting with May, the number of polytrauma cases increases, reaching a peak in autumn during October-November (13, 14). As a result, ED services are highly demanded during this period. If the victims are children, the timing of road accidents with polytrauma evidenced some peculiarities. Starting with May, there is an increase in outdoor activity during summer holidays. The children rashness and imprudence (6-14 years), unsupervised preschool children (3-6 years), but also excessive speed of drivers is the main causes of polytrauma (15, 16). Our study highlighted a significant difference in the number of children with polytrauma during summer months as compared with other seasons. As also reported in the literature (17), the distribution of polytrauma in our study showed a peak at the end of the week.

A multicentric study (18) conducted by the *Pan-African Pediatric Surgery Association* (PAPSA) was carried out for 31 days (May 1 - June 1, 2015) on pediatric trauma admissions, simultaneously, in 15 surgical centers, from 11 countries (3 high income, 5 middle income, 3 low income). The study revealed that there were 1,377 pediatric admissions for trauma, of which 1,295 in 10 centers in low-income countries and 84 in 5 centers in high-income countries. Children were 7 years old, of whom 62% were males. Road traffic accidents were the most frequently involved (41%), followed by falls (41%) and violence (11%).

In a study (19) conducted in 2009-2010 in ED Tîrgu-Mureș, Romania, one of four trauma patients was found to be due to a road accident, and of the 718 patients treated for trauma due to road accidents, most of them were young adults aged 18-29 (30.5%), and male gender represented more than two-thirds of patients (66.9%). Children aged 0-17 accounted for 33.3% of all injuries caused to pedestrians. Most injuries happened on national roads (71.4%), and rural roads were involved in 2.9% of cases.

Our study has certain limitations. The collected data may be representative for Galati County, but not for the entire country. Data was collected in a limited interval of time. It is possible to be cases that have been lost from the record. However, the results of our study might help ED management for pediatric cases admitted with polytrauma, and further research will complete the existing data.

CONCLUSIONS

Our study may be a starting point for establishing a medical and prevention strategy for polytrauma at pediatric age. Age of 8-10, male gender and rural area were more frequently demographic characteristics of study group. Road traffic accidents occurred in summer during the weekend was the most frequent pattern involved in pediatric polytrauma. These results may facilitate the ED management to be more adapted to pediatric patients, allowing a faster orientation of medical staff and resources. On the basis of the data obtained in our study, primary prevention measures regarding polytrauma may also be initiated.

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DIAGNOSTIC DILEMMAS IN PANCREATIC ADENOSQUAMOUS CARCINOMA. CASE REPORT

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DIAGNOSTIC DILEMMAS IN PANCREATIC ADENOSQUAMOUS CARCINOMA - CASE REPORT (Abstract): Adenosquamous carcinoma of the head of the pancreas (ASCAP) is a very rare tumor (1-2%), consisting of both squamous cell carcinoma (required 30%) and ductal adenocarcinoma. ASCAP is a malignant tumor with debatable histogenesis. We report the case of a 54-year-old man, with a history of more than half of a year of right upper quadrant abdominal pain, jaundice, with no anamnestic data of acute or chronic pancreatitis, or substance abuse. On gross examination the tumor was solid, white, poorly circumscribed, located in the head of the pancreas, with 4 cm diameter, and direct extension into ampulla of Vater, common bile duct, and peripancreatic soft tissue. Microscopically, the tumor was composed of squamous polygonal cells arranged in small glandular structures, with tightly packed pattern, and mild nuclear pleomorphism, along with small areas with Alcian blue positive intracellular mucin. Therefore, the differential diagnosis between ASCAP and pancreatic collision tumor, metastasis of adenosquamous carcinoma or mucoepidermoid tumor was compulsory. The immuno-histochemistry assay confirmed ASCAP as the final diagnosis, based on the intense positive reaction for cytokeratin 7 mainly in the glandular structures, whereas cytokeratin 5 and p63 were positive predominantly in the squamous component. The present report aimed to highlight the pathological characteristics of this rare subtype of pancreatic tumors and to point out the difficulty in differentiation between a pancreatic and an extra pancreatic (biliary tract or ampulla of Vater) tumor, due to the extensive tumor growth that interests, in advanced stages, the same anatomical space, given the different prognosis and therapeutic approach of these entities. **Keywords**: ADENOSQUAMOUS CARCINOMA, PANCREATIC TUMOR, COLLISION TUMOR.

Adenosquamous carcinoma of the pancreas is a malignant tumor consisting of both squamous cell carcinoma and ductal adenocarcinoma components (1). These entities can vary in amount and distribution, a 30% squamous differentiation being

required according to some authors for a positive diagnosis (2). It represents a rare entity, being described in 1-2% cases of exocrine pancreatic tumors (3). This paper presents a case and reviews the characteristics of a rare pancreatic malignancy.

CASE REPORT

We are presenting the case of a 57-year-old patient, with no medical history of acute or chronic pancreatitis, hospitalized in the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi with jaundice and acute calculous cholecystitis. He was admitted in the emergency room with right upper quadrant abdominal pain for about 2 weeks. The patient did not mention any relevant personal or familial medical history aside from an appendectomy. Physical examination outlined sclero-tegumentary jaundice and hyperchromic urines.

Laboratory tests at admission showed high WBC (10,360/µL) associated with elevated transaminases (ALT = 522 U/L, AST = 267 U/L), as well as other liver disease markers (GGT = 760 U/L, ALP = 556 U/L). The hepatic pathology was sustained by the critical levels of bilirubin, with a maximum of 21.86 mg/dL and the conjugated bilirubin of 9.27 mg/dL at admission starting to normalize and maintaining levels between 2 and 3 mg/dL.

The abdominopelvic CT revealed a nodular mass in the head of the pancreas (33/31/32 mm), well demarcated, hypodense, with anfractuous areas inside, being in close proximity to the superior mesenteric vein. Two lymph nodes of 7 mm and 6 mm, respectively, were discovered in the retroduodenopancreatic space as well as the atrophy of the neck, body and tail of the pancreas, and the dilatation of the pancreatic duct. The common hepatic duct and the intrahepatic bile ducts were dilated in both lobes and gallbladder hydrops was present. The right kidney had a cortical cyst of 16 mm and the prostate showed hypertrophy (34/49/46 mm). A solid retroperitoneal nodule (15/12/19 mm) was also detected in the left iliac fossa, adjoined to the psoas and iliac muscles. The exam concluded the diagnosis of a neoplasm located in the head of the pancreas cT2N1M1 with retroperitoneal metastasis.

The surgery included both duodenopancreatectomy and cholecystectomy, which revealed normal features of the gallbladder and a tumor in the pancreatic area. The gross specimen consisted of gastric tissue (3 cm), duodenal tissue (12 cm) and pancreatic tissue (5.5/4.5/3.5cm). A histopathological examination identified a large tumor located in the head of the pancreas (4.2/3.3/3.2 cm), showing firm consistency, white color, and being poorly demarcated, with direct extension into ampulla of Vater, common bile duct, and peripancreatic soft tissue (fig. 1).

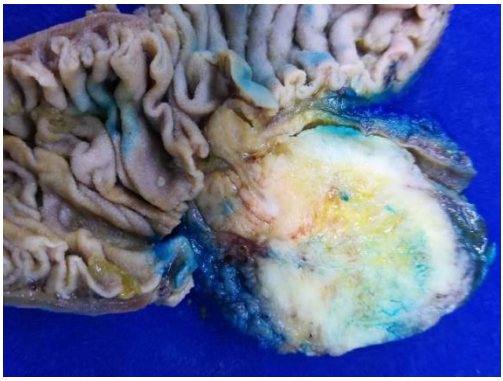

Fig. 1. White, poorly demarcated pancreatic tumor, with direct extension into common bile duct

Multiple sections were stained with hematoxylin and eosin (H&E) and Alcian blue (AB). Microscopic examination of the

pancreatic tumor in H&E showed an adenosquamous carcinoma with biphasic malignant growth, identified as squamous cells with keratohyalin granules and glandular structures with mild nuclear pleomorphism associated with small areas with AB positive intracellular mucin (figs. 2-5).

Perineural invasion, luminal necrosis, desmoplastic stroma, and necrotic areas were also identified. Pancreatic tumor showed diffuse invasion into the thickness of the biliary duct, without duodenal wall involvement. Peripancreatic lymph nodes were not dissected. Furthermore, the gallbladder revealed signs of chronic cholecystitis and the remainder of the pancreatic tissue was modified (atrophied acini, hyperplasia of the neuroendocrine islets, interstitial fibrosis, and mild chronic inflammation), suggesting chronic pancreatitis. Immunohistochemical staining was performed to demonstrate pancreatic biphasic tumor. Therefore, immunolabeling for cytokeratin 7 was positive mainly in the glandular structures, whereas cytokeratin 5 and p63 were positive predominantly in the squamous component (figs. 6-8).

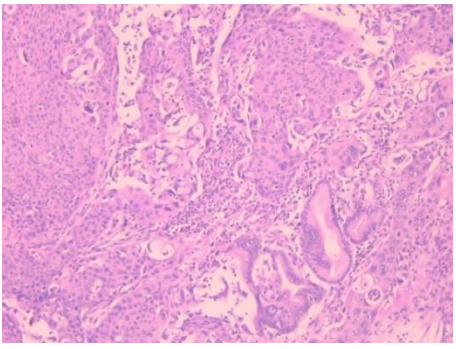

Fig. 2. Biphasic malignant tumor growth composed of moderately differentiated adenocarcinoma and well differentiated squamous cell carcinoma

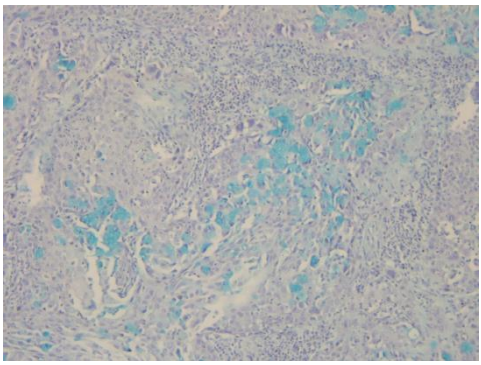

Fig. 3. The adenocarcinoma component of the tumor with glandular structures containing intracellular mucin (Alcian Blue positive staining)

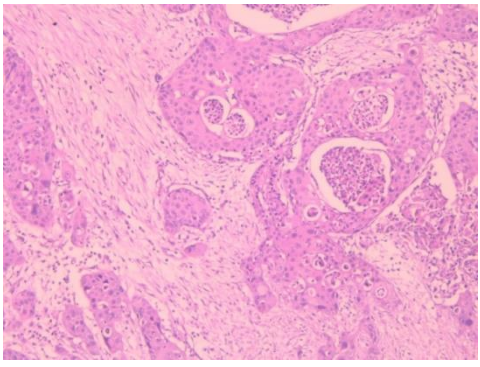

Fig. 4. Squamous differentiation with infiltrative nests of polygonal cell, intercellular bridges, eosinophilic cytoplasm, and variable degrees of keratinization

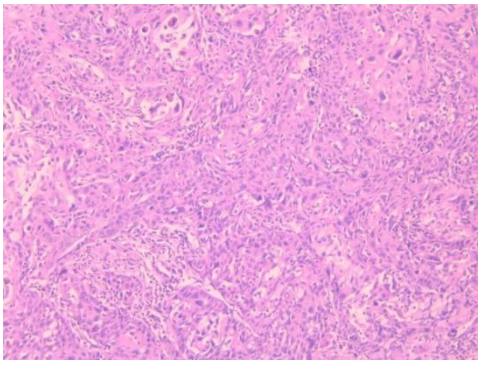

Fig. 5. Two different patterns intimately admixed with the tumor

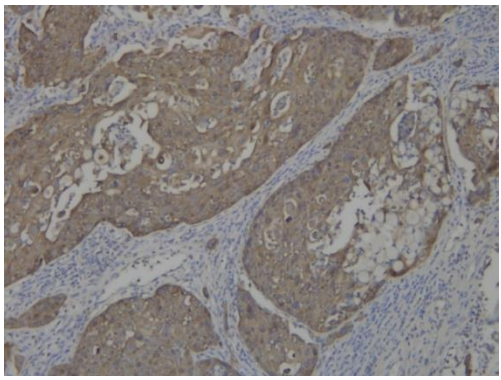

Fig. 6. Tumor cells positivity for cytokeratin 5 predominantly in the squamous cells and in few adenocarcinoma components

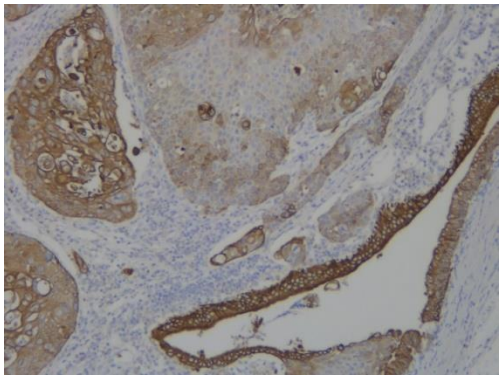

Fig. 7. Immunoreactivity for CK 7 restricted to the glandular component, while the squamous component is mainly negative

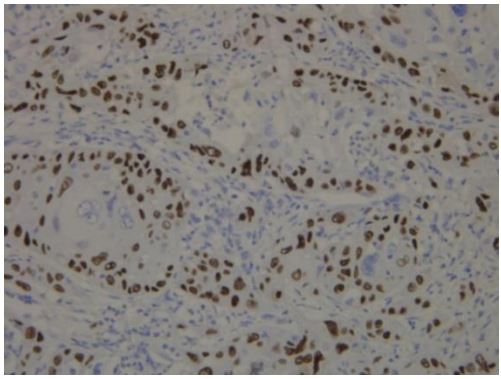

Fig. 8. Immunoreactivity for p63 is restricted to the squamous component, while the glandular component is negative

The patient had a difficult and complicated postoperative course, during which he also developed ascites and fluid in the right pleural cavity. The examination of the liquid showed an infection with *Enterobacter cloacae*, *Pseudomonas aeruginosa*, and *Candida spp*.

The wound was re-examined after two weeks and the results came back positive for *Klebsiella pneumoniae*. The patient was discharged 50 days after admission, when the infection was completely resolved.

DISCUSSION

The first known case of adenosquamous carcinoma was reported in 1907, when Herxheimer referred to this lesion as "cancroide" (4). Adenosquamous carcinoma is a rare entity on its own, making it an even more interesting case with the occurrence in the pancreatic tissue. The reported cases were seen more commonly in organs such as lungs, esophagus, colon, stomach or salivary glands (3). WHO 2010 classification synthesizes pancreatic cancers as ductal adenosquamous carcinoma, neuroendocrine neoplasms, cystic tumors, solid pseudopapillary tumors, acinar cell carcinoma, adenocarcinoma, squamous cell carcinoma, primary lymphoma, and metastatic lesions (2). A study published in 2012 by Boyd *et al*. (5) reviewed 415 patients with this kind of tumor and concluded that it is most commonly located in the head of the pancreas with 185 cases (44.6%), while the body/tail tumors appeared in 121 cases (29.2%). Males are slightly more affected than females (54.5% *vs.* 45.5%) with the mean age of incidence of 66 years and a racial distribution with Caucasian dominance (about 80% of patients) (5).

Adenosquamous carcinoma of the pan-

creas (ASCAP) has an even more aggressive tumor behavior than the ductal adenocarcinoma (DADC), with a median survival of less than one year (2), and its body or tail locations have a rare occurrence (3, 5).

The histogenesis of ASCAP remains uncertain, raising controversies regarding its origin, with several theoretical hypotheses launched nowadays. One of them proposes a preexisting adenocarcinoma undergoing squamous cell transformation, while another supports its origin from a heterotopic squamous epithelium transforming into a malignant entity. It is also possible that a stem cell population could be able of differentiation into either a squamous or glandular pattern, followed by malignant transformation (6, 7). However, the clinical and macroscopic aspects are general and very similar to other pathologies, including abdominal pain, weight loss, anorexia, and jaundice, making it difficult to diagnose (3, 8). CT imaging may show more conclusive aspects, including large infiltrative lesions and the presence of central necrosis. Nabae *et al*. (9) concluded that the presence of these features in a large pancreatic tumor seems to be suggestive of the diagnosis of adenosquamous carcinoma of the pancreas (9). Serum data and radiology examination are not very helpful, as this type of tumor has no differentiating aspects from the DADC (10).

Currently there are no guidelines for treating patients with ASCAP due to its rarity and diagnosis difficulties. Treatments are similar to those for adenocarcinoma and are mainly based on local resection, with three main surgical methods performed to ensure a long-lasting survival: pylorus preserving pancreaticoduodenectomy (PPPD), distal pancreatectomy (DP), and total pancreatectomy (TP) (7).

Adjuvant radiotherapy and chemotherapy does not play an important role in the prognosis of ASCAP. Literature highlights that the median survival period of patients treated with radiotherapy/chemotherapy is increased (13 months/7 months) compared to patients that have not received radiotherapy/chemotherapy (6 months/5 months) (10).

The prognosis of this type of cancer is poor, with a median survival range of 4.6 to 9 months. Nevertheless, the outcome after pancreatic resection shows improvement (5.2-10.8 months), with a survival rate of 33.4% after 1 year and 17.2% after 2 years *vs.* 25.5% and 14.0% in patients that had no pancreaticoduodenectomy (PD) (7).

The main diagnosis issue of ASCAP is derived from the difficulty in differentiation from a biliary tract or ampulla of Vater tumor, due to similar morphology and the extensive tumor growth that interests the same anatomical space. The differential diagnosis also took into consideration a pancreatic collision tumor, metastasis of an adenosquamous carcinoma or mucoepidermoid tumor from other organs. It is crucial to differentiate between this particular histological type from other malignancies, in order to evaluate the diagnosis consequences for the management of the patient.

CONCLUSIONS

Adenosquamous carcinoma of the pancreas remains a rare and complicated diagnosis to manage, with important difficulties in patients' treatment. From the clinical examination to the laboratory and imaging tests, it is crucial to differentiate between this particular histological type and other malignancies, such as adenocarcinoma,

being aware of the diagnosis consequences for the treatment options. With a poor prognosis even after resection of the tumor, more studies need to be conducted in order to fully understand the implications and the outcome of this disease.

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NEWS

FLUOROQUINOLONE USE AND RISK OF AORTIC ANEURYSM AND DISSECTION

The objective of this study was to investigate whether oral fluoroquinolone use is associated with an increased risk of aortic aneurysm or dissection. This is a retrospective study conducted using linked register data on patient characteristics, filled prescriptions, and cases of aortic aneurysm or dissection. Results: within the 60-day risk period, the rate of aortic aneurysm or dissection was 1.2 cases per 1000 person years among fluoroquinolone users and 0.7 cases per 1000 person years among amoxicillin users (amoxicillin is an antibiotic with no known association with aortic aneurysm or dissection). Fluoroquinolone use was associated with an increased risk of aortic aneurysm or dissection (hazard ratio 1.66 (95% confidence interval 1.12 to 2.46)), with an estimated absolute difference of 82 (95% confidence interval 15 to 181) cases of aortic aneurysm or dissection by 60 days per 1 million treatment episodes. In a secondary analysis, the hazard ratio for the association with fluoroquinolone use was 1.90 (1.22 to 2.96) for aortic aneurysm and 0.93 (0.38 to 2.29) for aortic dissection. Conclusions: in a propensity score matched cohort, fluoroquinolone use was associated with an increased risk of aortic aneurysm or dissection. This association appeared to be largely driven by aortic aneurysm. (Pasternac B *et al*. *BMJ* 2018; 360: 678).

THE IMPORTANCE OF THE NEUTROPHIL TO LYMPHOCYTE RATIO AS A PREDICTIVE FACTOR FOR THE OCCURRENCE OF INTESTINAL NECROSIS IN PATIENTS WITH INCISIONAL HERNIAS

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THE IMPORTANCE OF THE NEUTROPHIL TO LYMPHOCYTE RATIO AS A PREDICTIVE FACTOR FOR THE OCCURRENCE OF INTESTINAL NECROSIS IN PATIENTS WITH INCISIONAL HERNIAS (Abstract): The **aim** of the present study was to establish the importance as a predictive factor of the NLR (neutrophil to lymphocyte ratio) for the occurrence of intestinal necrosis in patients with incisional hernias. **Material and methods**: We conducted a retrospective study including 141 patients over a 3-year period in which we analyzed the values of NLR. **Results**: Thus, we noticed that there is a direct, statistically significant correlation between the NLR and the indication of small bowel resection in patients with incisional hernias. Depending on the binary variable resection yes or no, we set a cutoff threshold of 2.553 with predictive value, threshold for which we have the best values of sensitivity and specificity as performance parameters. Over this threshold the sensitivity increases, so the patients or the risk of resection is highly appreciated, AUC=0.853 (CI 95%=0.784 to 0.907), p<0.001. **Conclusions**: Increased NLR values may play a particularly important role in predicting the existence of intestinal necrosis in patients with incisional hernias. NLR values above 2,553 are commonly associated with the appearance of necrosis or ischemia in the small intestine in these patients. **Keywords**: INCISIONAL HERNIAS, NEUTROPHIL TO LYMPHOCYTE RATIO.

Incisional hernias are a common condition in patients with a history of abdominal surgery. Their incidence varies depending on the type of surgery performed, as well as the associated comorbidities of the patient, which varies between 9 and 20% (1).

The occurrence of incisional hernias in the vast majority of cases is frequently associated with the onset of abdominal pain syndrome, with the presence of intestinal transit disorders, these aspects usually requiring surgical treatment, sometimes even in emergency (2). Some of the main complications that can occur in patients with incisional hernias are strangulation, respectively incarceration or irreducibility

of bowel. The appearance of these post operatory complications can endanger the life of these patients and impose the emergency surgical treatment of these cases, mainly due to the risk of intestinal necrosis or of the peritonitis (3, 4).

In the last years, more and more emphasis has been placed on the specialty literature on the importance of systemic inflammatory syndrome as a prognostic factor in the pathogenesis of neoplastic or non-neoplastic disorders. Most commonly, the extent of the systemic inflammatory syndrome is quantified by calculating the ratio of serum neutrophils (NEU) value and serum lymphocytes (LYM) value (5, 6). The purpose of the present study was to establish the importance as a predictive factor of the value of the NLR ratio for the occurrence of intestinal necrosis in patients with incisional hernia, as well as to establish a cutoff value of the NLR with a predictive value in these cases.

MATERIAL AND METHODS

In order to achieve the proposed purpose, we conducted a retrospective observational study over a 3-year period between 01.01.2016 - 31.12.2018 in which we included 141 patients who were admitted to the Surgery Department of Onesti Municipal Hospital. As exclusion criteria from the study it should be mentioned that, we excluded from the study patients who at the time of admission had clinical signs of other inflammatory conditions. We also excluded patients with incarcerated incisional hernia who required resection of the large intestine.

In all the patients studied, at the time of routine hospitalization, routine blood tests were collected (complete blood count (CBC), serum biochemistry, coagulation). In the case of CBC, we were mainly interested in the serum level of the NEU, LYM, serum monocytes (MON), serum platelets (PLT) and serum white blood cells (WBC). Based on these, the value of the NLR levels was calculated in all patients.

RESULTS

From the descriptive analysis of the processed data we obtained a relatively uniform distribution over time for the cases registered in the statistics of the surgical department (tab. I). From the analysis of the age of the studied patients we observed that they were between 19 and 89 years old.

TABLE I

Distribution by year

YEAR				
		Frequency	Percent	Valid Percent
Valid	2016	46	32.6	32.6
	2017	39	27.7	27.7
	2018	56	39.7	39.7
	Total	141	100.0	100.0

Regarding the distribution by sex of the patients we noticed that, in the studied group 31 patients were male (22% of cases) and 110 patients were female (78% of cases). In all the patients studied, the timing of the surgery was decided according to the general medical condition of the patients and the local clinical examination of incisional hernias. Thus, in the case of patients presenting with clinical signs of

bowel obstruction or local clinical signs of incarcerated incisional hernia, it was decided that the surgery should be performed urgently. In this sense it can be observed that in 46 cases it was decided that the surgery should be performed urgently and in 95 cases the surgery was scheduled (tab. II).

TABLE II

The time of the surgery

EMERGENCY / SCHEDULED SURGERY				
		Frequency	Percent	Valid Percent
Valid	E	46	32.6	32.6
	S	95	67.4	67.4
	Total	141	100.0	100.0

At the time of surgery in 27 cases (19.1%) intraoperatively the trophic disorders (necrosis or ischemia) was found in the ring of the peritoneal diverticula, which determined the need for bowel resection (tab. III).

TABLE III

Incidence of intestinal resection

SMALL BOWEL RESECTION YES / NO				
		Frequency	Percent	Valid Percent
Valid	YES	27	19.1	19.1
	NO	114	80.9	80.9
	Total	141	100.0	100.0

From the descriptive analysis of the values of the serum hemogram in the studied patients we observed that the value of the serum hemoglobin (Hb) and the serum hematocrit (Ht) have a Gaussian distribution, this fact allowing their expression by means and standard deviation (SD). On the other hand, the value of NEU, LYM, MON, WBC, PLT and NLR respectively, did not have a Gaussian distribution, this fact determining their expression by median, minimum and maximum. In terms of NLR levels they ranged from 0.7055 to 18.4160 (tab. IV).

TABLE IV

Descriptive analysis of the distribution of serum hemogram and NLR values

	Age	HG	HTC	NEU	LYM	MON	PLT	WBC	NLR
Mean	64.55	13.6318	40.9064	5156.31	2012.62	507.59	249035.46	7903.97	3.041186
Median	67.00	13.6000	40.4000	4340.00	1910.00	440.00	239000.00	7000.00	2.270900
Std. Deviation	12.131	1.51099	4.16793	2812.367	824.489	268.323	88485.545	3097.283	2.5581158
Minimum	19	9.80	30.20	1630	480	100	56000	2600	.7055
Maximum	89	17.00	49.90	20210	6810	2290	797000	23900	18.4160

In the case of the multivariate statistical analysis of the correlation between the NLR value and the indication of intestinal resection in the studied patients we applied the Mann-Whitney test, which compares the difference between the medians. Thus,

we observed the existence of a direct, statistically significant correlation between the value of NLR and the indication of bowel resection in these patients ($p<0.0001$) (fig. 1).

Next, in the case of the subgroup with bowel resection (27 patients) we tried to establish the existence of a correlation between the value of the neutrophil to lymphocyte ratio and the hemogram parameters. In this regard, we applied Spearman - type correlations between all quantitative variables. Thus, we observed that in these patients the NLR value correlates with the level of leukocytes, neutrophils, lymphocytes and serum monocytes, but not with the level of serum platelets. The results are presented in table V (**: Correlation is significant at the 0.01 level - 2-tailed, *: Correlation is significant at the 0.05 level - 2-tailed).

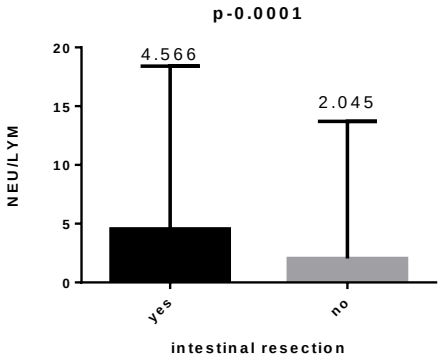

Fig. 1. Correlation between NLR value and indication of bowel resection

TABLE V

Spearman analysis of the correlation between NLR level and blood hemogram parameters

		HG	HTC	NEU	LYM	MON	PLT	NLR	WBC
Hg	Correlation Coefficient	1.000	.985**	.456*	.408*	.459*	.103	.257	.562**
	Sig. (2-tailed)	.	.000	.017	.035	.016	.610	.195	.002
Ht	Correlation Coefficient	.985**	1.000	.489**	.379	.496**	.188	.302	.588**
	Sig. (2-tailed)	.000	.	.010	.051	.009	.347	.126	.001
NEU	Correlation Coefficient	.456*	.489**	1.000	.085	.516**	.294	.866**	.962**
	Sig. (2-tailed)	.017	.010	.	.672	.006	.137	.000	.000
LYM	Correlation Coefficient	.408*	.379	.085	1.000	.095	.093	-.389*	.240
	Sig. (2-tailed)	.035	.051	.672	.	.636	.644	.045	.227
MON	Correlation Coefficient	.459*	.496**	.516**	.095	1.000	.309	.481*	.622**
	Sig. (2-tailed)	.016	.009	.006	.636	.	.117	.011	.001
PLT	Correlation Coefficient	.103	.188	.294	.093	.309	1.000	.240	.305
	Sig. (2-tailed)	.610	.347	.137	.644	.117	.	.227	.122
NLR	Correlation Coefficient	.257	.302	.866**	-.389*	.481*	.240	1.000	.757**
	Sig. (2-tailed)	.195	.126	.000	.045	.011	.227	.	.000
WBC	Correlation Coefficient	.562**	.588**	.962**	.240	.622**	.305	.757**	1.000
	Sig. (2-tailed)	.002	.001	.000	.227	.001	.122	.000	.

Forwards, through statistical analysis, we tried to establish the cutoff value of the NLR which correlates with the necessity of practicing intestinal resection in patients with incisional hernias. Depending on the binary variable resection yes or no, we set a cutoff threshold of 2.553 with predictive value, threshold for which we have the best values of sensitivity and specificity as performance parameters. Over this threshold the sensitivity increases, so the patients or the risk of resection is highly appreciated, AUC=0,853 (CI 95%=0.784 to 0.907), p < 0.001, Sensitivity 85.20%, Specificity 72.80%. The values are represented in figure 2.

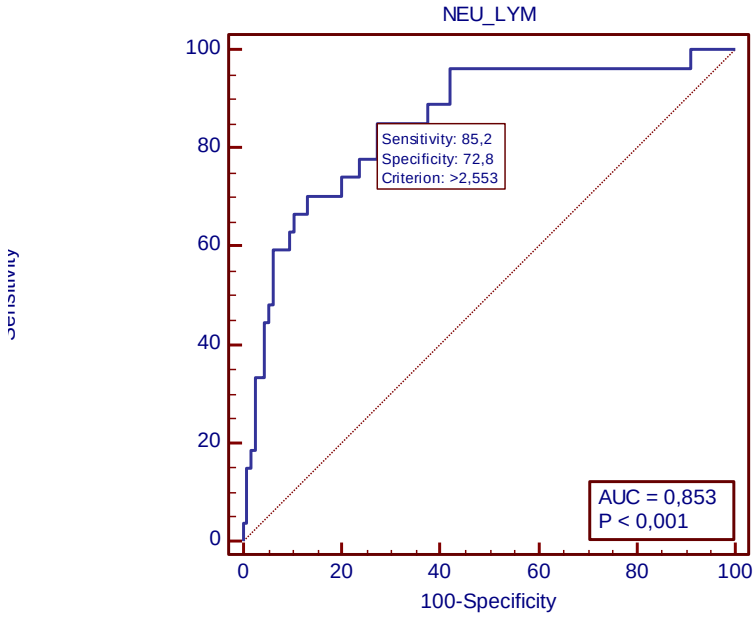

Fig. 2. Establishing the cutoff value of NLR that correlates with the indication of intestinal resection

DISCUSSION

In the vast majority of cases the appearance of a local complication in patients with incisional hernias (incarceration, strangulation) determines the appearance of a local inflammation which is frequently followed by the onset of a systemic inflammatory syndrome. Usually the factor that triggers systemic inflammatory syndrome is the activation of NEU (11). NEU activation occurs in most of the cases following their migration to the inflammatory outbreak. On the other hand, it is recognized at present that LYM play an inhibitory role in the migration of inflammatory cells as well as neoplastic cells. It has also been shown that increased LYM levels are a marker of immune system activation (12, 13).

The increase of the NLR value in the vast majority of cases denotes the increase of the serum level of the NEU accompanied by a relative decrease of LYM. Few studies in the literature have investigated so

far, the importance as a prognostic factor of NLR in patients with incisional hernias (14-16). In addition to the local inflammation that exists in patients with incisional hernias, another factor that may explain the increase of NLR in patients with intestinal necrosis is the existence of mesenteric ischemia, a fact also demonstrated by other authors in the literature (17-19).

Several studies have shown that the value of NLR correlates with the severity and prognosis of inflammatory syndrome in different conditions (20, 21). The major advantage of determining the value of NLR in patients with incisional hernias is that it does not require special tests, it is only necessary to perform blood hemograms, blood tests that are not expensive and are performed routinely in all patients admitted in different hospital units.

In our study we observed that the NLR median in patients who required bowel resection was 4.566 and in the case of patients who did not require bowel resection the NLR median was 2.045. Also, by statistical analysis we established the cutoff value of NLR that is predictive for the necessity of practicing an intestinal resection of 2.553 (sensitivity 85.20% and specificity 72.80%). In a study published in 2016 by Zhou in patients with incarcerated groin hernias, it was observed that the NLR median value that required intestinal resection was 6.5 (18, 22).

The present study has certain limitations due to the fact that it is a retrospective study, also being performed on a relatively small number of patients. Also, some clinical parameters (hemodynamic status, respiratory status of patients admitted to hospital) were not included in the study. Thus, further prospective, randomized studies are needed to establish the cutoff value of NLR that correlates with the risk of developing small bowel necrosis in patients with incisional hernias in much larger cohorts of patients.

CONCLUSIONS

Increased NLR values may play a particularly important role in predicting the existence of intestinal necrosis in patients with incisional hernias. NLR values above 2.553 are commonly associated with the appearance of necrosis or ischemia in the small intestine in these patients.

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A SHORT HISTORY OF THE HISTOLOGICAL PROCESSING TECHNIQUES FOR TOOTH AND SUPPORTING HARD TISSUES

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A SHORT HISTORY OF THE HISTOLOGICAL PROCESSING TECHNIQUES FOR TOOTH AND SUPPORTING HARD TISSUES (Abstract): Knowledge of the microscopic morphological tooth structure is compulsory for dental practice and research. The paper aims to present some major milestones in the evolution of hard tissue processing techniques, used in the histology laboratories for the analysis of the different specific tissues belonging to the tooth and supporting hard tissues. Decalcification is a mandatory step for processing these tissues. The decalcified tissues are subsequently impregnated with a suitable substance such as paraffin or celloidin. Despite the technical advances in medicine, the histopathological methods of tooth and supporting tissues processing are still difficult. The decalcification procedure can be replaced by non-decalcifying techniques. Technical problems are amplified by the fact that teeth and periodontal structures are extremely difficult to sample in humans under normal conditions. **Keywords**: TOOTH, DECALCIFICATION, EMBEDDING, PARAFFIN, CELLOIDIN.

Knowledge of the microscopic morphological tooth structure is compulsory for competent dental practice (1). Almost all dental specialties start from histology. The following examples are eloquent in this respect: cariology is directly related to the histoarchitecture of enamel and dentine, endodontics is related to the connective assembly specific to the dental pulp, periodontics and implantology are based on the morphological individuality of the periodontium, pedodontics shows the tooth germ developing elements, orthodontics integrates movements and forces inside the tooth attached to the periodontium.

On the other hand, research in the field of dentistry requires a good comprehension of tooth histology, whether it involves fundamental or clinical studies (1). However, the histological processing techniques for teeth and supporting hard tissues are difficult, due to the main characteristic of their structure, namely the hardness gained as a result of the mineralization process. Enamel is the hardest tissue in the body, with a degree of mineralization of 98-99%, followed by dentin, with a degree of mineralization of 70%, alveolar bone, with a degree of mineralization of 55% and cementum, with 45% mineralization (2, 3).

With strict reference to the deep periodontium, technical problems are doubled by structural peculiarities, the alveolar bone being part of the maxillary bone and, consequently, impossible to separate and taken from the human being, in normal conditions (3). This is confirmed by the fact that numerous periodontal studies are performed on experimental models, in animal species (4-7). Therefore, the periodontal research in humans is focused primarily on implants, following the elements of biocompatibility and bio integration that "mimic" a restoration of the components of the deep periodontium, close to the histoarchitecture resulting from the embryonic development and, subsequently, the eruption processes of the deciduous and permanent dentition. Other research directions target various bone grafting techniques and materials, such as bone augmentation and acceleration of recovery through the addition of natural or synthetic growth factors (8).

Within this context, our aim is to present some major milestones in the evolution of hard tissue processing techniques, used in the histology laboratories for the analysis of the different specific tissues belonging to the tooth and supporting hard tissues.

Decalcification is a mandatory step for processing these tissues.

One of the first solution for hard tissue decalcification was already known since 1882 – namely Perenyi's fluid composed by 10% nitric acid 40 mL, 0.5% chromic acid 30 mL, and absolute alcohol 30 mL (9). It is worth to mention that Perenyi's fluid is also used at the moment (10, 11). However, the first tooth processing techniques that include decalcification and paraffin/ celloidin embedding were reported starting with the 2nd decade of the 20th century (9, 12-14). A wide range of decalcification methods have been proposed, some of them having currently only a historical value (9, 12-15).

The removal of inorganic component (namely the calcium ions) from the hard tissue can be done with the help of strong mineral acids, weak organic acids or chelating agents (10, 11, 16, 17). The common strong mineral acids are hydrochloric acid (5-10% in distilled water) and nitric acid (5% in distilled water). A "classic" variant for this type of decalcifying solution is von Ebner's fluid, based on hydrochloric acid, saturated sodium chloride and distilled water (9, 13). All these reagents ensure a rapid decalcification, but the reaction time must be carefully monitored because the staining will be altered when the end-point is exceeded (10, 17). The class of weak organic acids class includes formic acid (10% in distilled water), trichloroacetic acid and picric acid (17). The third method relies on chelating agents that ensure the elimination of calcium ions from the hydroxyapatite crystals and, subsequently, diminishing their size. The main reagent is the ethylenediaminetetraacetic acid (EDTA). The major inconvenience of this method, the long processing time, is counteracted by significant advantages which derives from the fact that hard tissues preserve their organic structure (10, 17). For that reason, the usage of chelating agents is highly recommended for morphological and molecular studies (10, 17).

Table I summarizes some examples for old decalcification methods (9, 12-15).

The decalcified tissue is subsequently impregnated with a suitable substance such as paraffin or celloidin. The impregnation process is named embedding (11-16).

TABLE I

Historical aspects in decalcification

DECALCIFYING SOLUTION	WORKING TIMES, FEATURES
Schulz, 1922: saturated solution of chlorine dioxide (yellow gas, toxic) in acetic acid with trade name "Diafanol"	- washing - immersion in ethyl alcohol 70° - immersion in solution - dehydration - paraffin/celloidin embedding
Drahn, 1926: extemporaneous preparation, consisting of 10 parts saturated aqueous solution of chlorine dioxide (made in appropriately equipped chemistry laboratories), and one part concentrated sulfuric or nitric acid	- technique made in specially designed niches, or in sealed containers - immersion in solution - dehydration - paraffin/celloidin embedding
Gooding and Stewart 1932: solution composed of formic acid 5-25 mL, 40% formaldehyde 5 mL, distilled water 75 mL).	- immersion in solution - dehydration - paraffin/celloidin embedding
Murray, 1937: solution 1, consisting of equal parts absolute ethyl alcohol, chloroform and acetic acid; solution 2, consisting of equal parts of chloral hydrate and phenol, mixture melted at 60°C and then kept fluid	- immersion in solution 1 for 12 hrs. - immersion in solution 2 up to 1-6 days - dehydration - paraffin/celloidin embedding
Greep, 1948: 5% formic acid pure or in combination with 10% formalin, OR 2% formic acid and 20% sodium citrate in equal parts	- immersion in solution for 2-10 days - wash in tap water for 24 hrs. - dehydration - paraffin/celloidin embedding
Evans and Krajian, 1930: formic acid 25 mL, sodium citrate 10 g, distilled water 75mL	- immersion in formic acid buffered with citrate - dehydration - paraffin/celloidin embedding
Kristensen, 1948: formic acid 18 mL, sodium formate 3.5 g, distilled water 82 mL	- immersion in formic acid buffered with sodium formate - dehydration - paraffin/celloidin embedding
Clayden, 1952: 5% fuming nitric acid solution (7.5 cc fuming nitric acid (d 1.40) and distilled water up to 100 cc)	- immersion in fuming nitric acid 5% - immersion in 0.1% urea aqueous solution, with stabiliz ing role, or 0.1% formalin, to prevent collagen swelling - immersion in 5% sodium sulphate for 24 hrs. - wash in tap water for 24 hrs. - dehydration - paraffin/celloidin embedding
Freiman, 1954: EDTA in phosphate buffer with pH 7.4, associated with 10% formalin	- immersion in solution for 4-5 days - rinse in tap water or ethyl alcohol 70° - dehydration - paraffin/celloidin embedding
Lorch, 1964: solution: 14.7 g crystallized citric acid; 0.2 N 700 mL sodium chloride solution, 0.1 N 300 mL hydrochloric acid solution; 1% zinc sulphate solution 2 mL; chloroform 0.1 mL	- fixation in ethyl alcohol or cold acetone (0-4°C)for 24 hrs. - decalcifying in the Lorch mixture for 3-10 days - wash in tap water for 24 hrs. * indicated for histochemical methods
Lillie, 1965: 5% sulfuric acid solution	- does not require further treatment with sodium sulphate, but only a flush with running water for 24 hrs - dehydration - paraffin/celloidin embedding
Lillie, 1965: 15% ammonium citrate, pH 7-8	- the fluid is changed several times, for 6 days - dehydration - paraffin/celloidin embedding * indicated for histochemical methods

Both paraffin and celloidin impregnation follow the same standard stages: dehydration, sample immersion in specific solvent, sample immersion in paraffin/celloidin at higher concentrations, block casting and solidification (11-16).

The common procedure of dehydration, by using alcohols in ascending concentrations, is not suitable because paraffin and celloidin are not soluble in alcohol. Therefore, other solvents were advised based on their properties to dilute the embedding medium, namely xylol or oil of cedarwood for paraffin, and mixture of equal parts of ether and alcohol, or equal parts of absolute ethylic alcohol for celloidin (11-16). The length of time which is necessary for solvents to penetrate the specimens may range between 12-24 hours, depending upon size and permeability of the tissue (enamel, dentin, cementum, alveolar bone) (11-16).

The usage of paraffin for the hard tissue had several disadvantages, the more important being the fragility of the decalcified specimens due to the heating imposed by the method. Therefore, celloidin embedding has been strongly recommended because it produces less shrinkage, despite the prolonged working time (11-16).

Celloidin for histological use is a solid or soluble mixture of mono- and dinitrocellulosis. The solid form looks like flexible translucent plates, dry or imbued in alcohol.

The soluble form has a variable concentration (2-4-8%), is viscous like honey and is delivered already dissolved, resulting in a consistent elastic mass (11-16). Celloidin is highly inflammable, so all handling stages should take place in well ventilated areas, avoiding any contact with fire sources (11-16).

Data presented in Table II are interesting, as they reveal different olden versions of celloidin impregnation.

TABLE II

Historical aspects in celloidin embedding

METHODS	FEATURES
Apathy Method, 1912 (18)	
- dehydration in absolute ethyl alcohol (3 baths for 4 hrs.) - immersion in the mixture of absolute ethyl alcohol and anhydrous ethyl ether, in equal parts (4 baths for 6-12 hrs.) - immersion in 2-4% celloidin solution (2-4 days) OR 8% celloidin solution (1-2 weeks) - casting and hardening of the celloidin block	- for dehydration, small crystallizers or weighing capsules are used, both provided with a hermetic lid; they must be kept in an anhydrous atmosphere, in a sulfuric acid dryer or under a glass bell under which an anhydrous calcium chloride has been placed in a vessel - the volume of the 2-4% celloidin solution should exceed the specimens by 0.5-1 cm - for casting the blocks baths of 8% celloidin solution are used; it is necessary that, by a slow evaporation, the volume of the baths is reduced by 50%, the concentration of the celloidin solution thus reaching 16% - the definitive completion of the block requires 70° ethyl alcohol which is poured into a thin layer in the inclusion vessels of the crystallizer and covered with a tight lid for several hours for the appearance on the surface of the inclusion baths of a well-solidified celloidin crust, 70° ethyl alcohol is further added to the celloidin in the baths, which by the loss of absolute alcohol will solidify into a compact block in 24 hours, which will be extracted from the container, using a scalpel * The celloidin hardening can be done, under the same conditions, with chloroform or with a mixture of 70° ethyl alcohol and glycerin in a ratio of 2:1 - the blocks of celloidin are preserved in 70° ethyl alcohol

METHODS	FEATURES
Seki Method, 1937 (19)	
- dehydration with absolute methyl alcohol - immersion in celloidin solution with increasing concentrations: 2% (for 24 hrs.), 4% (for 2 days), 8% (for 4 days) - casting of the blocks: treating the celluloid bath with a squirt of vapor water until the solid crust is formed, then adding a solution of 70° ethyl alcohol ten parts and three parts chloroform which will be change 1-2 times.	- the use of methyl alcohol ensures a better penetration of the parts and consequently the shortening of the working time - the blocks solidify in 1-6 days, and are stored in 70° ethyl alcohol - sections smaller than 15 microns are difficult to obtain
Wolf Method, 1940 (20)	
- rinse in tap water - immersion in three regular glycerin baths (24 hrs.) - immersion in three baths of anhydrous glycerin (specific weight 1.26) (6-12 hrs.) - immersion in celloidin solution with 8% concentration - casting of the blocks	- it is recommended to use tall containers to ensure the su pension of the pieces at the top of the solution. - the duration of inclusion by this method is shorter.

The last decades of the 20th century brought important changes in the working methods specific to histopathology (21, 22), due to the technical advances in medicine.

The effectiveness of decalcification was improved by several approaches, including ion-exchange resins added to decalcifying solutions (23), electrolytic decalcification (24), sonication applied with chelating agents (25) or microwave processing (26-28). A number of papers showed that, compared to classical methods, microwave ensures a shorter decalcification time; despite this fact, the heat can damage the tissue morphology and can lead to defects in tissue staining (22, 27, 28).

Nevertheless, hard tissue processing – mostly tooth processing is still difficult, and the technologies available today for tooth and supportive tissue processing are extremely expensive – which is why the number of specialized laboratories for this approach is limited.

Reviewing the evolution of the histopathological working methods in hard dental tissues, we consider the non-decalcifying technique to be of interest. Three examples for these techniques are relevant. The first example refers to the Exakt system (Exakt, Hamburg-Norderstedt, Germany) designed and developed in the 80' (29, 30) allows the histological study of jaws with teeth and/or implants of metallic or ceramic materials (2-5) and, consequently, permits the analysis of the tooth and supporting hard tissue. According to the working protocol applied by the Hard Tissue Research Laboratory at the University of Minnesota School of Dentistry (31), the specimens are fixed in buffered neutral 10% formalin (the fixator volume being 10 times higher than the sample volume) minimum 24-48 hours. Afterwards, the tissue fragments are dehydrated, then impregnated and embedded in a type of synthetic resin, which is subsequently cut with special devices, capable of penetrating hard tissues (i.e. Exact band-cutting system based on a diamond or carborundum-coated cutting band). Vestibular-oral and mesio-distal sections, or cross-sections, can be performed. However, the section thicknesses are much larger (35-50 µm) compared to those obtained by de-

calcification processes (3-5 μm). The dehydration and impregnation process takes about one month. The specimens are colored with different stains (including fluorochrome labelling and fluorescence examination), which can differentiate between different degrees of bone maturity and between vital, non-vital, calcified and non-calcified elements. Computer-assisted image analysis is frequently used, leading to the accumulation of histomorphometry information, which can objectively assess the percentage of vital bone, non-vital graft bone, or non-bone materials (for example, various types of hydroxyapatite). Fluorochrome-labelled bone fluorescence microphotography is also common. In the analysis of the implant-tissue interface, the evaluation of the osseointegration of the bone with the metallic surface is possible. The most difficult is to collect the samples ensuring the presence of an area of alveolar bone, without damaging the maxillary structure. The second example refers to cryomicrotomy, technique based on the immersion of non-decalcified tooth in liquid nitrogen and subsequent sectioning with a cryostat using a heavy-duty freezing microtome (32, 33). By cryomicrotomy, the cellular and extracellular structures of hard tissues are well conserved and can be analyzed in light and electron microscopy, and also investigated by using immunohistochemistry. The third example consists in a petrographic technique, firstly applied in 1974 (34), for the histological processing of teeth (35). This method used dried teeth embedded in a resin (Epo-Tech 301 resin). The teeth were grounded down to half using 600 grit carborundum, thinned using a diamond bladed rock saw, grounded down to 40 μm thickness using 600 grit carborundum and then polished using 3 grades of diamond paste. Finally, the tooth specimen of 30 μm thick were analyzed in fluorescence microscopy in order to assess the tetracycline presence, as indicator for the tooth growth (35). Despite the complexity of the procedure, the obtained results sustain the method's potential to bring new data regarding the tooth hard tissues.

FINAL REMARKS

The histopathological methods of tooth and supporting tissue processing are difficult. The decalcification procedure can be replaced by non-decalcifying techniques. Technical problems are amplified by the fact that teeth and periodontal structures are extremely difficult to sample in humans under normal conditions.

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HEMOLYSIS - AT THE BORDER OF THE CONTROVERSY OR MALPRACTICE

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HEMOLYSIS - AT THE BORDER OF THE CONTROVERSY OR MALPRACTICE (Abstract)**:** Clinical laboratory medicine provides nowadays significant level of reliability of adequate measurement and testing, as well as a quality assurance in hemostasis assays through modern analyzers. However, many non-conforming samples are still recorded and reported due to events which are not always under the control of the laboratories. Numerous studies indicate that most sources of error in hemostasis laboratories occur with the blood collection procedure for prothrombin time (PT) and activated partial thromboplastin time (APTT) determination. The purpose of this study is to provide a better understanding upon the potential interference between hemolysis and in vitro coagulation mechanism. **Materials**: In order to create a comprehensive approach to the effect of hemolysis on hemostasis determinations, we performed a search in the main databases which included original studies reviewed by specialists in this field. **Results:** For the clinical laboratory, hemolysis is the most frequent error of the samples, accounting for 54-65% of all non-compliant samples. We have referred to studies in which hemolysis was mechanically provoked, studies that were based only on macroscopic identification, and last but not least, on studies in which plasma hemoglobin concentration was determined. **Conclusions:** This study could represent the premise for revising the policy of refusing the hemolyzed samples as a result of the controversial outcomes that are not considered clinically relevant. **Keywords**: hemolyzed plasma, rejection, prothrombin time, activated partial thromboplastin time.

The evaluation of hemostatic system function in clinical laboratories is based on screening tests: prothrombin time (PT) measuring the extrinsic pathway and activated partial thromboplastin time (APTT) that corresponds to the intrinsic pathway (1). In 2000, Carraro and colleagues pointed out that out of the total number of nonconformities, 60% of the rejected specimens were hemolyzed (2). Hence, concerning transmittance of the results it is suggested that clinicians should be aware about this, in order to exclude an *in vivo* hemolysis (2). In 2006, US pathologists recommended additional investigations for samples with hemolyzed plasma, for identifying possible *in vivo* hemolysis (3).In the same year, Lippi and colleagues concluded that in the case of *in vivo* hemolysis, *in vitro* hemolysis is difficile to be avoided (4).

During *in vivo* hemolysis, the process of red blood cells destruction triggers the release of hemoglobin (5-7), the process being either physiological or pathological, whenever is exceeding certain levels. The etiology of hemolytic syndromes is various

and represents a common cause of anemia syndromes (5-7). Clinically it manifests by icteric skin, while laboratory tests point toward evidence of anemia of various degrees, namely normochromic, normocytic, hyperbilirubinemia with predominance of the indirect bilirubin, increased LDH (lactate dehydrogenase) activity, reticulocytosis, and decrease of haptoglobin (5-7).

Congenital and acquired hemolytic anemias can be associated with thrombotic phenomena. Several studies revealed thus that particular autoimmune hemolytic anemia with warm antibodies are associated with thrombotic manifestations, with 15-33% of these patients experiencing venous thromboembolic events (8). Hence, there is a need for tests to investigate hemostasis which can accurately acknowledge this thromboembolic risk, in order to help the clinician in establishing appropriate treatment. One of the general conditions to be considered in a laboratory performing coagulation tests is related to the quality of the collected biological product. The plasma should not be hemolyzed or with fibrin, and the sample should not contain clots (9-11).

Hemolysis is one of the error sources in the pre-analytical phase, and could interfere with the manner of tests assessment in the analytical phase (12-14). Coagulation tests determination in hemolyzed plasma samples generates the activation of platelets (14) and *in vitro* coagulation (15), hence preventing clot detection through optical techniques, as a response to the reaction(1, 13, 16). The activation mechanism is not clearly understood, so that speculations are made based on the membrane phospholipids release as a result of erythrocyte lysis, which in turn could generate a shortening of the coagulation time (17). Because of these variables, the results of the coagulation tests determinations will reflect the accuracy of the pooled sample, and will not precisely reflect the clinical condition of the investigated patient (13). The consequences may sometimes be particularly serious for the patient (9) and equally for the health system in general (18), as these determinations are frequently considered as “diagnostic” (13). The incidence of hemolyzed plasma samples is a common phenomenon in clinical laboratories and is a reason for their rejection (9, 19, 20) due to the interference with the determinations. It is difficult to decide which of the recommendations is more effective as most studies argue based only on the results obtained on small group of patients, a larger study being therefore recommended (1).

The aim of our study is to shed some light on the effect of hemolysis upon the *in vitro* coagulation mechanism. The main objective is thus identification of the recommendations resulting from the literature based on the paraclinical studies, in order to adopt the appropriate conduct in relation to performing hemostasis determinations, from the hemolyzed samples.

MATERIALS AND METHODS

The progress of the current literature review was performed using the PubMed, Google Scholar and Cochrane database records, based on the following keywords: pre-analytical, post-analytical, hemostasis, hemolysis, incidence, hemoglobin, *in vivo*, *in vitro*, results, shortened, PT, APTT, rejected, controversy, investigations, malpractice, comments, information, diagram, visual, clinician, clot. In order to understand the *in vitro* mechanism, we scanned for more recent, peer-reviewed original studies describing the effect of the *in vitro* hemolysis on PT coagulation and APTT coagulation determinations. Study protocols, reported cases as well as opinion polls have been considered.

It was not necessary to obtain informed consent, as the research was based on the cases and protocols presented by colleagues in the literature, which describe the incidence and effect of hemolysis in the context of coagulation determinations.

RESULTS

This review has some limitations given the small number of papers that were referred. Despite this fact, the selected articles can be considered reliable as they describe the samples and the methods used according to the scientific criteria. Being accepted for publication, the selected studies shed some light upon the understanding of the effect of *in vitro* hemolysis on the determination of PT and APTT coagulation tests. After the initial review, the data were abstracted using a table and have summarized: the interpretation of hemolysis, as well as the mechanism of induction of *in vitro* hemolysis used by some authors, as described in the first table.

TABLE I

Relevant studies included

Authors	Year	Title
Hernaningsih Y, Akualing JS. (1)	2017	The effects of hemolysis on plasma prothrombin time and activated partial thromboplastin time tests using photo-optical method.
Arora S, Kolte S, Dhupia J. (9)	2014	Hemolyzed Samples Should be Processed for Coagulation Studies: The Study of Hemolysis Effects on Coagulation Parameters.
Laga AC, Cheves TA, Sweeney JD. (17)	2006	The effect of specimen hemolysis on coagulation test results.
Woolley A, Golmard JL, Kitchen S. (20)	2016	Effects of haemolysis, icterus and lipaemia on coagulation tests as performed on Stago STA-Compact-Max analyser.
Wan Azman WA, Omar J, Koon TS, Ismail TST (41)	2019	Hemolyzed Specimens: Major Challenge for Identifying and Rejecting Specimens in Clinical Laboratories.

On the other hand, relevant studies which present the hemolysis color chart, as well as the interpretation of the hemolysis degree depending on the hemoglobin concentration in the supernatant have been identified (tab. II).

TABLE II

Policies and recommendations regarding the interpretation of hemolysis

Authors	Year	Title	Interpretation
Binzari E, Zaharia M, Barbu S, Oprea OR, Dobreanu M. (30)	2019	Hemolysis has no influence on routine coagulation tests in subjects without anticoagulant therapy - a referral Romanian emergency hospital laboratory experience	- hemoglobin concentration
Woolley A, Golmard JL, Kitchen S. (20)	2016	Effects of hemolysis, icterus and lipemia on coagulation tests as performed on Stago STA-Compact-Max analyzer	- hemoglobin concentration
Arora S, Kolte S, Dhupia J. (9)	2014	Hemolyzed Samples Should be Processed for Coagulation Studies: The Study of Hemolysis Effects on Coagulation Parameters	- color chart

Authors	Year	Title	Interpretation
Lippi G, Plebani M, Favaloro EJ. (29)	2013	Interference in Coagulation Testing: Focus on Spurious Hemolysis, Icterus, and Lipemia	- hemoglobin concentration
Plumhoff EA, Masoner D, Dale JD. (28)	2013	Preanalytical laboratory errors: Identification and prevention	- color chart - hemoglobin concentration
Zhao J, Kan Q, Wen J, Li Y, Sheng Y, Yang L *et al.* (27)	2012	Hemolysis of Blood Samples has no Significant Impact on the Results of Pharmacokinetic	- color chart - hemoglobin concentration

DISCUSSION

The incidence of hemolyzed plasma is common in medical laboratories performing coagulation tests, being a reason for refusing the determinations based on these samples (1, 13, 16). During our research, we found studies in which the authors triggered the *in vitro* hemolysis by aspirating 1 ml of blood from the primary sample, followed by strong expulsion in the tube through a 23 G needle (9). The experiment was repeated by Hernaningsih and Akualing who used 3ml of blood aspirated in a syringe, creating thus hemolysis by expulsion. This operation was performed twice or thrice (1). The results obtained from non-hemolyzed samples compared to those acquired after induction of the *in vitro* hemolysis from the same sample (9) were included as reference data. We also referred to studies involving macroscopically identified hemolysis specimens (17), as well as those in which the *in vitro* hemoglobin concentration has been measured (2). In their study, Woolley *et al.* obtained hemolyzed plasma by adding cells that were mechanically hemolyzed (20).

Carraro *et al.* highlighted the importance of clinicians' awareness upon such aspects (2). Their results recorded the following: 5 out of 16 cases confirmed by *in vivo* hemolysis would not have been suspected by clinicians, in the absence of the essential laboratory contribution which flagged these critical situations, contributing thus to a significant improvement of the medical decisions (2). Moreover, by performing an observational study to identify the possible shortened PT tests as a result of the interference of the *in vitro* tissue factor (FIII) with the determination, during 4 months we recorded 544 tests with results below the minimum normal range (10"-14") [21]. Furthermore, upon repeating the PT determination from the same sample, 200 tests were corrected, falling within the normal range (21). Of the remaining 344 shortened tests, 27 tests (21) were correlated with the elevated values of bilirubin (22), suggesting thus a potential *in vivo* hemolysis, in agreement with Carraro's and collaborators research (2). In their study, Bonini *et al.* stated that more than 60% of all laboratory errors come from the pre-analytical phase (23). Guder mentioned that the diagnosis accuracy is given by the performance of the analyses in the field of medical laboratories (24), recalling the 60's and 70's when the quality assurance of the analytical procedures was introduced, as well as the extra-analytical variables which influence the precision of the laboratory results (25). In order to avoid hemolysis caused by environmental factors during transport, starting with the 70'sthe glass tubes for biological sampling have been replaced with capped specimens (24). Although the measurement of plasma hemoglobin concentration is practiced in some laboratories, it is often macroscopically

assessed by laboratory specialists (9,17), by coloring the plasma or serum from pink to red (26).

In some studies, a color chart was used to assess plasma hemolysis such as that provided by Arora *et al.*, Plumhoff *et al.*, and Zhao *et al.* (9, 27, 28). The numbers indicate the extent of hemolysis expressed in mg/dl (20, 28-30). Hemolysis is visible at a serum hemoglobin concentration of 0,2-0,3 g/l (17, 20, 31). Laga and colleagues measured the value of the supernatant hemoglobin in the specimens included in the study, reporting hemolysis at values above 30 mg/dl, assuming there was no hemolysis at ranges such as 20 mg/dl (17). The interval between 20-30 mg/dl was left to the appreciation of the person interpreting the hemolysis (17). The results obtained by Hernaningsih and Akualing (1) were consistent with the studies performed by Laga (17) and Arora *et al.* (9), clarifying the clinically insignificant influence of the *in vitro* hemolysis on the PT test if the patient's results are within the normal reference range (1). They claimed that one cannot refer to these conclusions for patients with lower or upper limit of this range, as they could have clinically significant implications (1). In the case of the APTT test, in order to validate quantitatively the hemolysis interference, the authors referred to the existing research in which differences that could not be explained were found between the results obtained from hemolyzed and non-hemolyzed plasma samples (1).

In the research performed by Hernaningsih and Akualing in subjects with initial test results within the normal reference range, a shortening of the APTT test from the hemolyzed plasma samples have been recorded (1). The results agreed with the study by Lippi *et al.* (4). In 2006, Laga *et al.* correlated the prolongation of APTT in healthy volunteers, and shortened APTT in study group patients with low levels of FVIIa (proconvertin) (17). As the mechanism of shortening the test in the hemolyzed samples was not constant, it was correlated with the possible release of intracellular substances from leukocytes, of phospholipids from erythrocytes or with platelet-induced activation of coagulation (16,32). Among the literature data, we identified a study by Favaloro *et al.* that describes the decrease of PT and fibrinogen level following the activation of the coagulation cascade (13). The APTT prolongation or shortening test is influenced by the fibrinogen loss or fibrinogen activation (13). Two years later, Arora *et al.* agreed with Laga's study and used photo-optical instruments to determine APTT from hemolysed samples for healthy volunteers, recording prolongation of the test (9,17). Hernaningsih and Akualing did not investigate the effect of hemolysis on PT and APTT prolongation in patients that were administered oral anticoagulant therapy (1), although previous studies suggested that the PT test in hemolyzed samples could be determined through electromechanical or optical instruments (17,33) From the clinician's point of view, the accuracy of the coagulation tests results is of critical importance, the promptness of the diagnosis and the appropriate treatment depending on it.

Thus, if the hematologist picks up the results of the APTT coagulation tests in such cases, without any awareness from the laboratory, he is entitled to regard the results as to be real and continues the investigations in order to provide a proper diagnosis. For a prolonged APTT, the following will be considered: the presence of a LA (*Lupus Anticoagulant*) or a genetic or acquired coagulation factor deficiency. If the

latter is advised, then the investigations will assume in the first stage the plasma composite test, followed by evaluation of the activity of coagulation factors and inhibitors that modulate APTT: factor VIII (anti-hemophilic A), factor IX (anti-hemophilic B), antibodies anti-factor VIII, and anti-factor IX. If APTT is shortened, the investigations will continue to identify the pathological conditions associated with an increased thrombotic risk, knowing that a shortened APTT reveals an increased thromboembolic risk (34). In both situations, we are discussing about complex investigations with a considerable financial impact on the hospital budget, which adds to the patient's discomfort facing the unnecessary investigations.

The laboratory should inform the clinician upon the possibility of coagulation investigation tests interference with hemolysis, because the orientation may be different: it may draw the attention of the physician to complete the investigations for a proper diagnosis of hemolytic anemia (reticulocytes, total and direct bilirubin, lactate dehydrogenase, haptoglobin, peripheral blood smear), especially in case of a mild anemic syndrome without clinically significant changes. Due to the fact that studies have pointed that hemolytic anemia is associated with increased thromboembolic risk, in case of the presence of hemolysis secondary to an *in vivo* hemolysis and shortened APTT, the therapeutic response will be marked by anticoagulant therapy to prevent the occurrence of thrombotic incidents (35). In this situation cut down APTT is intended to alert the physician regarding this risk. In order to study the effect of hemolysis on PT and APTT tests, cell lysis was mechanically obtained, this being similar to factors inducing *in vitro* hemolysis (1, 9, 36). The prolongation or shortening of PT and APTT tests also depends on tissue factor release due to mechanical cell lysis (1).

Starting with 2015, we also pay special attention to the incidence of hemolyzed plasma samples (37), intending an approach that could turn this in patient's favor. Although previous studies recommend rejecting these samples, in our laboratory, we determine PT and APTT tests from hemolyzed samples and the results are accompanied by a textual explanation, being in agreement with Carraro and collaborators, attracting the attention of hemolysis possible interference: ***"Sample with hemolyzed plasma, possible interference with the determination"***. Favaloro *et al.* recommend for these samples verification by different methods, to exclude the accidental presence of the clot in the erythrocyte sediment (13). Starting with 2014, we implemented a re-verification and post-analytical procedure based on transvasation, which allows us to highlight the clot (38).

In the case of an intensely hemolyzed plasma, we inform the clinician about this aspect, recommending resampling and textually explaining the non-compliance: "Repeat the sampling. Highly hemolyzed plasma sample". This behavior is in accordance with the recommendations in the national specialized literature, where it is mentioned that the rejection of a hemolyzed plasma sample without obligatory information from the clinician can be a proof of malpractice (39,40). As long as the result is not accompanied by information that assists the clinician in the correct interpretation of the results, it is quite a challenge for the medical practitioner to issue proper decisions (40). In a very recent study, differentiating *in vitro* from *in vivo* hemolysis is even imposed for laboratory

specialists, motivated by the fact that the sample is admitted in the laboratory without seeing the patient (41). Referring to previous studies, Wan Azman *et al.* consider the rejection of these samples to be inappropriate, and in order to perform proper differentiation they recommend blood smear examination (41), also supporting the addition of a brief comment suggestive for the reluctance in the interpretation of the results (41-43). As a necessity, the authors pay attention to the need for a laboratory guide to focus on standardizing how to interpret hemolyzed samples, so that essential information to be provided to the clinicians for further proper management of the patients (41). Wan Azman *et al.* urge upon the bidirectional communication between laboratory specialists and clinicians, as being of paramount importance in seeking solutions based on each patient condition (41).

To reduce pre-analytical errors, the development of quality indicators (QI) can serve as tool for pre-analytical activities control and monitoring (44). In agreement with Magnette's study, by educating the medical personnel involved in the biological product sampling process, we can help reduce, understand and raise awareness of the effects of pre-analytical variables on the coherence and reliability of coagulation screening tests (45,46).

CONCLUSIONS

Considering all the mentioned aspects, a link between the medical laboratory and clinicians should be issued, in order to exclude any *in vivo* hemolysis that could be disregarded as a result of the rejection of samples with hemolyzed plasma. Performing routine screening tests from specimens with a certain degree of hemolysis is accepted, and clinicians may receive reports about the outcomes that fall within the normal reference range. For patients which follow anticoagulant therapy, the results should be reported with caution, and the possible interference of hemolysis with the determination need to be explained. The policy of rejecting hemolyzed specimens should be reconsidered, as duplicated sampling creates patient's discomfort, delayed decision making in critical situations, and last but not least, can trigger additional costs for the hospitals' budget and the health sector in general.

Further research on a large scale samples would be requisite, further testing in order to study the coagulation factors which fall under the influence of *in vitro* hemolysis that is directly reflected in the quantification of PT and APTT tests, being recommended.

ABBREVIATIONS

PT - prothrombin time

APTT - activated partial thromboplastin time

LDH - lactate dehydrogenase

FIII - tissue factor

FVIIa - proconvertine

LA - Lupus Anticoagulant

factor VIII - anti-hemophilic A

factor IX - anti-hemophilic B

QI - quality indicators

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AUTHORS' CONTRIBUTIONS

All authors made equal contribution to the paper, to that of the first author.

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CANCERUL MAMAR LA BĂRBAT

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BREAST CANCER IN MALE (Abstract) : The aim of this study is to point out the late diagnosis and initiation of treatment in male with breast cancer. At the same time, to show the importance of the correlation between different markers in assessing the prognostic, as well as the treatment for the patient. **Material and methods** : Retrospective study on a group of 15 males with breast cancer, out of 1043 patients with the disease, in a period of 10 years. Eight patients were stage III of disease, 2 were stage II, one was stage I, and in other 3 cases the evaluation of the tumor and of the axillary lymph nodes was performed only by echography, considered stage II. **Results** : All patients underwent radically modified Madden mastectomy ; 4 patients needed a partial resection of the great pectoralis muscle. Adjuvant chemotherapy was performed in 9 patients, and neoadjuvant chemotherapy in 2 cases. Three patients refused the chemotherapy, and one patient chose an alternative paramedical treatment. The treatment with Tamoxifen was done in 11 patients with high values of Progesterone and Estrogen Receptors (PR, ER). At the date of our study, 8 patients were alive, without clinical signs of disease (free of disease), while in 3 patients, alive, clinical signs of disease were detected (recurrence). Survival rate couldn't be evaluated in 4 patients. **Conclusions** : Breast cancer in male is usually discovered in locally advanced stages, although most of the patients are regularly screened for chronic hepatitis. Use of biological markers allows a more accurate evaluation of the aggressiveness of the tumor, as well as a more specific treatment for each patient. Modified radical mastectomy type Madden remains the preferred surgical approach. Surgical approach should be considered even in locally advanced cases, as well as in elder patients. **Key words** : BREAST CANCER IN MALE, BIOLOGICAL MARKERS, MADDEN MASTECTOMY

THE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN A HOSPITAL FROM NORTH-EAST OF ROMANIA

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THE PREVALENCE OF *HELICOBACTER PYLORI* INFECTION IN A HOSPITAL FROM NORTH-EAST OF ROMANIA (Abstract). This study aims to analyze the prevalence of *Helicobacter pylori* infection in a hospital from North-East of Romania. **Material and methods**: *H. pylori* infection was investigated in 2,048 patients at Emergency County Hospital Bacău. *H. pylori* infection was diagnosed by fecal antigen test. The patients were analyzed based on demographic criteria, and by presenting pathology. **Results**: Out of 2,048 patients tested in one year, 819 patients (39.9%) were positive for *H. pylori*. No statistically significant differences were identified between men and women (40.3% *vs.* 39.8%, p=0.821). Patients from rural areas had a significantly higher prevalence of *H. pylori* infection than urban areas (47.3% *vs.* 36.8%, p=0.00001). A direct correlation was noted between *H. pylori* infection and age. There was a significantly higher average age in patients with *H. pylori* infection (*H. pylori* (+): 51.1±22.3 *vs. H. pylori* (-): 49.1±21.3, p=0.0323). The highest prevalence was in patients aged 31-40 years (54.3%) and the lowest in patients aged 0-6 years (25.8%). Regarding the wards where patients were admitted to, the highest prevalence was on the Internal Diseases ward (50.71%), while on the Gastroenterology ward the prevalence of *H. pylori* infection was not significantly different from other sections with less cases of digestive pathologies (Gastroenterology 41.2%, Endocrinology 42.9%, Nephrology 48.6%). **Conclusion**: The prevalence of *H. pylori* infection in people who presented at the Emergency County Hospital Bacău is lower than the figure estimated for Romania in a recent meta-analysis (2017) performed globally. **Keywords**: H. PYLORI , PREVALENCE, FECAL ANTIGEN TEST.

Helicobacter pylori (*H. pylori*) infection is one of the most widespread infections worldwide, being a real public health issue (1). About 30-80% of world's population is infected with *H. pylori*, the prevalence varying from one country to another depending on economic status. General data shows that prevalence of *H. pylori* infection differs in developing countries (approximately 70%) from developed countries (approximately 34.7%) (1, 2).

These prevalence differences are also influenced by socio-economic status, degree of urbanization, and access to running

water and medical services of those countries. Even across the same country the prevalence of *H. pylori* infection can vary (2, 3). For developing countries, including Romania, prevalence of *H. pylori* infection in middle-aged adults is approximately 80%. Although no national study has been completed, a recent meta-analysis estimates a prevalence of *H pylori* infection in Romania of 65.6%-71.5% (1, 4).

Considering different age groups, the literature (1, 2) shows a lower prevalence in children (less than 20%). A systematic review by Ibrahim et al (4) of the data from 244 studies revealed male gender is more commonly associated with *H. pylori* infection in both children (OR=1.06, 95% Cl: 1.01 - 1.12, I^2 (total heterogeneity / total variability)=43.7%) and adults (OR=1.12, 95% Cl: 1.09 - 1.15, I^2=68.5%).

The present study analyses the prevalence of *H. pylori* infection in the population who presented at Emergency County Hospital Bacău. Thus, the presence of *H. pylori* infection was investigated in 2048 patients tested between 1st June 2018 and 31st May 2019.

The presence of *H. pylori* was tested using fecal antigen test, a non-invasive method with 90% specificity and 88-89% sensitivity (5, 6).

The method can be used both to detect infection and to control its eradication. Factors that may influence its sensitivity are diarrhea (in loose stool, the fecal antigen for *H. pylori* is diluted), use of antibiotics and proton pump inhibitors (5). Also, in patients with upper gastrointestinal bleeding there is a low sensitivity of fecal antigen test for *H. pylori* . Other factors that may influence the diagnosis are temperature and the time interval between harvest and analysis. Stool samples should be stored at low temperatures and tested soon after collection. To prolong antigenicity, stool samples must be stored at -80°C (6).

MATERIALS AND METHODS

This study includes 2,048 patients aged 0-90 years, from both rural and urban areas, who presented at Emergency County Hospital Bacău between 1st June 2018 and 31st May 2019. All patients were tested for *H. pylori* using fecal antigen test. Patients were grouped and analyzed based on demographic criteria (age, gender, environment: urban/rural areas), as well as on the ward where they had been hospitalized.

Statistical analysis

The categorical variables were presented by number and proportions and compared with the *Chi-square* test. Kolmogorov-Smirnov test was used to assess whether the continuous data shows a normal distribution. Continuous variables (normally distributed) were expressed by mean ± standard deviation (SD) and compared using the ANOVA test. The general prevalence of *H. pylori* infection was calculated as follows: (all individuals with a positive *H. pylori* test) / (all individuals who underwent *H. pylori* testing). A stratified analysis of *H. pylori* prevalence was performed by age groups on genders and by background (urban/rural). The Joinpoint regression model was applied to evaluate the correlation between age and prevalence with *H. pylori*. The junction points where prevalence of *H. pylori* assessed for all age groups began to increase or decrease were identified. For this, we constructed the univariable regression of a connection point in which relationships between age and *H. pylori* prevalence were analyzed. The annual percentage change (year of age) (APC) was calculated with the corresponding 95% confidence interval (CI) for each effect estimate.

Finally, a logistic regression analysis (univariate / multivariate) was created to assess the relationship between *H. pylori* infection, gender, and patient background. The odd ratio (OR) was calculated with 95% CI. *p*-values lower than 0.05 were considered statistically significant. The statistical analysis was performed using the trend analysis software *Joinpoint Regression Program version 4.8.0.1*, 2020 (National Institutes of Health, Maryland, United States) and *SPSS version 24.*

Ethical approval

The present study was approved by the Ethical Committee of Emergency County Hospital Bacău. All patients provided informed consent for inclusion in the present study (parents or legal guardians signed the informed consent for children). For all data obtained, personal identifying information was in accordance with the laws of privacy protection in Romania and hospital regulations.

RESULTS

During the analyzed period, out of 2,048 patients tested, 819 were positive for *Helicobacter pylori* (fig. 1).

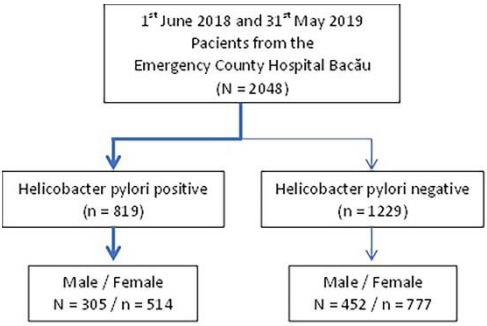

Fig. 1. Flow chart of patients included in the study

The overall prevalence of *H. pylori* infection was 39.9%. The presence *of H. pylori* did not show a significant association (p=0.8217) with gender (male / female: 40.3% / 39.8%). Patients with *H. pylori* infection had a significantly higher mean age (p = 0.03239) (51.1±22.3) compared to those who tested negative (49.1±21.3). The prevalence of *H. pylori* was significantly (p=0.00001) increased in patients from rural areas (47.3% *vs.* 36.8%) (tab. I).

TABLE I

Demographic characteristics of the analyzed patients

	All groups	*Helicobacter pylori*		Statistic test	p-value
		Yes (n=819)	No (n=1229)		
		39.9%	60.01%		
Age (years old) mean ± SD		51.1±22.3	49.1±21.3	4.124 †	0.03239*
Gender (male/female), n (%)	757/1291 (36.96/63.03)	305/514 (40.3/39.8)	452/777 (59.7/60.2)	0.045152 §	0.82172
Environment (U/R), n (%)	1442/626 (69.43/30.57)	523/296 (36.8/47.3)	899/330 (63.2/52.7)	19.82294 §	0.00001*

§ Pearson Chi-square test; † ANOVA test; (*) Marked effects are significant at p<0.05

Analyzing the prevalence of *H. pylori* infection by age groups, it was showed that in children under 18 the prevalence was 38.6% (114/295). Thus, between 0-6 years the prevalence was 25.8% (23/89), between 7-13 years 45.9% (45/98), and between 14-18 years it was 42.6% (46/108). The highest prevalence was noted in the 31-40 age group with 54.3% (89/164) (tab. II, fig. 2).

TABLE II

***H. pylori* infection prevalence based on age and gender**

Age (years)	Total cases (n=2048)			Total cases with *H. pylori* (n=819)		
	All	male	female	*H. pylori* total	male	female
0-6 years	**89**	38	51	**23**	8	15
7-13 years	**98**	56	42	**45**	21	24
14-18 years	**108**	37	71	**46**	15	31
19-30 years	**112**	48	64	**51**	15	36
31-40 years	**164**	67	97	**89**	42	47
41-50 years	**288**	125	163	**121**	52	69
51-60 years	**354**	133	221	**155**	61	94
61-70 years	**508**	164	344	**171**	59	112
71-80 years	**279**	76	203	**99**	26	73
81-90 years	**48**	13	35	**19**	6	13
Totals	**2048**	**757**	**1291**	**819**	**305**	**514**

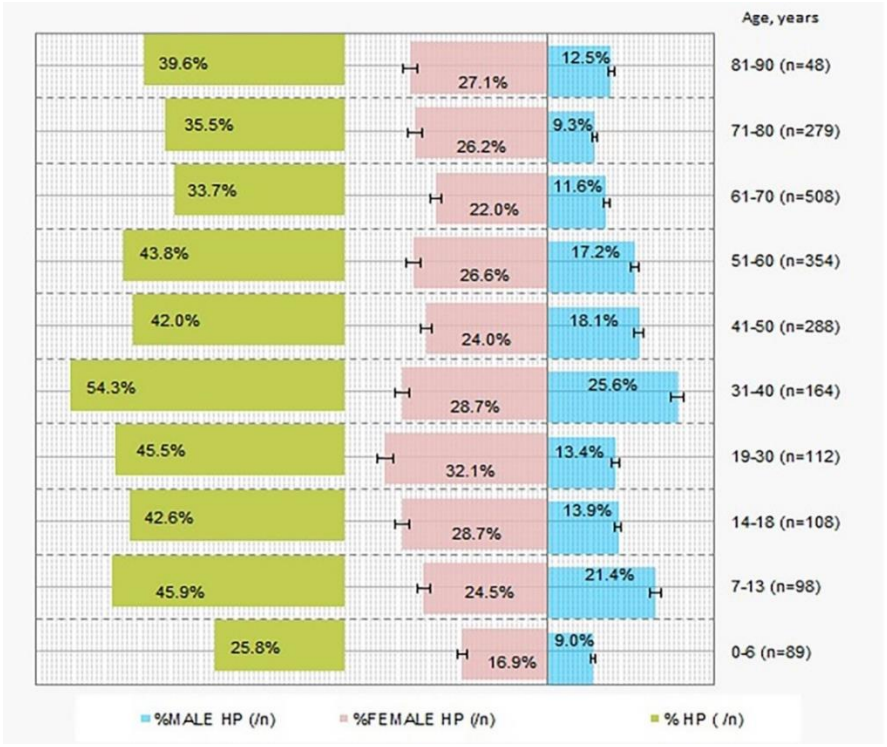

Fig. 2. *H. pylori* infection prevalence according to age and gender

Both univariate and multivariate analysis indicated that age and rural origin are the predictive factors for presence of *H. pylori* infection. Therefore, for age, an OR value of 1.48 was calculated (95%CI: 1.276–2.387, p<0.0001). For patients from rural areas, the risk of *H. pylori* infection was 2.84 times higher compared to that of patients from urban areas (OR=2.849; 95%CI: 2.186 – 3.713, p <0.0001) (tab. III).

TABLE III

Univariate and multivariate logistic regression analysis used for identifying predictive factors for *H. pylori* infection

***H. pylori* infection**	**Univariate logistic regression**				**Multivariate logistic regression**			
	Odd ratio	95% confidence interval		p-value	Odd ratio	95% confidence interval		p-value
		Lower	Upper			Lower	Upper	
Age	1.996	1.292	2.687	0.043*	1.481	1.276	2.387	<0.0001*
Male	1.020	.849	1.225	0.832	1.007	.835	1.214	0.943
Rural	1.542	1.275	1.865	<0.0001*	2.849	2.186	3.713	<0.0001*

(*) Marked effects are significant at p<0.05

Based on *Joinpoint* regression model, a nonlinear association was observed between patients' ages and prevalence of *H. pylori* infection (fig. 3). In patients younger than 7 years (33 cases) there was a significant association between increased age (≤7 years) and high prevalence of *H. pylori* (APC=21.34%; 95%CI: 5.8 – 39.2, p=0.0062), reaching a peak at age 7, with an observed gross rate of 47.1% (modelled gross rate: 48.3%). In patients aged 7-28 years, prevalence of *H. pylori* infection did not change significantly with age but presented a slightly decreasing trend as age progressed (APC= -1.28%; 95%CI: -3.4 – -0.8, p = 0.2528) (tab. IV, fig. 3).

The regression analysis for the next age group, 28-31 years showed an increasing, although insignificant, prevalence trend. This indicated an APC of 12.85% (95%CI: 2.8 - 21.8, p=0.7671) which suggests an insignificant positive linear regression of prevalence in the 28-31 years age group (tab. IV, fig. 3).

TABLE IV

APC - annual percentage change for the regression slopes of patients ages and *H. pylori* infection prevalence

	Age(years)		**APC**	**APC**		**Test Statistic (t)**	**p-value**
	Lower Endpoint	**Upper Endpoint**		**Lower CI**	**Upper CI**		
Slope 1	1	7	**21.34**	5.8	39.2	2.813585	0.006291*
Slope 2	7	28	**-1.28**	-3.4	0.9	-1.152507	0.252873
Slope 3	28	31	**12.85**	2.8	21.8	0.297245	0.767123
Slope 4	31	79	**-1.13**	-1.7	-0.5	-3.672413	0.000455*
Slope 5	79	83	**19.91**	-20.0	79.8	0.892708	0.374947
Slope 6	83	90	**-13.37**	-22.3	-3.5	-2.641793	0.010083*

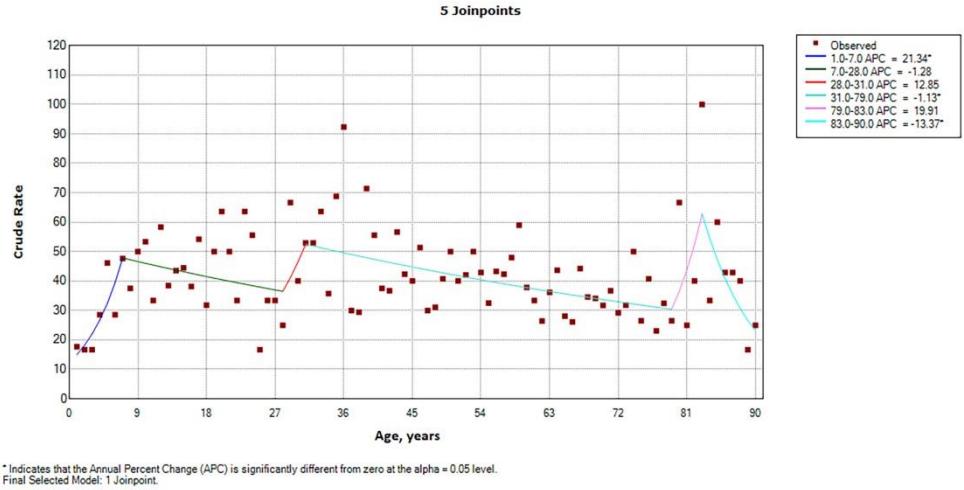

Fig. 3. Correlation between patients' age and *H. pylori* infection prevalence

In the age group 31-79 years, the prevalence of positive *H. pylori* cases showed a significant negative linear regression. Thus, in this age interval, the increase in age brought about a decrease in positive cases (APC= -1.13, 95%CI: -1.7 – -0.5, p=0.0004). The gross rate observed at 79 years was 31.4%, and gross modelled rate was 29.9%. For the 79-81 age group there was a marked increase in prevalence, but it is insignificant (p=0.3749). However, a significant decrease in prevalence with age was observed for the 83-90 years group which registered an increased APC value of -13.37 (95%CI: -22.3 – -3.5, p=0.01). This result clearly indicates a significant decrease in positive cases for the 81-90 age group (Table IV, Fig. 3). Overall, the gross prevalence of *H. pylori* infection did not show significant differences between women and men (p=0.8217). Out of 2,048 patients included in the study, 967 patients were admitted to various wards for different pathologies. The request for *H. pylori* testing was made by gastroenterologists, or by doctors who considered it necessary to test for *H. pylori* infection in patients with extradigestive disorders. For these patients, the prevalence of *H. pylori* infection was 43%. Thus, there is a significant association (p <0.0001) of *H. pylori* infection in patients with nephrological pathology (48.6%), endocrinological pathology (42.9%), diabetes or nutritional diseases (37.9%). Less cases were identified in patients with dermatological or allergic conditions (28.6%).

DISCUSSION

The present study regarding the *H. pylori* infection in patients at Emergency County Hospital Bacău, shows a prevalence of 39.9% of infection. This prevalence is considerably lower than the national estimate from a meta-analysis done in 2017 (65.6-71.5) (1). However, the value falls within the prevalence interval of other Central or East-European countries (1, 7). One explanation would be that testing in our study was done in the general population, without differentiating between previously treated (experienced) and naive pa-

tients. The large number of patients tested (2048) made such a specification difficult. The result obtained (39.9%) may entail that some of these patients had undergone an effective treatment to eradicate *H. pylori* infection, as showed by a negative fecal antigen test.

Considering the different age groups presented, lowest prevalence of *H. pylori* was recorded in children (38.6% in children between 0-18 years), a category presenting varying prevalence values if further subdivided in smaller groups: 0-6 years - 25.8%, 7-13 years - 45.9%, 14-18 years - 42.6%. The overall prevalence value in children comes in contrast with another study, conducted in North-West Romania (8) where prevalence of *H. pylori* infection in those aged 0-18 years was 24.49%. The contrasting results could be due to different methods of testing for *H. pylori* infection. The study from North-West Romania used an invasive method of testing, the gastroscopy with biopsy collection from the gastric antrum, followed by identification of the bacterium by electron microscopy with specific staining. This method has a specificity of 95-100% (9, 10), but sensitivity is usually low. In our study we used the results of fecal antigen testing, which has a specificity of 90% and sensitivity of 88-89% (5). Another explanation for the difference in outcomes could be related to socio-economic differences between the two centers (11, 12). The higher prevalence of *H. pylori* infection in children aged 0-18 years from a center in North-Eastern Romania compared to those in the North-Western side of the country could be correlated with the lower socio-economic level of the first region. Regarding the results in adult population (over 18 years old), our study found a prevalence of *H. pylori* infection of 40.2%. By age groups, the highest prevalence was in people aged 31-40 years (54.3%). This result does not come in accordance with the literature which shows that prevalence of *H. pylori* infection increases with age, being equal to age in people over 60 years (12, 13). One explanation could be that many people over the age of 60 had already been effectively treated for *H. pylori* infection (14, 15).

Comparing the data obtained in our study with an epidemiological study performed between 1st September 2006 and 10th October 2008 (11) which aimed, among others, to identify the prevalence of *H. pylori* infection in patients admitted to a County Hospital, it can be noted that the latter study found a higher prevalence of infection (59.03%), with a maximum 78% in the 41-50 age group (11). These dissimilarities can be the result of the effective treatments performed for *H. pylori* in a large part of the population, especially following the emergence of the Maastricht V Consensus (2). Also, the *Helicopat Epid Study* reported a prevalence of 59.03%, based on the results of the urea breath test, a method that has a sensitivity and specificity of 95-97% (16). Therefore, the differences are not due to testing method for the bacterium.

Based on genders, our study did not identify significant differences (p=0.8217) between *H. pylori* infection in males (40.3%) and females (39.8%), supporting the data in the literature (1).

Regarding the environment, our study found a significantly higher prevalence (p = 0.00001) in rural areas than in urban areas, which further supports the involvement of socio-economic factors in the spread of *H. pylori* infection. Focusing on *the prevalence of infection on different*

hospital wards, there was a significantly higher number of H. pylori infections on the internal diseases ward (50.7%), while the gastroenterology ward recorded a prevalence of 41.21%, similar percentage to other wards without digestive pathologies. An explanation could be that the Department of Gastroenterology, although it has a much larger number of patients tested compared to other medical departments, has an extensive number of patients who had previously undergone effective eradication treatments. Furthermore, majority of cases admitted on the gastroenterology ward are digestive hemorrhages which can interfere with the correct identification of the fecal *H. pylori* antigen.

CONCLUSIONS

The present study conducted at Emergency County Hospital Bacău reveals a downward trend in prevalence of *H. pylori* infection in North-Eastern part of Romania, results which come close to reaching the levels of *H. pylori* prevalence found in more socio-economically advanced countries. Although the study was conducted on a limited population from one regional hospital, the decrease in prevalence of *H. pylori* infection is clearly illustrated. This can possibly be due to previous successful treatments, but also due to an increasing socio-economic level and a better understanding and application of hygiene norms amongst the general population.

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NEWS

UPDATES IN GENETIC BASIS OF LYNCH SYNDROME

Lynch syndrome is a cancer-predisposing syndrome inherited in an autosomal-dominant manner, wherein colon cancer and endometrial cancer develop frequently in the family, it results from a loss-of-function mutation in one of four different genes (*MLH1*, *MSH2*, *MSH6*, and *PMS2*) encoding mismatch repair proteins. Being located immediately upstream of the *MSH2* gene, *EPCAM* abnormalities can affect *MSH2* and cause Lynch syndrome. Mismatch repair proteins are involved in repairing of incorrect pairing (point mutations and deletion/insertion of simple repetitive sequences, so-called microsatellites) that can arise during DNA replication. *MSH2* forms heterodimers with *MSH6* or *MSH3* (*MutSα*, *MutSβ*, respectively) and is involved in mismatch-pair recognition and initiation of repair. *MLH1* forms a complex with *PMS2*, and functions as an endonuclease. If the mismatch repair system is thoroughly working, genome integrity is maintained completely. Lynch syndrome is a state of mismatch repair deficiency due to a monoallelic abnormality of any mismatch repair genes. The phenotype indicating the mismatch repair deficiency can be frequently shown as microsatellite instability in tumors. Children with germline biallelic mismatch repair gene abnormalities were reported to develop conditions such as gastrointestinal polyposis, colorectal cancer, brain cancer, leukemia, etc., demonstrating the need to respond with new concepts in genetic counseling. In promoting cancer genome medicine in a new era, such as by utilizing immune checkpoints, it is important to understand the genetic and genomic molecular background, including the status of mismatch repair deficiency (Tamura K, Kaneda M, Futagawa M, *et al.* Genetic and genomic basis of the mismatch repair system involved in Lynch syndrome *Int J Clin Oncol* 2019; 24(9): 999-1011).

INTRACEREBRAL HEMORRHAGE IN SARS-CoV-2 INFECTION. CASE REPORT

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INTRACEREBRAL HEMORRHAGE IN SARS-CoV-2 INFECTION. CASE REPORT (Abstract): Beyond pulmonary infection, SARS-CoV-2 can cause dysfunction of multiple other organs and system, including neurovascular complications such as intracerebral hemorrhage, about which we have now few information. We report the case of a 36-year young COVID-19 patient with intracerebral hemorrhage. As a particularity of this case, we mention the onset of coronavirus disease through a neurological manifestation, to which was later added the respiratory symptoms characteristic of SARS-CoV-2. In conclusion, we consider it important to be aware that COVID-19 patients may present with these onset findings, without initially pulmonary symptoms. Subsequent studies remain to confirm this presumed causality between intracerebral hemorrhages and COVID-19. **Keywords:** INTRACEREBRAL HEMORRHAGE, COVID-19, SARS-CoV-2 INFECTION.

In December 2019, new cases of unexplained pneumonia appeared in Wuhan (China), which later spread very quickly to other regions of China, then to Europe and also Romania, North America, and Asia. This outbreak has been confirmed as a new coronavirus infection (CoV) (1). The new coronavirus has been reported later to produce symptoms similar to those of severe acute respiratory syndrome CoV (SARS-CoV-2) (2).

Subsequently, the virus was named SARS-CoV-2, and in February 2020, the World Health Organization named the disease: coronavirus disease 2019 (COVID-19) (3). In addition to respiratory symptoms and typical clinical manifestations including fatigue, fever, cough, anorexia, throat and abdominal pain or diarrhea, neurological manifestations have been reported, with an increasing proportion of patients presenting with extrapulmonary

symptoms (3). Moreover, starting with the first months of 2020, some authors reported the increase of cerebrovascular events associated with COVID-19 (3-5), the patterns being occlusion of large vessels, venous thromboembolism, multiple territorial infarcts, raised inflammatory markers, severe systemic inflammation, younger age of presentation and antiphospholipid antibody production (6-9). On the other hand, however, the clinical features of intracerebral hemorrhage (ICH) in COVID-19 patients have been much less described (8, 10-12). The aim of this paper is to provide a clinical and imaging description of a young patient with ICH in association with COVID-19, in which the onset symptoms were extrapulmonary. We will also discuss the possible pathophysiological mechanisms and their association with the patient's neurological pathology.

CASE REPORT

A 36-year-young female without prior medical history, presents for important headache, seizures and left homonymous hemianopsia. In the emergency department, the patient was not febrile, with normal oxygen saturation and nonspecific laboratory workup. Initial head CT scan on admission and further head MR imaging showed a right temporal hematoma without other findings (fig. 1). The SARS CoV-2 PCR test performed on admission was positive.

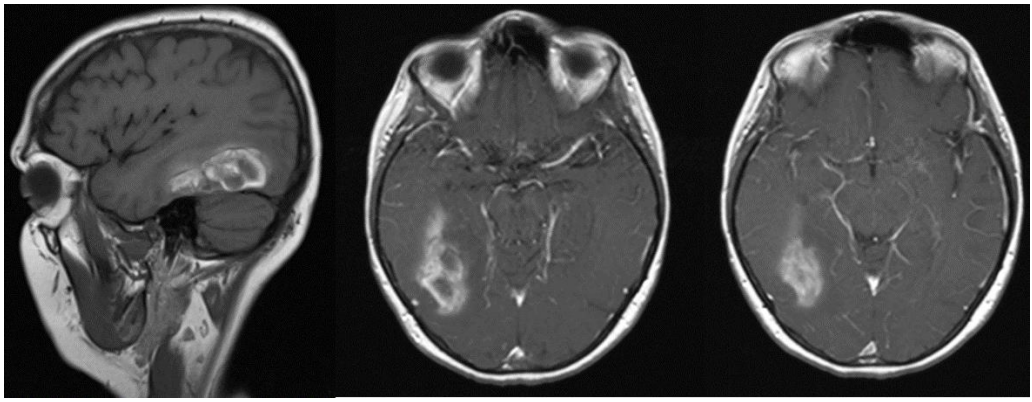

Fig. 1. Sagittal and axial enhanced T1-weighted MR imaging showing hemorrhage in right temporal lobe (*C. Toader's personal collection*)

Subsequent cerebral angiography ruled-out vascular pathology. After 12 days, the patient's neurological condition worsens, and head CT re-examination reveals increase in size of the hematoma. Urgent evacuation was carried out and no unusual brain tissue findings were reported. The postoperative evolution was good, with the appearance on the second day after surgery of dyspnea. The patient was transferred to intensive care for O_2-therapy and further management. Chest radiography and CT showed bilateral opacities consistent with multifocal pneumonia (fig. 2B and fig. 2C). The patient died a few days later, due to pulmonary complications.

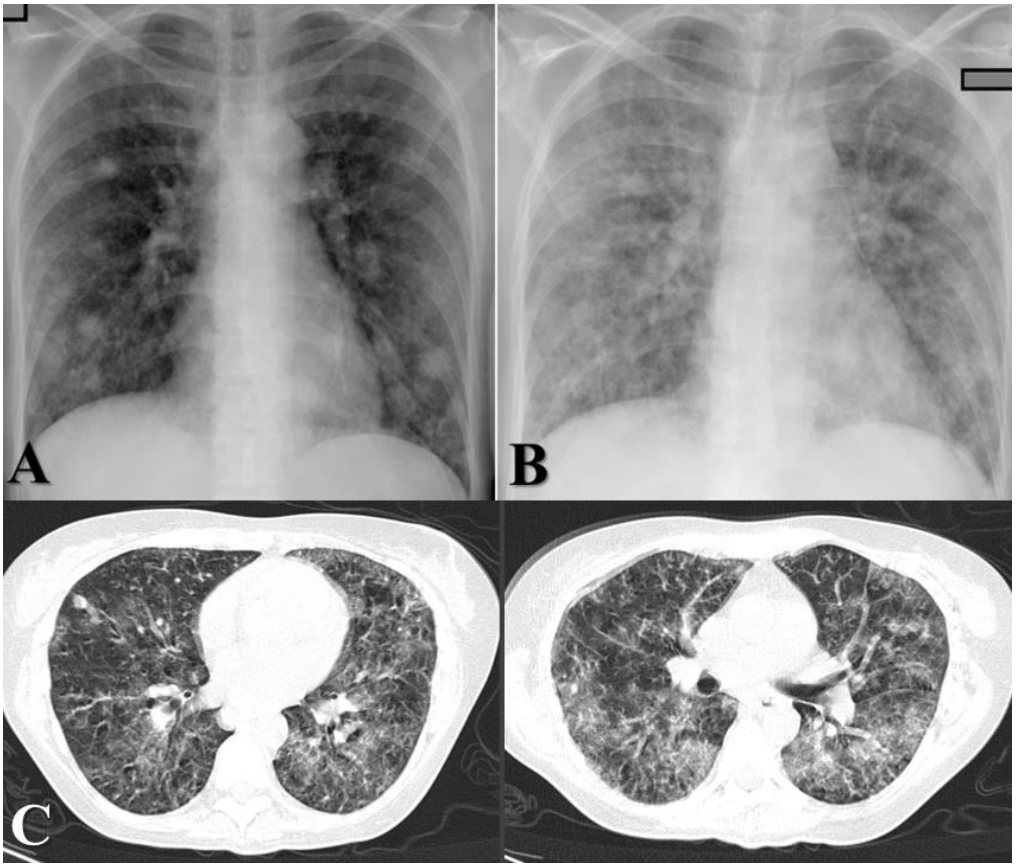

Fig. 2. Chest radiographs at 12 days (A) and at 19 days (B) after admission showing the exacerbation of bilateral pulmonary opacification; Chest CT (axial images) showing important ground-glass opacities of the lungs (C) (*C. Toader's personal collection*)

DISCUSSION

This case highlights the clinical features of a COVID-19 patient with ICH. Our patient was young, much younger than expected for conventional ICH (13, 14). In this regard, a meta-analysis that studied all types of cerebrovascular events in COVID-19 patients identified a mean age range between 45-67 years (15). Up to present, ICH in young COVID-19 patients has been reported in the literature in (16). Regarding the onset of neurological symptoms, studies reported an average delay of 32 days (range 14-38 days) between the time of COVID-19 symptoms onset and the time of occurrence of ICH. As a particularity of our case, the patient initially presented with neurological symptoms and after that the symptoms of SARS-CoV-2 disease occurred. These aspects confirm the increasing incidence of extrapulmonary symptoms at the beginning, reported in the literature (3). The location of the hematoma was lobar (fig. 1), according to some authors who reported a higher frequency of ICH in the lobar territories in COVID-19 patients (6). As in similar cases reported in the literature (6), our patient did not shown

underlying vascular abnormality on cerebral angiography. In a retrospective study of 5,227 patients with COVID-19, Altschul *et al.* observed in the 35 patients with ICH of some kind, that multi-focal and multi-compartmental ICH was more likely to be present in patients with severe pulmonary complications (17).

Although a causal link between COVID-19 and occurrence of ICH has not yet been demonstrated (17), however, numerous studies have shown that in the case of SARS-CoV-2 infection, a prothrombotic states, multi-system organ failure, cytokine storm, but also disseminated intravascular coagulopathy may occur, which would be a predisposition, at least theoretically for brain hemorrhages (3,18-20). Regarding the spread of the virus into the nervous system, two routes were considered: neuronal retrograde neurotropic route through the olfactory tract and hematogenous spread through the blood brain barrier (21, 22).

On the other hand, Benger *et al*. proposed two possible mechanisms of ICH in COVID-19 patients (6). He first considers that initial direct endothelial cell invasion occurs, and subsequently, a combination of systemic factors occurs such as inflammatory cytokine production, prothrombotic factors and activation of coagulation cascades. Ultimately, disruption of tight junction protein complexes will lead to blood brain barrier compromise and occurrence of ICH. As a second mechanism, the authors suggest that disruption of the renin-angiotensin system plays an important role in ICH in COVID-19 patients. It is well known that the renin-angiotensin system has distinct regulatory pathways in the brain, and these may be affected by SARS-CoV-2 through down flow dysautoregulation (6, 23, 24).

CONCLUSIONS

ICH in COVID-19 patients is a rare but serious way in which this new disease may manifest. It is important for neurosurgeons to become aware that patients with COVID-19 infection may present with these findings without initially pulmonary symptoms. Subsequent studies remain to confirm this presumed causality between ICH and COVID-19, as well as to determine the incidence, mechanisms, and characteristics of brain hemorrhage in COVID-19 patients.

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ODONTOGENIC ORBITAL CELLULITIS. CASE REPORT AND LITERATURE REVIEW

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ODONTOGENIC ORBITAL CELLULITIS. CASE REPORT AND LITERATURE REVIEW (Abstract): Orbital cellulitis, a relatively infrequent infection of the ocular adnexa and orbital adipose tissue, is an ophthalmic emergency. In the absence of prompt anti-infective treatment, the spread of the infectious process can quickly lead to serious complications: vision loss, cavernous sinus thrombophlebitis, meningitis, intracranial septic spreads, and even death. In the adult population, the frequency of orbital complications from sinus infection ranges from 0.5% to 3.9%, and that of odontogenic orbital inflammation is even more infrequent. We report the case of a 56-year-old female patient with type 2 diabetes admitted in emergency for left exophthalmos, with painful limitation of eye movements and blurred vision in her left eye (LE). Fundus examination revealed in the LE significant optic disc edema, flame-shaped peripapillary hemorrhages, and dilated tortuous retinal veins. Due to the relative subacute course of proptosis and the mild orbital inflammatory signs, the diagnostic challenge was to differentiate between orbital cellulitis and a pseudo inflammatory sign that could be associated to an orbital lymphoma or other malignancies. Imaging findings were suggestive of odontogenic orbital cellulitis. A prompt intravenous antibiotic therapy was initiated, followed by ENT surgery of the infected tooth and maxillary sinusitis. The outcome was favorable, with progressive recovery of visual function. Conclusion: Odontogenic orbital cellulitis is an ocular emergency that is best managed by a multidisciplinary team consisting of an ophthalmologist, otolaryngologist, infectious diseases specialist and neurosurgeon. Prompt multimodal therapeutic approach, including imaging investigations, antibiotic therapy, and surgical treatment can avoid serious complications and lead to full recovery. **Keywords**: EXOPHTHALMOS, ORBITAL ABSCESS, MAXILLARY SINUSITIS, ODONTOGENIC.

Orbital cellulitis, a relatively infrequent infection of the ocular adnexa and orbital adipose tissue, is an ophthalmic emergency. In the absence of prompt anti-infective treatment, the spread of the infectious process can quickly lead to serious complications: vision loss, cavernous sinus thrombophlebitis, meningitis, intracranial septic spreads, and even death. Vision loss may be due to increased intraocular pressure caused by the mass effect of the orbital infectious process, but also by direct spread of infection to the optic nerve, resulting in optic neuritis or even ischemic necrosis after staphylococcal infection.

In adults, the causes of orbital cellulitis are trauma, orbital foreign body retention, eye surgery, dacryocystitis, while in chil-

dren sinusitis accounts for about 90% of the etiology. In adult population, the frequency of orbital complications from sinus infection ranges from 0.5% to 3.9%, and that of odontogenic orbital inflammation is even more infrequent (1-3).

Clinical forms of orbital cellulitis were defined by Chandler (1) according to the severity and extension of the pathological process in: 1) preseptal cellulitis; 2) diffuse orbital cellulitis, 3) subperiosteal abscess, 4) orbital abscess, 5) cavernous sinus thrombophlebitis. Separating them based on clinical examination is difficult. However, proptosis, painful limitation of eye movement, and decreased vision are signs of severity when the infectious process progresses beyond the orbital septum. Magnetic resonance imaging (MRI) scans are particularly important in differentiating the clinical forms described by Chandler, indicating the presence of an associated sinusitis, and monitoring the effectiveness of therapy.

CASE REPORT

We present the case of a 56-year-old woman admitted in emergency for left exophthalmos, with painful limitation of eye movements and blurred vision in her left eye. She was known with type 2 diabetes mellitus and arterial hypertension. The patient declared she had some dental problems neglected due to COVID-19 pandemic. She noticed a mild protrusion of the eyeball and blurred vision about 2 weeks before presentation. As the symptoms became worse and associated left hemicrania, the patient presented to the emergency room for further diagnostic and treatment.

Ophthalmological examination revealed that the best corrected visual acuity was normal in the right eye, and 0.4 in her LE. Applanation tonometry revealed normal intraocular pressure of 16 mmHg in her right eye (RE) and slightly elevated in LE (IOP 17 mmHg (RE) and 25 mmHg (LE). The patient presented left proptosis (exophthalmometry: RE: 16 mm; LE: 24 mm), with painful limitation of eye movements, dilated episcleral veins, and mild conjunctival hyperemia. The right eye was normal.

Fundus examination revealed in the LE significant optic disc edema, flame-shaped peripapillary hemorrhages, and dilated tortuous retinal veins (fig. 1a), further confirmed by optic coherence tomography examination (OCT Zeiss). Computerized visual field testing was normal for RE and revealed peripheral nasal narrowing, and paracentral scotomas in LE (fig. 1b).

Due to the relatively subacute course of proptosis and the mild orbital inflammatory signs, the diagnostic challenge was to differentiate between orbital cellulitis and a pseudo inflammatory sign that could be associated with orbital lymphoma or other malignancies.

Laboratory investigations suggested an inflammatory syndrome: leukocytosis 12,000/mm^3, with 80.6% neutrophils, ESR 40 mm/hr., and fibrinogen 400 mg/dL.

MRI exam revealed a 27/31/32 mm poorly defined intraconal mass, located in the inferior and internal quadrant, without demarcation line with the medial and inferior rectus muscles, and with the inferior and medial orbital walls. MRI findings were suggestive of odontogenic maxillary sinusitis (fig. 3a).

The associated maxillary and left ethmoid sinusitis seen on MRI scan oriented the clinical diagnosis towards an infectious etiology. Optic nerve involvement was considered a consequence of intraorbital compression, as well as of direct extension of inflammation.

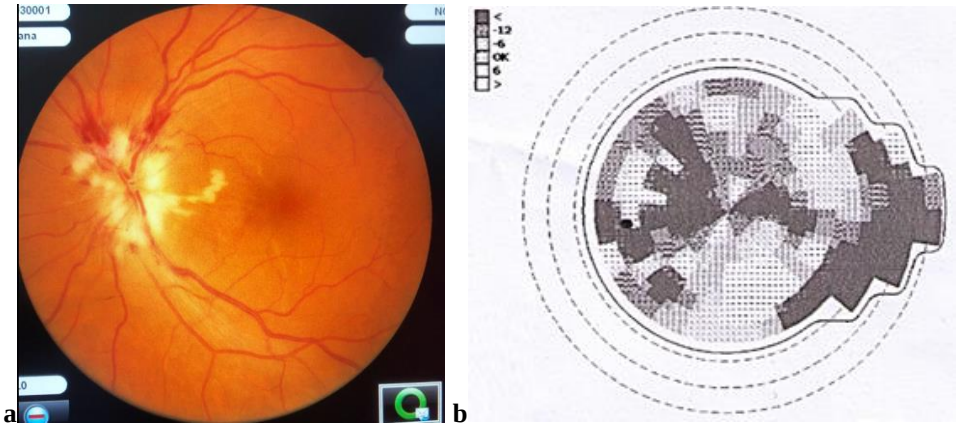

Fig. 1. a) Fundus appearance of the LE at admission;
b) Computerized visual field test (Optopol PTS-910) at admission:
multiple superior and inferior paracentral scotomas, nasal narrowing;

Fig. 2. MRI scan: a) Thickening of the left maxillary sinus mucosa with minimal fluid accumulation; thickening of ethmoid cell mucosa; fluid blockages at the level of the supraalveolar recess of the right maxillary sinus and of the left t of the sphenoid compartment; b) The pseudotumoral process encircles the optic nerve, causing mass effect and superolateral deviation. The orbital mass exhibits heterogenous pseudo nodular_contrast enhancement; diffuse edematous infiltration of the orbital fat and rectus muscles.

ENT assessment confirmed the diagnosis of odontogenic maxillary sinusitis. LE: Exophthalmos. Orbital cellulitis in observation. The treatment plan and possible com-

plications that can happen in any kind of surgery (4) were discussed with the patient and the signed informed consent was obtained (5).

General intravenous broad-spectrum antibiotic and anti-inflammatory treatment was immediate initiated: cefuroxime 3g/day and metronidazole 1g/day, and dexamethasone 8 mg/day, with slowly favorable outcome. The patient was transferred to the ENT department for the rehabilitation of sinus and dental infection foci. Surgery was performed by endoscopic approach and enucleation of the cystic transformation of the infected mucosa, evacuation of secretions from the maxillary sinus. The infected alveolar cysts were also treated surgically. Multiple biopsies were taken from the nasopharyngeal mucosa to rule out a possible lymphoma. Histopathological and immunohistochemical results showed the nonspecific reactive nature of the lymphoid infiltrates, suggestive of chronic rhinopharyngitis.

Treatment outcome was favorable, with remission of optic nerve edema and exophthalmos and recovery of visual function. At discharge visual acuity was of 0.9 in her LE and visual field examination showed the decrease in intensity and surface of paracentral scotomas. The patient was discharged with the recommendation to continue the treatment with antibiotics - cefaclor 500 mg twice daily- and with anti-inflammatory drugs - ibuprofen 200 mg/day, for 10 days, and return for follow-up visits after one week and then at one month, resulting in a progressive remission of the ophthalmological pathological findings.

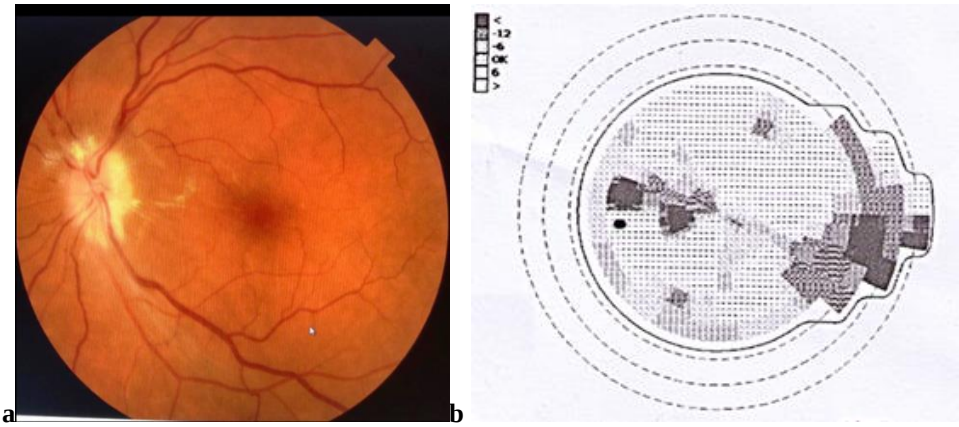

Fig. 3. a) Fundus appearance of the LE 7 days later: diminished optic nerve edema, resolved peripapillary hemorrhages; b) Computerized visual field (Optopol PTS-910): paracentral scotomas decreased in number, intensity, and size.

DISCUSSION

In the current conditions of development of antibiotic and surgical therapy, maxillary sinusitis is a condition that progresses without special complications. In most cases it is the consequence of an upper respiratory infection with *H. influenza, Pneumococcus, Staphylococcus, Streptococcus spp.* Odontogenic orbital cellulitis is a rare and severe cause of orbital infection and is associated with high risk of vision loss (2).

Orbital cellulitis most commonly occurs when bacterial infection spreads from the paranasal sinuses, most often from the ethmoid sinus. Lamina papyracea, part of the medial wall, is paper-thin and perforated by numerous foramina for nerves and blood vessels, which makes easy the contiguous spread from the ethmoid sinuses to the orbit (6). Less frequently, frontal or maxillary sinusitis can generate orbital septic complications by spreading the infection through the floor, respectively the orbital ceiling. Another mechanism described by Rothschild *et al.* (7) is that the infection spreads through the maxillary sinus, the canine fossa with a thrombophlebitis of the angular vein or the pterygopalatine fossa and infra-temporal fossa both reaching the orbit by means of the inferior orbital fissure. The involved flora is mixed, consisting of aerobes and anaerobes, and the diversity of flora and the presence of anaerobes in maxillary sinusitis increase with age (1, 8). Commonly reported bacteria in orbital abscesses include *Staphylococcus aureus, Staphylococcus epidermidis, Streptococci, Diphtheroids, Haemophilus influenza, Escherichia coli,* and multiple species including aerobes and anaerobes. Li et al. (9) reported a case of necrotizing orbital cellulitis secondary to odontogenic *Streptococcus constellatus*. The presence of *methicillin-resistant staphylococci* has been found in a relatively large number of cases, reason why these germs must be considered as possible infectious agents when planning antibiotic therapy.

Therapeutic management should be initiated urgently when orbital cellulitis is suspected, due to the formidable complications, such as acute vision loss, cavernous sinus thrombosis, or septic intracranial dissemination (7-15). High-dose intravenous antibiotic therapy will be initiated with broad-spectrum, Gram-positive, Gram-negative, and anaerobic antibiotic combinations. Analgesics such as NSAIDs and acetaminophen can be used alone or in combination to achieve effective pain control in patients with orbital cellulitis (16).

The most commonly used therapeutic combinations are vancomycin (15 to 20 mg/kg per day IV every 8 to 12 hours) + second or third generation cephalosporins (Ceftriaxone 2 g per day IV) + metronidazole (500 mg IV or orally every 8 hours) (1, 2, 16-18). In case of cephalosporin allergy, fluoroquinolones may be used (Ciprofloxacin 400 mg IV twice a day, levofloxacin 500 mg IV twice a day). There are no controlled trials to define the ideal duration of antimicrobial therapy in orbital cellulitis or when to switch to oral from intravenous treatment (16). Therapy is initiated intravenously and can be continued orally after at least 3-5 days, in case of favorable clinical course, in the absence of fever and signs of decreased inflammation and proptosis. Oral antibiotic therapy options are cephalosporins or combinations of penicillin + beta-lactamase inhibitor.

Surgery may be indicated in cases with poor response to medical treatment and imaging evidence of orbital abscess. According to Harris and Garcia (19), the indications for surgical approach must consider the location of the orbital abscess, visual status, presumed response to antibiotic therapy, sinus involvement, and the risk of intracranial spread of infection. The main indications for surgery are compromised vision by retinal and optic nerve lesions, large abscesses or extensive superior or inferior abscesses (that might not resolve quickly, even if sinusitis is medically cleared) and increased risk of intracranial complications. After the initiation of intra-

venous antibiotic therapy, surgery may be necessary if there is no improvement after 48-72 hours, suggesting that bacteremia is not responding to the choice of antibiotics or relative afferent pupillary defect develops (20). Streptococcus spp can cause necrotizing lid disease and systemic manifestation such as fatal streptococcus toxic shock syndrome, with multiorgan failure, even in previously healthy subjects. The virulence is related to the production of M proteins and exotoxins A and B, that mediate tissue necrosis by causing massive release of cytokines (18), such as tumor necrosis factors and interleukins, as general biomarkers of inflammation.

Imaging of the orbit and eye are extremely useful in the differential diagnosis of optic disk edema, including angiofluorography with dyes like sodic fluorescein and indocyanine green – now a wide-open used dye, in many surgical specialties, including general surgery (21), documenting a possible sinus origin of the infection and the presence of complications. In these cases, a multidisciplinary approach is required, with dental extraction, trans alveolar drainage, and ENT surgery of the infected sinuses.

COVID-19 pandemic has a deep impact on the social and economic life worldwide and is considered the most severe sanitary crisis since the Spanish flu, a hundred years ago (22). In the present case, diabetes, the delayed dental treatment due to the fear of getting infected with COVID-19, as well as the relative delays in getting appointments to specialized outpatient clinics, in the presence of insufficient and incomplete antibiotic self-medication caused the development of a torpid infectious process (23). imposed the differential diagnosis with an orbital malignancy, particularly an orbital lymphoma.

The management of anti-infective therapy in a diabetic patient has particularities. Blood-sugar levels are more difficult to control during the infectious process, associate a higher risk of complications, sepsis including, and the response to usual therapy may be altered (24, 25). The arterial hypertension is a frequent associated condition with diabetes, leading in early phases to issues of differential diagnosis of the flame shaped retinal hemorrhages and mild optic disk edema.

CONCLUSIONS

Odontogenic orbital cellulitis is an ocular emergency that is best managed by a multidisciplinary team consisting of ophthalmologist, otolaryngologist, infectious disease specialist, and neurosurgeon.

Prompt multimodal therapeutic approach, including imaging, antibiotic therapy, and surgical treatment, can avoid serious complications and lead to full recovery.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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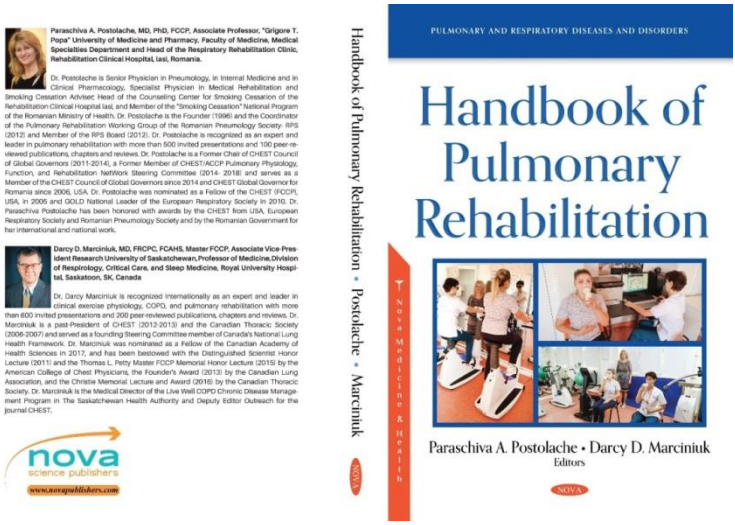
BOOK REVIEW

HANDBOOK OF PULMONARY REHABILITATION

Editors: Paraschiva A. Postolache (Romania), Darcy D. Marciniuk (Canada)
Nova Science Publishers, Inc., New York, USA



In the conditions of the current COVID-19 pandemic, we welcome the publication of the volume: Handbook of Pulmonary Rehabilitation, an editorial novelty for Romania. The idea of this volume has crystallized in the last 20 years of intense activity in this field of Associate Professor Dr. Paraschiva A Postolache. The manual for the rehabilitation of the patient with respiratory diseases is a reference work, which places pulmonary rehabilitation, a fundamental part of patient management, in the wider framework of the sick human body. The book emerged as a necessity for the management of chronic lung disease whose incidence is constantly increasing worldwide and at the right time in the current context of COVID-19 disease pandemic, for which this therapy is essential.

Pulmonary rehabilitation is not a completely new concept, but this book is the first to focus on the quality of life of the patient, not only on the medical act itself and its safety. In the last three decades, pulmonary rehabilitation has become a cornerstone in the comprehensive management of COPD and other chronic lung diseases, on a solid physiological basis, as a result of extensive scientific evidence, randomized controlled trials, meta-analyzes and guidelines. Pulmonary rehabilitation is today an extremely important tool of treatment, a source of information for respiratory pathophysiology, but has also added therapeutic methods and measuring tools in patient care. We focus on the future !

The volume provides a scientific and practical framework for all those involved in the care of the chronic lung patient, pulmonologists, geriatrics, family physicians, nurses, physiotherapists, nutritionists, social workers, occupational therapists, psy-

chologists and bioengineers. Those interested have access to all the elements necessary to set up a Clinic or Department of Pulmonary Rehabilitation.

The two editors of the Handbook, Assoc. Prof. Dr. Paraschiva A. Postolache (Romania) and Prof. Dr. Darcy D. Marciniuk (Canada) coopted a team of internationally renowned specialists in the field of pulmonary rehabilitation and related medical fields.

Assoc. Prof. Dr. Paraschiva A Postolache has a rich international activity, being President of the CHEST Council of Global Governors and member of the CHEST Board of Regents from 2011 to 2014, member of the Steering Committee of the Pulmonary Physiology, Function and Rehabilitation Network of the American College of Chest Physicians (ACCP)/CHEST between 2014 and 2018 and member of the CHEST Council of Global Governors since 2014 and CHEST Global Governor for Romania since 2006. Dr. Postolache has been nominated as a Fellow of CHEST (FCCP), USA, in 2005 and National Leader GOLD of the European Respiratory Society (ERS) in 2010, being honored with awards granted by CHEST (U.S.A.), ERS (Europe), the Romanian Society of Pneumology (Romania) and the Romanian Parliament, for its international and national activity. Dr. Postolache is the founder (1996) and Coordinator of the Respiratory Rehabilitation Working Group of the Romanian Society of Pneumology, being recognized as an expert and leader in pulmonary rehabilitation.

Dr. Darcy D. Marciniuk is a University Professor in Canada and has a focus on pulmonology, critical care and sleep medicine. Dr. Marciniuk is also the medical director of the Chronic Disease Management Program: COPD Live Well. Dr. Marciniuk is internationally recognized as an expert and leader in the exercise physiology, chronic obstructive pulmonary disease (COPD) and lung rehabilitation, with over 420 international guest presentations and 200 peer-reviewed publications, chapters and reviews (Google Scholar H-index 51). Dr. Marciniuk is former President of CHEST (2012-2013) and the Canadian Thoracic Society (CTS) (2006-2007) and President of the International Respiratory Society Forum. He served as a member of the founding Steering Committee of the National Lung Health Framework of Canada (2007-2011) and a member and chair of the Royal College of Physicians and Surgeons of Canada and the Pulmonology Examination Committee (1999-2007). Dr. Marciniuk has been the lead author of three clinical practice guidelines in COPD, published by CTS, a member of the International Group of Clinical Practice Guidelines in COPD (ACP/ACCP/ATS/ERS), Cardiopulmonary Stress Testing (ATS/ACCP), pulmonary rehabilitation (ATS/ERS) and prevention of acute exacerbations of COPD (CTS/CHEST), as well as in many clinical practice guidelines for the management of COPD, published by CTS. As an associate vice president of research, he led the development of the University of Saskatchewan's international plan and currently chairs the institution's pandemic response and recovery team. Dr. Marciniuk is a member of the Canadian Academy of Health Sciences and deputy editor (Outreach) for CHEST Journal. He was awarded the Distinguished Scientist Honor Lecture (2011) and Thomas L. Petty Master FCCP Memorial Honor Lecture (2015) by the ACCP, Founder's Award (2013) by the Canadian Lung Association, Christie Memorial Lecture and Award (2016)) by the CTS and the Distinguished Researcher Award (2013) by the University

of Saskatchewan.

The book was reviewed by Prof. Dr. Nicolino Ambrosino, M.D., FCCP, from the University of Pisa, Italy, Editor-in-Chief of the Italian Journal of Pulmonology, Consultant Researcher at Maugeri Clinical Institutes, Pavia, Italy, international expert in lung rehabilitation and awarded by the ERS for educational merits; Prof. Dr. Panagiotis Behrakis, M.D., Ph.D. (McGill), FCCP, Greece Pulmonologist - Intensivist, Athens Medical Center, Director, Institute of Public Health, American College of Greece, Principal Investigator of the Biomedical Research Foundation of the Athens Academy, Associate Professor at the Universities of Athens and Harvard) and Prof. Dr. Mihai Berteanu ("Carol Davila" University of Medicine and Pharmacy, Bucharest; Head of the Medical Rehabilitation Clinic at the "Elias" University Emergency Hospital; President of the Specialized Commission of Physical Medicine and Rehabilitation of the Ministry of Health and Delegate of Romania to UEMS-PRM).

The book has 24 chapters, 410 pages, reviewing the fundamentals and the practical applications of pulmonary rehabilitation. After a short history of pulmonary rehabilitation internationally and in Romania, are presented the general aspects of pulmonary rehabilitation, then the specific aspects of complex evaluation of patients to be included in the program, correct pharmacological therapy, smoking cessation, followed by aspects practices of education, nutrition, psychotherapy, ethics, prescribing the lung rehabilitation program with emphasis on the training of general and respiratory muscles, saline therapy, telerehabilitation, robots and artificial intelligence, all for returning to the socio-professional environment as early as possible, etc. The following are particular aspects of pulmonary rehabilitation in other chronic lung diseases besides COPD, including COVID-19 disease, the last chapter addressing the future of pulmonary rehabilitation.

Among the authors we highlight Prof. Dr. Nicolino Ambrosino, from Italy. Prof. Ambrosino, in the chapter on Telemedicine and Rehabilitation, highlighted that there is a great potential of telerehabilitation to reduce existing barriers for patients to have easy access, and in any conditions, to the pulmonary rehabilitation programs, such as there are also in the current COVID-19 pandemic, that there are still many barriers to the expansion of telerehabilitation and that research to date has not explored the true effectiveness of telerehabilitation.

A new methods of pulmonary rehabilitation is the training of respiratory muscles, approached by Oliver Christian Göhl, Ph.D., from Germany, Executive member of the Board of Directors of "AG-Lungensport in Deutschland eV", Member of the Scientific Advisory Board of "Selbsthilfegruppe Mailingliste Lungenemphysem COPD Deutschland eV" and "Deutsche Emphysemgruppe e.V.", Eicklingen, Germany and the Romanian team consisting of Paraschiva A. Postolache, Gabriela Jimborean, Edith Simona Ianosi and Mioara Szatmary. The training of the respiratory muscles, independently or associated with the respiratory rehabilitation program, has the effect of reducing the symptoms, increasing the capacity for effort and the quality of life, being achieved with different types of devices and techniques.

Rehabilitation can be applied starting from the newborn, useful information can be found in the chapter "Rehabilitation of infants with chronic neonatal lung disease according to clinical guidelines" of the Romanian team: Andreea Avasiloaiei and Maria Stamatin, continued in children with

chronic lung diseases, extremely important aspects being addressed by Prof. Dr. Paul H. Sammut from U.S.A. and then to adults, the most important aspects related to this subject being developed in the rest of the 24 chapters, which address the newer aspects, according to the international guidelines, of the pulmonary rehabilitation.

Also noteworthy is the chapter addressing the role of pulmonary rehabilitation in surgical lung diseases written by Prof. Dr. Francis J. Podbielski from U.S.A., patients undergoing surgery to reduce lung volumes, with operable lung cancer or lung transplant requiring pre- and postoperative lung rehabilitation for a correct and complete management, with remarkable results anywhere in the world.

Ethical aspects of the application of pulmonary rehabilitation at all ages (Cristina Gavrilovici and Liviu Oprea from Romania and Teodora Manea from UK), return to the socio-professional environment (Ioana Soare, Romania) and future directions of pulmonary rehabilitation (Paraschiva A. Postolache and Andreea -Daniela Meca from Romania and Darcy D. Marciniuk from Canada, co-editor of the book) point out the current trends in lung rehabilitation around the world.

This book provides a theoretical and practical scientific framework to help mainly the students and residents, the future medical practitioners, but also the specialists. The pulmonologist, explorationist, geriatrician, family doctor, nurse or physiotherapist, nutritionist, social worker, occupational therapist, bioengineer, psychologist, etc., all interested in alleviating the suffering of the chronic lung patient, now have access to all the elements necessary to establish a Clinic or Department of Pulmonary Rehabilitation.

The Handbook of Pulmonary Rehabilitation for patients with respiratory diseases has a contemporary, holistic multidisciplinary approach. This comprehensive reference book places pulmonary rehabilitation, a fundamental part of the complex management of patients, in the wider framework of the sick human body, in all its aspects. In the 24 chapters, the book includes new sections on the development of rehabilitation as a global discipline and new aspects of the results of pulmonary rehabilitation, such as socio-professional reintegration. Older approaches to nutrition, psychotherapy, etc. were reconsidered because they also interfere with the patient's well-being in the rehabilitation process.

Pulmonary rehabilitation has hardly been imposed among effective therapeutic methods, but now its place is well established, and the treatment of the chronic respiratory patient can no longer be conceived without the integration of rehabilitation, as is currently practiced in most centers in the developed world.

The book was published by Nova Science Publishers, Inc., New York, one of the most important scientific, technical and medical publishing houses in the world, founded in 1985 by Frank Columbus, with over 1,800 titles published annually. It is considered the second largest multidisciplinary publisher in the world, based on BKCI coverage from 2005 to 2012.

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"SOMMO POETA" - 700 YEARS SINCE HIS DISAPPEARANCE

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Dante Alighieri (1265-1321), often simply called Dante, was an Italian poet, writer and philosopher of Tuscany. His famous work, "Divine Comedy", originally called Comedia and later christened "Divine" by Giovanni Boccaccio (1), is widely considered one of the most important works of the Middle Ages and the greatest literary work in Italian; thus, we can say that the work "Divine Comedy" is the nucleus around which the Italian language crystallized, as we know it today (2).

Dante is known for promoting and establishing the use of the vernacular Italian language in literature at a time when most of his publications were written in Latin, which was accessible only to educated readers. "De vulgari eloquentia" (On eloquence in vulgar language) was one of the first literary appearances of the Italian vernacular. The use of the Tuscan dialect for works such as "New Life" (1295) and "Divine Comedy" helped to form and concretize what is the standardized Italian language today. His work set a precedent that would be repeated by important Italian Renaissance writers such as Petrarca or Boccaccio.

Dante played a key role in the formation and establishment of Italian literature. He is considered the "father" of the Italian language, and in Italy he is often called il Sommo Poeta ("Supreme Poet"). Dante, Petrarca and Boccaccio are considered to be the "three crowns" of Italian literature. His literary descriptions of Hell, Purgatory, and Evil have inspired the next generation of artists and writers, especially influencing Western literature. He is considered a source of influence on English writers such as Geoffrey Chaucer, John Milton and Alfred Tennyson, among many others; moreover, the use of a form of rhyme (third rhyme) with three interlaced lines is dedicated to him (3).

Dante Alighieri was born in the Republic of Florence, Tuscany, Italy around 1265. This can be deduced from the autobiographical allusions in the "Divine Comedy". The

first book, Hell, begins, "Nel mezzo del cammin di nostra vita" ("In the middle of our life's journey"), suggesting that Dante was about 35 years old, if we look at the average lifespan of that period, of 70 years; and since his imaginary journey into the world of hell took place in 1300, he was most likely born around 1265. Some verses in the "Paradiso" section of the "Divine Comedy" also give a possible indication that he was born in Gemini sign, because in that year, the sun was in Gemini between about May 11 and June 11.

Dante claimed that his family had its roots in ancient Rome (Hell, XV, 76), but the most distant member of the family he could remember (Paradiso, XV, 135) was Cacciaguida degli Elisei (Italian crusader, foreigner Dante's great-grandfather), born around 1100. Dante's father, Alighiero di Bellincione, was affiliated with the "white Guelph" political movement but did not suffer significant retaliation after the Ghibellines were victorious in the Battle of Montaperti of the middle of the 13th century). This suggests that Alighiero and his family had a certain prestigious social status; although some sources indicate otherwise, the fact that Alighiero, politically inactive, was so fallen that he was not considered worthy of being sent into exile or punished (4).

Politically, Dante and his family were loyal to the Guelphs, a Florentine political alliance that had the support of the papacy and was in opposition to the alliance of the Ghibellines, the latter being supported by the Emperor of the Holy Roman Empire. Regarding Dante's family, his mother, Bella, came from the Abbati family, and there is not much information about her influence on Dante, because she passed away when Dante was less than ten years old. After his mother's death, Dante's father, Alighier, remarried a woman, Lapa di Chiarissimo Cialuffi. Some sources indicate that he did not formally marry, he grows with it, because during the Middle Ages the remaining widows were socially limited in this respect; but it is certain that their relationship resulted in two children, Dante's sister and half-brother, Tana and Francesco, respectively (Gaetana) (5).

Related to the main muse of Dante's creation, Beatrice, he relates that he first met Beatrice Portinari at a very young age, about eight or nine years old, mentioning that he fell in love with her at first sight, without having to at least talk to her. Unfortunately, at the age of only twelve, the young Dante was promised in marriage by his father, a marriage that would take place with Gemma di Manetto Donati, a descendant of the powerful Donati family. Dante relates in his writings that he had frequent meetings with Beatrice on the streets of Florence, meetings that were limited to some exchanges of formal greetings; he never got any closer to Beatrice.

Following these encounters, about eight to nine years after his marriage to Gemma, Dante dedicated several sonnets to Beatrice, but none to Gemma - which he does not even mention in his writings of this kind. Many other details about Dante's marriage to Gemma are missing, but we can say for sure that the marital relationship between the two resulted in three children - Pietro, Antonia and Jacopo (6).

In order to have the right to participate in public life, Dante had to be a member of one of the many craft / trade guilds in Florence, so he became a member of the guild of doctors and pharmacists. In the years following this affiliation, he occasionally participated by speaking or by voting in certain councils of the city-republic of Florence from that

period. Due to the irreversible damage or loss of some of the archives of the meetings between 1298-1300, years in which Dante was an active member of the republican councils, we cannot appreciate the true extent of Dante’s participation in public life in those years.

There is not much relevant information about the education Dante received as a child; most likely he studied in a Florentine church / monastery school or at home. From his work, more precisely from Purgatorio - XXVI, we know that he greatly appreciates Tuscan poetry and the compositions of the Bolognese poet Guido Guinizelli, calling the latter his “father” during the period when the Sicilian Poetic School was gaining as much ground as possible. cultural-artistic scene from the Tuscany region. Moreover, he was passionate about the poetry of Daniel Arnaut, as well as the great old classics of antiquity, such as Virgilius and Ovid.

The relationship between Dante and his muse, Beatrice, was representative of “courtly love”, an unshared love whose socio-emotional characteristics were first cataloged in French poetry of the eleventh and thirteenth centuries. Dante’s emotional experience with such love was typical, but its expression in his work was completely unique. In the name of this love, Dante left his mark on dolce stil novo (“the sweet new style”, a term that Dante himself coined) and would join other contemporary poets and writers to explore aspects of love that were never stressed (7). Love for Beatrice (as Petrarch would express it for Laura, but somewhat differently) would be his leitmotif; and more, for the reason of living, together with political passions. In many of his poems, she is described as semi-divine, constantly watching over him and giving spiritual instructions. When Beatrice died in 1290, Dante sought refuge in Latin literature. Convivio reports that he read “De consolatione philosophiae” by Boethius and “De Amicitia” by Cicero.

He then devoted himself to philosophical studies in religious schools, such as the Dominican one in Santa Maria Novella. He took part in the disputes that the two great mendicant orders (Franciscan and Dominican) held publicly or indirectly in Florence, the former explaining the doctrines of the mystics and St. Bonaventure, the latter explaining the theories of St. Thomas Aquinas (8).

At the age of 18, Dante met Guido Cavalcanti, Lapo Gianni, Cino da Pistoia and, in a short time, Brunetto Latini in Florence; together they became the leaders of dolce novo style. Later, Brunetto received a special mention in the Divine Comedy (Inferno, XV, 28) for what he had taught Dante: his eminent comrades. About 50 such poetic commentaries, known as Rime, have been published, much of which are included in Convivio and Vita Nuova.

Like much of the citizens of Florence at that time, Dante took part in the political conflict between the Guelph and Ghibelline factions; participating in the Battle of Campaldina in 1289 on the side of the Guelphs, and later to join Charles of Anjou in 1294. In the current situation, in order to continue his political activity and public life, Dante became a pharmacist, but without intend to practice this trade. (He was forced to make this professional choice because a law that came into force in 1925 required the nobility, who wanted to have such activities, to belong to a guild within the “Corporazioni delle Arti e dei Mestieri”). As a politician he did not have an exhaustive activity, but he managed to hold important positions during his years of activity; considering also the extremely unstable political context from

that date (9).

Following the victory over the Ghibelline faction; the Guelph faction suffered a schism that led to the formation of two new political factions - the White Guelphs (to which Dante would join) and the Black Guelphs (a faction patronized by Corso Donati). Separated at first only on dynastic lines, schims began to deepen ideologically, especially on papal influence in the internal and external affairs of the Republic of Florence. The White Guelphs wanted to achieve higher gradients of independence from the pope, implicitly from Rome, while the Black Guelphs supported the papal faction and satellite approach to Rome. As a result of these ideological differences, the White Guelphs managed to emerge victorious, initially exiling members of the rival faction in Florence.

Following these events, Pope Boniface VIII launched a military campaign against the Florentine Republic, culminating in the military occupation of the city; despite peace efforts through Carol de Valois (brother of the King of France at the time) - named pacifier of the conflicts in the Tuscan region. (1301) The city council chooses to delegate Dante to Rome in an attempt to negotiate with the Papal authorities (10).

Arriving in Rome with the delegation of the White Guelphs, Dante is asked by the Pope to stay with him in Rome, dismissing the other delegates sent with Dante. While Dante was with the Pope in Rome, Florence was attacked by Black Guelph forces, led by Carol of Valois; As a result of these actions, most of the white Guelphs in Florence are killed or exiled. A new government takes over the city, led by Cante dei Gabrielli da Gubbio, a government that accuses Dante of corruption (by joining the White Guelph faction) and forces him into exile for two years, along with a fine. The new government will also accuse Dante of financial evasion during his tenure as city prior (1300). Fortunately, the Pope, although a supporter of the Black Guelphs, suggests that Dante stay in Rome to avoid the repercussions he would suffer if he returned to Florence; In the months that followed, Dante was declared a deserter by the city-state authorities (11).

Next, Dante refused to pay a fine, firstly because he considered himself innocent of all the purely politically motivated accusations brought against him, and secondly because he could not afford to pay it, all his possessions remaining in Florence, confiscated. by the Black Guelphs. Thus, he was sentenced to life in exile; if he dared to return to Florence without paying a fine, being in danger of being executed. In 2008 - almost 700 years after the great writer's death - the city council of Florence decided to annul Dante's sentence by a motion of annulment.

In the years following his sentence, Dante continued to participate in numerous attempts by the White Guelph faction to retake the city and regain its leadership, unfortunately all these attempts failed, most often due to betrayals within the faction. Disappointed by all this, he decides to give up any kind of political affiliation, disgusted by the vile behavior of both his enemies and his allies; thus, he swears that he will never again be politically affiliated with any faction (12).

He was to be the guest of Moroello Malaspina, between the years 1306-1307, at Lunigiana. At the end of his stay in Lunigiana, he left as a guest of Bartholomew I in Verona, then continued his journey to Liguria, where he settled in Sarzana. He is about to set off again, this time for Lucca, where

he will meet a woman - Gentucca - who will help him settle there for a while. (He mentions it showing his gratitude in Purgatory, XXIV). Other sources indicate that he visited Paris (1308-1310), some even speculating that it reached Oxford, England - statements taken from Boccaccio’s book; book published a few decades after Dante’s death. Most of these speculations can be justified by the admiration of readers in France and England at that time who were impressed by Dante’s writings. In Dante’s Immensa Dei dilentione testante to Henry VII of Luxembourg, he indicated his residence as in the region of the Arno springs, near the region of Tuscany - 1311. Regardless of the multiple residences in which he was, it is certain that the works of the great Sommo Poets were influenced by exile, which now had much more time to deepen philosophy, history, poetry and prose; his literary and cultural interests becoming much more prominent in this period of his life (13).

Henry VII of Luxembourg entered Italy in 1310, along with more than 5,000 soldiers; on hearing this news, Dante sees in him a new Charlemagne who will reunite the Tuscan region with the Holy Roman Empire, thus bringing to Florence the glory of yesteryear by removing the Black Guelphs. In this sense, Dante will personally write to Henry and more Italian princes and rulers, thus hoping to help liberate the city of Florence.

Henry attacked Florence and defeated the Black Guelphs in 1312, but Dante did not participate directly in this battle; largely due to the fact that he was no longer affiliated with the White Guelphs. Unfortunately, Henry died shortly after this victory, just a few months later, in 1313, and with his death came the death of Dante’s last hope of seeing Florence again. On his return to Verona in 1313, Cangrande I will offer him shelter and security to lead a relatively prosperous life; Dante later mentioning it in Paradiso XVII (14).

The politico-military agitation continued in Florence, in 1315 Ugoccione della Faggiuola took over the city and demanded a general amnesty for those in exile, including Dante, but the government of Florence still demanded that he pay a very large fine. Dante refused to pay this fine, choosing to remain in exile. Moreover, Ugguccione changed Dante’s death sentence, provided he came to the city and declared that he would never enter Florence again. Again, Dante completely refused this orphanage, and his death sentence would be extended to his sons. He still hopes late in life that he will be able to return to Florence on honorable terms (15).

The last days of the life of the “father” of the Italian language are spent in Ravenna, where he lived since 1318, being the guest of Prince Guido gives him Polenta. Dante Alighieri dies of malaria (which he contracted in Venice) on 14 September 1321, at the age of 56; his three children, most likely his wife, Gemma, and many of his admirers from neighboring towns attended the funeral. He was buried in Ravenna, at the Church of San Pier Maggiore (now called the Basilica di San Francesco). In 1483, the praetor of Venice, Bernardo Bembo, erected a tomb for Dante Alighieri (16).

On the tomb, a verse by Bernardo Canaccio, a friend of Dante, is dedicated to Florence: *“Parvi Florentia mater amoris”.*

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NEWS

MIDGUT VOLVULUS - DIAGNOSIS AND TREATMENT

Midgut volvulus is a complication of malrotated bowel, can occur at any age but most frequently appears within a month of birth. Typically, the neonate is entirely normal for a period before suddenly presenting with bilious vomiting. Plain radiographs can appear normal, can reveal bowel obstruction, double bubble sign or pneumoperitoneum. Gastrointestinal contrast study can identify the volvulus, the malrotation and sometimes can spontaneous perform reduction of the volvulus. Ultrasound findings include clockwise whirlpool sign, abnormal superior mesenteric vessels, dilated fluid-filled loops of small bowel, free intra-abdominal fluid. CT is often performed in older patients, in which presentation is non-specific and will depict whirlpool sign of twisted mesentery, malrotated bowel configuration, inverted SMA/SMV relationship, bowel obstruction, free fluid or gas. Urgent surgical repair (Ladd procedure) is required to prevent ischemia or to resect infarcted bowel loops. If resection is performed stomas are usually created. In some instances, surgical fixation of the duodenum and cecum may be performed although it is unclear if this is of benefit in preventing recurrence. Normal anatomical positioning is not achieved; the duodenum and small bowel remain on the right, and the cecum and colon are on the left side of the abdomen. (B Rasuli, F Gaillard, *et al*. Midgut volvulus. https://radiopaedia.org/articles/midgut-volvulus?lang=us).

FORMULATION AND STUDY OF RECTAL IBUPROFEN MUCOADHESIVE HYDROGELS

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FORMULATION AND STUDY OF RECTAL IBUPROFEN MUCOADHESIVE HYDROGELS Abstract): The rectal route represents an alternative to oral administration in pediatrics. Ibuprofen is a non- steroidal anti-inflammatory agent, used in all age groups to reduce pain, fever, and inflammation. **Aim:** to develop and characterize ibuprofen-containing mucoadhesive rectal gels. **Material and methods:** Carbopol 940, sodium alginate, and low viscosity hydroxypropyl methylcellulose HPMC were used as gel-forming agents and propylene-glycol and glycerin as permeation enhancers. 5% of ibuprofen was incorporated in all formulations. Three gels have been prepared containing 1% Carbopol, 0.5% Carbopol and 5% HPMC, and 5% sodium alginate. The influence of formulation variables on the pharmaco-technical parameters (adhesivity, pH, and diffusion) was studied. The amount of API released through the membrane using a Franz cell in the acceptor media was measured. Mathematical models were applied to model the release of the active substance. **Results:** The pH of gels ranged between 4.2-5.0. Viscosity varied between 165 and 212 mPas. The detachment force was from 0.02 to 0.12 N. All three gels were formulated suitable for children and adults. **Conclusions:** The obtained experimental results showed a superior release of ibuprofen from the 5% sodium alginate gel. **Keywords**: IBUPROFEN, RECTAL GEL, ADHESION, FRANZ CELL.

The oral route presents the main route of administration for all age categories. For children (from birth to 16 or 18 years) an alternative administration route is rectal, having a series of advantages such as avoiding swallowability and taste problems and administration for vomiting or unconscious children (1). This population group needs specific formulation to meet their needs in concordance with age-specific development (cognitive and motor skills). Challenges also may be associated with this administration route; patient education is a key factor to better acceptance. The rectal route may be used for both, local and systemic effects. Rectal formulations have systemic effects intended to treat pain, fever, nausea, vomiting, allergies, depression, and reflux (2-6).

Rectal pharmaceutical forms present bioadhesion, the adhesion of biological tissue, and a pharmaceutical form (7), pre-

senting enhanced bioavailability (8). Rectal gels present type 3 bioadhesion (9), an interaction between a mucin surface and a polymer. Mucoadhesive substances are characterized by numerous hydrophilic groups.

Polymers like Carbopol - polyacrylic acid, hydroxypropyl-methylcellulose (HPMC), and sodium alginate present carboxylic or hydroxyl groups and are included in the category of excellent bioadhesion properties (10). In contact with water, these polymers tend to swell, forming bonds with the mucosa (10, 11).

Ibuprofen, a propionic acid derivate, is a widely prescribed anti-inflammatory drug among pediatric patients for its antipyretic and analgesic effect (12). It is the most prescribed drug for pain and inflammation (13) being available as a prescription and over-the-counter drug (14) and approved for children above the age of 3 months (15). Ibuprofen figures on the Essential Drug List of the World Health Organization (16). It is a non-selective inhibitor of both cyclooxygenase enzymes COX1 and COX2 and is mainly metabolized by the liver (17).

Our study aims at the formulation and characterization of ibuprofen, as an active pharmaceutical ingredient (API), containing rectal gels. As gel formers, Carbopol 940, sodium alginate, and low viscosity HPMC were used. Propylene-glycol and glycerin were used as permeation enhancers. 5% of ibuprofen was incorporated in all formulations.

MATERIAL AND METHODS

Ibuprofen was a kind gift from BASF (Germany). Carbopol 940 was purchased from Lubrizol (USA), sodium alginate from FoodINC (China), and low viscosity HPMC JRS Pharma (Germany). Sodium hydroxide, ethanol, and glycerin were from Chemical Company (Romania). Propylene-glycol used was from Scharlau Chemie SA (Spain).

Table I presents the composition of the three formulated gels.

TABLE I.
Gel compositions

Component	Amount (g)			Role of component
	F1	F2	F3	
Ibuprofen	5	5	5	API
Ethanol	10	20	10	API solvent
Carbopol 940	1	0.5	-	Gel forming agent
Sodium Hydroxide solution 10%	0.4	0.2	-	Neutralizer for Carbopol
Sodium alginate	-	-	5	Gel forming agent
HPMC LV	-	5	-	Gel forming agent
Propylene-glycol	2	2	2	Penetration enhancer
Glycerin	10	10	10	Hydrating agent
Distilled water	71.6	57.5	68	Solvent

Gel preparation method. Ibuprofen was dissolved in ethanol. The Carbopol 940 gel was prepared by polymer hydration and neutralization with NaOH 10% solution. Sodium alginate and HPMC were hydrated at room temperature with water. Glycerin and propylene-glycol were added followed by homogenization. As the last step, the

dissolved ibuprofen was incorporated.

Consistency was determined using a manual *penetrometer* (VEB Feinmess Dresden) equipped with a penetration cone. The penetration rate was recorded in mms. The penetration depth was measured at room temperature. *Extensometry* was measured as the surface covered by 1 g of gel after the addition of increasing weights. *Viscosity* was determined with a Fungilab Viscolead Adv apparatus using an R4 stem, completely emersed in the sample at room temperature.

Evaluation of adhesive properties Adhesion strength was measured, in terms of the force needed to detach gels with an in-house assembled apparatus. Samples were introduced into a tubular cell with a semi-permeable membrane. The balance system determined the mass needed to detach the sample from the cell. The detachment force was calculated as F= Gxg, where G is the necessary mass for detachment and g=9.81 m/s^2. The maximum adhesive forces were the average of three measurements.

pH measurement: 25 mL solutions were prepared by dissolving 1.0 g of gel in distilled water. The pH value of each sample was measured after 10 minutes at 25°C using a pH meter (Consort C831 multi-parameter analyzer).

In vitro release study

Used apparatus: Franz cells (Perme-Gear, Germany), clear jacketed with flat ground joint, 14 mL receptor volume, pinch clamp, and stir bar. The acceptor phase was phosphate buffer pH 7.4, maintained at 37°C±1°C. From the acceptor compartment of the Franz cell, samples of 1.0 mL were withdrawn at 15 minutes for a period of 2 hours. The acceptor phase was immediately replaced with fresh phosphate buffer also maintained at 37°C ± 1°C. Hydrated Teknokroma nylon membrane (0.45 µm thickness, 25 mm diameter) was used.

The released amount of ibuprofen was determined with a UV spectrophotometer (Shimadzu UV 1800, Japan) at 273 nm wavelength.

Mathematical modeling of drug release was realized with the DDSolver Add-In Program, Microsoft-Excel software by model-dependent methods (18). Model-dependent analysis was performed with 4 most used models (tab. II) for hydrogels (19).

TABLE II.

Mathematical models and equations

No.	Mathematical model	Equation
1.	0 order kinetic	$F=k_0*t$
2.	First order kinetic	F=100*(1-Exp(-k1*t))
3.	Higuchi	F=kH*t^0.5
4.	Korsmeyer-Peppas	$F=k_{KP}*t^n$
F= % dissolved active ingredient at time "t"; k = release constant;		

RESULTS AND DISCUSSION

The obtained hydrogels are homogenous, semisolid preparations (fig. 1). F3 is colored in light yellow brown.

The texture of the hydrogels is influenced by the gel-forming agent. The highest spreadability was observed for F3, based on sodium alginate. Adding low viscosity HPMC to the Carbopol-based hydrogel modified spreadability. Penetration depth had a maximum of 13 mm for F3 (fig. 2).

Fig. 1. The appearance of the three formulated gels (A - F1, B - F2, C - F3)

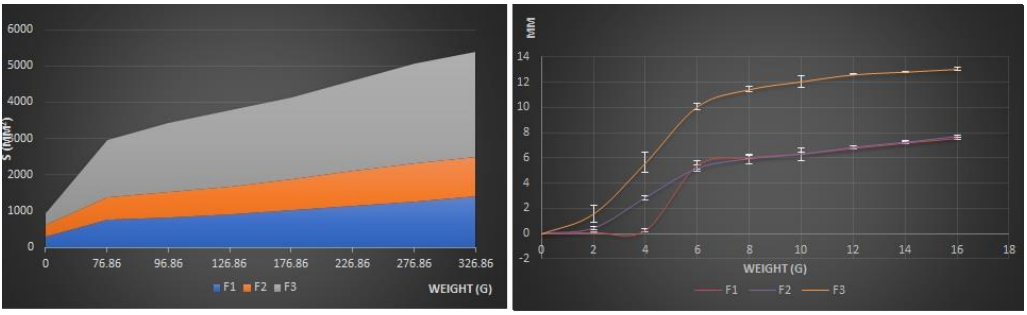

Fig. 2. Consistency measurement (extensometry and penetration)

F1 and F2 have closed pH values, for F3 pH are slightly higher. The adhesion force confirms the findings of consistency evaluation, values are closed to each other for the two Carbopol-based gels, and HPMC addition increased the detachment force. Viscosity values show the same pattern (tab. III).

Drug release had been followed for 120 minutes in a phosphate buffer at pH 7.4. After 120 minutes in all cases above 8% of ibuprofen was released. F3 hydrogel released the maximum amount of API (fig. 3).

Mathematical modeling of the diffusion profiles intends to characterize and explain drug release from the hydrogel matrix. Of the most used four models the highest R^2 values were observed in the case of the Korsmeyer Peppas model (with values over 0.98). The Korsmeyer-Peppas constant of the mathematical model describes the diffusion from the gel and n is the release exponent. If n is above 0.5, a non-Fickian drug diffusion takes place. In our case n is above 0.790 for all formulations, meaning that ibuprofen can diffuse through the hydrogel mesh, probably because it's smaller than the mesh size (tab. IV).

TABLE II.I

pH, adhesion force, and viscosity values

Formulation	pH	F (N)	Viscosity (mPa)
F1	4.72	0.104	212
F2	4.84	0.124	234
F3	5.00	0.026	165

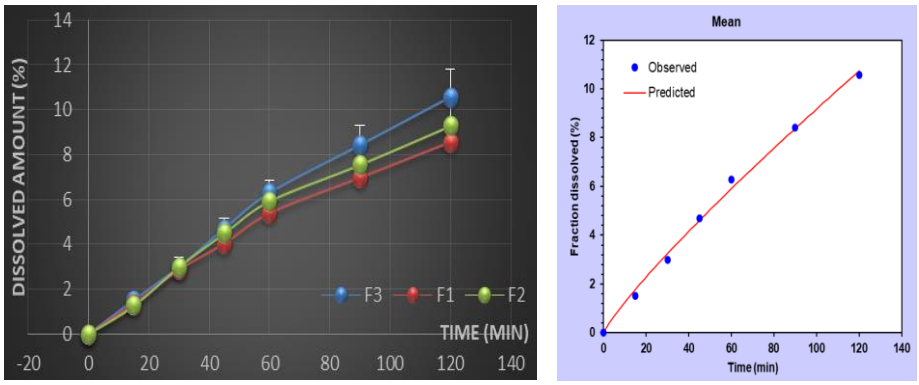

Fig. 3. Release profiles of the three hydrogels and the best fit model for F3

TABLE IV.
Values of the mathematical models

Mathematical model	Parameters	F1	R^2_adj	F2	R^2_adj	F3	R^2_adj
0 order kinetic	K_0	0.077	0.9691	0.084	0.9673	0.093	0.9862
First order kinetic	K_1	0.001	0.9757	0.001	0.9757	0.001	0.9909
Higuchi	K_H	0.693	0.9265	0.753	0.9183	0.828	0.9004
Korsmeyer-Peppas	K_{KP} and n	0.198 and 0.790	0.9946	0.207 and 0.799	0.9894	0.173 and 0.863	0.9954

F1 and F2 hydrogels are both Carbopol 940 based, probably, presenting similar mesh sizes, and similar released drug amounts. Sodium alginate hydrogel presents a superior drug release after 120 minutes, but also a disadvantage of poor mechanical strength and possible microbial contamination (19).

CONCLUSIONS

Hydrogels are versatile drug delivery systems for several administration routes. These highly hydrated networks enable the rectal administration of small molecules, such as ibuprofen. 5% API (100 mg ibuprofen in 2 g or 200 mg in 4 g of hydrogel) is intended for children, but also suitable for adult patients. The formulated mesh networks presented suitable consistency, viscosity, pH, and adhesion force for rectal administration. A superior release of ibuprofen from the 5 % sodium alginate gel was determined, the release is non-Fickian.

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CENTENARY OF THE BIRTH OF PROF. Dr. DOC. GRIGORE TEODOROVICI

Dana Baran, E. Târcoveanu, A. Ivan
"Grigore T. Popa" University of Medicine and Pharmacy Iasi

"*May the human model illustrated by Professor Grigore Teodorovici remain always present in the memory and tradition of the Iași medical school*."
Valeriu Rusu

The Society of Physicians and Naturalists of Iași, its publications and first collaborators

In 1830 "The Society of Physicians and Naturalists of the Principality of Moldavia" (SPN) was founded in Iași as a *Medical Reading Circle*. It was the first scientific association in the Romanian Countries, "the first European academy of the national spirit" or, according to Gustav Otremba, "the oldest Medical Society in the Orient". The activity of this Society took shape through: the establishment of the first medical and naturalistic library which contained materials of great documentary value (1830); the creation of the first Museum of Natural History (1834); the establishment of the first botanical garden in the Romanian Principalities (1856); promotion in Moldavia of economic and technological progress, including industrialization, but especially modernization of agriculture; this latter was due to Ion Ionescu de la Brad, organizer of model farms, remarkable statistician and professor of agriculture, who, in 1841, obtained the first Romanian champagne. As early as 1835, SPN initiated the first group scientific expeditions to explore flora, fauna, minerals, hydrologic and spa resources of Moldavia (Slănic Moldova spa resort was also scientifically analyzed). SPN supported pioneering geological (Repedea) and archaeological (Cucuteni) studies. It also contributed to the establishment of the first medical-military institutions, such as hospitals (1831) and a scientific association. SPN purchased a telescope in order to set up the first astronomical observatory (1850). It strove for national emancipation and Union of the Principalities (1859) and was directly involved in the foundation of pre-university and university institutions, including the establishment of the Faculty of Medicine in Iași (1879). Concurrently it stimulated the use of the Romanian language in science. SPN supported the medicalization process of the country, stimulating sanitary organisation, Western European type of medical and pharmaceutical assistance and health education. In the SPN history, the appearance of the first medical-pharmaceutical, economic and agricultural popularization publications in Romanian edited by a scientific society can be considered one of its most important achievements. The first attempts date back to 1844 illustrated by "The Advisor of Health and Economics. Periodical leaflet for the Romanian People" (*Povățuitorul sănătății și a economiei.*

Foaie periodică pentru poporul românesc), followed, in 1851, by the „*Leaflet of the Society of Physicians and Naturalists of the Principality of Moldavia*" (*Foaia Societății de Medici și Naturaliști din Principatul Moldovei*), both under the direction of Dr. Costache Vârnav. Since 1859, *The Society of Physicians and Naturalists of the Principality of Moldavia* turned into *The Society of Physicians and Naturalists of Iași*, being integrated into the young State of the United Principalities.

On November 28th, 1886, owing to the efforts of several hearty doctors and naturalists, most of them members of the teaching staff of the University of Iași (Faculty of Sciences and Faculty of Medicine), the decision was made to be published, both in Romanian and French, "*The Bulletin of the Society of Physicians and Naturalists of Iași*", intended to contribute to the dissemination of modern knowledge from the various fields of science and to the publication of papers authored by members of the Society. At the same time, it was meant to render Romanian research known in foreign countries and enable the exchange of the publication of the Society of Physicians and Naturalists with periodicals from abroad.

The appearance of the "*Bulletin of the Society of Physicians and Naturalists of Iași*" since *January 1, 1887,* which, from 1924, changed its name to "*The Medical-Surgical Journal of Iași. Bulletin of the Society of Physicians and Naturalists*" (French title: "*Revue médico-chirurgicale de Jassy / Bulletin de la Société des Médecins et des Naturalistes*"), generated by the increasingly rich academic life and the permanent interest of the Iași intellectuals in the development of a climate favorable to research activities. The existence of the SPN periodical has continued to this day, with some interruptions, due to the two World Wars, polarizing around it valuable collaborators from Iași, from all the medical centers of the country, as well as from abroad.

Since its early years, in the editorial board of the *Journal* one could always find prominent scientific figures of Iași, professors at the city University: the chemist Petru Poni, the geologist Grigore Cobălcescu, the philosopher Vasile Conta, the physiologist Leon Cosmovici, the parasitologist Nicolae Leon, Dr. Anastasie Fătu, Dr. Aristide Peride, Dr. Eugen Rizu and many others. Over the years, other great medical figures of Iași and SPN presidents, like Professors Ioan Ciurea, Emanoil Riegler, George Bogdan, Ernest Juvara, Constantin Bacaloglu, Constantin I. Parhon, Nicolae Hortolomei, Vasile Rășcanu, Mihai Ciucă, Alexandru Slătineanu, Grigore T. Popa, Gheorghe Tudoranu, Ion Nicolau, Alexandru Moruzi, Gheorghe Năstase, Iuliu Nițulescu, Ion Enescu, Theodor Economu, Nicolae Bumbăcescu, Gheorghe Popovici, contributed, through *The Medical-Surgical Journal* (*Revista Medico-Chirurgicală*), to the advancement of medical sciences, amplifying the torch of creative thought and multidisciplinary research (1).

Regrettably, the instauration of communism in Romania immediately after Second World War, caused the suppression of both *SPN* and *The Medical-Surgical Journal* from 1948 to 1956.

Academic milestones in the life of Professor Dr. Doc. Grigore Teodorovici (1922-1992)

Studies and beginning of his career

Among our contemporaries, it is appropriate to pay posthumous tribute to the

personality of Professor doctor docent Grigore Teodorovici, the longest-serving editor - over 30 years - of *The Medical-Surgical Journal*, the one who played an essential part in preserving its continuity and in maintaining its prestige. During its existence of 126 years *"The Society Bulletin"* (later called *"The SPN Medical-Surgical Journal")* was the forum for the most advanced ideas of the time, being appreciated in national medicine as a periodical of high standards and scientific rigor.

Grigore Teodorovici was born one hundred years ago, on April 15th, 1922, in Faraoanele commune, Vrancea County (2). He went through all the stages of pre-university education of the time, after which he attended the courses of the Faculty of Medicine of the University in Iași (1942-1948), during a difficult period that covered World War II. He was possibly also counseled and supported by one of his uncles, a famous surgeon. From the beginning he attracted attention due to his dedication to the profession he chose. He started his career in microbiology as a IVth year student, at the "Alexandru Slătineanu Bacteriological Institute". In 1946, after the refuge, the young demonstrator (*preparator)* participated in the regrouping of the Iași medical school. By then research and practice were synchronized with Western science in the creative spirit of the national school of bacteriology, virology inframicrobiology, infectious diseases, hygiene and epidemiology represented, among others, by Professors Mihai Ciucă, Petru Condrea, Ion Nicolau, Constantin Bart, Ion Alexa, Alexandrina Gaiginschi, Maria Franche, Dumitru Cornelson. In 1952, under Prof. Condrea's direction, the Iași branch of *Dr. Cantacuzino Institute of Serums and Vaccines* in Bucharest was inaugurated, and particularly efficient drugs started to be synthesized (vaccines, immunostimulants – such as *Polidin*).

Directly or indirectly, most of these scientists belonged to the Cantacuzino school of microbiology and the same was true for Professor Teodorovici. Endowed with a remarkable intelligence, with a thirst for knowledge and for deepening scientific truths, from an early age Dr. Teodorovici was noticed and appreciated by his teachers, particularly by the eminent and demanding Professor Dr. Petru Condrea. Consequently, on June 19, 1948, he was appointed assistant professor of microbiology.

The Antibiotics Factory in Iași and industrial microbiology

Educated in his Master's laboratory, Teodorovici became an elite bacteriologist, being concerned, among other things and among the first specialists in our country, with the issue of antibiotics: biosynthesis, purification and dosage, bacterial resistance (3). Recommended by Condrea, from 1952 to 1954 Dr. Teodorovici gave microbiology courses to the biologists of the Antibiotics Factory in Iași, which opened in 1955. Head of the microbiology section of the *Institute for Antibiotic Research* from 1957 until 1960, he trained the first industrial bacteriologists, and contributed to the production of the first batch of Romanian penicillin G, the first such antibiotic obtained in South-East Europe as well (3). All his life he remained connected to this pioneering Romanian Factory, where he was a professor and a permanent adviser on the problems of industrial microbiology. He intuited that the excessive use of antibiotics would generate bacterial mutants and recombinants, further leading to multiple bacterial resistance. Hence the stalemate of

antibiotic therapy was to be soon reached. Among the first, he paid attention to nosocomial infections that he generally characterized as "infectious hospitalism".

From Microbiology to Epidemiology

In 1959 he left the chair of Bacteriology for the vacant chair of Epidemiology, feeling attracted to this discipline, which, thanks to his work and talent, soon became the most appreciated in the country. Teacher, scientific researcher in the laboratory of this department, practical guide of the medical and sanitary structures in the counties of Moldavia, author of interesting articles in the field of epidemiology that have kept their validity until the present times, Dr. Teodorovici developed and consolidated prophylactic medicine in Moldavia, becoming a model for the entire country. He strengthened the team spirit, cooperating with all those who could intervene in the prevention and combat of epidemic diseases that, at the time, occurred almost in a chain, one after another, on the territory of Moldavia: exanthematical typhus, typhoid fever, diphtheria, malaria, meningitis, tularemia and Q fever and others were present. Gifted with initiative and organizational skills, Professor Teodorovici was much admired by his coworkers at the Iași Institute of Hygiene and Public Health, at the Sanitary-Antiepidemic Centers (CSAs) on the territory of Moldavia, at the "Isolation" Hospital for Contagious Diseases in Iași and at other hospitals in the territory, because, at the time, he managed to control the entire specific activity, identifying and containing the spread of epidemics in Moldavia.

In 1961, together with Prof. Dr. Eugenia Duca, he highlighted the *Friedlander's pneumobacillus* multiple resistance to all antibiotics used by then and its frequency in children hospitals. Another critical situation emerged in 1968, when Prof. Teodorovici, fruitfully cooperating with Prof. Dr. Eugenia Duca, proved, for the first time worldwide, the nephritogenic capacity of *Streptococcus zooepidemicus* (2). Teodorovici's epidemiological surveys and diagnoses were flawless. Similarly, in 1970, he epidemiologically and clinically diagnosed an outbreak of enterovirosis that subsequently laboratory tests confirmed to have been caused by *Coxsackie B6* virus. Interestingly, Prof. Teodorescu's very accurate methods for assessing sensitivity and specificity indexes of diagnostic efficacy were internationally validated only eight years later by Ransohoff and Feinstein (3).

Promoting teamwork and understanding the interdisciplinary nature of epidemiology, Prof. Dr. Doc. Grigore Teodorovici carried out successful long-term collaborations with various disciplines of the Institute of Medicine and Pharmacy (today, the University of Medicine and Pharmacy) from Iași, such as those coordinated by Prof. Dr. Alla Vâţă, Prof. Dr. Mihai Duca, Prof. Dr. Eugenia Duca, Prof. Dr. Gheorghe Zamfir, Prof. Dr. Lucia Alexa, Prof. Dr. René Duda, or Prof. Dr. Marcel Burdea, a famous pediatrician.

At the Iași Institute of Hygiene and Public Health, he worked with famed specialists, like, Dr. Nicolae Constantinescu, Dr. Olga Zavate, Dr. Copel Bercovici, but he also cooperated with renowned physicians from various health directions or Antiepidemic Sanitary Centers (CSA) of Moldavia, such as Dr. Constantin Oană, Dr. Ioan Buzdugan, Dr. Gheorghe Brebenel (2).

At the same time, he cooperated with the *Cantacuzino Institute of Sera and Vaccines* and the *Institute of Inframicrobiology* (today: *Ștefan S. Nicolau Institute of Virol-*

ogy) in Bucharest, as well as with epidemiologists specialized in agricultural, veterinarian and military issues. The discussions or debates initiated by Prof. Teodorovici were real lessons, always animated by comradeship and proceeding in harmony.

Grigore Teodorovici's School of Epidemiology in Iași

Thus, Prof. Dr. Grigore Teodorovici created a valuable school of epidemiology, and with his skills, goodwill and proverbial friendship, he attracted worthy collaborators who, over time, became his professional followers and successors: Prof. Dr. Aurel Ivan and Prof. Dr. Doina Azoicăi, who preserved the prestige of the Department of Epidemiology, reaching, on a didactic, scientific and practical level, the measure of their training Master.

Grigore Teodorovici, a sensitive man, lover of art, music and of the city of Iași, also encouraged and helped young artists to accomplish their career. Rodica Postolache, professor of French and acknowledged visual artist, is among them.

Professor Teodorovici had a noteworthy publishing activity, too. In 1950, using Professor Condrea's typed notes, as well as some materials from the similar university departments in Bucharest and Cluj, along with those of his coworkers, Prof. Teodorovici already set up the "engine" for the appearance of the first didactic material of Bacteriology and Virology, in three lithographed volumes, which remained available to students for more than half a century. The following year, in 1951, he contributed to the appearance of the first notebook of "Practical Works" which, in 1957, was edited by the Medical Publishing House in Bucharest. The treatise on *The Epidemiology of Transmissible Diseases* published in 1978 - the first on this topic in the country - illustrated a reference material for specialists for a very long time. In 1981, *The Epidemiology of Non-Communicable Diseases* was printed at the Bucharest Medical Publishing House - once again, a national priority -, with Prof. Dr. Grigore Teodorovici and his close disciple and successor at the chair, Prof. Dr. Aurel Ivan, as coauthors. The same year, the work entitled "Guidelines in Contemporary Epidemiology" was published at Junimea Publishing House in Iași.

He was a prominent teacher and a demanding researcher, with an impressive accuracy and intuition of the epidemic phenomenon, qualities that imposed him as a high-class expert among his specialist colleagues in the country and abroad. He was a role model for many doctor generations, he valued his direct collaborators, and was a supporter of interdisciplinary activities, addressing the most important and difficult community health problems. In this framework, the lifetime tandem he formed in 1959 with Professor Mihai Duca, in studying the epidemiology of influenza and acute respiratory infections, led to theoretical and practical research, followed by publications of great value, which determined the establishment, in 1964, at the Virology Department of the Institute of Medicine and Pharmacy from Iași of the "World Health Organisation National Center for Influenza Surveillance". Its investigations yielded reference results (3).

He also directed postgraduate education in preventive medicine in Iași (1972-1976). In fact, for a while, he also led the Institute for the Improvement and Specialization of Doctors and Pharmacists that belonged to the Institute of Medicine and Pharmacy of Iași.

University Professor Dr. Doc. Grigore Teodorovici and *The Medical-Surgical Journal*

Academician Prof. Dr. Vasile Râșcanu, the personality who in 1948 saved from extinction both The Society of Physicians and Naturalists and its periodical, which was later edited by Professor Dr. Teodorovici, signed in the first issue of ***The Medical-Surgical Journal*** from 1956, the article "The historical-scientific significance of *The Medical-Surgical Journal* of Iași", being aware that its success will only be real if the journal could prove its vitality. That was why, in his capacity as editor-in-chief, Prof. Rășcanu worked with an extremely strong editorial team, made up of two assistant chief-editors - Professors Șerban Brătianu and Vladimir Bușureanu -, four editorial secretaries and 50 members of the editorial board, all outstanding specialists. The results were to be seen in a short time: the four issues of the *Journal* in 1956 gathered 1,097 pages, constituting the most productive year in the entire history of the publication.

Starting with first number of 1960, Professor Grigore Teodorovici was appointed *editorial secretary*, and the *Journal* became his own life. In 1966, ten years after the resumption of the post-war publication, in the editorial "Ten years after", the balance sheet of *The Medical-Surgical Journal* looked incredible: 20 million pages had been printed, there were 2,000 annual subscriptions due to exchanges with 44 countries, 157 foreign journal titles were received. Over time, the noblest goal of the Society of Physicians and Naturalists, already formulated in the draft statute from 1832, was happily fulfilled: "To follow the progress of the medical sciences and to contribute as much as possible to them".

The Journal evolved both quantitatively and qualitatively. In 1971, thanks to Dr. Teodorovici, the first color advertisement for antibiotics appeared in the SPN Journal, and starting with second number of 1972, when he became deputy editor-in-chief, Prof. Dr. Doc. Grigore Teodorovici improved the publication's graphics and created the first color cover, in a formula that has been maintained to this day (3).

Professor Dr. Doc. Grigore Teodorovici was the one who determined the fate of the Journal, as he required value and originality for all accepted articles, but he also had to be very careful about the censorship of the time. He was an innate editor, devoted to the Journal, a fact that was evident by the way he cared about the quality of the articles various authors submitted. He reworded unclear or confusing sentences, sometimes rewrote entire passages, improving the scientific style of medical articles, all from his passion for science and professional responsibility. He solicited innovative articles from his contributors gifted with writing he trained himself, systematically trying to mobilize them, either through letters or personal discussions.

With frequent materials - documents on the history of medicine being published in the *Journal*, he equally promoted the evocation of traditions of the Iași medical school. He got involved in the celebration of 100 years of University medical education in Iași in 1979, co-authoring four articles included in the *Proceedings of the Scientific Sessions* (volumes I and II). Two focused on historical aspects: "Representative figures and stages in the field of hygiene and epidemiology at the Institute of Medicine and Pharmacy of Iași" and "The development of postgraduate education at the Institute of Medicine and Pharmacy of

Iasi". Two others pointed out epidemiological studies and surveys the Institute of Medicine and Pharmacy of Iași successfully supported: "Research directions in the epidemiology of transmissible diseases on the territory of Moldova. Twenty years of experience" and "Contributions of the Institute of Medicine and Pharmacy in Iași to the problem of bacterial etiology in the diarrheal disease in children of the young age group". He revered his surviving Masters whom he continued to ask for advice sometimes. Professor Ion Alexa was one of them.

Another hard part was working in the printing house. A special relationship was created between the Professor and the typesetters, since he conquered them with the bonhomie, gentleness, and kindness he showed to these persons whom he respected and helped every time they needed it.

With no. 1 of 1975, Prof. Dr. Doc. Grigore Teodorovici was appointed *editor-in-chief* of the periodical and did not part with the *Journal* until his death. In 1980 he coauthored a short history of the Society of Physicians and Naturalists (4) and in November 1986 - January 1987, he had the chance and joy to celebrate, in an anniversary issue (*Rev Med Chir Soc Med Nat Iași, Supplement 1986*), 100 years since the appearance of the periodical of the *Society of Physicians and Naturalists of Iași* on January 1, 1887. This supplement summed up the whole centennial history of the SPN periodical, and at the same time reflected the evolution and specific involvement of the SPN members and collaborators over the decades. Concurrently, on November 28, 1986, a plenary session, reuniting all the scientific sections of the SPN, took place at the SPN headquarters in Iași.

Professor Teodorescu's editorial duties spanned the communist era from 1956 to 1990, when, as paradoxically as it may seem today, *The Medical-Surgical Journal* went through a scientifically flourishing lapse of time (1). Its post-war period was one of "transition and reaffirmation" (6).

The whole medical university life in Iași and Moldavia revolved again around the Society and its publication, *The Medical-Surgical Journal*, whose prestige and role had increased significantly. The Society's projects were now mainly aimed at epidemiological actions, hygiene and public health whereas the other specialized sections encompassed the medical, surgical, pediatric, pharmaceutical and dental fields. The auxiliary health personnel, students gathered in two distinct sections. The *Journal* was not limited to university staff alone. Besides, it tightly synchronized to the evolution of medical education in Iași: the establishment of The Institute of Medicine and Pharmacy after 1948, with its Faculties - General Medicine, Pediatrics, Hygiene, Pharmacy and Dental Medicine - and closely mirrored new directions of medical education, research and assistance. Communication sessions of various types were mainly carried out within the Society and in its historical headquarters. In 1992, a supplement to the *Journal* appeared briefly, belonging to the SPN student section, formally established in 1974, which redefined itself in September1991 (6).

As an editor-in chief, Prof. Dr. Doc. Grigore Teodorovici understood his mission was to continue all his predecessors' efforts and adapt the publication to his day: «During the 100 years since the appearance of its first issue, the "Bulletin of the Society" later entitled "*The Medical-Surgical Journal*" - became the tribune of the most advanced ideas of the time, being appreci-

ated in the national and world scientific life as a periodical of high-quality and scientific rigor. In fact, in 1932 the Hygiene Section of the League of Nations considered "*The Medical-Surgical Journal* of Iași" as one of the "great medical publications"»… "In the last 30 years, the medical periodical from Iași experienced a very special development. Today, due to its rich and varied content, as well as technical editing conditions, it ranks alongside important and prestigious scientific publications from the country and abroad. In the current conditions of the development of medicine in Iași, *"The Medical-Surgical Journal"* reflects - first of all - the scientific concerns of the departments and circles of *"The Society of Physicians and Naturalists"* and of the teaching staff of the Institute of Medicine and Pharmacy in Iasi, their thinking and experience, the best results of national scientists, as well as the trends in contemporary medicine".

In the pages of the *Journal,* various sections are inserted, the content of which is closely related to the realities and progress of medical and pharmaceutical sciences: general reports, studies on different topics, medical news, practical medicine notes, letters to the editor, medical chronicles, the graduate forum, syntheses of some scientific manifestations, reviews. A number of articles signed by Romanian or foreign physicians are published in widely circulated languages" (1). Professor was satisfied with his work: "…our *Journal*… is accepted for exchange by over 275 periodicals from 43 countries, being sent for review and indexed by 22 publications and institutions specialized in this direction" (1).

Unfortunately, after only five years since the *Journal* centennial, severely ill, Prof. Dr. Doc. Grigore Teodorovici corrected the issues 1-2 of the 1992 *The Medical-Surgical Journal* on his hospital bed, the spirit of responsibility being a *modus vivendi* for him. His passing away in 1992, came as a shock to everyone, including the regular contributors to the publication he had led so dedicatedly for so many years. The consequences stemmed out immediately: from 240 pages, included in the first issue of the Journal in 1991, with a circulation of 1,500 copies, in no. 1 of 1993 dropped to 156 pages and to a print run of 800 copies.

Contemporaries' Tribute to Professor Grigore Teodorovici

In 2002, 10 years after his disappearance, fourteen prominent members of the Iași medical academic community, who knew him closely, commemorated him and authored laudatory articles in Supplement 1, to no.1, vol.106 of *The Medical-Surgical Journal* of the mentioned year (May 24th , 2002) (3).

Of these 14 personalities, only two are still alive, but the stories of all of them continue to testify until today lesser-known aspects of Professor Dr. Doc. Grigore Teodorovici's life, activity and time.

We retain a few appreciations and memories recorded at that moment, for their pertinent observations regarding Professor Dr. Teodorovici's personality.

Prof. Dr. Aurel Ivan: "For all of us, those who knew him and valued him for the way in which he gave himself with all his being to his work and family, Prof. Dr. Doc. Grigore Teodorovici will forever remain a role model teacher, researcher, animator of *The Medical-Surgical Journal* and Romanian epidemiology. His long and efficient didactic and scientific activity

along with his guidance of the epidemiological network of the Moldavian counties, as well as his service as editor-in-chief of *"The Medical-Surgical Journal",* placed forever Prof. Dr. Doc. Grigore Teodorovici, amongst the prominent people of the "Grigore T. Popa" University of Medicine and Pharmacy of Iași, from Moldavia and from Romania" (3).

Prof. Dr. Doc. Alla Vâță: "Professor Grigore Teodorovici dedicated himself to epidemiology, while remaining faithful to microbiology, which was his life's passion. The treatise on *The Epidemiology of Communicable Diseases* published in 1978 (the first in the country) was a reference material for specialists. He was generous to all collaborators, sharing with them his theoretical knowledge and his rich experience as an epidemiologist. Clear mind, he quickly grasped the essence of the epidemiological phenomena he studied. He wrote with great ease in a style as clear as his mind. As editor-in-chief of *The Medical-Surgical Journal*, with national and international circulation, he contributed to the appearance of this periodical, being concerned with maintaining the prestige of this representative publication for the research activity of the Iași university medical center" (3).

Prof. Dr. Dumitru Buiuc: "I was attracted by the passion and dedication with which he worked. I followed his extensive epidemiological investigations in which there was no winter, no summer, no distances. In the heat of the summer or in the whirlwind of the blizzard, he was always on the road: from Brodina in the Bucovina Obcine to Câmpuri in the Vrancei Mountains; between two roads he was always engaged in teaching activities, processing data from field and laboratory investigations, receiving friends in the evening and the collaborators with whom he was thinking about other and other investigations. He edited, issue after issue, *The Medical-Surgical Journal* that was so dear to him" (3).

Prof. Dr. Valeriu Rusu, former Editor-in-chief of *The Medical-Surgical Journal* and Dean of the Faculty of Medicine: "The Journal was his very life. Professor Grigore Teodorovici was the main author of the entire Journal for so many years. Through his scientific work, he contributed to the evolution of Romanian epidemiology. In an article *"Guidelines to contemporary epidemiology"* from no. 1/1968, new ideas concerning epidemiology were mentioned, including the study of non-infectious diseases, and the new methodology was clearly defined: its clinical, experimental, epidemiological basis. We recognize there the contours of what today is evidence-based medicine" (3).

Prof. Dr. Doc. Gheorghe Zamfir: "Having a special training in general medicine and also mastering, along with epidemiology, the fields of microbiology and virology, as well as those of general hygiene and social medicine, Prof. Dr. Doc. Grigore Teodorovici was able to respond, with particular effectiveness, to the multiple episodes and epidemic conjunctures that our province was constantly haunted by"... "An aspect of Prof. Teodorovici's activity, to which, all his lifetime, he paid particular attention was that of editor of *The Medical-Surgical Journal of Iași*. In this respect, Prof. Teodorovici identified himself with the Journal in such a way that anyone who uttered the words *"The Medical-Surgical*

Journal" meant Prof. Teodorovici. Carefully and extremely meticulously, the Professor checked every paper intended for publication, regardless of the profile (internal medicine, surgery, pediatrics, gynecology, hygiene), detecting with amazing ease any typographical or any other type of error. For this *Journal*, Prof. Teodorovici had collaborators who work hard and contributed to the prestige of the *Journal* (Gheorghe Popovici, Gheorghe Scripcaru, Traian Baran, Mihaela Groll, *et al.*)" (3).

Dr. Ioan Buzdugan: "The perfect combination of his literary and oratory talent, with a remarkable ability to synthesize scientific and practical results in the fields of laboratory, clinic and prophylactic actions, were concretized in "postulates" for modern Romanian epidemiology, so that through his presence at the University of Medicine and Pharmacy in Iași, he commanded the appreciation and respect of other university collectives in our country and even abroad... Through his exceptional efforts, *The Medical-Surgical Journal of Iași* experienced a new period of editorial and scientific glory, while he was the coordinator of this secular medical periodical of Iași" (3).

Prof. Dr. Gheorghe Scripcaru: "In the field of scientific research that he affirmed as a teacher, as well as Editor of the oldest scientific journal in the country, he expressed the conviction that you are not a convincing teacher if you do not participate in creating the science you teach, if you don't live in it, if you don't lead a common life with science, so as not to become a simple science hunter, as Grigore T. Popa said. He thus achieved the initial desire of *The Medical-Surgical Journal* to rely on the University's point of view, as science should be. The rigor of verifying and validating scientific truth was for him a cure for any disappointments, probably also a solution to despair, weapons with which he defeated the risk of easy satisfaction."

Prof. Dr. Traian Baran: "Professor Teodorovici's name came, thus, in the end, to be identified with a prosperous period in the long and arduous life and survival of *The Medical-Surgical Journal*, a period of stability, in which exchanges with over 300 similar publications on a world level definitely meant an apogee. And this, in an era when the flow of information of any kind was constantly viewed as a potential danger and systematically restricted. The constant updating of the Journal, along with the care for the representation of all the Faculties and Disciplines of the University, all the research groups, the acceptance and invitation of external collaborators, the quality of the inserted materials were priorities of the editors and, in this regard, Professor Teodorovici's discernment and tact in relations with colleagues, with people in general, his understanding, his gentle but firm character, morality and spirit of justice played a decisive role."

The message of testimonies

Professor Teodorovici undoubtedly strengthened the prestige of the University of Medicine and Pharmacy from Iași, of the Society of Physicians and Naturalists and of its famous *Journal.* The documentary and affective testimonies reveal the impact he exerted on his contemporaries, colleagues and coworkers. A man like him is rarely born, to fill with the kindness of his heart the souls of those he helped, regardless of their social and intellectual position, and with the light of his mind the minds of those who were lucky enough to have him as a teacher, mentor and role model.

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NEWS

ORBITAL RECONSTRUCTION USING MODERN TEHNOLOGY BASED ON 3D MODEL PRINTING

The use of digital assistance in reconstruction is increasingly used due to the advantages it implies, one of the most used technologies being the one based on stereolithography. The aim of this study is to analyze the advantages of this modern method used in the orbital fracture reconstruction. The study was performed on a group of 34 patients with unilateral orbital fractures. In order to reconstruct the orbit, a printed model was made based on stereolithography, the affected area being digitally reconstructed by mirroring of the healthy area. After that a titanium meshes were preoperatively shaped using the stereo lithic model, and then used during the reconstructive surgery. The reconstruction resulted in a satisfactory restoration of facial symmetry and good functional outcomes. Also, the duration of surgery was significantly reduced, and the precision of the reconstruction was greatly increased. This modern method based on the three-dimensional printed model used to repair the defects involving the orbit reduces the intraoperative time, making the postoperative result closer to the operator's expectations, and depending on the complexity of the case to restore the functionality of region (Blumer M, Pejicic R, Gander T, *et al*. Customized Titanium Reconstruction of Orbital Fractures Using a Mirroring Technique for Virtual Reconstruction and 3D Model Printing. *Oral Maxillofac Surg*. 2021; 79(1): 200.e1-200.e9 / doi: 10.1016/j.joms. 2020. 09.001).

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ORAL HEALTH STATUS AND RELATED BEHAVIOR IN ADULTS FROM NORTH-EASTERN REGION OF ROMANIA

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ORAL HEALTH STATUS AND RELATED BEHAVIOR IN ADULTS FROM NORTH-EASTERN REGION OF ROMANIA (Abstract): Oral health represents a standard for the quality of life of the individual in a society. Good oral health allows an individual socio-economic integration as well as personal development to the maximum capacity, thus making him useful to society. The **aim** of the study was to evaluate the oral health status as well as the behavioral factors that influence this status in the adult population from the N-E region of Romania. **Material and methods**: The retrospective study data were gathered via questionnaires completed by 765 participants in the "Prophylaxis Caravan" annual event between March 2022 - April 2023. We collect demographic data, information about type of medical insurance they have, the monthly income, the type of dental office they visit, about oral hygiene habits (number of daily tooth brushings, toothbrush changing, frequency of dental check-ups) and oral health status using DMFT index and CPITN index. SPSS 26.0 was used for statistical analysis, $p<0.005$. **Results:** 64.3% of the subjects brush their teeth more than once a day, and 75.8% change their toothbrush 1-3 times/per year. 35.8% go to the dentist "when needed", 59.3% of subjects go for check-ups or treatment. DMFT = 11.4, consisting of DT= 2.38, MT=6.20 and the FT=2.49. **Conclusions**: The oral health of adults in the N-E region of Romania is influenced by an important carious experience, dental edentacy having an increased value. Health promoting behavior as well as monthly income are elements that influence the quality of oral health of the population. **Keywords**: DMFT, CPITN, ADULTS, N-E REGION OF ROMANIA.

Oral health represents a standard for the quality of life of the individual in society. Good oral health allows an individual socio-economic integration as well as personal development to the maximum capacity, thus making him useful to society. The presence of untreated oral diseases produces great pressure on society, affecting the functioning in normal parameters due to the presence of pain, discomfort, deformity, and sometimes death (1). These problems can be readily avoided if patients seek dental treatment on time. Early diagnosis of oral disorders, as well as fast clinical treatments, are promoted by good oral health-seeking behavior (2).

In Romania, even though there were socioeconomic disparities across groups, oral

health in adults has improved in recent decades. This condition can be explained by inequalities in individual oral status, as well as the impact of social variables such as economic, environmental, and lifestyle factors (3).

A low socioeconomic and education level, in association with reduced access to dental services and poor oral hygiene behavior, will result in a higher frequency and severity of dental caries and periodontal disease (4-7). The studies carried out so far in the adult population in Romania indicate an increased prevalence of oral diseases, the prevalence of dental caries varying between 70-90% (8,9) and periodontal disease between 60-70%, values that can be compared with those of neighboring countries (10).

A study on the oral health of the population in the Member States of the European Union conducted in 2010 of the percentage that in Romania, a percentage of 30% of the population declared that they are all natural. Among those who no longer have all their natural teeth, 14% are partially or totally edentulous. 32% have difficulty chewing due to dental lesions, and approximately 16% have experienced dental or periodontal pain. 16% were embarrassed by the aesthetic appearance of their teeth - the first place in Europe, while 81% believe they can reach a dentist within 30 minutes from home or work if necessary. The results of a study carried out in Iasi in 2019 regarding the level of knowledge and skills regarding oral health, showed that 35% of the participants chose toothpaste with a whitening effect, and almost 25% considered the type of toothpaste teeth rather insignificant (11).

The socioeconomic and cultural factors associated with the level of knowledge and attitudes accumulated regarding oral health have an impact on oral status. Thus, the aim of the study was to evaluate the oral health status as well as the behavioral factors that influence this status in the adult population from the N-E region of Romania.

MATERIAL AND METHODS

After receiving clearance from the Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa" Iasi, Romania (no.322/08.06.2023), this retrospective observational study was undertaken among adults from the N-E area of Romania. The information was gathered via questionnaires presented to participants in the "Prophylaxis Caravan" oral health screening annual event.

The following criteria were used to select participants: adults over the age of 18 from Romania's N-E region, who signed informed consent and completed the full questionnaire after receiving explanations about the study under the condition of anonymity. We utilized the calculation procedure with a confidence level of p = 95% to an adult population of 3.7 million people (12), and the sample size was 385 adults (13). Because adults completed 765 of the total surveys completed at the previous activity, we chose all questionnaires completed by adults between March 2022-April 2023.

In addition to demographic questions (age, gender, residence, occupation), the questionnaire included questions about the type of medical insurance they have, their monthly income, the type of dental office they visit, and questions about oral hygiene habits (number of daily tooth brushings, toothbrush changing, frequency of dental check-ups.

The DMFT index was used to evaluate the oral health status, measured by adding

the number of decaying (DT) teeth, the number of missing (MT) teeth, and the number of filled (FT) teeth (D) (14).

To evaluate the periodontal status, we used the Community Periodontal Index of Treatment Needs (CPITN) as a method of screening that evaluates the presence or absence of periodontal pockets, calculus, and gingival bleeding. Scores from 0 to 4 are given for each tooth examined, and finally, the need for periodontal treatment is established according to the score obtained on each sextant (15).

SPSS Software Version 26.0 (*SPSS*® Inc., Chicago, IL, USA) was used to produce descriptive statistics and evaluate the data gathered. Frequency, percentages, averages, and standard deviations were used to show descriptive data. The most essential characteristics that can define person's attitudes about oral health are age, gender, occupation, monthly income, and the frequency of visits to the dentist. To assess the differences between variables, t Student test was utilized. A multivariate regression analysis was performed to assess the predisposition for an affected oral health condition. A p-value of 0.05 or less was judged statistically significant.

RESULTS

First table shows the analysis results regarding the study group's demographic data. Thus, of the total of 765 participants, 53.7% are female subjects, 81.6% come from the urban zone and 55% are unemployed (student, unemployed, retired). The average age was 47.68±17.42 years (min. 19 - max. 82 years) (tab. I).

Regarding the distribution by socio-economic 31.9% of participants have a high level, 49.7% a medium level, and 18.4% a low level. From a financial point of view, 63.5% of the participants have a monthly income > 501 €, and 64.2% are self-financed, followed by 35.8% of those who have state insurance. 78.8% of the participants frequent private clinics (tab. I).

TABLE I.

Distribution (%) of subjects according to sociodemographic (N= 765).

		No.	%
Age	47.68±17.42 (min.19, max.82)		
Sex	Female	411	53.7
	Male	354	46.3
Residence	Urban	624	81.6
	Rural	252	18.4
Occupation	Employee	344	45
	Unemployed (student, unemployed, retired)	421	55
Socio-economic level	High level	244	31.9
	Medium level	380	49.7
	Low level	141	18.4
Monthly income	EUR <500	279	36.5
	EUR >501	486	63.5
Payment for dental services	State insurance	274	35.8
	Private insurance and self-funded	491	64.2
Preferred dental clinic	Private clinic	603	78.8
	State clinic	162	21.2

The evaluated oral health habits indicate that 64.3% of the subjects brush their teeth more than once a day, and 75.8% change their toothbrush 1-3 times/per year. Regarding the number of visits to the dentist, 35.8% go to the dentist "when needed" and 34.1% "once a year". The reasons to visit a dentist were for 59.3% of check-ups or treatment and for 40.7% for dental emergencies (tab. II).

TABLE II.
Oral health habits

		No.	%
Oral hygiene habits			
Number of brushing/days	1 toothbrushing /day	273	35.7
	>1 toothbrushing /day	492	64.3
Number of toothbrushes/years	1-3 times/year	580	75.8
	>3 times/year	185	24.2
Number of visits to the dentist/ year	Less than 1 year	43	5.6
	Once a year	261	34.1
	Once in 5 years	187	24.4
	When needed	274	35.8
Reasons to visit a dentist	Check-up or treatment	454	59.3
	Emergency	311	40.7

The evaluation of the dental status achieved by determining the DMFT index indicates an important carious experience, DMFT having an average value of 11.4, consisting of DT (decayed teeth)= 2.38, MT(missing teeth)=6.20 and the FT (filled teeth) component=2.49. The increased value of the extracted teeth component is observed. In relation to certain socio-demographic variables, dental status is more affected in the case of male subjects (DMFT=12.16), in those with a low socio-economic level (DMFT=15.65), and in those unemployed (DMFT=12.68). The recorded differences were statistically significant for all evaluated variables (p=0.000) (tab. III).

In the case of the periodontal status evaluated through the CPITN index, the evaluation indicates an increased frequency of subjects presenting dental calculus, followed by those with superficial pockets (up to 5mm deep) and those with gingival bleeding. The distribution of the results in relation to the socio-demographic variables shows that male subjects (25.7%), with a high socioeconomic level (26.6%), employed (31.1%) show increased values for the dental calculus, while female subjects (16.8%), with medium socio-economic level (16.1%), unemployed (21.9%) show increased values for superficial periodontal pockets. The recorded differences were statistically significant (p=0.000) (tab. III).

Certain sanogenic habits as well as income can influence the adult's oral status. Thus, those who brush their teeth once a day have higher DMFT values (13.67) compared to those who brush more than once a day, the difference being statistically significant (p=0.000). The same trend is recorded in the case of those who go to the doctor "when needed" (toothache) (12.42) but also in those who go to the doctor only once a year (11.82). Although monthly incomes >501 euros/month, the value of

the DMFT index was higher (11.64) compared to the value recorded in the case of those with incomes < 500 euros/month (9.99) (tab. IV).

TABLE III.

Oral health status *vs.* gender, socioeconomic level and occupation

	Mean value	Gender		Socio-economic level			Occupation	
		Female	Male	High level	Medium level	Low level	Employee	Unemployed
Odontal status								
DT	2.38	2.14	2.65	2.00	2.31	3.21	2.44	2.32
MT	6.20	5.75	6.71	3.95	6.13	10.26	3.57	8.35
FT	2.49	2.22	2.49	2.61	2.47	2.32	3.02	2.06
DMFT	11.04	10.07	12.16	8.56	10.92	15.65	9.03	12.68
p		0.000**		0.000**			0.000**	
Periodontal status - CPITN								
Healthy	51.0%	47.9%	54.5%	54.1%	49.7%	48.9%	55.2%	47.5%
Bleeding	6.8%	8.5%	4.8%	9.0%	6.8%	2.8%	8.7%	5.2%
Calculus	23.5%	21.7%	25.7%	26.6%	23.4%	18.4%	31.1%	17.3%
Shallow pockets	14.0%	16.8%	10.7%	8.2%	16.1%	18.4%	4.4%	21.9%
Deep pockets	4.7%	5.1%	4.2%	2.0%	3.9%	11.3%	0.6%	8.1%
p		0.017*		0.000*			0.023*	

*t student test, **ANOVA test, statistically significant differences when p<0.05

TABLE IV.

Factors related to oral health habits and dental service's frequency of utilization

	Odontal status				***P***
	DT	MT	FT	DMFT	
Number of brushings/day					
1 toothbrush /day	3.07	7.89	2.79	13.67	0.000
> 1 toothbrush/day	1.99	5.26	2.32	9.58	
Number of visits to the dentist/ year					
less than once a year	3.30	4.28	2.33	9.91	0.000
once a year	2.65	6.46	2.71	11.82	
twice a year	1.71	3.57	2.89	8.17	
when needed	2.42	8.05	2.03	12.42	
Monthly income					
< 500 euro	1.52	6.76	1.72	9.99	0.000
> 501 euro	2.87	5.88	2.93	11.64	

ANOVA test, Statistically significant differences when p<0.05

TABLE V.

Multivariate regression between gender, occupation, monthly income (independent variables), and dependent dental status and hygiene attitudes.

	B	Std. Error	Sig.	OR	95% Confidence Interval for Exp(B)	
					Lower Bound	Upper Bound
DMFT >10	.869	.215	.000			
[Gender=Male]	-.492	.161	.002	.611	.446	.838
[Occupation=employed]	-.759	.179	.000	.468	.329	.665
[Socio-economic level=high]	-.994	.238	.000	.370	.232	.590
[Socio-economic level=medium]	-.863	.208	.000	.422	.281	.635
[Monthly income=<500 euro]	-.634	.183	.001	.531	.371	.759

The multivariate logistic regression (table 5) shows us that in the case of the number of tooth brushings, male subjects have a 0.611 greater predisposition to have a DMFT>10 than female subjects (p = 0.002, OR = 0.611), the same positive trend being recorded and in the case of those employed (p = 0.000, OR = 0.468), those with high socio-economic level (p = 0.000, OR = 0.370) and medium (p = 0.000, OR = 0.422) as well as for those with monthly income <500 euros (p=0.001, OR=0.531).

DISCUSSION

Oral health, which has become a serious public health issue in every country, regardless of the degree of development, is an indication of individuals' standard of life and education (16-18). Socioeconomic and cultural factors impact the oral health of Romanians, who, despite the assistance of the medical insurance system and an increased number of dentists, have poor oral health (19-23).

The aim of our study was to evaluate the oral health status and the behavioral factors that influence this status in a group of adult patients from the N-E region of Romania. For this purpose, we analyzed the indicators of the oral status (dental, periodontal) and the behavioral factors that affect attitudes related to oral hygiene, visits to the dentist, reasons for going to the doctor or monthly income, factors that influence access to medical services

The analysis of the collected data showed us that more than half of the subjects brush their teeth more than once a day, change their toothbrush 1-3 times/per year and less than half of participants go to the dentist "when needed" or "once a year". The reasons to visit a dentist were for 59.3% of check-ups or treatment and for 40.7% of dental emergencies. This kind of behavior can explain the high carious experience, with a DMFT index having an average value of 11.4. The increased value of the extracted teeth component is observed. The dental status is more affected in the case of male subjects, those with a low socio-economic level, and unemployed. The mean DMF index in this study was

higher than in other previously studies (24-28).

In the case of the periodontal state, as measured by the CPITN index, the results show an increased incidence of participants with dental calculus, followed by those with superficial pockets (up to 5mm deep) and gingival bleeding. The distribution of the results in relation to socio-demographic variables reveals that male subjects with a high socioeconomic level who are employed have higher values for dental calculus, whereas female subjects with a medium socioeconomic level who are unemployed have higher values for superficial periodontal pockets. These findings vary from those of other research in which female respondents have superior periodontal health due to higher levels of dental care knowledge and attitudes (29, 30).

Certain sanogenic habits, as well as income level, can influence the adult's oral status (31-33). In our study those who brush their teeth once a day have higher DMFT values (13.67) compared to those who brush more than once a day. The same trend is recorded in the case of those who go to the doctor "when needed" (toothache) (12.42) but also in those who go to the doctor only once a year (11.82). The results are like those of the studies carried out so far (34-36).

The income level is linked to oral diseases and a poor quality of life in terms of oral health (38, 39). In our study, although monthly incomes >501 euros/month, the value of the DMFT index was higher (11.64) compared to the value recorded in the case of those with incomes <500 euros/month (9.99). The studies carried out so far support the fact that socioeconomic factors might impact dental service use. In Romania, dental treatments are generally supplied in private dentist offices, and patients pay for treatment out of their own pockets and access to these services is influenced by many factors (40).

Inequalities in access to medical services are found in all countries of the world, the pattern is similar in all European countries. Improving access to medical services can be done through better education regarding oral health as well as financial support for dental treatments, with the stimulation of preventive attitudes related to oral health. Currently, the costs for oral health care amount to significant values because patients in Romania present themselves to the doctor at an advanced stage of the disease, which entails high treatment costs (41, 42).

CONCLUSIONS

The results of our study show us that the oral health of adults in the N-E region of Romania is influenced by an important carious experience, dental edentulism having an increased value. Sanogenic behavior as well as monthly income are elements that influence the quality of oral health of the population.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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IN SILICO SCREENING OF SOME OLD DRUGS FOR NEW APPROACHES IN HEART FAILURE

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IN SILICO SCREENING OF SOME OLD DRUG FOR NEW APPROACHES IN HEART FAILURE (Abstract): **Aim**: The aim of the present study is to discover new approaches for old drugs, targeting heart failure (HF), using *in silico* studies. **Material and methods**: Several old drugs, belong different chemical and therapeutical classes: oral hypoglycemic drugs (sulfonylurea), proton pump inhibitors, nonsteroidal anti-inflammatory drugs, statins, H_1/H_2 antagonists, antineoplastic drugs, alfa-adrenergic agonists, vitamins and antioxidants, were tested for affinity to glycogen synthase kinase 3 beta (GSK-3β), using *in silico* molecular docking studies. Indirubin-3'-monoxime (I3M) and adenylyl-imidodiphosphate (AMP-PNP), were used as GSK-3β reference inhibitors. Moreover, the drugs with the best docking score for GSK-3β, were included in an ADME-Tox study to predict their pharmacokinetic profile. **Results:** The used computational method was validated using RSMD variation (less than 2 Å) recorded for GSK-3β inhibitors (I3M and AMP-PNP). The results showed that the binding of drugs to GSK-3β involve direct hydrogen bonds and van der Waals interactions with key amino acids from active site, Lys85, Asp133, Val135, Glu137, Arg141, Gln185, Asp200 and Arg220. **Conclusions**: Based on *in silico* results, in term of affinity for GSK-3β and ADME Tox profile, several drugs have theoretical premises to have beneficial effects in HF and could be propose for repurposing approach, which look of finding new clinical indications for existing drugs. **Keywords**: IN SILICO, HEART FAILURE, OLD DRUG, GSK-3β, REPURPOSING.

Heart failure (HF), a final common pathway of many cardiovascular diseases, develops when the heart is no longer able to provide adequate blood flow/pressure in response to the body's needs. This in turn induces the activation of several compensatory mechanisms, initially beneficial, which over time contribute to disease progression. These compensatory mechanisms include salt and water retention by the kidneys, activation of neurohormones, and triggering intracellular signaling cascades in the heart and vasculature, resulting in alterations of the cellular and organ morphology and function, with myocardial loss, left ventricular hypertrophy, or a combination of both (4). HF is characterized by severe morbidity and mortality, increasingly prevalence, poor

quality of life, and a high burden on health care systems in terms of resource use and expenditures (10). With an alarming statistic of more than 64 million people worldwide (19) suffering from HF, this syndrome is a major and growing public health problem worldwide.

In 2019, The Heart Failure Association Atlas survey observed that the median incidence of HF in Europe was 3.20 cases per 1000 person/year, ranging from ≤2 in Italy and Denmark to >6 in Germany; the prevalence was even more variable, ranging from ≤12 in Greece and Spain to >30 in Lithuania and Germany (6, 13). In the USA, the estimated 2020 prevalence of HF was 6.9 million which is expected to increase by 24% to nearly 8.5 million in 2030 (19). In Romania, approximately 4.7% of individuals aged 35 and older are affected by HF, which translates to a total of around 560.000 patients (9).

Despite significant improvement in the current therapies, patients with advanced HF have very few options other than cardiac transplantation. This is firstly due to extremely limited regenerative potential of the adult mammalian hearts. Due to this poor regenerative capacity, the injured heart has no alternate to adapt and compensate (adverse ventricular remodeling) to maintain the pump function for the time being (7). Unfortunately, adverse remodeling leads to a significantly scarred hearts, which will fail over the time. The proliferation of pre-existing cardiomyocytes (CMs) represents the primary mechanism to promote the cardiac regeneration (16). Therefore, strategies to stimulate the proliferation of pre-existing CMs are the most cited approaches to repair the injured heart. To achieve this, the research has focused towards identifying the growth factors and signaling pathways capable of enhance endogenous cardiac regenerative potential. It was identified several key pathways to take back the CMs proliferative capacity, which include (i) inhibition of Hippo pathway, (ii) direct activation of cell cycle regulators, (iii) activation of Neuregulin (NRG)-1/ErbB signaling, and (iv) inhibition of glycogen synthase kinase (GSK)-3 signaling (20).

GSK-3 is a widely distributed serine/threonine kinase initially recognized for its role in controlling glycogen synthase activity and maintaining glucose balance. It exerts control over various cellular functions, including cell growth, metabolic processes, programmed cell death, and embryonic development (17). The GSK-3 family consists of two isoforms, namely GSK-3α and GSK-3β. These isoforms have been demonstrated to exhibit both overlapping functions and isoform-specific unique roles in maintaining organ homeostasis and contributing to the development of various diseases (18). Several recent studies have highlighted the cardioprotective properties of various GSK-3β inhibitors in counteracting myocardial injury induced by hypoxia-reperfusion. Additionally, phosphorylation of GSK-3β has shown promising effects in alleviating myocardial damage caused by myocardial hypertrophy, cardiac fibrosis, cardiomyopathy, and parasympathetic dysfunction (15). Furthermore, Lin et al. explored the potential of lansoprazole (a proton pump inhibitor) in protecting against cardiac hypertrophy and HF, the fundamental mechanism involving the deactivation of the Akt/GSK-3β/β-catenin pathway, which effectively attenuated cardiac remodeling (8)

Discovery of new drugs is a very slow process, time consuming, very expensive and often it fail in best results, and related to these aspects the finding new ways to successfully convert novel ideas into day-to-day practice is crucial to improve healthcare. The new cited strategy consists in *drug repurposing*, which is a strategy used to discover new

applications for existing drugs (2).

The aim of this paper is to discover new approaches for old/existing drugs for HF, using *in silico* molecular docking studies, targeting the affinity of the drugs to GSK-3β enzyme. Moreover, the drugs with the best docking score for GSK-3β, were included in an ADME-Tox study to predict their pharmacokinetic profile.

MATERIAL AND METHODS

In silico GSK-3β docking study

For this research it was used AutoDock-GPU software, which enables fast calculations with the AutoDock4.2.6 scoring function and which facilitates the docking of very large libraries of molecules, as well as modeling of molecules of increasing size and complexity (7, 12). A large library of conventional drugs, which are currently not use for treatment of HF, was screening in terms of affinity for GSK-3β, as follow: (i) oral hypoglycemic drugs (sulfonylureas), (ii) proton pump inhibitors; (iii) nonsteroidal anti-inflammatory drugs (NSAIDs); (iv) statins; (v) H_1/H_2 antagonists; (vi) antineoplastic drugs; (vii) alfa-adrenergic agonists; (viii) vitamins; (ix) antioxidants. The results, expressed as Gibbs free energies values (ΔG), were compared to indirubin-3'-monoxime (I3M) and adenylyl-imidodiphosphate (AMP-PNP), used as reference GSK-3β inhibitors.

Generate the receptor coordinate file (RCF)

X-ray crystallographic structure of the GSK-3β was selected in terms of the quality of the model obtained from RCSB Protein Data Bank and was processed prior to docking (7). From downloaded experimental structures, was removed the ligands, the water molecules, cofactors and ions that should not be included in the receptor by a text editor. After that, each receptor was converted to PDBQT format file using AutoDock4.2.6 by reading the coordinates, adding charges, merging nonpolar hydrogens and assigning appropriate atom types.

Generate the ligand coordinate file (LCF)

Dimensional structures of the tested drugs were sketched in ChemDraw and after that were converted to PDB coordinate files using Chimera 1.14. Each LCF contain special keywords recognized by AutoDock4.2.6 like ROOT, ENDROOT, BRANCH, and ENDBRANCH that establish a rigid set of atoms and rotatable groups of atoms that are connected to the rigid root. As well, TORSDOF was used in estimating the change in free energy caused by the loss of torsional degrees of freedom upon binding. Each structure was energy minimized and converted to PDBQT format file. To study the interaction between a single ligand with a single receptor, with explicit calculation of affinity maps, a docking method was used. The receptors were kept rigid, and the ligands were allowed to be flexible (12).

Preparing the Grid Parameter File (GPF)

The GPF specifies the parameters for generating the atomic affinity maps and the PDBQT files for the receptor. For GSK-3β complex was used a grid box of 95×86×81 points with a spacing of 0.375 Å between grid points and the grid box center was put on x = 19.614, y = 11.232 and z = −6.299. The binding site of GSK-3β ligands was identified by using protein visualization software such as PyMOL, Chimera 1.14 and DSvisualizer.

Preparing the docking parameter file (DPF)

The DPF consist of which grid map files to use, which ligand molecule to dock, what is its center and number of torsions, which docking algorithm to use and how many runs

to do. For conformation search, the Lamarckian Genetic Algorithm (LGA) was applied, with the following parameters: number of runs for each docking procedure (200), number of individuals in the population (300), the maximum number of 27,000 generations simulated during each LGA run, the maximum number of evaluations at 25,000,000, a mutation rate of 0.02 and a cross over rate of 0.80, while the remaining docking parameters were set to default (7). After performing molecular docking simulation of the selected ligand molecules against the GSK-3β, the best ligand molecules were evaluated on the basis of their score against the GSK-3β receptor. All the results obtained by molecular docking simulation were evaluated on the basis of hydrophilic and lipophilic interactions obtained between the binding residues present in the active ligand binding site of the macromolecule and ligand. The dockings experiments were clustered with a root mean square deviation (RMSD) of 0.5 Å and evaluated by PyMol software.

In silico ADME-Tox study

ADME-Tox (Absorption, Distribution, Metabolism, Excretion and Toxicity) study offers important data to predict the pharmacokinetic properties and toxicity degree of new candidates, during the drug discovery process (5). The ADME-Tox profile of the tested compound was predicted using *pkCSM-pharmacokinetics* (http://structure.bioc.cam.ac.uk/pkcsm) and *SwissADME* (http://www.swissadme.ch) tools. *SwissADME* provide an overall assessment of the pharmacokinetics profile of small molecules by most relevant computational methods (14) and *pkCSM-pharmacokinetics* is a new method based on graph signatures and experimental data (11). The methods have been selected for robustness, but also for ease of interpretation to enable efficient translation to medicinal chemistry. For these *in silico* tools, the methods design description, the methods validation information and the datasets used, are provided in the literature.

RESULTS AND DISCUSION

In silico GSK-3β docking results

To predict the binding mode of the tested drugs with the GSK-3β, an X-ray crystallographic structure was selected in term of the quality of the atomic model obtained from the crystallographic data. The PDB 1J1B file was selected to study the predicted binding mode of the ligands with GSK-3β enzyme. GSK-3β was described as the kinase structure with the highest resolution to date, 1.80 Å, which means that there is a more confidence in the location of atoms in the electron density map. Furthermore, the reliability factor (R-value) was less 0.25, indicating a strong agreement between the crystallographic model and the experimental X-ray diffraction data.

The used computational method was validated using RSMD variation (less than 2 Å) for I3M and AMP-PNP, based on their affinity to the active site of GSK-3β, and it was found the same binding cleft residues reported in literature (12). Moreover, the docking results showed that both reference inhibitors have polar interactions with the catalytic site of GSK-3β, bounding deeply by specific interaction with Lys85, Asp133 and Val135. According to reported data (7), the residues Lys85, Asp133, Val135, Glu137, Arg141, Gln185, Asp200 and Arg220 are key amino acids for interactions with the binding pocket of GSK-3β.

In order to calculate the Gibbs free energies (ΔG) of tested drugs, each ligand-receptor complex was subjected to careful analysis for ideal docked position, based on the least binding energy scores and maxi-

mum number of cluster conformations, and the results are presented in first table.

The analysis of the results revealed that the estimated free binding energy of the reference GSK-3β inhibitors was less than -7 kcal/mol (AMP-PNP -8.2 kcal/mol, and I3M -7.34 kcal/mol). Based on these values, it can appreciate that compounds which obtained lowest docking score need more less energy to stabilize the compound at the ligand binding center of GSK-3β receptor and have theoretical premises to be more potent inhibitors than references. The analysis of the results revealed that there are several GSK-3β inhibitors, belong different chemical structures and therapeutic classes, more potent than references, such as: (i) sulfonylureas (oral hypoglycemic drugs): gliquidone (-11.78 kcal/mol), glimepiride (-10.25 kcal/mol), glibenclamide (-9.72 kcal/mol), glipizide (-9.41 kcal/mol), gliclazide (-8.22 kcal/mol); (ii) statins (cholesterol-lowering drugs): atorvastatin (-9.67 kcal/mol), simvastatin (-9.48 kcal/mol), fluvastatin (-9.12 kcal/mol), lovastatin (-9.08 kcal/mol), rosuvastatin (-7.88 kcal/mol); antioxidants (polyphenolic compounds): rutin (-9.41 kcal/mol), curcumin (-8.92 kcal/mol), resveratrol (-7.43 kcal/mol); vitamins: vitamin E (-8.89 kcal/mol), riboflavin (-7.59 kcal/mol); NSAIDs: indomethacin (-8.68 kcal/mol), ketoprofen (-7.77 kcal/mol); proton pump inhibitors: omeprazole (-8.47 kcal/mol), rabeprazole (-8.33 kcal/mol), lansoprazole (-7.82 kcal/mol).

Referring to the binding mode of tested drug to GSK-3β it was observed that all compounds interact by direct hydrogen bonds and van der Waals interactions with amino acids from active site. For example, the binding of gliquidone in the cleft formed between the N– and C– terminal lobes of GSK-3β, is based on interactions of sulfonylurea moiety with Asn64, Lys183, Gln185, Asn186 and Asp200 and of 1,3-dioxo-2-isoquinolinyl moiety with Arg141 *via* hydrogen bonds (fig. 1).

TABLE I.

The Gibbs free energies (ΔG) for GSK-3β of tested drugs compared to the values recorded for AMP-PNP, I3M, as GSK-3β inhibitors

Ligand	GSK-3β ΔG (kcal/mol)	Ligand	GSK-3β ΔG (kcal/mol)
Gliquidone	-11.78	Sitagliptin	-7.73
Glimepiride	-10.25	Riboflavin	-7.59
Glibenclamide	-9.72	Resveratrol	-7.43
Atorvastatin	-9.67	Coenzyme Q10	-7.38
Simvastatin	-9.48	**I3M**	**-7.34**
Rutin	-9.41	Diclofenac	-7.22
Glipizide	-9.41	Pantoprazole	-7.08
Fluvastatin	-9.12	Cetirizine	-6.98
Lovastatin	-9.08	Dipyridamole	-6.85
Ezetimibe	-8.99	Biotin	-6.85
Linagliptin	-8.94	Naproxen	-6.79
Curcumin	-8.92	Famotidine	-6.78
Vitamin E	-8.89	Ibuprofen	-6.78
Demethoxycurcumin	-8.88	Naphazoline	-6.63
Indomethacin	-8.68	Xylometazoline	-6.6

Ligand	GSK-3β ΔG (kcal/mol)	Ligand	GSK-3β ΔG (kcal/mol)
Bisdemethoxycurcumin	-8.62	Antazoline (Phenazoline)	-6.59
Omeprazole	-8.47	Tetryzoline	-6.51
Methotrexate	-8.47	Phenytoin	-6.48
Rabeprazole	-8.33	Oxymetazoline	-6.36
Gliclazide	-8.22	Dexmedetomidine	-6.27
Epalrestat	-8.21	Tramazoline	-6.24
AMP-PNP	**-8.2**	Ranitidine	-6.24
Pioglitazone	-8.11	Vitamin B1	-6.21
Saxagliptin	-8.11	Lipoic acid	-6.1
Tolrestat	-8.09	Pantothenic acid	-5.62
Retagliptin	-8.06	Cimetidine	-5.61
Rosiglitazone	-7.91	Nizatidine	-5.61
Rosuvastatin	-7.88	Vitamin B6	-5.48
Alogliptin	-7.84	Cytarabine	-5.46
Vildagliptin	-7.83	Ascorbic acid	-4.81
Lansoprazole	-7.82	Metformin	-4.79
Ketoprofen	-7.77	Adenine	-4.74
Sitagliptin	-7.73	Flucytosine	-4.44

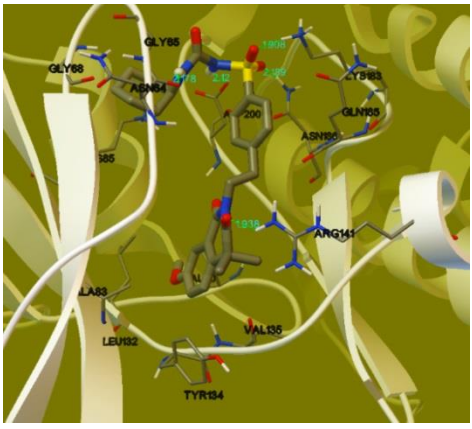

Fig. 1. The binding mode of gliquidone to the active site of GSK-3β.

***In silico* ADME-Tox results**

For ADME-Tox evaluation were selected the drugs with the best docking score. The distribution properties, based on the blood brain barrier (BBB) permeability, the volume of distribution (VDss) and the fraction unbound in plasma protein descriptors were predicted and the results are summarized in second table.

For tested drugs, the calculated values of VDss ranged from -1.918 to 1.663 and of fraction unbound ranged from 0 to 0.676. It is know that only the unbound fraction of the drug is active whereas the high binding to proteins can affect both the pharmacokinetic and pharmacodynamic profile of drugs (14, 15). In order to establish the statistical significance of the difference between the VDss and fraction unbound while removing ligand variances from the overall error variance, a two-way ANOVA analysis was applied. The results established there is no statistically significant difference between the two parameters ($F(0.5, 1, 38) = 17.202$, $p = 0.001$, Fcrit = 4.098). So, the lower VDss values are in agreement with lower value for the fraction of drug molecules in the plasma which is unbound (free) to proteins.

The BBB permeability is assessed using

two parameters, one qualitative (BBB) and other one quantitative (logBB). Depending on the BBB parameter value, the drugs are classified as drugs with high and low permeability, respectively. More specific, a logBB value higher 0.3 means that drug readily cross the BBB while a value less than −1, indicates a reduced permeability for this barrier (54). The BBB permeability values of tested drugs were predicted to range from -0.876 to −1.899 which means that are poorly distributed to the brain.

The drugs which have the best volume of distribution (VDss<0.5) and poorly blood brain barrier permeability (BBB<−1) belong from different chemical classes, such as: (i) benzimidazoles (proton pump inhibitors): lansoprazole (VDss = -1.944, BBB = -0.162); (ii) sulfonylureas (oral hypoglycemic drugs): glibenclamide (VDss = -1.918, BBB = -1.162), glipizide (VDss = -1.228, BBB = -0.908), gliclazide (VDss = -0.667, BBB = 0.108); (iii) polyphenolic compounds (antioxidants): demethoxycurcumin (VDss = -1.633, BBB = -0.563); resveratrol (VDss = -0.974, BBB = -0.961); (iv) 1,3-thiazolidines (oral hypoglycemic drugs): rosiglitazone (VDss = -1.347, BBB = -1.671); (v) cyanopyrrolidine (oral hypoglycemic drugs): saxagliptin (VDss = -1.013, BBB = 0.245). The values recorded for GSK-3β references inhibitors were VDss = -1.605, BBB = 0.236 (I3M) and VDss = 0.284, BBB = -0.912 (AMP-PNP), respectively.

TABLE II.

Distribution profile of tested drugs, predicted using pkCSM-pharmacokinetics and SwissADME tools

Ligand	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
Gliquidone	-0.339	0.22	-0.978	-2.737
Glimepiride	-0.218	0	-1.01	-2.674
Glibenclamide	-1.918	0.089	-1.162	-2.916
Atorvastatine	0.2	0.098	-0.26	-2.095
Simvastatine	1.663	0.187	-1.899	-5.178
Rutin	-0.249	0.247	-1.043	-3.366
Glipizide	-1.228	0	-0.908	-2.454
Fluvastatin	0.182	0.125	-0.265	-2.187
Lovastatin	-0.436	0	-0.017	-1.992
Ezetimibe	0.26	0.236	-1.509	-2.958
Linagliptin	-0.215	0	-0.562	-2.99
Curcumin	0.709	0	0.876	-1.669
Vitamin E	-0.075	0	-0.337	-2.458
Desmethoxycurcumin	-1.633	0.077	-0.563	-2.026
Indomethacin	0.139	0.045	-0.089	-2.271
Bisdemethoxycurcumin	0.28	0.251	-0.288	-2.622
Omeprazole	-0.097	0.259	-1.617	-3.794
Methotrexate	0.126	0.238	0.067	-2.806
Rabeprazole	0	0.278	0.079	-2.344
Gliclazide	-0.667	0.186	0.108	-2.323
Epalrestat	-0.007	0.017	-0.591	-2.496

Ligand	VDss (human)	Fraction unbound (human)	BBB permeability	CNS permeability
AMP-PNP	0.284	0.558	-0.912	-4.593
Pioglitazone	0.043	0.603	-0.227	-2.69
Saxagliptin	-1.013	0.057	0.245	-2.195
Tolrestat	0.207	0.416	-1.576	-3.387
Retagliptin	-0.183	0.078	-0.727	-2.785
Rosiglitazone	-1.347	0.044	-1.671	-3.674
Rosuvastatin	0.555	0.405	-0.603	-2.746
Alogliptin	0.586	0.569	-0.652	-3.129
Vildagliptin	0.086	0.245	-0.01	-2.452
Lansoprazole	-1.944	0.075	-0.162	-2.156
Ketoprofen	0.154	0.544	-0.339	-3.186
Sitagliptin	-0.196	0.676	-1.552	-4.144
Riboflavin	0.296	0.166	-0.048	-2.067
Resveratrol	-0.974	0.146	-0.961	-1.176
Coenzyme Q10	0.095	0.096	-0.926	-1.72
I3M	-1.605	0	0.236	-1.97
Diclofenac	0.219	0.318	-0.798	-3.473
Pantoprazole	0.832	0.177	0.211	-2.54

CONCLUSIONS

The repurposing or repositioning of existing drugs is a new drug discovery strategy, which gaining increasing interest over the traditional de novo drug discovery, because is faster and less expensive than the last one. Using AutoDock-GPU software, which enables fast calculations with the AutoDock4.2.6 scoring function, more than 60 existing drugs, from several chemical and therapeutic classes, were assessed for their affinity to GSK-3β, as new approach in HF management. The most promising drugs, in term of affinity to GSK-3β, expressed as Gibbs free energy (ΔG), are gliquidone, glimepiride (antidiabetic drugs), simvastatine, atorvastatine (cholesterol-lowering drugs), curcumin (a powerful antioxidant) and indomethacin (an important NSAIDs). For these drugs, the value recorded for ΔG ranged between -11.78 kcal/mol (gliquidone) and -8.68 kcal/mol (indomethacin), much better than the values recorded for GSK-3β reference inhibitors, AMP-PNP (-8.2 kcal/mol), and I3M (-7.34 kcal/mol). These drugs showed also a good predictable pharmacokinetic profile, having best volume of distribution (VDss<0.5) and poorly blood brain barrier permeability (BBB<−1). The most favorable drugs will be included in large study, based on *in vitro* and *in vivo* assays, in order to prove the *in-silico* results.

CONFLICT OF INTEREST. Authors declare that there is no conflict of interest.

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THE DEVELOPMENT OF ALGINATE-BASED MATRICES LOADED WITH RIFAMPICIN FOR WOUND HEALING

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THE DEVELOPMENT OF ALGINATE-BASED MATRICES LOADED WITH RIFAMPICIN FOR WOUND HEALING (Abstract): The proposed study aims to develop polymeric systems based on alginate and N-vinylcaprolactam (NVCL) coated with poly(D,L-lactide-co-glycolide) (PLGA) as vehicles for Rifampicin (RIF) as active substance. Rifampicin is an agent used for tuberculosis treatment, but the present study proposes to be used for wound healing. The proposed topic is a complex study comprising a lot of data; herein information on Rifampicin loaded matrix and its antimicrobial activity against the main strains of the following bacteria: *S. aureus*, *MRSA*, *E. coli*, *P. aeruginosa*. **Materials and methods**: Alginate and NVCL were used as main materials for polymer preparation then coated with PLGA; the polymeric matrix was loaded with Rifampicin as active substance. The preparation of polymer consisted of various steps such as polymerization technique, water in oil in water emulsification and then matrix lyophilization for characterization in solid state. Chromatography was used to indicate the presence of the drug (TLC) and then HPLC was used to quantify the amount of it. The antimicrobial activity was tested by culturing the tested substances against some bacteria e.g. *S. aureus*, *MRSA*, *E. coli*, *P. aeruginosa*. **Results:** The tested matrix showed positive results in terms of drug loading and antimicrobial activity and will be further investigated for IC50 and cytotoxicity. **Conclusions**: Based on the obtained results, the proposed rifampicin-loaded alginate-based matrix shows potential in wound treatment, including surgical wound care. **Keywords**: RIFAMPICIN, ALGINATE MATRICES LOADED, WOUND HEALING.

Advancements in medical technology have led to improvements in patient care, compliance, and outcomes. Significant strides have been made in the development of materials used during or after surgical procedures. These procedures typically

involve incisions with specialized instruments in an operating theatre, often requiring anesthesia and/or respiratory support. Surgical materials, including adhesives, sealants, hemostatic agents, wound covers, absorptive sponges, and sutures, are now expected to meet specific criteria. They should be non-toxic, biocompatible, and supportive of cellular proliferation for tissue regeneration, while also possessing sufficient mechanical and physical properties for durability. Naturally sourced biopolymers, which are biological macromolecules metabolized within the body, have gained recognition for their compatibility with the biological environment. Unlike synthetic polymers, they typically do not provoke chronic inflammatory or immunological reactions or toxicity due to their similarity to the extracellular matrix. Furthermore, modifying the structure or processing conditions of biopolymers can enhance their functionality, physical-mechanical properties, regenerative capabilities, adsorptive properties, and more. Biopolymers, which are naturally occurring polymers, are typically produced by living organisms and can be sourced from microbial systems, extracted from plants, or synthesized chemically from basic biological systems. They offer several advantages over synthetic polymers, including a well-defined and more intricate structure, biocompatibility, functionality, degradability, and renewability. However, they also have drawbacks such as poor mechanical properties, lower producibility, and a dependence on various environmental factors. Nonetheless, with appropriate functionalization, their properties can be significantly enhanced. The most utilized biopolymers in surgical materials include chitosan, alginate, hyaluronic acid as polysaccharides, collagen, gelatine, fibroin as proteins, and biocompatible polymers like polylactic acid derivatives. In the subsequent section, we will outline the general properties of these key biopolymers and their roles in surgical applications. Alginic acid, a polysaccharide extracted from brown seaweeds, possesses distinct physical properties that make it valuable as a rheology modifier in various applications such as food products, paper consumables, printing inks, and biomaterials for medical and pharmaceutical preparations. Its biocompatibility and biodegradability have sparked significant research interest. Like chitosan, alginates are copolymers comprised of repeating units of guluronic acid (G) and β-D-mannuronic acid (M), linked via 1,4 glycosidic bonds. In molecular terms, they are C5 epimers of each other. The orientation of the carboxyl group (–COOH) on the C5 carbon of the six-membered saccharide ring is above the plane of the ring in the M unit and below the plane in the G unit. The polymer chain of alginate contains blocks of guluronic acid, mannuronic acid, and alternating sequences thereof. The viscosity and gel-forming capacities are key characteristics of alginate, heavily influenced by the block structure and chain length.

Among the polymers exhibiting a Lower Critical Solution Temperature (LCST) close to physiological conditions (32-34°C) are poly(N-vinylcaprolactam) and poly(N-isopropylacrylamide), rendering them suitable for biomedical applications. Poly(N-vinylcaprolactam) (PNVCL) is a water-soluble, non-toxic, thermosensitive, and biocompatible polymer categorized under N-vinylamide polymers. In comparison, although poly(N-isopropylacrylamide) (PNIPAAm) is extensively studied as a thermosensitive polymer, PNVCL exhibits even higher biocompatibility due to the

direct linkage of the amide group to the hydrophobic backbone chain (C–C). Consequently, the hydrolysis of the amide group in PNVCL does not yield small amide compounds, which are undesirable for biomedical applications and limit the biocompatibility of PNIPAAm (1, 2, 3).

The combination with biocompatible monomers such as N-vinylcaprolactam with alginate can lead to functional biocompatible materials which impart both the characteristics of alginate and NVCL. The present study aimed to prepare and characterize the nano/microparticles of alginate grafted from NVCL and covered with a shell of PLGA for applications like wound healing, particularly, healing after a surgical operation. Within the present study, there will be reported the loading of the selected model drug i.e. Rifampicin and the antimicrobial activity against 4 strains of bacteria such as *S. aureus*, *MRSA*, *E. coli*, *P. aeruginosa*.

MATERIALS AND METHODS

Chemicals

Alginic acid (AgA, Sigma Aldrich product) was utilized, along with N-vinylcaprolactam (NVCL), as monomer, achieved from Sigma Aldrich. The initiator system comprised ammonium persulphate (APS, Sigma Aldrich) and 50% hydrogen peroxide solution. The reaction was carried out in ultra-pure distilled water as the solvent. To create the nanoparticle suspension, a 0.5 wt.% acetic acid glacial solution was employed. Purification of the copolymer was achieved using a dialysis bag with a cut-off of 3.5 kDa. Rifampicin (RIF), 99% purity, with a molecular weight (Mw) of 822.95 g/mol sourced from S.C. Antibiotice S.A. Iasi, Romania. Poly(D,L-lactide-co-glycolide) 50:50 (PLGA), Sigma Aldrich product was used to form the Nano/microparticles. All other chemicals and reagents used in this study met analytical grade standards or exceeded them and were procured commercially.

Preparation of grafted biopolymer

The grafted biopolymer i.e. alginate grafted N-vinylcaprolactam (NVCL) was synthesized by using the free radical polymerization method in water by using as initiating systems of azobisisobutyronitrile (AIBN) under nitrogen flow within a ratio of 0.1% against the monomer (NVCL). The proposed mechanism was represented within the Fig. 1. The structure identification was assessed by FTIR spectroscopy.

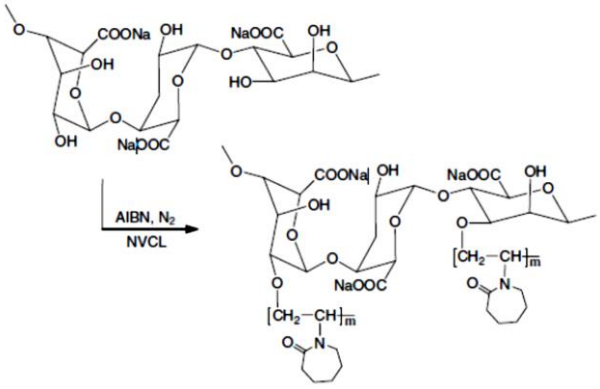

Fig. 1. The proposed mechanism for the synthesis of the polymeric matrix

Preparation of PLGA coated alginate grafted from NVCL

The prepared biopolymeric matrix was used further to synthesize nano/microparticles without and containing Rifampicin, the selected drug model for the post-surgery wound healing.

An amount of prepared biopolymer was dissolved in water within a concentration of 0.1 wt.% and then Rifampicin was added to the polymeric solution to ensure a good blending and formation of electrostatic bonds with the hydrophilic groups of the biopolymer. The theoretical amount used for the Rifampicin loading within the matrix was 0.5 wt.% against the polymer amount. The mixture was stirred for couple of hours then lyophilized.

The biopolymeric matrix without Rifampicin was dissolved in twice distilled water within a concentration of 0.1 wt.%. The oil phase solution was prepared by dissolving 0.1 g PLGA (50/50) in dichloromethane (DCM) and Span 80 was added to ensure a good stability of the emulsion. The oil phase was probe sonicated for 10 minutes then the water phase was added dropwise under strong stirring under vortex to prepare water-in oil emulsion. The first emulsion was again probe sonicated then added dropwise in the second water phase consisting of 5 wt.% polyvinyl alcohol low molecular weight, Tween 80 and salt to give the final water-in-oil-in water (W/O/W) dispersion. The solvent was evaporated at room temperature, and the system was kept under stirring overnight. The final dispersion was then centrifugated at 15000 rpm under a temperature of 5°C to collect the nano/microparticles of N-vinylcaprolactam grafted alginate (AgA-g-NVCL) coated with PLGA. The schematic representation of the procedure was illustrated within second figure.

The formation of particles containing Rifampicin was performed similarly except the fact that in the water was dissolved the matrix loaded with Rifampicin. The drug content was determined by means of high-performance liquid chromatography (HPLC).

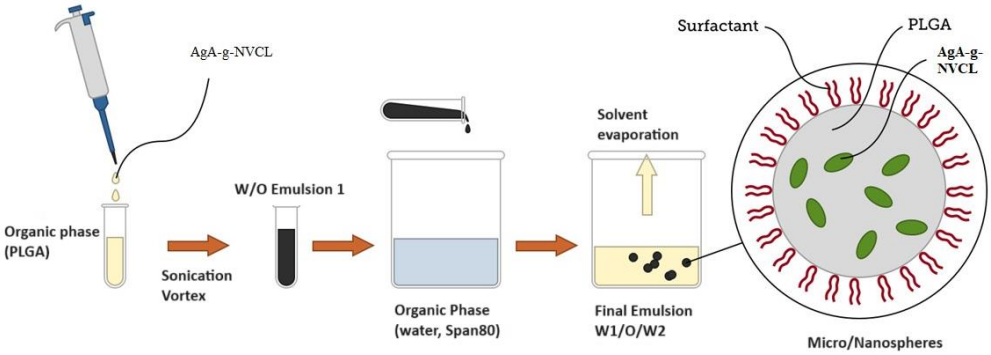

Fig. 2. Preparation scheme of Micro/Nanospheres of grafted alginate-NVCL coated with PLGA shell

Fourier transform infrared spectroscopy (FTIR) analysis

The structure was confirmed by FT-IR spectra of the copolymer films which were recorded with a Perkin-Elmer Spectrum-100 ATR-FTIR instrument by scanning in

the range of 600-4000 cm^{-1}.

Thin layer chromatographic chemical screening (TLC)

Thin Layer Chromatography (TLC) was used to separate components in the analyzed compounds. After separation, each compound shows horizontal bands at different points; each band has a specific retention factor (Rf) which be determined by using equation (1):

$$\mathbf{Rf} = \frac{\text{distance traveled by sample}}{\text{distance traveled by solvent}} \quad (1)$$

The biopolymer loaded Rifampicin was dissolved in ethanol with a concentration of 1 mg/mL. Chloroform: methanol (9:1) solvent mixture used as mobile phase to screen and determine the separation. Approximately 10 mL of mobile phase was placed into a rectangular glass tank and covered with a glass lid; the solvent-based mixtures were allowed to saturate for 10 min before use. 2 µL of each sample were placed with a capillary tube on the DC Kieselgel 60 F_{254} silica gel (0.2 mm thickness was purchased from Merck), total 5 drops. As a reference sample was used the pure drug marked as standard and placed onto silica gel as well. After separation, spots were detected at 254 nm.

HPLC method

The amount of Rifampicin was analyzed by using an Agilent 1260 high-pressure liquid chromatograph (HPLC) system. The system was equipped with UV detector and Chrompack C18 (250×4.6 mm) column, Santa Clara, CA, USA. UV detection was performed at 238 nm for Rifampicin and the HPLC conditions were applied according to the HPLC method developed by Chellini *et al.* (4). Gradient elution was carried out with a mobile phase A of 20 mM monobasic sodium phosphate buffer with 0.2% triethylamine (pH 7.0) and acetonitrile and mobile phase B was acetonitrile, at a flow rate of 1.5 mL/min. The total run time was 12 min, and the re-equilibration time was 5 min. Gradient elution was carried out as follows: 100% mobile phase A was first held for 5 min, then mobile phase B was raised up to 52% in 7 min, mobile phase B was held at this level until 12 min, and at 12.01 min, mobile phase A was switched back to 100% until 17 min (re-equilibration). The flow rate was 1.5 mL/min, and the injection volume was 25 µL. Limit of detection (LOD) and limit of quantification (LOQ) were evaluated. For the determination of Rifampicin amount, a calibration curve was done by preparing serial standard solutions within a concentration range of 0-1000 µg/mL and recorded the absorbance obtained at 238 nm, specific for RIF. For this purpose, approximately 20 mg polymeric matrix loaded with Rifampicin was weighted, then sonicated and dissolved in 250 µL CH_3OH; the solution was filtrated and 100 µL were transferred to a new vial and injected 25 µL into the chromatographic column.

Antimicrobial activity

There were used 4 bacterial strains (*S. aureus, MRSA, E. coli, P. aeruginosa*) isolated from patients with common infections. The pure and fresh culture (18 hours) of each of the 4 bacterial strains were tested against Rifampicin-loaded nanoparticles (RNPs), Rifampicin pure substance (RIF) and unloaded nanoparticles (NPs), following the Kirby-Bauer disk diffusion protocol (10^8 CFU/mL bacterial suspension, homogenous inoculation of Mueller Hinton blood

agar (5). The tested antimicrobial substances were prepared to respect the classical antibiotic discs: 6 mm in diameter. The diameter of inhibition area was measured in mm, in transmitted light, after 18-24 hours incubation at 35°C.

RESULTS AND DISCUSSION

Matrix characterization

The structure of the synthesized biopolymer was analysed by means of FTIR spectroscopy and FTIR spectrum of the prepared polymer was represented within fig. 3.

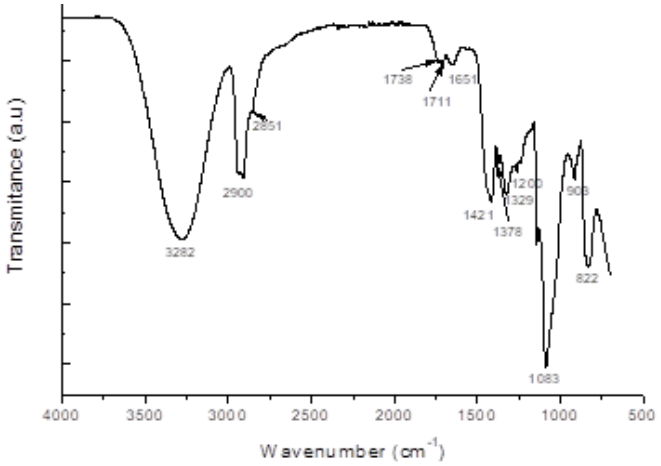

Fig. 3. FTIR spectrum of AgA-g-NVCL

During the synthesis procedure, NVCL as monomer, containing vinyl groups was polymerized and connected with alginate via ester bonds. Exploration of the FTIR spectra of the final grafted copolymer is in fact verifying whether the monomer has transformed into polymer and ensuring that the characteristic peak of vinyl groups at 1626 cm^{-1} attributed to the –C=C–stretching vibration has disappeared, showed that polymerization occurs by opening the carbon-carbon double bond without any change in the caprolactam ring. The peaks at 2926 cm^{-1} and 2856 cm^{-1} correspond to the aliphatic C–H stretching. The peak of C–N stretching vibration was observed at 1421 cm^{-1} and the peak at 3282 cm^{-1} can be assigned to N–H stretching vibrations. Similar results were found in the case of a NVCL homopolymerization, the spectrum showing similar trendline (6).

Thin layer chromatography chemical screening (TLC)

The qualitative assessment of the drug loading was performed by TLC chemical screening. The loaded sample was compared with the pure drug as standard. The Fig. 4 described the UV images recorded at 254 nm of the sample with Rifampicin and pure Rifampicin as standard. Using a silica gel plate and ethyl acetate as the development solvent, the pure drug as standards and by comparison, the loaded sample with Rifampicin showed spots having Rf's of 0.55 and 0.58, and 0.52 the latter one. Comparing these Rf polymeric matrix loaded with Rifampicin with the Rf's of standard, a similarity between the elution spots could be observed; the difference being just in the intensity due to the concentration differences.

Considering the hydrophobic character of Rifampicin, the identification of the spot belonging to the drug was facile, the results confirming the presence of Rifampicin within the polymeric matrix. Similar results were found by Abdelwahab *et al.* (7), where values of 0.44 for Rf were determined but similar trendline of elution column.

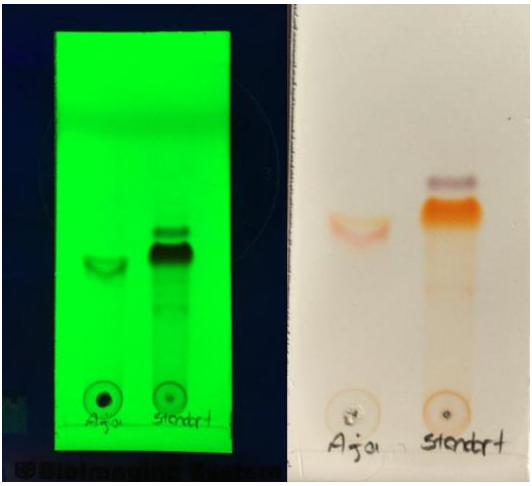

Fig. 4. The chromatographic separation of the standard substance (Rifampicin) and the loaded matrix wit Rf (left). UV images at 254 nm

HPLC method

The quantitative assessment of the drug loading was performed by HPLC analysis by using the method described with the section 2. The retention time of Rifampicin using the described method was found to be around 12.6 minutes (fig. 5).

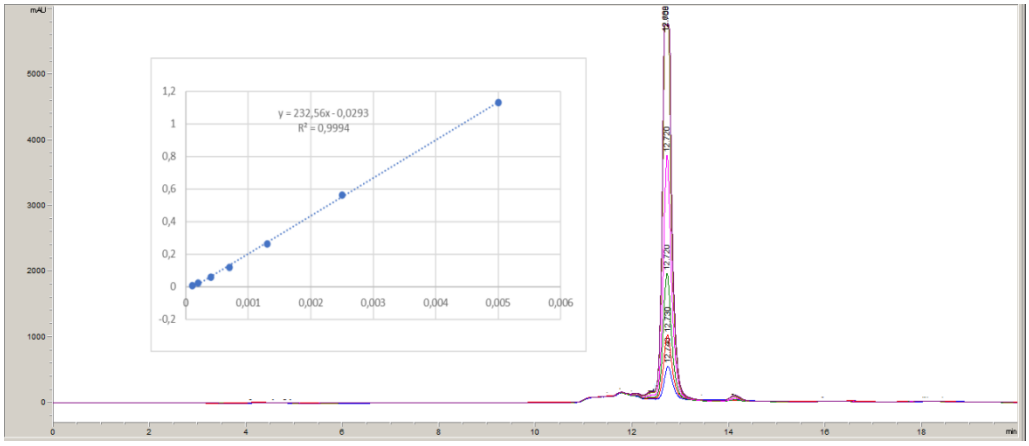

Fig. 5. Representative chromatogram of Rifampicin with its characteristic peak and the calibration curve based on HPLC analysis

The determined limit of the detection LOD and limit of quantification LOQ were found to be 0.7132 and 2.1623 µg/mL respectively. The amount of Rifampicin found in 20 mg was determined by using the calibration curve of being 160 µg/mL, a 26.66% loading as compared with the amount loaded initially. This result was considered for further studies *in vitro* (e.g. cytotoxicity, IC50 determination, etc.).

Antimicrobial activity

S. aureus, MRSA, E. coli, P. aeruginosa are bacterial strains with a high potential to produce infections difficult to be treated, if only *mecA* gene of *S. aureus* and extended-spectrum β-lactamase (ESBL)-producing *E. coli* is good to be mentioned. Other reports are mentioning the high prevalence of infected wounds with similar strains as we tested (8, 9, 10), which underline the importance of developing new therapeutical strategies. Our results are promising, but to be implemented in clinical practice we should validate our developed therapeutical drugs using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline (11).

The antimicrobial activity was evaluated and RNPs was found to have a good antimicrobial action against all 4 microbial strains. Fig. 6 described the images of cultured blood agar plates inoculated with *S. aureus, MRSA, E. coli, P. aeruginosa*, tested at RNPs, RIF and NPs.

The diameters of inhibition areas are summarized in first table.

As observed both from table I and figure 6, the greatest area of inhibition is against *MRSA* and comparable to those against *S. aureus* and *P. aeruginosa*. NPs have a good antimicrobial action against *MRSA* and *E. coli*, which recommends this type of matrix for its use in therapy after loading with antimicrobial substances, to which it will give added efficacy. It should be noted that on *P. aeruginosa*, neither Rifampicin has antimicrobial action and even less so NPs, which was to be expected, while RNPs have good antimicrobial action, and so they are particularly valuable for use in healing superinfected wounds or for preventing superinfection in immunocompromised patients.

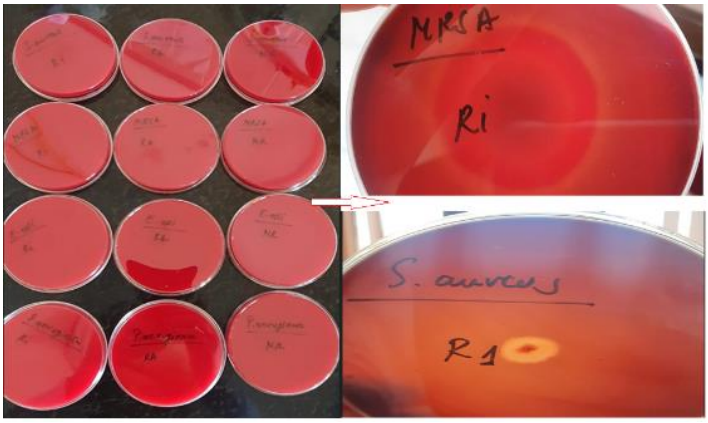

Fig. 6. Images of blood agar plates inoculated with *S. aureus, MRSA, E. coli, P. aeruginosa*, tested at RNPs, RIF and NPs

TABLE I.

The diameters of inhibition areas for the Rifampicin-loaded nanoparticles (RNPs) comparative with the Rifampicin pure substance (RIF) and the unloaded nanoparticles (NPs)

	RNPs		RIF		NPs	
	24 h	48 h	24 h	48 h	24 h	48 h
S. aureus	35	40	complete inhibition	complete inhibition	5	10
MRSA	41	45	1/2 inhibition	1/2 inhibition	15	26
E. coli	24	26	30	30	18	18
P. aeruginosa	32	36	-	-	-	-

RIF was expected to have very good efficacy, as proven on *S. aureus* (complete inhibition) and *MRSA* (1/2 inhibition), and against *E. coli*, but the side effects of high concentrations of Rifampicin are not to be neglected and therefore RNPs are a viable and highly effective alternative in the care of postoperative wounds/sores/burns in general.

In all cases the zone of inhibition increased slightly after 48 hours, which indicates maintenance of the antimicrobial activity for a long time, from which it follows that the application to the wound of the pharmaceutical form with RNPs (as ointment, dressing, patch, etc.) can be done at a long interval and will thus improve patient compliance to proper wound care.

CONCLUSIONS

The study had the purpose to prepare materials suitable for the treatment of post-surgical wounds by using Rifampicin as the active substance. The first step within this complex study was to select a proper matrix, i.e. polymeric matrix which has the role not only to protect the active substance but also to release it at the target site without creating a burst delivery. Moreover, the matrix should be biocompatible and non-toxic for the human body. Considering the data from literature a copolymer of alginic acid and N-vinylcaprolactam seemed a good choice and it was tested from the perspective of loading and matrix compatibility with Rifampicin and its antimicrobial activity against very powerful bacteria commonly found in post-surgical infections (e.g. *S. aureus*, *MRSA*, *E. coli*, *P. aeruginosa*). Rifampicin loading in the PLGA-coated alginate and PNVCL copolymer matrix was demonstrated qualitatively by TLC and quantitatively the drug loading was found to be 26.66% using the HPLC method. As regarding its antimicrobial activity, it was found out that the inhibition against the bacterial agents increased slightly even after 48 hours, indicating the persistence of the antimicrobial activity for a long time, making the systems suitable for wound healing, including post-surgical wound treatments, when this time of 48 hours is extremely important.

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CONFLICT OF INTEREST AND FUNDING

The authors declare no conflicts of interests. This research did not receive external funding.

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THE MEDIATING ROLE OF SLEEPING PROBLEMS, BURNOUT SYNDROME, STRESS, DEPRESSIVE SYMPTOMS, AND SELF-EFFICACY ON THE RELATIONSHIP BETWEEN ATTITUDES TOWARDS APPEARANCE AND BODY DISSATISFACTION AMONG ROMANIAN YOUNG PEOPLE

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THE MEDIATING ROLE OF SLEEPING PROBLEMS, BURNOUT SYNDROME, STRESS, DEPRESSIVE SYMPTOMS, AND SELF-EFFICACY ON THE RELATIONSHIP BETWEEN ATTITUDES TOWARDS APPEARANCE AND BODY DISSATISFACTION AMONG ROMANIAN YOUNG PEOPLE (Abstract): The **aim** of the study was to evaluate the presence of anxiety, depression, burnout, and dissatisfaction with body image among young people in Romania. **Materials and methods:** A total of 432 respondents aged 18-30 were included in the research, and several instruments were used: SES-SQ evaluated the socioeconomic level; DASS 21 for the severity of central symptoms in the sphere of depression, anxiety, and stress; the BSQ items assessed body dissatisfaction and concern over body shape; the GLQ questionnaire identified scores related to physical activity, cognitive activities, social activities, other leisure activities, and other behaviors that significantly influence health; and the SATAQ-4 assessed socio-cultural attitudes towards appearance by measuring the pressure from peers, family, and the media to attain the ideal appearance, internalization of the thin/low body fat and internalization of the muscular or athletic body image. The statistical analyses were performed using IBM *Statistical Package for Social Sciences (SPSS) for Windows, version 24* (SPSS Inc, Chicago, IL, USA). **Results**: Sex, the presence of a chronic disease, marital and socioeconomic status was found to be important in depression, anxiety and satisfaction with the body image. Age was found to be related to some of the variables: the older the respondent was, the lower the levels of stress, depression or anxiety. Additionally, age correlates negatively with burnout and sleeping problems, in the sense that as one ages, burnout levels decrease, and sleep disturbances become less prevalent. Negative correlations were also identified between age and the pressure felt from the media. Young people with chronic diseases had a statistically significantly higher level of internalization of thin/low body fat. **Conclusions:** The results showcased a significant correlation between factors such as age, gender, marital and socio-economic status and the impact of stress, depression, anxiety on the perception of body image and related levels of dissatisfaction. **Keywords**: SLEEPING PROBLEMS, BURNOUT SYNDROME, STRESS, DEPRESSIVE SYMPTOMS, SELF-EFFICACY, BODY DISSATISFACTION.

INTRODUCTION

Contemporary society places significant emphasis on self-perception and the manner which body image is perceived by the social group. Numerous studies have highlighted the link between mental health and satisfaction with body image among children and adolescents, but a lack of body satisfaction is very prevalent among adolescents. While this pertains more to weight or the existence of physical abnormalities in children, it frequently involves a desire for aesthetics and group approval among adolescents and young adults; thus, we are discussing group pressure and social pressure.

Media channels and social networks reinforce this desire to have a perfect body, and virtual reality makes it easier to satisfy the desire to look the way everyone wants: selfie posts, photo editing, or video manipulation are common.

The lack of satisfaction with body image and appearance has negative consequences on the physical, mental, and social health. According to Fox *et al.* (1), girls and young women report the feeling of pressure to conform to gender and beauty norms more frequently, and they are more likely to post photos that show them as more attractive. The position of self-objectivation (seeing themselves as a third party) is associated with poor health, shame, low self-esteem, anxiety, depression, sleep-related problems, eating disorders, and low cognitive performance (2, 3). Several studies have indicated that individuals who have low body image satisfaction are more critical of themselves when they are in a negative mood (4). Furthermore, the literature does not distinguish between men and women in this regard. For example, Matias and his team (5) showed that body dissatisfaction is associated with psychological concerns among both genders, but these concerns do not represent a motivational incentive to adopt healthy behaviors. Therefore, many researchers mentioned that the psychological impact of body dissatisfaction should represent a public health concern due to its negative consequence on psychological balance and general quality of life, including sleep disorders (6), depression (7), eating disorders (8), physical exercises (9), burnout syndrome (10), and self-efficacy (11, 12). Self-efficacy is one of the most important factors related to the process of changing behaviors and adopting healthy ones, referring to the subjective prediction of one's ability to accomplish a certain task or fulfill a specific goal (13, 14).

The aim of the present study was to identify the mediating role of sleeping, burnout syndrome, stress, depressive symptoms, and self-efficacy on the relationship between attitudes towards appearance and body dissatisfaction among Romanian young people.

MATERIALS AND METHODS

Participants. The study was carried out between September and November 2023, and the questionnaires were distributed online to young people on social networks (Facebook, WhatsApp). Participants were informed about the purpose of the study, about the fact that they could withdraw from the study whenever they wanted, without consequences, and about the confidentiality of data. No incentive was given to respondents. The inclusion criteria were young people aged between 18 and 30 years old who agreed to participate in the study and filled out the questionnaire before the deadline. The exclusion criteria were incomplete questionnaires, in which the young people

did not express their consent to participate, and questionnaires filled in by people over 30 years old. After applying the inclusion and exclusion criteria, a total of 432 questionnaires were included in the research.

Data collection. The online questionnaire was developed using the Google Forms application (Alphabet, Mountain View, CA, USA), to obtain detailed information about satisfaction with body image, acceptance of dominant socio-cultural standards for appearance, as well as the influence of stress, depression, and anxiety on these variables.

The first part of the questionnaire gathered socio-demographic information, such as age, gender, marital status, living environment, educational level, the presence or absence of chronic disease diagnosis, the type of diagnosed disease (if applicable), and socioeconomic status). The body mass index (BMI) was calculated considering the weight and height declared by the participants.

Some items investigated cultural and religious characteristics and nutrition-related habits as well as physical activity, such as the frequency of consuming home-cooked meals, preference for foods that require little preparation time, frequency of dining at restaurants, the importance of consuming locally-produced food, the adherence to religious fasting, the consumption of food supplements or slimming teas, as well as performing physical exercises to maintain body shape. Additionally, a few items investigated the respondents' availability to accept minimally invasive aesthetic procedures or aesthetic surgeries to beautify or change the facial appearance, as well as the use of photo editors or filters when displaying photos on various social networks (e.g, Facebook, Instagram etc.).

Socioeconomic characteristics were evaluated using SES-SQ developed by Roohafza *et al*. (15). The SES-SQ consisted of six items. As the authors sustained, the evaluation of socioeconomic status is a major predictor of health and nutritional status as well as mortality and morbidity related to many diseases.

The next part of the questionnaire included the following psychological instruments:

- DASS 21 - is a set of three self-assessment scales built to assess negative emotional states and the severity of central symptoms in the spheres of depression, anxiety, and stress (16). The reliability of DASS-21 showed that it has excellent Cronbach's alpha values of 0.81, 0.89, and 0.78 for the subscales of depression, anxiety, and stress, respectively;

- sections from the Copenhagen Psychosocial Questionnaire (COPSOQ) related to individual health and well-being, which includes several factors: 1 – self-assessed health, 2 – sleep disorders, 3 – burnout, 4 – stress, 5 – somatic stress, 6 – cognitive stress, 7 – depressive symptoms, 8 – self-efficacy (17);

- The BSQ 16-item is a refined self-report questionnaire developed by Evans and Dolan (18) to assess body dissatisfaction and concern over body shape. Participants rate the items based on a six-point Likert scale, ranging from 1 = *never* to 6 = *always* with higher scores indicating greater body shape dissatisfaction. This instrument has been found to be a reliable and valid measure of body image satisfaction as it has demonstrated excellent internal consistency (0.95);

- SATAQ-4 is a 22-item questionnaire assessing socio-cultural attitudes towards appearance, measuring media, family, and

peer pressure to attain the ideal appearance; internalization of the thin/low body fat; internalization of the muscular or athletic body image. SATAQ-4 subscale scores demonstrated good internal consistency (0.84 or higher) (19);

- The General Lifestyle Questionnaire (GLQ) questionnaire (20) which includes five subscales related to physical activity, cognitive activities (attention, memory and reasoning), social activities, other leisure activities (including activities that we practice mainly for relaxation or personal enrichment, for example, meditation) and other behaviors that significantly influence health (eating habits, alcohol consumption, tobacco consumption, and sleep quality). GLQ scores are calculated using a 5-point Likert scale (score 1 = never, 2 = several times in the last year, 3 = several times a month, 4 = several times a week, 5 = every day or almost every day). Overall, Cronbach's alpha coefficients were satisfactory, with an overall Cronbach's alpha score of 0.72.

Statistical analysis

The statistical analyses were performed using IBM Statistical Package for Social Sciences for Windows, *SPSS version 24* (SPSS Inc, Chicago, IL, USA). The results for descriptive statistics were expressed as means (M) and standard deviations (SD). The normality of the data distribution was tested using the Kolmogorov-Smirnoff test. Given the fact that all data are normally distributed, parametric tests will be applied. To assess comparative results considering gender, living environment, and the presence or absence of chronic diseases, the Independent T Test was performed. Comparative results considering marital status, weight status, and socio-economic status were assessed using the ANOVA One Way Test to determine if there were statistically significant differences between more than two groups of an independent variable or a quantitative dependent variable. The Pearson correlation was used to test the relationship between variables. A p-value < 0.05 was considered statistically significant.

RESULTS

The average age of the participants was M = 20.71 ± 2.60 years old, with most of them being women (81.7%, N = 353). About half of the participants were single (48.8%, N = 211), less than half of them were in a relationship (46.5%, N = 201), and the fewest were married (4.6%, N = 20).

The participants of the study had a medium level of education, with most of them having high school education (83.3%, N = 360), and a very small part of them having completed university studies (11.3%, N = 49). Additionally, most of the participants reported having an average financial situation (76.6%, N = 331).

The SES-SQ instrument, which measures socio economic status, showed that more than half of the participants had a medium economic level (55.1%, N = 238), and more than a third had a high economic level (39.9%, N = 172). The score of SES-SQ ranged from 3 (0.5%, N = 2) to 17 (2.1%, N = 9), with the average of the participants being M = 11.61 ± 2.76. As indicators of health status, weight and the presence of chronic disease were considered. More than half of the participants were of normal weight (63.2%, N = 273), while more than a quarter were either underweight (15.7%, N = 73) or overweight (16.9%, N = 68). The smallest proportion of the participants were obese (4.3%, N = 18).

Although most of the participants did not suffer from chronic diseases (92.1%, N = 398), among those diagnosed with such a disease (7.9%, N = 34), most suffered from gastrointestinal diseases (1.6%, N = 7), autoimmune diseases (1.4%, N = 6), diabetes (1.2%, N = 5), skin diseases (0.7%, N = 3) etc.

Most of the participants reported consuming home-cooked food daily (74.1%, N = 320), while more than a quarter of them at eat a restaurant once every 2-3 months (36.6%, N = 158) or 2-3 times a month (34.7%, N = 150). About half of the participants preferred to buy fresh ingredients for cooking over frozen options quite often (48.8%, N = 211). Less than half of the participants opted for food from local producers or farmers' markets instead of supermarkets quite rarely (41.9%, N = 181). More than a third of the participants occasionally (38.0%, N = 164) or frequently (35.0%, N = 151) consumed traditionally prepared preserved foods such as mashed vegetables, pickles and jam.

More than half of the participants stated that they did not resort to various food diets to lose weight (60.9%, N = 263), or consumed various weight-loss teas or food supplements (86.3%, N = 373). Less than a quarter of the participants occasionally (24.1%, N = 104) or rarely (22.0%, N = 95) engaged in excessive exercise for weight loss or body shape maintenance. Over half of the participants (69.0%, N = 298) reported not utilizing various photo filters or photo editors for the pictures they post on various social media platforms. Other items are detailed below, (tab. I), where the answers were indicated on a Likert scale ranging from 1 (total disagreement) to 5 (total agreement).

TABLE I.
Self-rated items[1]

Items	1	2	3	4	5	M±SD
I buy fresh ingredients for cooking (e.g, meat, vegetables, etc.) to cook over frozen options.	1, (0.2)	39, (9.0)	91, (21.1)	211, (48.8)	90, (20.8)	3.81 ± 0.87
I buy food from local producers or farmers' markets rather than from supermarkets.	16, (3.7)	126, (29.2)	181, (41.9)	91, (21.1)	18, (4.2)	2.93 ± 0.90
It is important to me that the food I eat is prepared the way my family cooked it.	91, (2.1)	27, (6.3)	180, (41.7)	167, (38.7)	49, (11.3)	3.51 ± 0.85
I prefer foods that are prepared quickly rather than those that take more time to cook.	15, (3.5)	61, (14.1)	203, (47.0)	121, (28.0)	32, (7.4)	3.22 ± 0.90
I eat canned food, traditionally prepared by myself or family members.	6, (1.4)	61, (14.1)	164, (38.0)	151, (35.0)	50, (11.6)	3.41 ± 0.91
It is important to me that the food I eat comes from my own country.	31, (7.2)	69, (16.0)	208, (48.1)	84, (19.4)	40, (9.3)	3.08 ± 1.00
I read food labels.	11, (2.5)	77, (17.8)	145, (33.6)	132, (30.6)	67, (15.5)	3.39 ± 1.02
Since abiding by religious fasts is important to me, my eating behavior undergoes important changes during these periods.	120, (27.8)	83, (19.2)	129, (29.9)	68, (15.7)	32, (7.4)	2.56 ± 1.25

[1]Means and standard deviations (M±D), frequency and percentages (%)

Approximately half of the participants reported a good state of health (46.5%, N = 201), with an average evaluation of health of M = 7.68 ± 1.56 on the Likert scale ranging from 1 (very bad) to 10 (very good).

The DASS-21 demonstrated strong reliability in our study, with Cronbach's alpha values of 0.87, 0.87, and 0.85 for the stress (M = 18.50 ± 9.02), depression (M = 13.10 ± 8.58) and anxiety (M = 15.56 ± 9.27) subscales, respectively.

The interpretation of these subscale values can be found in second table.

TABLE II.
DASS Severity Classification[1]

	Normal	Mild	Moderate	Severe	Extremely severe
Stress	163 (37.7)	78 (18.1)	86 (19.9)	73 (16.9)	32 (7.4)
Depression	146 (33.8)	89 (20.6)	120 (27.8)	43 (10.0)	34 (7.9)
Anxiety	91 (21.1)	23 (5.3)	107 (24.8)	69 (16.0)	142 (32.9)

[1]Frequency and percentages (%)

An independent-samples t-test was run to determine if there were significant differences between women and men on this scale. The results showed that men (M = 15.11 ± 8.52) reported lower stress levels than women (M = 19.26 ± 8.97, $t(430)$ = -3.754, $p <$ 0.001. Moreover, men (M = 11.84 ± 8.00) reported lower levels of anxiety than women (M = 16.39 ± 9.22); $t(430)$ = -4.004, $p <$ 0.001.

This study found that people living in rural areas had statistically significantly higher levels of anxiety (M = 14.15 ± 8.00) compared to people residing in urban areas (12.45 ± 8.87), t(430) = 2.016, p= 0.044. There was a statistically significant difference between groups in terms of marital status as determined by the one-way ANOVA ($F(2,429)$ = 7.313, p = 0.001). A Tukey post hoc test revealed that married people (M = 11.50 ± 8.82) had a statistically significantly lower level of stress compared to single people (M = 19.39 ± 9.18), p = 0.001 and people who were in a relationship (M = 18.27 ± 8.59), p = 0,004.

A one-way ANOVA was performed to compare the level of depression on marital status and revealed that there was a statistically significant difference in marital between the three groups (F(2, 429) = 10.053, $p<$ 0.001). Tukey's HSD Test found that married people (M = 6.40 ± 7.85) had statistically significantly lower level of depression compared to single people (M = 14.48 ± 9.24), $p<$ 0.001 and to people who were in a relationship (M = 15.21 ± 8.30), p = 0,008. Additionally, results showed that single people (M = 14.48 ± 9.24) had statistically higher levels of depression compared to people who were in a relationship (M = 12.32 ± 7.48), p = 0,029. A one-way ANOVA was also performed to compare the level of anxiety on marital status and it revealed that there was a statistically significant difference in marital status between the two groups (F(2, 429) = 4.263, p = 0.015). Results showed that single people (M = 16.38 ± 9.93) had a statistically higher level of anxiety compared to married participants (M = 10.30 ± 9.78), p = 0,015.

There was a statistically significant difference between groups in terms of socioeconomic status as determined by the one-way ANOVA ($F(2,429)$ = 5.128, p = 0.006). A Tukey post hoc test revealed that people with a middle socioeconomic status (M = 19.73 ± 9.37) had a statistically significantly higher level of stress compared to people with a high socioeconomic status (M = 16.87 ± 8.46), p = 0.005. A one-way

ANOVA was also performed to compare the level of depression with socioeconomic status and revealed that there was a statistically significant difference ($F(2, 429) = 5.155$, $p = 0.006$). Results showed that people with a middle socioeconomic status ($M = 14.11 \pm 8.81$) had a statistically significantly higher level of depression compared to people with a high socio-economic status ($M = 11.50 \pm 8.13$), $p = 0.007$.

A one-way ANOVA was performed to compare the level of anxiety on socioeconomic status and revealed that there was a statistically significant difference ($F(2, 429) = 6.597$, $p = 0.002$). Tukey's HSD Test found that people with a high socioeconomic status ($M = 13.59 \pm 8.50$) had a statistically significantly lower level of anxiety compared to people with a middle socioeconomic status ($M = 16.86 \pm 9.60$), $p = 0.001$.

The **COPSOQ wellbeing scales** consisted of several items collected with a five-point Likert scale (categories ranging from "never" to "always"). The single items of the COPSOQ scales were transformed to a theoretical range from 0 (representing the lowest possible value of the aspect under investigation) to 100 (representing the highest possible value). Scale values are presented as mean values. Cronbach's Alpha was employed to evaluate the internal consistency of the scales, demonstrating good reliability: 0.74 for sleep disorders, 0.85 for burnout, 0.79 for general stress, 0.74 for somatic stress, 0.85 for cognitive stress, 0.85 for depressive symptoms, and 0.84 for general self-efficacy. Table III presents the results according to gender, for the seven COPSOQ wellbeing subscales.

TABLE III.
Gender differences for COPSOQ wellbeing subscales.[1]

Subscales	Men	Women	Differences
Sleep disorders	36.15 ± 17.55	39.53 ± 19.57	p = 0.158
Burnout	50.07 ± 25.14	56.92 ± 21.02	p = 0.012
General stress	43.45 ± 21.99	48.44 + 21.05	p = 0.060
Somatic stress	25.31 ± 20.67	33.76 ± 18.47	p = 0.000
Cognitive stress	38.44 ± 20.80	43.89 ± 22.03	p = 0.046
Depressive symptoms	42.32 ± 25.88	45.57 ± 22.16	p = 0.255
General self-efficacy	72.46 ± 18.49	65.21 ± 17.86	p = 0.001

[1] Means and standard deviations (M±D), frequency and percentages (%)

An independent-samples t-test was run to determine if there were significant differences between people living in urban areas and those living in rural areas on the COPSOQ scale. The results showed that people who lived in urban areas ($M = 37.17 \pm 19.86$) reported lower levels of sleeping troubles than people who lived in rural areas ($M = 41.71 \pm 17.91$). This difference was significant: $t(430) = 2.401$, $p = 0.017$. Additionally, the results showed that people living in urban areas ($M = 30.16 \pm 19.25$) reported lower levels of somatic stress than people living in rural areas ($M = 35.50 \pm 18.57$) , $t(430) = 2.839$, $p = 0.005$.

The results of the research showed that people diagnosed with chronic diseases had statistically a significantly higher level of burnout ($M = 63.41 \pm 17.95$) compared to people who did not have such a diagnostic

(55.00 ± 22.17) , t(430) = 2.152 , p= 0.032.

There was a statistically significant difference between groups in terms of marital status, as determined by the one-way ANOVA (F(2,429) = 5.759, p = 0.003). A Tukey post hoc test revealed that single people (M = 58.26 ± 21.46) had a statistically significantly higher level of burnout compared to married people (M = 42.18 ± 22.56), p = 0.005. Cronbach's Alpha score for the BSQ scale was 0.94. The overall BSQ score averaged at M = 43.43 ± 19.20, with scores ranging from 16 (0.7%, N = 3) to 96 (0.2%, N = 1). An independent-samples t-test was conducted to determine if there were significant differences between women and men on this scale. The results showed that men (M = 34.18 ± 14.05) reported a lower level of body dissatisfaction compared to women (M = 45.50 ± 19.60). This difference was significant t(430) = -5.972, p < 0.001.

This study found that people diagnosed with chronic diseases had a statistically significantly higher level of body dissatisfaction (M = 51.76 ± 18.62) compared to people who did not have such a diagnostic (42.72 ± 19.11) , t(430) = 2.653, p= 0.008.

A one-way ANOVA was performed to compare the level of body dissatisfaction on weight status and revealed that there were statistically significant differences in the three groups (F(3, 428) = 20.080, p< 0.001). Tukey's HSD Test found that normal-weight people (M = 42.85 ± 18.51) had a statistically significantly higher level of body dissatisfaction compared to underweight people (M = 32.62 ± 10.98), p< 0.001. Results showed that normal-weight people (M = 42.85 ± 18.51) had a lower level of body dissatisfaction compared to overweight people (M = 50.52 ± 20.96), p = 0.007. Additionally, results showed that overweight people (M = 50.52 ± 20.96) had a lower level of body dissatisfaction compared to underweight participants (M = 64.11 ± 19.37), p = 0,026.

For the **SATAQ scale**, Cronbach Alpha was 0.91 for total scale, 0.85 for Internalization (thin/low body fat) 0.87 for Internalization (Muscular or Athletic), 0.95 for Pressures-Media, 0.87 for Pressures-Family and 0.89 for Pressures-Peers. For SATAQ subscales we obtained the following results:

TABLE IV.

Gender differences for SATAQ scale and subscales.[1]

Subscales	Men	Women	Differences
Internalization Thin / Low Body Fat	12.49 ± 4.42	14.89 ± 5.39	p = 0.000
Internalization Muscular / Athletic	16.41 ± 5.00	13.44 ± 4.68	p = 0.000
Pressure Media	8.12 ± 4.88	11.24 + 5.49	p = 0.000
Pressure Family	8.22 ± 3.77	8.19 ± 4.38	p = 0.956
Pressure Peers	7.70 ± 3.62	7.32 ± 4.05	p = 0.440
General SATAQ score	52.97 ± 13.99	55.11 ± 17.13	p = 0.302

[1] Means and standard deviations (M±D), frequency and percentages (%)

This study found that people diagnosed with chronic diseases had statistically significantly higher levels of internalization of thin/low body fat (M = 16.88 ± 4.41) compared to people who did not have such a diagnostic (14.25 ± 5.33), t(430) = 2.796 , p= 0.005. Moreover, results showed that people diagnosed with chronic diseases had

statistically significantly higher levels of socio-cultural pressures from media (M = 13.50 ± 4.82) compared to people who did not have such a diagnostic (10.43 ± 5.50), t(430) = 3.145, *p*= 0.002.A one-way ANOVA was conducted to compare SATAQ subscale levels among different marital status groups, uncovering statistically significant differences in the internalization of thin, low body fat levels (F(2, 429) = 3.835, p = 0.022). The Tukey's HSD Test found that single people (M = 14.82 ± 5.28) had a statistically significantly higher level of internalization of the thin, low body fat compared to married people (M = 12.90 ± 5.95), *p* = 0.041. Additionally, one-way ANOVA results for the pressure from the media subscale (F(2, 429) = 5.470, *p* = 0.005) showed that married people (M = 6.85 ± 3.68) had a lower level of *socio-cultural* pressure from the media compared to single people (M = 11.07 ± 5.58), *p* = 0,003.

A one-way ANOVA was performed to compare the level of internalization of the thin/low body fat on socioeconomic status and revealed that there was a statistically significant difference (F(2, 429) = 4.930, *p* = 0.008). Tukey's HSD Test found that people with a high socioeconomic status (M = 14.85 ± 4.86) had a statistically significantly higher level of internalization of the thin, low body fat compared to people with a middle socioeconomic status (M = 13.34 ± 4.79), *p* = 0.001. For the present study, the Cronbach Alpha score was 0.77. **General lifestyle scores,** as measured with the GLQ, ranged from 94 up to 190 (M = 143.83 ± 15.14). The following results were obtained for GLQ subscales: *physical activity*- M = 28.40 ± 6.84, *cognitive activities* - M = 18.72 ± 6.16, *social activities*- M = 32.53 ± 3.97, *other leisure activities* – M = 23.64 ± 3.56, *eating habits, alcohol consumption, tobacco consumption, and sleep quality* - M = 36.39 ± 4.86.

A one-way ANOVA was performed to compare the levels of GLQ subscales on marital status, and revealed that there were statistically significant differences in terms of physical activity levels (F(2, 429) = 5.066, *p* = 0.007). The Tukey's HSD Test found that single people (M = 29.43 ± 7.08) had a statistically significantly higher level of physical activity compared to people who were in a relationship (M = 27.55 ± 6.55, *p* = 0.015).

A one-way ANOVA was performed to compare the levels of GLQ scale and subscales on socioeconomic status. There was a statistically significant difference (F(2, 429) = 5.751, *p* = 0.003) on GLQ scale. Tukey's HSD Test found that people with high socioeconomic status (M = 146.72 ± 15.49) had a better overall lifestyle compared to people with middle socioeconomic status (M = 142.17 ± 14.96), *p* = 0.008. There was a statistically significant difference between groups on GLQ subscale measuring social activities (F(2, 429) = 16.599, *p*< 0.001). The Tukey's HSD Test demonstrated that people with high socioeconomic status (M = 33.67 ± 4.00) participated in more social activities compared to people with low socioeconomic status (M = 29.59 ± 3.73), *p*< 0.001 and to people with a middle socioeconomic status (M = 31.97 ± 3.72), *p* <0.001. Furthermore, results found that people with a low socioeconomic status (M = 29.59 ± 3.73) engaged in fewer social activities compared to people with a middle socioeconomic status (M = 31.97 ± 3.72), *p*= 0.017.

There was a statistically significant difference between groups in terms of weight status and *Sleep, diet and consumption subscale* as determined by the one-way

ANOVA ($F(3,428) = 3.860$, $p = 0.010$). A Tukey post hoc test revealed that underweight people (M = 37.81 ± 4.79) had a statistically significantly higher level on this scale compared to overweight people (M = 35.22 ± 4.93), $p = 0.008$.

The correlational analysis showed that there were significant associations between the participants' age and certain variables. We identified that the older the age, the higher the BMI (r = 0.121*, p = 0.012). The results showed that age correlated negatively with stress (r = -0.186**, p < 0.001), depression (r = -0.115*, p = 0.016), and anxiety (r = -0.204**, p < 0.001) in the sense that the older the age, the lower the levels of stress, depression, or anxiety. Furthermore, age correlated negatively with burnout (r = -0.196**, p < 0.001) and sleeping problems (r = -0.179**, p < 0.001) variables in the sense that the older the age, the lower the levels of burnout, and fewer problems with sleep appear. Negative correlations were identified between age and the pressure felt from the media (r = -0.104*, p = 0.031), in the sense that the older the age, the lower the level of *socio-cultural* pressure from the media. Moreover, age correlated negatively with social activity (r = -0.099*, p = 0.039), in the sense that the older the people get, the fewer social activities they perform.

The Pearson correlation results showed that there were positive correlations between BMI and the BSQ total score (r = 0.396**, p < 0.001), in the sense that the higher the BMI, the higher the body dissatisfaction.

The results showed that BMI correlated with SATAQ subscales such as Internalization of body fat (r = 0.204**, p < 0.001), Pressure from Media (r = 0.163*, p = 0.001) Pressure from Family (r = 0.441**, p < 0.001)and Pressure from Peers (r = 0.355**, p < 0.001), in the sense that the higher the BMI, the higher the internalization of a thin, low body fat, and the more *socio-cultural* pressures from the media, family, or peers were felt. Furthermore, there were negative correlations between BMI and the GLQ total score (r = -0.101*, p = 0.036), in the sense that the higher the BMI, the less healthy the lifestyle of the participants.

The correlational analysis showed that there was a positive correlation between socioeconomic status and the GLQ total score (r = 0.188*, p < 0.001), in the sense that the higher the socioeconomic status, the healthier the lifestyle of the participants. The results showed that socioeconomic negatively correlated with cognitive stress (r = -0.160**, p = 0.001), somatic stress (r = -0.187**, p < 0.001), burnout (r = -0.149**, p = 0.002), sleeping problems (r = -0.102*, p = 0.034), anxiety (r = -0.161**, p = 0.001) and depression (r = -0.169**, p < 0.001) in the sense that the higher the socioeconomic status, the lower the participants' levels of cognitive stress, somatic stress, burnout, depression, anxiety, and the fewer sleep problems appear. Other correlational results are presented in Table V.

A multiple regression was run to predict body dissatisfaction from depression, anxiety, and stress. This resulted in a significant model, $F(3, 248) = 27.309$, $p < 0.001$, $R2 = 0.161$. Further analysis of the individual predictors revealed that depression ($t = 3.075$, $p = 0.002$) and anxiety ($t = 2.435$, $p = 0.015$) were significant predictors but stress was not ($t = 0.584$, $p = 0.559$). Another multiple regression was run to predict *socio-cultural* attitudes towards appearance from sleeping problems, burnout, depressive symptoms,

self-efficacy, somatic stress, and cognitive stress. This resulted in a significant model, $F(6, 425) = 9.373$, $p < 0.001$, $R2 = 0.342$. Further analysis of the individual predictors revealed that depressive symptoms ($t = -4.313$, $p < 0.001$) and self-efficacy ($t = -1.885$, $p = 0.006$) were significant predictors but, sleeping problems ($t = 0.444$, $p = 0.657$), burnout ($t = -0.564$, $p = 0.573$), somatic stress ($t = 1.336$, $p = 0.182$), and cognitive stress ($t = -0.928$, $p = 0.354$) were not.

TABLE V.
Correlation results

	BSQ	SATAQ_ Int_body fat	SATAQ_ Int_Muscular, Athletic	SATAQ_ Pressure_ M	SATAQ_ Pressure_ F	SATAQ_ Pressure P	Stress	Depression	Anxiety
BSQ	1								
SATAQ Int Body fat	r=0.680 p=0.000**	1							
SATAQ Int Muscular, Athletic	r=0.153 p=0.001**	r=0.342 p=0.000**	1						
SATAQ Pressure Media	r=0.578 p=0.001**	r=0.469 p=0.001**	r=0.158 p=0.001**	1					
SATAQ Pressure Family	r=0.527 p=0.000**	r=0.372 p=0.000**	r=0.169 p=0.000**	r=0.388 p=0.000**	1				
SATAQ Pressure Peers	r=0.530 p=0.000**	r=0.377 p=0.000**	r=0.157 p=0.001**	r=0.414 p=0.000**	r=0.682 p=0.000**	1			
STRESS	r=0.341 p=0.000**	r=0.188 p=0.000**	no correlation	r=0.264 p=0.000**	r=0.188 p=0.000**	r=0.197 p=0.000**	1		
DEPRESSION	r=0.377 p=0.000**	r=0.206 p=0.000**	no correlation	r=0.283 p=0.000**	r=0.213 p=0.000**	r=0.219 p=0.000**	r=0.763 p=0.000**	1	
ANXIETY	r=0.361 p=0.000**	r=0.197 p=0.000**	no correlation	r=0.271 p=0.000**	r=0.190 p=0.000**	r=0.223 p=0.000**	r=0.742 p=0.000**	r=0.707 p=0.00**	1

* p < 0.05; ** p < 0.001.

The study assessed the *mediating role of sleeping problems, burnout, general stress, cognitive stress, somatic stress, depressive symptoms, and self-efficacy on the relationship between attitudes towards appearance and body dissatisfaction*. The results revealed a significant indirect effect of impact of attitudes towards appearance on body dissatisfaction through sleeping problems(b = 0.832, t = 3.783, p = 0.002), burnout (b = 1.018, t = 4.067, p = 0.001), general stress (b = 0.629, t = 3.440, p = 0.006), somatic stress (b= 0.950, t = 4.363, p < 0.001), cognitive stress (b = 1.058, t = 4.250, p < 0.001), depressive symptoms (b = 1.779, t = 7.071, p < 0.001), and general self-efficacy (b = -1.262, t = -4.060, p < 0.001).

The study also found a significant indirect effect of the impact of body dissatisfaction on attitudes towards appearance through sleeping problems (b= -0.093, t = -2.335, p = 0.002), somatic stress (b= 0.043, t = 2.258, p = 0.002), depressive symptoms (b= 1.731, t = 3.404, p = 0.007), and general self-efficacy (b= -0.079, t = -2.330, p = 0.021). Furthermore, the direct effect of

attitudes towards appearance on body dissatisfaction in the presence of the mediators was also found to be significant ($b = 0.730$, $p < 0.001$). Hence, sleeping problems, somatic stress, depressive symptoms, and general self-efficacy partially mediated the relationship between attitudes towards appearance and body dissatisfaction.

DISCUSSION

The present research showed that sex, age, marital status, socioeconomic level, the presence of a chronic disease, and BMI are important variables. The analysis of the data revealed that men were less stressed and anxious, and they were less likely to have body dissatisfaction compared to women. These results are congruent with the scientific literature and can be explained by the fact that, usually, men are less accurate in evaluating their weight compared to women. Young women were more accurate in estimating their BMI, so they could be more objective in expressing dissatisfaction with their body weight. Additionally, failing to estimate overweight could generate a low level of involvement in dieting or the practice of healthy behaviors (21-23). Dye and his team (24) also showed in their study that women reported higher preoccupation with weight, dieting, and eating restraint than men.

Age. The research showed that the older the respondent was, the lower the levels of stress, depression, or anxiety. Additionally, age correlated negatively with burnout and sleeping problems. Negative correlations were identified between age and the pressure felt from the media and social activity, in the sense that the older the people got, the fewer social activities they performed, and the less pressure from the media they felt. Some other authors maintain that age plays a significant role in the relationship between body dissatisfaction and BMI. This is because BMI increases across the transition from adolescence to young adulthood. The studies of Bucchianeri *et al.* (25) and Bully *et al.* (26) identified opposite results regarding the fact that youth become increasingly dissatisfied with their bodies. The first research team identified that young females were more dissatisfied with their body image, while Bully and his team found that there were not important differences throughout the years.

Marital status was found to be an important factor, the study revealing that married people presented statistically significantly lower levels of stress, depression, anxiety, and burnout syndrome compared to single people or those in a relationship. The results of the research identified that married respondents presented a lower level of *socio-cultural* pressure from the media and a lower level of internalization of the thin, low body fat compared to single subjects. Single people had a statistically significantly higher level of physical activity compared to people who were in a relationship.

Rosenqvist *et al.* (27) identified that in case of married women, satisfaction with body image is related to the level of education. These differences in body dissatisfaction based on educational level were found to be important in public health actions aiming to reduce socioeconomic inequalities in health and well-being.

Socio-economic status. There was a statistically significant difference between groups in terms of socioeconomic status. Results showed that people with a middle socioeconomic status had a statistically significantly higher level of depression, anxiety and stress compared to people with

a high socioeconomic status. We also found that people with a high socioeconomic status had a statistically significantly higher level of internalization of the thin, low body fat compared to people with a middle socioeconomic status. Subjects with a high socioeconomic status had a better overall lifestyle and had better social activity compared to respondents with middle socioeconomic status. The data showed that the higher the socioeconomic status, the healthier the lifestyle of the participants and the lower the participants' levels of cognitive stress, somatic stress, burnout, depression, anxiety, and fewer sleep problems. Therefore, health policies prioritize addressing socioeconomic factors to ensure that individuals from all socioeconomic backgrounds have equal access to recreational, medical, psychological, and recovery health services. These policies also aim to promote changes in nutritional and physical activity behaviors that contribute to overall health, while emphasizing the importance of high-quality food.

Chronic disease. People who were chronically ill had statistically significantly higher levels of burnout syndrome, and they registered higher levels of body dissatisfaction compared to young people with no chronic disease. Young people with chronic diseases obtained statistically significantly higher levels of internalization of the thin, low body fat and a significantly higher level of *socio-cultural* pressure from the media, compared to people who did not have a diagnostic. Our results are congruent with those of the scientific literature, but this variable should also be interpreted considering the type of chronic disease. For example, Markey *et al*. (28) identified that the acceptance of pain was associated with reports of body appreciation in case of young people with chronic pain; Wabich *et al*. (29) showed that 87% of patients with inflammatory bowel disease reported body dissatisfaction; and Darukhanavala *et al*. (30) proved that the rate is about 21% among men and 66% among women with cystic fibrosis.

BMI. The analysis of the answers identified that the higher the BMI, the higher the body dissatisfaction. We found that normal-weight young people had a statistically significantly higher level of body dissatisfaction compared to underweight people, but a lower level of body dissatisfaction compared to overweight people. We also found that underweight people obtained a statistically significantly higher level on the scale *Sleep, diet and consumption* compared to overweight respondents.

In order to predict body dissatisfaction from depression, anxiety, and stress, the multiple regression showed a significant model, and indicated that depression and anxiety were significant predictors, but stress was not.

We also found that sleeping problems, somatic stress, depressive symptoms and general self-efficacy partially mediated the relationship between attitudes towards appearance and body dissatisfaction.

CONCLUSIONS

In this study, by using a specific questionnaire to collect relevant information related to socio-economic and psychological factors, we assessed the impact of sleeping problems, burnout syndrome, stress, depressive symptoms, and self-efficacy on the relationship between attitudes towards appearance and body dissatisfaction among Romanian young people. The study was conducted on people aged between 18-30 years old. The results showcased a significant correlation between

factors such as age, gender, marital and socio-economic status and the impact of stress, depression, anxiety on the perception of body image and related levels of dissatisfaction.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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THE USE OF NURSING WORK INDEX AS A PRACTICE CONDITIONS ASSESSMENT FOR THE ROMANIAN NURSE'S CASE

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THE USE OF NURSING WORK INDEX AS A PRACTICE CONDITIONS ASSESSMENT FOR THE ROMANIAN NURSE'S CASE (Abstract): The work conditions of nurses have a significant impact on the practice of nursing. Suboptimal working conditions can be attributed to insufficient workplace structure, substandard organization of tasks, inadequate training, and unsuitable staffing policies. **Materials and methods:** This research sought to analyze the factors that influence the work conditions of nurses by employing the practice conditions scale of the Nursing Work Index (NWI). The validation of the NWI was conducted, utilizing nurse-reported job features as the independent variables. A total of 746 nurses from hospitals located in North - East Region of Romania were included in the sample. The Nurse Forecasting (RN4CAST) protocol was implemented for data collection. **Results:** The NWI construct was accounted for by 42.9% through factors such as "Advancement opportunities", 'Learning opportunities', 'Satisfaction with current job', 'Professional status', and 'Level of education'. An analysis of the NWI revealed the presence of distinct variables within a five-factor structure. The mean score achieved on the Nursing Work Index was observed to be among the least favorable in comparison to earlier survey findings. **Conclusions:** It is imperative for nurses to be acknowledged as peers within the healthcare sector, thereby requiring empowerment for professional growth and access to career advancement prospects. Furthermore, the significance of inter-professional relationships and equitable participation of nurses in hospital matters cannot be understated. **Keywords**: NURSES WORK CONDITIONS, NURSE WORKFORCE, ASSESSMENT OF THE PRACTICE CONDITIONS, NURSING WORK INDEX.

The Nursing Work Index (NWI) is a widely used tool to evaluate the work conditions of nurses. Developed by Dr. Marlene Kramer and colleagues in the 1980s, it assesses the features of the nursing work conditions that contribute to satisfaction at work and the quality of patient care. The NWI helps in identifying the strengths and weaknesses within the nursing practice conditions and is instrumental in guiding improvements (1, 2).

Using this tool can be measured the several dimensions of the nursing work conditions, such as nurse participation in hospital operations, nursing fundamentals for high-quality healthcare, and nurse-physician collaboration (3, 4). Implementing the NWI involves surveying nursing

staff using a validated questionnaire. The responses are then analyzed to produce scores for each component, highlighting areas of strength and opportunities for improvement. Hospitals can then develop targeted interventions to address the identified issues.

In addition, studies (3, 4) using the NWI have consistently shown that better work conditions, as indicated by higher NWI scores, are associated with improved nurse satisfaction at work, lower burnout rates, and better patient outcomes. For example, research has demonstrated that hospitals with supportive management, adequate staffing, and good nurse-physician relationships have lower patient mortality rates and higher levels of patient satisfaction. This shows that by focusing on key areas, the NWI provides a comprehensive picture of the factors influencing nurse satisfaction and patient care quality (5, 6, 7).

Referring to applications and benefits, hospitals use the NWI for quality improvement, identifying areas needing improvement and implementing strategies for enhancing the work conditions. Also, the NWI allows for comparison across different units or hospitals, helping to benchmark performance and identify best practices. Not least, the NWI is frequently used in research to study the impact of work conditions on nurse outcomes, such as satisfaction at work, retention, and patient care quality.

The Nursing Work Index (NWI) is structured around several key components that collectively assess the quality of the nursing work conditions. Each component focuses on different aspects of the work conditions that are crucial for nurse satisfaction and effective patient care. Those key components are: *the involvement of nurses in hospital operations* (3, 6); *nursing fundamentals for high-quality healthcare* (4, 7); *capacity, support, and leadership of nurse managers* (5, 6); a*ppropriate staffing and resources* (6, 7) and *nurse-physician collaboration* (3, 6).

As additional components can be considered *Flexibility Of work schedule* (this includes the ability to balance work and personal life, which can significantly affect satisfaction at work and performance) and *professional development* (opportunities for further education and career growth are essential for maintaining a motivated and skilled nursing workforce).

Overview of research literature

As seen above, the Nursing Work Index (NWI) is a widely utilized tool in nursing research to evaluate and enhance the work conditions for nurses. The NWI's application in research studies, highlighting its impact and findings. For example, (8) suggest that NWI can be used to investigate the relationship between nurse staffing levels, nurse burnout, job dissatisfaction, and patient mortality. The study found that better nurse staffing was associated with lower patient mortality, reduced nurse burnout, and higher satisfaction at work. It emphasized the importance of adequate staffing and supportive work conditions in improving patient outcomes. Other researchers (9) used the NWI to examine the association between the nursing work conditions and patient safety outcomes, including the incidence of adverse events. The research highlighted that positive work conditions, as measured by the NWI, were linked to lower rates of adverse events and better overall patient safety. Another relevant study (10) explored the impact of the nursing work conditions on satisfaction at work, stress, and burnout among nurses

using the NWI. It was found that better work conditions significantly reduced stress and burnout, while increasing satisfaction at work among nurses. Also, (11) focused on adapting and validating the NWI for use in different cultural contexts, particularly in non-Western countries. The study confirmed that the NWI is a reliable and valid tool across various cultural settings, making it useful for international research and practice improvements.

Recent studies have continued to explore and validate the Nursing Work Index (NWI), focusing on various aspects of the nursing work conditions and its impact on nurse satisfaction and patient care quality. Thus, (12) aimed to update and revise the NWI to better reflect contemporary nursing practice. The research included a cross-sectional survey of hospital nurses to assess the relevance of the existing items and the addition of new ones. he revised instrument, PES-v2021, included several new items and removed outdated ones. The study confirmed that the revised scale is valid and reliable for assessing current nursing work conditions. (13) re-validated the NWI-Revised (NWI-R) in Finnish hospitals aspiring for Magnet status. It aimed to identify organizational features that influence nurse autonomy and satisfaction at work. The study found that components like nurse management and leadership, staffing and resource adequacy, and nurse-physician collaboration are crucial for a favorable nursing practice condition. The NWI-R demonstrated good internal consistency and validity. (14) evaluated the work conditions of nurses in Slovenian hospitals using the NWI, aiming to understand how job features affect nurses' perceptions of their work conditions. The study found that staffing and resource adequacy, nurse manager ability, and leadership significantly impact the perceived quality of the work conditions. The reliability of the NWI was confirmed, though some subscales showed varying levels of reliability. (15) reviewed the translation and cultural adaptation of the NWI across different countries and languages. It aimed to identify gaps and ensure the tool's applicability in diverse cultural contexts. The research identified translations into 24 languages across 35 countries, highlighting the need for further translation efforts in Africa, Asia, and South America. The study emphasized the importance of robust translation processes to maintain the tool's reliability and validity.

The purpose of our research study is to characterize and investigate the factors that influence nurses' work conditions by using the Nursing Work Index (NWI). The objectives of this research are: to analyze the regional context of nurses' work conditions in public hospitals from North-East Region of Romania using the NWI scale; and to evaluate the impact of factors that can affect nurses' self-evaluation of their work conditions, which managers can utilize to enhance nurses' working circumstances.

MATERIALS AND METHODS

Design - To get the data required to investigate areas with limited knowledge or to understand particular aspects better, a cross-sectional study was carried out. To collect the data needed was used the research protocol applied in RN4CAST (16). STROBE checklist was utilized to report the cross-sectional study.

Instrument - The NWI (2) scale was used. This scale consists of several subscales that assess different aspects of the nursing work conditions. Each subscale

includes specific items that nurses rate based on their experiences.

The first part of the scale contains 8 items that refer to respondent's socio-demographic features and professional membership: gender (0 - Male, 1 - Female), age, education level (1- Vocational health school, 2 - High school studies, 3 - Post-secondary studies, 4 - University studies, 5 - Postgraduate studies or equivalent (e.g., master programs, PhD, or advanced professional training), the department where the respondent works, whether they occupy or not a management position, and the geographic location.

The second part contains 81 items designed to assess the healthcare management dimensions and nursing practice conditions such as the involvement of decision factors; the management of human resources (selection of human resources, training of the nursing staff, the involvement of medical personnel); process management (simplifying administrative procedures for patients' admission in hospital, administrative support and clinical output, clinical outcomes of healthcare); supporting services provided by the hospital; focus on the healthcare services; focus on the employee; evaluation of indicators for hospital performance; system of hospital information; errors, safety, and risk management; the hospital organizational culture; quality management of healthcare and sustained improvement of the healthcare services, governance and social responsibility.

The third part includes 23 items related to factors that can create problems for the nurses' ability to fulfil their professional role. The list of items for each of the selected items of the Nursing Work Index is given in, Appendix.

For the items established for our questionnaire, scales were chosen to reflect the frequency of certain opinions. Thus, for the items in the first section of our questionnaire, we generally used the binomial scale and nominal scale. Since the scale with six or more points is valid and distinctive, a 7 - point Likert scale was used for the items in the second part of the proposed questionnaire (where: 1 - the lowest possible score; 2; 3; 4; . . . .7 - the highest possible score; quality), and a 10-point Likert scale was used for the items in the third part of the questionnaire (where: 1 - creates no problems at all; 2; 3; 4; ….10 - causes the most important problems).

Sample size, composition and data collection - Considering the RN4CAST protocol (16), all hospitals units form the North-East Region of Romania were asked to join in the study (N = 76). Of these, just 10 hospitals confirmed their participation. Eight regular hospitals and two larger hospitals that provide tertiary care.

All employed nurses at the included hospitals (N = 2021) were invited to take part to our study. Nurses who were absent due to illness, pregnancy leave, or holiday were excluded (N = 657). In total, 746 nurses participated to our study. Nurses are defined as those matching the European Union definition of trained and licensed nurses according to Directive 2005/36/EC (17).

The data collection period began in April and will last until June 2023. Each hospital has two weeks to collect data. The research coordinator employed a paper-and-pencil and delivered printed questionnaires. Participants submitted them in a sealed box to the agreed-upon pickup site.

Ethical considerations - Permission to conduct the study in hospitals from North-

East Region of Romania was obtained from the Hospitals Medical Ethics Committee (for example, No. 30/E3333, March 31st, 2023). All techniques were carried out in line with applicable standards and laws, including the standards for Research Ethics in the Social Sciences and Humanities (18) and the Helsinki Declaration (19). Each hospital approved its involvement by deciding on the ethics committee. Participants received written information about many parts of the study; their rights to participate voluntarily and removal from the study at any time were addressed, as were their privacy and confidentiality rights. Participants also indicated their approval of participating in the study and authorization to utilize national data for professional and scientific objectives.

Data analysis. The data underwent examination through the utilization of the statistical software *SPSS version 26*. Various forms of statistical analysis, including univariate and bivariate approaches, were conducted alongside the application of descriptive statistics and multivariate analysis. Due to the quantification of each item on a scale ranging from 1 to 7, the mean composite score for each subscale of NWI, in addition to the overall composite score, was established. The assessment of the reliability of the scales was carried out using Cronbach's alpha coefficient. Furthermore, the construct reliability of the measurable scales was evaluated through the use of Confirmatory Factor Analysis (CFA), and factor analysis. Principal Component Analysis (PCA) and Principal Axis Factor Analysis (PAF) were used to generate the most related structure of the instrument. The minimum threshold for communalities was established at 0.400. Subsequent to the identification of correlations among factors, the rotation method involving Keiser normalization was implemented. Utilization of Bartlett's test ($p < 0.05$) and KMO test (> 0.8) demonstrated adequacy of the sample size (20). A linear regression model was employed, with significance set at the p-value < 0.05.

RESULTS

Study participants

The population of nursing staff working in hospitals that participated in our study ranged from 48 to 1266. The response rate was 36.91% (n = 746), hospital response rates ranged from 20.1 to 67.2%. The lowest and highest number of responses per hospital were 49 and 264, respectively. Respondents included 654 (87.7%) females and 92 (12.3%) males.

The sample included 262 nurses with a professional health school (35.1%), 143 bachelor's degree (19.2%), 131 post-secondaries (17.6%), 193 university studies (25.9%), and 13 postgraduate studies (1.7%). The higher length of respondent's turnover was between 5 and 10 years (38.9%) and more than 10 years (36.1%). Almost all (97%) were employed full time.

Explorative results

The mean levels of satisfaction degree regarding the existing job, career, and work conditions are illustrated in (tab. I, fig.1) of the Appendix These findings provide insight into the self-evaluation outcomes concerning various job aspects. Among these aspects, the least satisfactory was the salary, followed by growth opportunities. An examination of the data revealed no apparent link between gender, educational attainment, and age in the nursing profession and the average value of the five dimensions. Conversely, a notable association was identified between the five dimen-

sions and job-related attributes. Furthermore, the established dimensions exhibited a significant relationship, as indicated in of the Appendix. The majority of these relationships fell within the medium range (r = 0.300 to 0.600).

TABLE I,
Examining job features and exploratory analysis between variables

Variables	N	M (SD)	F1	F2	F3	F4	F5
Satisfaction- current job	746	5.78 (7.715)	0.414**	-0.330**	0.310**	-0.410**	-0.205**
Satisfaction - career	746	4.93 (0.976)	0.428**	-0.460**	0.290*	0.220*	0.396**
Aspects of job							
Flexibility of work schedule	746	4.82 (0.925)	0.361**	-0.342**	0.475**	0.420**	-0.433**
Advancement opportunities	746	5.42 (6.952)	0.512**	0.471**	-0.397**	0.455**	0.402**
Work independence	746	5.74 (4.928)	0.321**	-0.255**	0.425**	0.222*	0.372**
Professional status	746	4.27 (0.826)	0.238**	-0.416**	0.402**	0.426**	0.471**
Salary	746	4.14 (0.957)	0.279**	0.343**	0.225**	-0.309**	0.370**
Learning opportunities	746	4.17 (4.193)	0.514**	-0.272**	0.381**	0.317**	0.417**
Annual leave	746	4.24 (4.361)	0.319**	-0.265**	0.347**	0.319**	0.316**
Factors of NWI							
F1 - Quality of management process	746	4.91 (4.950)	1				
F2 - Challenges of nurse's professional role	746	7.38 (14.279)	0.328	1			
F3 - Nurse manager proficiency in providing support to nurses	746	4.48 (4.255)	0.725	0.517	1		
F4 - Nurses' involvement in hospital management	746	5.32 (5.137)	0.016	0.029	-0.528**	1	
F5 - Nurses' development	746	5.00 (0.666)	0.081	0.371	0.513**	-0.418**	1

Note. N - Number of answers, M - Mean (7-point scale), SD - Standard deviation,
**Correlation is significant at the 0.01 level (2-tailed)

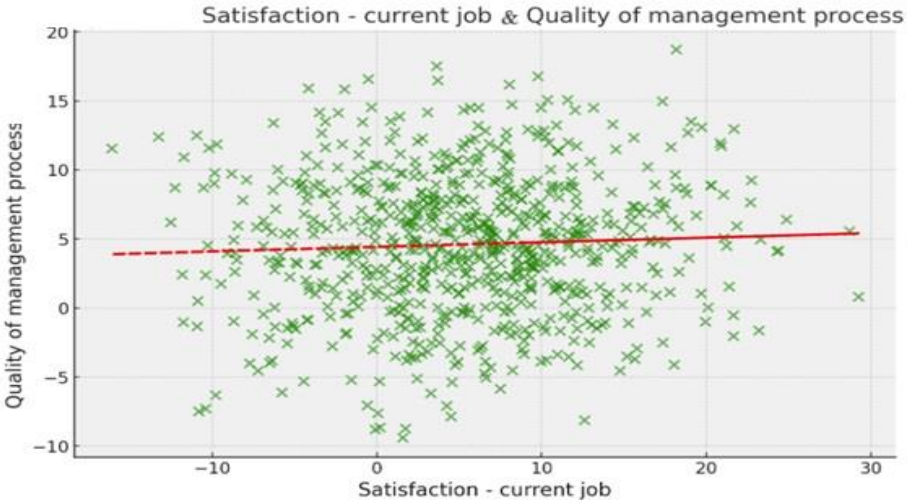

Fig. 1. Examining job features and exploratory analysis between variables.

TABLE II.

The effect of job characteristic on NWI scale and developed factors

Variables	Regression model 1		Regression model 2									
	NWI (all items)		F1		F2		F3		F4		F5	
	(R^2 = 0.429)		*(R^2 = 0.414)*		*(R^2 = 0.218)*		*(R^2 = 0.358)*		*(R^2 = 0.328)*		*(R^2 = 0.371)*	
	β	p	β	p	β	p	β	p	β	p	β	p
Satisfaction- current job	0.162	**< 0.001**	0.056	0.092	-0.193	**< 0.001**	0.169	**< 0.001**	0.137	**< 0.001**	0.159	**< 0.001**
Satisfaction - career	0.013	0.735	0.030	0.351	0.014	0.788	0.058	0.599	0.041	0.728	0.069	0.361
Aspects of job												
Flexibility of work schedule	0.026	0.076	-0.004	0.904	0.082	0.621	0.103	**0.005**	0.017	0.539	0.162	0.0729
Advancement opportunities	0.250	**< 0.001**	0.251	**< 0.001**	-0.118	**< 0.001**	0.083	**0.021**	0.228	**< 0.001**	0.317	**< 0.001**
Work independence	0.039	0.074	0.172	0.917	-0.036	0.736	0.116	**0.005**	0.051	0.949	0.041	0.747
Professional status	0.183	**< 0.001**	0.059	0.631	-0.055	0.498	0.129	0.661	-0.025	0.318	0.023	0.514
Salary	0.032	0.572	0.000	0.995	-0.041	0.647	0.029	0.701	-0.013	0.428	0.041	0.381
Learning opportunities	0.157	**< 0.001**	0.297	**< 0.001**	-0.015	**0.042**	0.217	**< 0.001**	0.277	**< 0.001**	0.339	**< 0.001**
Annual leave	0.002	0.839	0.002	0.724	0.011	0.429	0.032	0.739	0.133	0.420	0.071	0.821

Note. R^2 = Adjusted R-Squared, β = Standard regression coefficient, p = P-value. F1 - Quality of management process, F2 - Challenges of nurse's professional role, F3 - Nurse manager proficiency in providing support to nurses, F4 - Nurses' involvement in hospital management, F5 - Nurses' development **Correlation is significant at the 0.01 level (2-tailed)

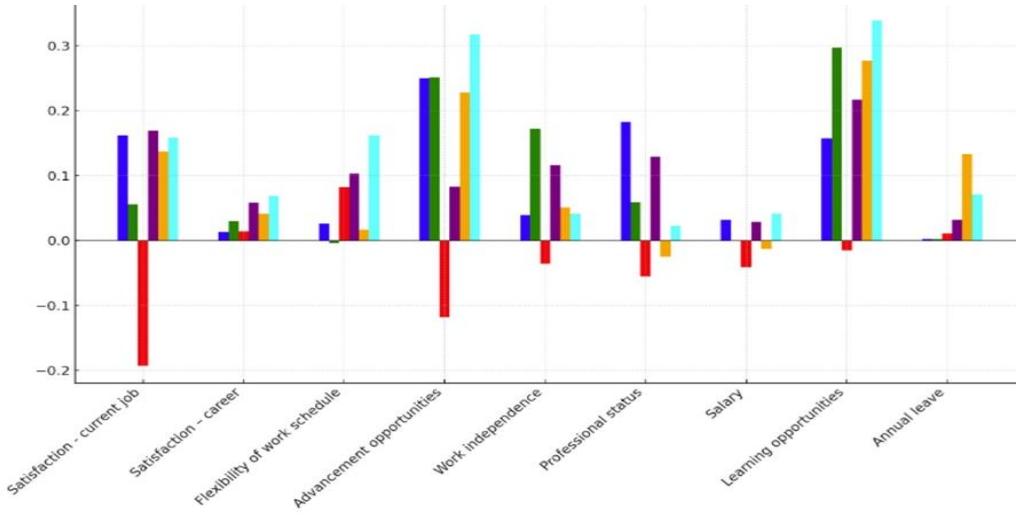

Fig. 2. The effect of job characteristic on NWI scale and developed factors.

Table II and second figure presents findings from two linear regression models. The initial model, Regression Model 1, indicates that 42.9% of the variance in the NWI scale, encompassing all items, can be accounted for. Noteworthy explanatory factors include 'Advancement opportunities', 'Satisfaction with current job', 'Learning opportunities',

and 'Professional status', with 'Level of education' being a less influential factor. In the subsequent Regression Model, Factor 1 ('Quality of management process') is associated with 41.4% of the variance, primarily explained by beta coefficients of 'Advancement opportunities' and 'Learning opportunities', followed by 'Level of education'. Factor 2 ('Challenges of nurse's professional role') is linked to 21.8% of the variance by beta coefficients such as 'Satisfaction with current job', 'Professional status', 'Advancement opportunities', and 'Learning opportunities'. Factor 3 ('Nurse manager proficiency in providing support to nurses') demonstrates an explained variance of 35.8%, with beta coefficients including 'Learning opportunities', 'Satisfaction with current job', 'Work independence', 'Advancement opportunities', and 'Flexibility 0f work schedule'. Factor 4 ('Nurses' involvement in hospital management') is connected to 32.8% of the variance, primarily through beta coefficients of 'Satisfaction with current job', 'Advancement opportunities', and 'Learning opportunities'. Lastly, Factor 5 ('Nurses development') is associated with 37.1% of the variance, primarily explained by beta coefficients of 'Satisfaction with current job', 'Advancement opportunities', and 'Learning opportunities'.

DISCUSSION

Prior studies have confirmed that the variables identified in the regression model of our research influence the work conditions of nurses. However, our study delves deeper into these variables by utilizing the NWI scale as a dependent variable. In a literature review by (21), the predictors linked to NWI are summarized, with each study highlighting only two to three significant variables related to self-assessed workplace features and their connection to NWI (such as nurse power, satisfaction at work, and organizational commitment). Another literature review involving nine studies by (22) revealed that findings regarding nursing workplace features were significant, either through composite scores or subscale scores of NWI. While some associations were significant at one analytical level, they did not hold at another level. Our study aims to showcase the intricate nature of nurse workplace features, providing valuable insights for managers in hospitals from North-East Region of Romania to enhance the work conditions for nurses.

Implications for managers

Our research delineates the workplace setting of two professional cohorts and elucidates the features of the work conditions that can serve as a valuable resource for managers to enhance the working conditions of nursing staff in North - East Region of Romania. Given the shortage of Bachelor of Science in Nursing (BSN) graduates in Romania, nursing services are delivered by a healthcare team with varying levels of educational background, necessitating vigilant monitoring of the impact of such services. As posited by (8), the substitution of registered Nurses with individuals possessing lower educational credentials heightens the potential risk for patient mortality.

The primary discovery in our research highlights that the satisfaction levels regarding wages, annual leave, and career selection do not impact the self-evaluation of the work conditions by nursing staff. According to (23), wages play a role in nurse outcomes, but the work conditions and staffing are even more significant. Our regression analysis, along with its components, indicates that opportunities for career advancement and further education are vital for survey participants, reflecting a response to obstacles in their career progression. These two factors largely accounted for the NWI scale and its respec-

tive dimensions. Subsequently, satisfaction at work elucidated two out of the three dimensions of the work conditions. (24) discovered a close relationship between satisfaction at work among hospital nurses and various factors such as the work conditions, empowerment culture, organizational commitment, professional commitment, job-induced stress, patient satisfaction, and patient-nurse ratios. Alongside the established predictors (career development opportunities and Learning opportunities), our study identifies satisfaction at work as a significant predictor influencing the work conditions of nurses.

The cultivation of collegial relationships may cultivate positive atmospheres that enhance nurse satisfaction (25). Enhancing the relationship between nurses and physicians, as well as nurses and leaders, can enhance equal engagement and the professional development of nurses in hospital administration. A similar impact was noted on the managerial skills of nurses, leadership qualities, and the support they receive. The interaction between nurses and physicians and the behavior exhibited towards nurses by physicians are critical, shaping the professional standing of nurses, providing educational prospects, and nurturing the evolution of nursing as a vocation and field, which are also deemed essential predictors in our study and others (22, 26, 27, 28, 29).

It is evident that nursing administration across various levels of healthcare facilities needs to establish a supportive condition for professional practice in order to ensure workforce stability and deliver optimal care, a notion previously acknowledged in prior studies utilizing the NWI scale (30). Enhancing the work conditions for nurses is imperative, encompassing the promotion of continuous education, advanced degrees, professional growth, and fostering a sense of camaraderie among peers (31). The creation of an ideal work setting for nurses stands as a critical responsibility for both managers and leaders. According to (28), implementing interventions aimed at enhancing the work conditions proves to be an effective strategy in enhancing satisfaction at work. Retention of nurses in their positions is encouraged through three pivotal elements: proficient leadership, opportunities for empowerment, and avenues for professional advancement (27). Equally significant is the imperative for national healthcare policies to regard nurses as equivalent healthcare professionals, recognizing nursing as both a profession and a field of science.

We acknowledge that nursing practice conditions are intricate and contextually dependent; certain aspects of the work conditions may have more pronounced impacts across different levels of analysis and contextual settings (25). Nurturing nurse empowerment, fostering engagement, and cultivating positive interpersonal relationships within the workplace are fundamental for fostering a favorable work condition and ensuring high-quality patient care (26).

CONCLUSIONS

The study suggests a decreased level of self-assessment regarding the work conditions. Some attributes of the work setting have been pinpointed as potential enhancements that hold significant value for nursing administration, hospital governance, and policymakers involved in the enhancement of nursing personnel in North-East Region of Romania. A pivotal discovery from our analysis pertains to the two factors that displayed the most substantial impact in enhancing the nursing work conditions: opportunities for career advancement and educational prospects. Emphasis should be placed on acknowledging nurses as peers within the healthcare

domain who necessitate empowerment for professional growth and access to career progression pathways. The establishment of inter-professional relationships and equitable participation of nurses in hospital operations are paramount. It is imperative for national healthcare policies to regard nurses as equivalent healthcare professionals and to recognize nursing as both a vocation and a scholarly discipline. Further longitudinal and interventional research endeavors are imperative for a more comprehensive comprehension of the working conditions of nurses.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi (No. 128/24.11.2021) for studies involving humans.

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RELATIONSHIPS BETWEEN CLINICAL MANIFESTATIONS AND HISTOPATHOLOGICAL FEATURES IN ENDOMETRIOSIS: IMPLICATIONS FOR PRECISION DIAGNOSIS AND TREATMENT

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RELATIONSHIPS BETWEEN CLINICAL MANIFESTATIONS AND HISTOPATHOLOGICAL FEATURES IN ENDOMETRIOSIS: IMPLICATIONS FOR PRECISION DIAGNOSIS AND TREATMENT (Abstract): Endometriosis is a chronic inflammatory disease affecting reproductive-age women, often associated with pain and infertility. **Aim:** This study aims seeks to investigate the relationships between clinical and paraclinical presentations, and histopathological features in endometriosis, with the objective of improving diagnostic accuracy and guiding personalized treatment strategies. **Materials and methods**: A prospective study was carried out involving 40 patients evaluated at "Cuza-Vodă" and "Elena-Doamna" Obstetrics and Gynecology Clinical Hospitals, Iasi, between September 2019 to August 2024, of whom 40 met the inclusion criteria. Key clinical data and biomarker levels, including CA-125, neutrophil-to-lymphocyte (NLR), platelet-to-lymphocyte ratios (PLR) and systemic immune inflammation index (SII) were analyzed for correlations with histopathological findings, as well as with rASRM staging and the EFI index. **Results:** Compared to the control group, the association of elevated CA-125 levels with systemic inflammatory markers (NLR, PLR, SII) highlights the chronic inflammatory process characteristic of this pathology. In terms of systemic inflammatory markers, the rASRM classification identifies lower values in stage IV compared to stage III, while the EFI classification does not reveal any significant changes. **Conclusions:** The study highlights the importance of correlating clinical and histopathological data in endometriosis for a more nuanced staging and personalized treatment approach. The association of CA-125, systemic inflammatory markers, and rASRM staging may provide the foundation for the identification of novel non-invasive biomarkers, as well as potential prognostic factors for endometriosis**.** **Keywords:** ENDOMETRIOSIS, HISTOPATHOLOGY, INFLAMMATORY MARKERS, INFERTILITY, PERSONALIZED TREATMENT.

INTRODUCTION

Endometriosis is a persistent inflammatory disorder that occurs when tissue similar to the endometrium located outside the uterus, impacting around 10% of women of reproductive age globally (1). This condition often presents with chronic pelvic pain, painful menstrual cycles (dysmenorrhea), pain during intercourse (dyspareunia), and infertility, collectively diminishing the quality of life for those affected (2). Endometriosis is increasingly recognized as a systemic condition influenced by hormonal, immune, and genetic factors (3).

The underlying causes of endometriosis remain under investigation, with several theories proposed. This encompasses the retrograde menstruation theory, proposing that menstrual blood moves back into the pelvic cavity, along with mechanisms such as coelomic metaplasia and embryonic cell migration, each offering distinct pathways for ectopic tissue formation (4, 5). In addition to physical symptoms, endometriosis has been linked to psychological and social challenges, along with comorbidities such as anxiety and depression, highlighting the importance of comprehensive management strategies (6). Endometriosis is staged in conformity with the *revised American Society for Reproductive Medicine* (rASRM) classification system, which ranks the disease from stage I to severe stage IV based on lesion size, location, and the presence of adhesions (7). Nonetheless, the relationship between ASRM staging and clinical severity - particularly in terms of pain and infertility-remains unclear, encouraging further exploration of histopathological characteristics that could elucidate differences in symptom presentation (8). Utilizing data from the rASRM classification, the EFI score can serve as a predictor of the likelihood of spontaneous pregnancy within three years postoperatively in patients with endometriosis (6).

This study seeks to delve into the interplay between clinical presentations-such as pain intensity, reproductive challenges, and other symptomatic expressions-and the histopathological features of endometriotic lesions, including tissue structure, depth, and hormonal receptor presence. By mapping these correlations, this research aims to deepen the understanding of endometriosis as a multifaceted disease, potentially enhancing diagnostic accuracy and recommended tailored therapeutic approaches that consider both the clinical profile and tissue-specific pathology of each patient.

MATERIALS AND METHODS

Sample selection

To achieve findings that reflect the demographics of Iasi's female population (n = 394,274), a representative sample was determined, allowing for a margin of error of 14.6% at 45 participants.

From 2019 to 2023, the number of hospitalized cases of ovarian endometriosis has shown a rising trend, represented by the equation ($y = 79 + 9x$), with projections indicating around 119 cases for 2024. Similarly, the trend for endometriosis cases that underwent surgery during the same period is also increasing ($y = 57 + 6x$), forecasting approximately 87 cases for 2024, which constitutes 73% of the total predicted cases for that year (fig. 1). Between January and September 2024, there were 64 hospitalizations for ovarian endometriosis, out of which 33 cases (51.6%) resulted in surgery, accounting for nearly half of all hospitalizations related to this condition.

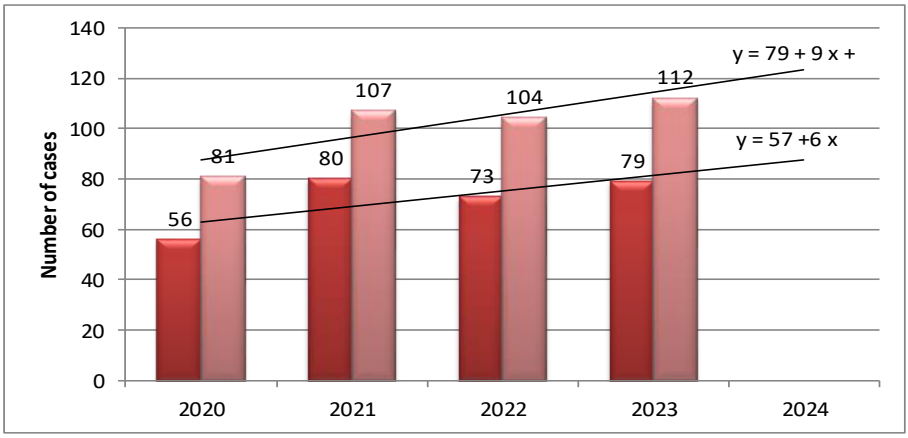

Fig. 1. Distribution of endometriosis cases that underwent surgery between 2020-2023.

Inclusion and exclusion criteria

A prospective study was carried out involving a total of 40 patients assessed at "Cuza-Vodă" and "Elena-Doamna" Obstetrics and Gynecology Clinical Hospitals, Iași between September 2019 to August 2024 of whom 40 met the inclusion criteria and had been underwent surgery. Participants were organized into two groups: an endometriosis group, comprising 35 patients with confirmed endometriosis (30 cases of ovarian endometriosis and five cases with parietal endometriosis), and a control group of 5 patients presenting inflammatory symptoms without histopathological confirmation of endometriosis.

The exclusion criteria applied were lack of signed consent, incomplete follow-up assessments, and absence of histopathological confirmation.

Statistical analysis

This prospective case-control study utilized *SPSS software version 18.0* and data were initially organized and summarized, followed by analyses to derive additional indicators through comparative techniques. Key statistical methods employed included the Student's t-test, the Chi-Square (χ^2) and the Kruskal-Wallis test. The Pearson Correlation Coefficient (r) evaluated the magnitude and direction of associations between variables, and the ROC curve was used to examine the balance between sensitivity and specificity.

The research was carried out in compliance with the Declaration of Helsinki and received approval from the ethics committees of both of the hospitals involved ("Cuza-Vodă" Obstetrics and Gynecology Clinical Hospital No. 130002/24.11.2020, "Elena-Doamna" Obstetrics and Gynecology Clinical Hospital No. 390/13.01.2023), but also of the ethics committees of the "Grigore T. Popa'' University of Medicine and Pharmacy Iasi (No. 401/15.02.2024). Due to the study`s prospective nature, patient consent was necessary. The study's design and data management procedures followed both national and international standards for research involving human participants.

RESULTS

Epidemiological data

Among the participants in the study co-

hort diagnosed with endometriosis, the median age was similar to the mean age (33 years *vs.* 33.03 years), and the Skewness Test results (value <2) indicated a consistent age distribution, justifying the application of statistical significance tests to continuous variables. In patients diagnosed with ovarian endometriosis, aged ranged from 25 to 42 years, with the mean age being higher than that of the other group analyzed, though the difference was not statistically significant, compared to the parietal group (33.60 years vs 32.20 years; p=0.532), and the control group (33.60 years *vs.* 30.40 years; p=0.203).

Demographic characteristics

Demographic analysis of the 40 study participants revealed no significant differences in characteristics between those with endometriosis (n=35) and the control group (n=5). Age distribution was homogeneous across groups (p=0.740), with 20-30% of cases in each group being older than 35 years. In conjunction with the median age of 30 years in the control group, this threshold was chosen for subsequent significance test: 73.3% of patients with ovarian endometriosis and 80% of those with parietal endometriosis were older than 30 years (p=0.747). Urban residency was common with similar distribution across both groups (70% in the ovarian endometriosis cases, 100% in the parietal endometriosis cases and 80.0% in the control patients; p=0.189). Marital status was also relative comparable, married women are including 83.3% of the ovarian endometriosis group, 80.0% parietal endometriosis group and 60.0% of the comparison group (p=0.340). Additionally, the percentage of patients with higher education attainment reported: 63.3% in the ovarian endometriosis cases, 80% in the parietal endometriosis group and 80.0% in the control group (p=0.604). Employment rates were high showing no significant variation between the groups (86.7% for ovarian endometriosis, 100% for parietal endometriosis and 80.0% for control; p=0.454). Smoking prevalence was in the control group 60.0% by comparison with other groups (36,9% ovarian endometriosis and 60% parietal endometriosis) (p=0.436). These balanced demographic characteristics provided a robust basis for comparative analysis.

Clinical characteristics of study participants

The clinical profiles of the patients revealed various features commonly associated with ovarian endometriosis. Primary infertility was present in 53.3% of cases, with a mean duration of infertility exceeding two years in 63.3% of ovarian endometriosis. Hormonal treatment was commonly utilized, particularly among those initially diagnosed with ovarian endometriosis, were 53.3% received treatment for a duration of 1 to 3 years. A smaller percentage, 26.7%, had undergone hormonal therapy for less than one year, while 20.0% had received treatment for a period of 3 to 5 years.

Laboratory findings

Biochemical and hematological assessments revealed significant differences between the two groups. CA-125 levels were markedly higher in those with ovarian endometriosis (67.87±23.61 U/mL) relative to parietal endometriosis (23.32±2.11 U/mL) and controls (22.58±4.8 U/ml, p=0.001). Among inflammatory markers, lymphocyte counts were lower in endometriosis, whereas eosinophil counts, red cell distribution width (RDW) and platelet count were higher in the control group. Although,

the values of the systemic immune-inflammatory biomarkers (neutrophil-to-lymphocyte ratio -NLR-, systemic immune inflammation index -SII, platelet-to-lymphocyte ratio -PLR-) in endometriosis patients were within the range typically observed in the international average scale, the following values were recorded: NLR: 1.76 (0.83-3.92), PLR: 120 (61-239), SII: 459 (189-1168) (tab. I) (9).

TABLE I.

Systemic immune-inflammatory markers

Parameter	Control Group n=5	Ovarian Endometriosis Group n=30	Parietal Endometriosis n=5
NLR	1.96±0.30	3.82±2.07 (p=0.040)	4.17±1.14 p=0.026) p=0.918)
PLR	110.31±23.19	183.18±54.62 (p=0.012)	166.75±30.11 p=0.188) p=0.776)
SII	696.15±193.84	1034.50±623.08 (p=0.044)	964.88±258.75 p=0.050) p=0.964)

In patients with ovarian endometriosis, the correlation of mean values for the inflammatory parameters according to the rASRM staging revealed significantly lower values in stage IV for the neutrophil-to-lymphocyte ratio (NLR) (2.68 *vs.* 4.81; p = 0.003), platelet-to-lymphocyte ratio (PLR) (156.70 *vs.* 206.35; p = 0.01), and systemic immune inflammation index (SII) (721.54 *vs.* 1308.34; p = 0.008) (tab. II).

TABLE II.

Association of inflammatory markers with rASRM staging in patients with ovarian endometriosis

Parameter	Stage III (rASRM) (n=16)	Stage IV (rASRM) (n=14)	t-Student test p
NLR	4.81±2.01	2.68±1.51	**0.003**
PLR	206.35±62.89	156.70±26.00	**0.010**
SII	1308.34±661.82	721.54±403.57	**0.008**

In patients with recurrent ovarian endometriosis, the correlation of mean values for inflammatory parameters according to rASRM staging demonstrated significantly lower values in stage IV for the neutrophil-to-lymphocyte ratio (NLR) (2.80 *vs.* 6.17; p = 0.027), platelet-to-lymphocyte ratio (PLR) (160.25 *vs.* 257.61; p = 0.046), and systemic immune inflammation index (SII) (760.63 *vs.* 1870.95; p = 0.034) (tab. III).

TABLE III.

Association of inflammatory markers with rASRM staging in patients with recurrent ovarian endometriosis

Parameter	Stage III (rASRM) (n=2)	Stage IV (rASRM) (n=3)	t-Student test p
NLR	6.18±1.53	2.80±0.29	**0.027**
PLR	257.61±83.03	160.25±5.38	**0.046**
SII	1870.95±709.14	760.63±175.01	**0.034**

Among the 30 patients with ovarian endometriosis, following surgical intervention, only 27 retained the potential for spontaneous conception. The correlation of the mean values of systemic inflammatory parameters according to the EFI index was not statistically significant ($p > 0.05$).

Additionally, the coagulation profile showed shorter activated partial thromboplastin time (APTT) and prothrombin time (PT) in the endometriosis group, while only a minor variation in urinary density was observed.

TABLE IV.

Association of systemic inflammatory markers with the EFI index

Parameter	EFI< 5 n=10	EFI = 5-7 n=14	EFI ≥8 n=3
NLR	4.50±1.99	3.20±2.04 p=0.319)	4.65±2.96 p=0.993) p=0.537)
PLR	178.16±56.86	211.39±86.36 p=0.958)	211.39±86.36 p=0.784) p=0.662)
SII	1270±612	817±528 p=0.203)	1457±1055 p=0.891) p=0.255)

Histopathological findings

By examining tissue characteristics and structural depth, histopathology helps categorize endometriosis into three clinically relevant subtypes. Superficial peritoneal endometriosis manifested as small, shallow implants on the peritoneum and was generally linked to milder symptoms and lower stages (10). Ovarian endometriomas, often identified as cysts containing old hemorrhagic material, were characterized by endometrial epithelial lining on the cyst wall (fig. 4). For instance, one case (fig. 2) displayed an ovarian cyst wall lined with endometrial stroma and glands (H&E x4), while another (fig. 3) showed endometrial gland inclusion within the tubal wall, highlighting tubal involvement (H&E x4). DIE lesions were marked by infiltration deeper than 5 mm into pelvic structures, including areas such as the rectovaginal septum, and were often correlated with severe pain and elevated inflammatory markers, evidenced by higher CA-125 levels and increased ASRM stages. Hormone receptor analysis indicated elevated estrogen receptor expression in DIE lesions, possibly contributing to their invasive behavior and reduced responsiveness to hormonal therapies in advanced cases.

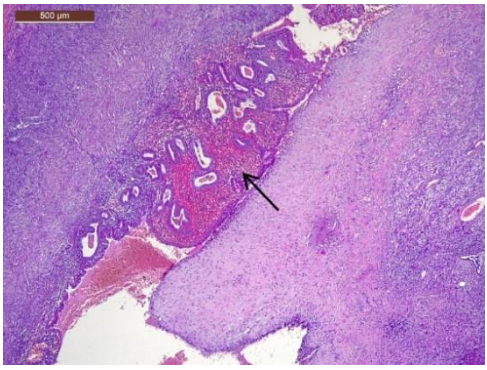

Fig. 2. Ovarian cyst wall showing cortical layer lined with endometrial stroma and glands (arrow) (HE, x4)

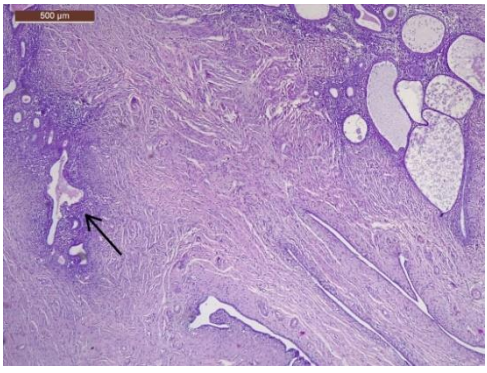

Fig. 3. Tubal wall of the same patient, showing inclusion of endometrial glands and stroma in the tubal wall (arrow) (HE, x4)

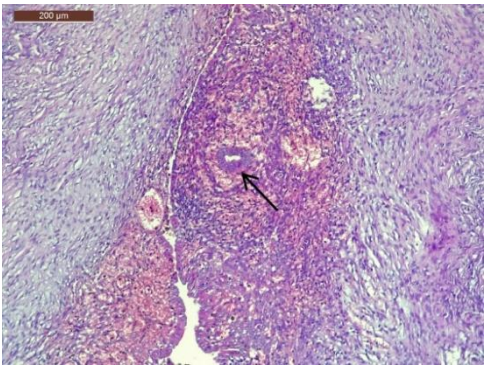

Fig. 4. Ovarian cyst lined with endometrial stroma and gland (arrow) (HE, x10).

DISCUSSION

Endometriosis continues to be a complex and multifaceted condition, significantly impacting both physical and mental well-being. This study provides insights into the intricate relationships between clinical symptoms and histopathological features, adding to the existing literature and offering a detailed perspective on diagnosis and management strategies for endometriosis. Consistent with previous studies, our results suggest that demographic factors, including age and lifestyle, although statistically nonsignificant, may influence disease severity and symptom presentation (11, 12). The association observed between infertility and elevated CA-125 levels aligns with findings from Horne *et al.* (2022), underscoring the relevance of inflammatory biomarkers in diagnosing and predicting endometriosis outcomes (6). In 2018, Fest *et al.* in *Nature*, associated chronic inflammatory pathologies with novel prognostic markers (NLR, PLR, SII) and provided their average reference values in a cohort of 8,711 patients aged up to 45 years (9). High CA-125 levels, along with inflammatory markers such as the NLR and PLR, further highlight the systemic inflammatory nature of endometriosis, emphasizing its immunological aspect. This finding is in line with recent research on emerging biomarkers by Zondervan and Becker, which supports the inclusion of biomarker analysis in routine clinical practice (13). Additionally, the prevalence of primary infertility and extended hormonal treatment durations highlights the necessity for more individualized therapeutic strategies, Current guidelines support a patient-specific approach based on clinical staging and hormonal profiles, as noted by Zondervan *et al.* (2020) (1). Integrating these findings with current recommendations, particularly concerning the timing and dosage of hormonal treatments, could enhance reproductive outcomes for patients at various stages of the disease. The absence of a straightforward correlation between rASRM staging and symptom severity remains a subject of debate, as similar inconsistencies have been noted in other studies. This lack of direct alignment suggests that staging should be supplemented with histopathological assessments, including hormone receptor analysis, to improve predictions of treatment responses and long-term outcomes (14). Emerging literature advocates for this approach, proposing that histopathological data could serve as valuable adjuncts to refine the rASRM classification, especially in distinguishing between deep infiltrating and ovarian endometriosis subtypes (15). In our study, in terms of systemic inflammatory markers, the rASRM classification identifies lower values in stage IV compared to stage III, while the EFI classification does not reveal any significant changes.

This study highlights the value of correlating clinical and histopathological findings in endometriosis, supporting a multidisciplinary and personalized approach to treatment. Future research should focus on

non-invasive diagnostic tools and further exploration of the inflammatory and immunological pathways involved, potentially leading to new options for targeted therapy.

CONCLUSIONS

This study emphasizes the significance of examining both clinical characteristics and histopathological features in understanding endometriosis. The associations observed between inflammatory biomarkers-such as elevated levels of CA-125, NLR, PLR, and SII-compared to the control group, suggest that endometriosis involves a systemic inflammatory component, rather than being limited to localized pathology (13, 15). These findings endorse the use of biomarkers as supplementary diagnostic tools, which could potentially enable earlier and more precise diagnoses in clinical practice.

The inconsistency between ASRM staging and symptom severity underscores the need for an expanded diagnostic framework. By incorporating histopathological evaluations, including lesion morphology, the accuracy of staging can be improved, allowing for a more tailored treatment approach (14). Such an individualized approach in endometriosis is essential both for fertility management and for the patient's quality of life.

Moreover, the association of CA-125, systemic inflammatory markers, and rASRM staging may provide the foundation for the identification of novel non-invasive biomarkers, as well as potential prognostic factors for endometriosis.

In summary, the findings of this study advocate for a comprehensive diagnostic approach to endometriosis that integrates clinical, histopathological, and inflammatory markers. Future research should prioritize the development of non-invasive diagnostic methods and further investigate the immunological mechanisms underlying endometriosis, which could open new avenues for effective, targeted therapies (13). The endometriosis continues to pose significant surgical challenges; however, the integration of advanced artificial intelligence techniques in ultrasonography offers the potential to enhance the precision of subsequent surgical decision-making (17-20).

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CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this article.

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EFFICACY AND SAFETY OF IMAGING-GUIDED TRANSTHORACIC NEEDLE LUNG BIOPSY – A REAL-LIFE STUDY

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EFFICACY AND SAFETY OF IMAGING-GUIDED TRANSTHORACIC NEEDLE LUNG BIOPSY - A REAL-LIFE STUDY (Abstract): Despite global efforts, the challenge of early-stage diagnosis of lung cancer persists. To improve diagnose of peripheral lung tumors, a less invasive, cost-effective, and time-efficient sampling technique is essential. Percutaneous imaging-guided transthoracic lung biopsy meets these criteria. **Aims:** This study aims to determine the efficacy of imaging-guided transthoracic needle lung biopsy and to identify periprocedural complications and diagnostic success rate in a tertiary pulmonology center. **Materials and methods:** We conducted in "Marius Nasta" Institute of Pulmonology, from Bucharest, Romania, a retrospective, observational study on consecutive patients diagnosed with lung, pleural or chest wall tumors that underwent transthoracic Computed-Tomography (CT)-scan guided needle biopsy or ultrasound (US)-guided needle biopsy. The efficacy and safety of these procedures were assessed. **Results**: We enrolled 280 patients, with 261 undergoing CT-scan transthoracic guided percutaneous needle biopsy and 19 undergoing US-guided needle biopsy, between 2021 and 2023. Histopathological results were obtained in 267 cases (95.35%) of lung biopsies, with only 13 (4.64%) inconclusive specimens. The reports identified 225 (80.35%) malignant tumors and 42 (15%) other diseases. Out of 225 malignant tumors, 193 (85.77%) were NSCLC. Out of NSCLC 103 were lung adenocarcinomas (53.36%). Periprocedural complications rate was 23.22%, mainly pneumo-thorax, hemoptysis and alveolar hemorrhage. No fatal outcome was recorded. **Conclusions**: The study underscores the efficacy of percutaneous CT-guided or US-guided transthoracic needle lung biopsy in clinical practice, with an affordable risk of complications. The implication of a multidisciplinary team is essential for the best outcome. **Keywords**: LUNG CANCER, HISTOPATHOLOGICAL TYPE, CT-GUIDED TRANSTHORACIC LUNG BIOPSY, US-GUIDED TRANSTHORACIC LUNG BIOPSY.

INTRODUCTION

In 2022, according to the European Cancer Information System, the European Union (EU27) alone estimated a total number of 319,236 new cases of lung cancer (incidence rate 71.5 per 100,000). It is the

second most diagnosed cancer in men (incidence rate 93 per 100,000) after prostate cancer and the third after breast and colorectal cancers in women (incidence rate 50.9 per 100,000). It ranks as the second most frequent malignancy with an estimated total number in the EU27 of 803,644 cases (prevalence rate 170.4 per 100,000). In Romania 11,716 new cases were diagnosed (incidence rate 61.5 per 100,000), with a significant difference between men (incidence rate 92 per 100,000) and women (incidence rate 32.8 per 100,000). The prevalence rate in Romania was 143.6 per 100,000 (27,016 total cases). Additionally, lung cancer stands as the leading cause of cancer-related mortality, with a mortality rate of 18 per 100,000 people (1).

Despite these staggering statistics and world-wide efforts to improve survival rates, early-stage diagnosis remains challenging. Early diagnosis and treatment of small, localized lung tumors is highly recommended. To facilitate this, a tumoral lung biopsy and histopathology confirmation are mandatory. The sampling technique should be less invasive, cost-effective, with a lower complication rate, and more time-efficient for medical staff compared to classical surgical methods. While ultrasound (US)-guided transbronchial lung biopsy is efficient for contiguous lesions, it faces challenges for peripheral ones (limited availability of endobronchial-navigation equipment). Despite their high diagnostic success rate, open lung biopsy and video-assisted thoracoscopic surgery (VATS) come with restriction related to poor clinical status of some patients and greater morbidity and complications (2). A proposed alternative solution meeting the outlined criteria is imaging-guided transthoracic needle lung biopsy.

This study aims to determine the efficacy of imaging-guided transthoracic needle lung biopsy in "Marius Nasta" Institute of Pulmonology, Bucharest, Romania, a tertiary pulmonology center, and to identify periprocedural complications and diagnostic success rate.

MATERIALS AND METHODS

We conducted a retrospective, observational study on consecutive patients diagnosed with lung, pleural or parietal tumors that underwent transthoracic Computed-Tomography (CT)-scan guided needle biopsy or ultrasound (US)-guided needle biopsy, between 2021 and 2023. All patients were hospitalized. Coagulation tests (Prothrombin Activity, Activated Partial Thromboplastin Clotting Time, International Normalized Ratio), along with a total blood cell count and other routine analyses, were conducted before the procedure. Additionally, medications for bleeding control and platelet function were adjusted or ceased according to international guidelines.

For all subjects, bronchoscopy was performed previously and endobronchial or transbronchial biopsies were not possible or were inconclusive for histopathology diagnosis. All cases were selected by a team consisting of a pulmonologist, a radiologist and a thoracic surgeon. The procedure was made by a specialized radiologist, in the presence of a thoracic surgeon prepared for immediate intervention, if needed.

We made a rigorous patient selection, considering the following indications:

- New or growing solitary nodules (more than 8 mm in diameter) or masses on chest imaging that are not amenable to diagnosis by bronchoscopy or are unlikely to be accessible by bronchoscopy;
- Multiple nodules in a patient not known to have malignancy or who has experienced prolonged remission or has more than one primary malignancy;

- Persistent focal infiltrates or consolidations, either single or multiple, for which no diagnosis has been made by sputum or blood culture, serology, or bronchoscopy;

We used 16-slice computed tomography scanner Neusoft (CT-guided) and a 5 MHz curvilinear (ultrasound guided). Regarding the needles, Medax Medeasy semi-automatic spring-loaded biopsy system was used, with 16, or 18 gauge.

Informed consent was obtained from all patients after an extensive discussion. Relevant blood tests and radiological exams were reviewed prior to the procedure. In order to facilitate the safest and most effective needle pathway, the patient was positioned accordingly: supine, prone or lateral decubitus. A first CT-scan acquisition of the thorax was performed to locate the target lesion and to determine the entry level, where a dotted line of radio-opaque markers was applied on patient's skin. A second volumetric CT scan was done, centered on the markers and including the entire target lesion. A specific entry point from the line was chosen. Then, on the disinfected area (using povidone-iodine) local anesthesia with 1% lidocaine was performed. Using measurements and repeated CT scans of that range the introducer needle was positioned in the target lesion. Biopsies were performed using a 16G or 18G coaxial semiautomatic biopsy device with adjustable sample notch (10 mm/20 mm). Needle size, sample number and length depend on lesion dimensions, degree of necrosis, risk of complications and patient status. The number of specimens collected ranged between 1 and 8 fragments. Fragments were placed in a labelled recipient containing 4% formaldehyde solution and sent to the histopathology lab.

For ultrasound guided procedures, suitable patients were selected, and a 5 MHz curvilinear probe was used for biopsy guidance. A 16G or 18 G semiautomatic biopsy needle was used directly without the coaxial to remove the samples.

All patients were monitored: CT-scan immediately after procedure and a chest X-ray 2 hours after. If stable, elective patients without signs of complications were discharged in the same day. The others were monitored for 24 hours in the hospital.

All data were collected from the hospital's electronic medical record database and personal identification data of the patients were anonymized.

Patients consented for using the data for scientific purposes with the condition of anonymity. The approval of the study by the Ethics Committee of the Institute was obtained.

Statistical correlations were generated in *SPSS version 29.0.2.0* (IBM), while the database was collected and organized in Microsoft Office Excel. The results were expressed as mean values ± standard deviation or as an absolute number (percentages). The results were considered statistically significant for a value of $p<0.05$, and were represented using graphs, histograms and tables.

RESULTS

A number of 280 consecutive patients that underwent CT-guided or US-guided transthoracic needle biopsy for lung, pleural or chest wall tumors were included.

Regarding the types of guidance for transthoracic needle approach, we used two types of imaging guidance: computed tomography (261 cases) and ultrasound (19 cases). Ultrasound guidance was more frequently used for pleural histopathology, chest wall involvement and peripheral lung lesions (3).

From the total number of patients, 189 were males (67.5%) with a gender ratio was M: F = 2.07: 1. The mean age was

62.99 years ± 10.92 SD (fig. 1).

Regarding smoking status, 135 patients were smokers or former smokers (48.21%), 20 patients were non-smokers (7.1%), and for 125 patients (44.6%), we were not able to find data about smoking history in database. Occupational exposure was seen in 71 patients (25.35%).

Regarding comorbidities, 132 patients (47.14%) had respiratory diseases (fig. 2).

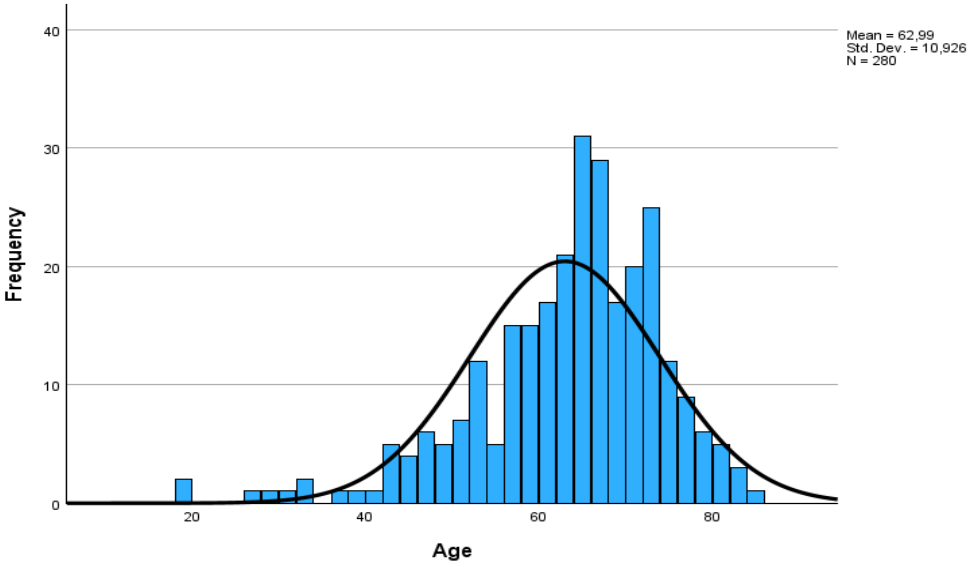

Fig. 1. Age distribution of the patients

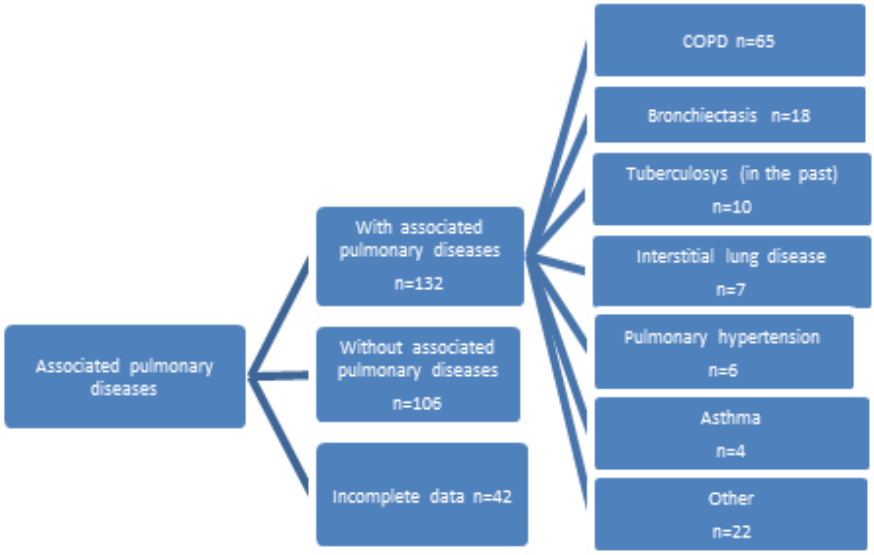

Fig. 2. Distribution of pulmonary comorbidities

The main pulmonary disease was chronic obstructive pulmonary disease (65 patients). A number of 191 subjects had other comorbidities, mainly cardio-vascular and metabolic disorders (fig. 3).

Anemia is only present in 12 patients, while 50 subjects had no other diagnosis.

Out of 280 imaging-guided transthoracic needle biopsies, 267 were diagnostic (95.35%). Only 13 biopsies were inconclusive. Positive results for malignant tumors were obtained in 225 cases (84.26%). Lung cancer was the most frequent (194 cases) with only one case of Small-Cell Lung Cancer (SCLC) and 193 cases of Non-Small-Cell Lung Cancer (NSCLC), with the following subtypes: adenocarcinomas (103 cases, 53.36%), NSCLC-no other specified (NOS) (47 cases, 24.35%), Squamous Cell Carcinoma (32 cases, 16,58%) and undifferentiated carcinoma (11 cases, 5.69%).

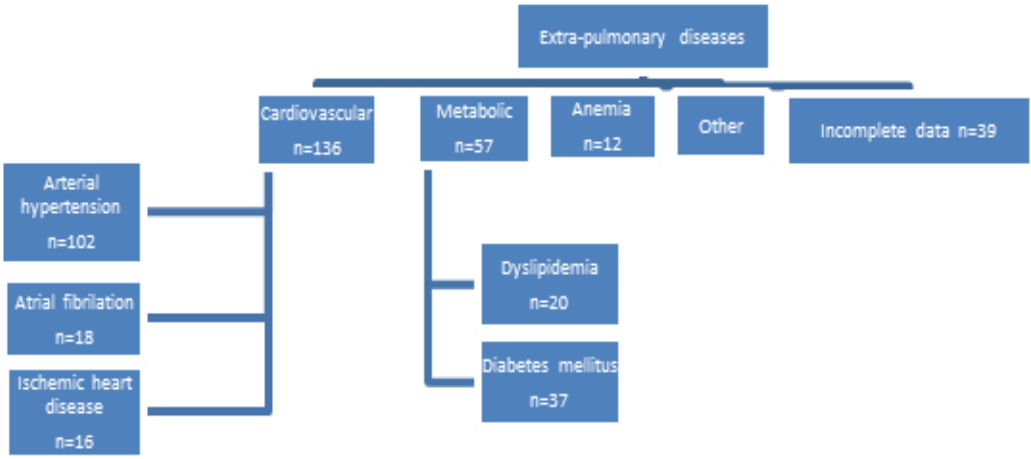

Fig. 3. Extra pulmonary comorbidities distribution

In other 31 cases, malignancy was confirmed, as following: 12 metastases from other distant malignant tumors, 4 neuroendocrine origin tumors other than SCLC, 5 Non-Hodgkin lymphomas, 3 lymphoproliferative malignant disorders, 1 sarcoma, 1 plasmacytoma and 5 only malignant tumoral aspect without definite histopathological pattern.

Among the remaining results, from the 267 conclusive results, 28 cases (10.48%) had “no tumoral aspect”. Additionally, 14 cases (4.86%) were benign lesions: 8 chronic inflammation, 2 pulmonary tuberculosis, 2 fibrinoid pleural mass, 1 organizing pneumonia, and 1 case of thymoma (fig. 4).

Post-procedural complications were seen in 23.22% of procedures (65 cases): 51 cases of small to mild pneumothorax (18.21%), 10 alveolar hemorrhage (3.57%), 7 hemoptysis (2.5%), and 2 subcutaneous emphysema (0.71%) (fig. 5). No severe nor fatal outcome occurred.

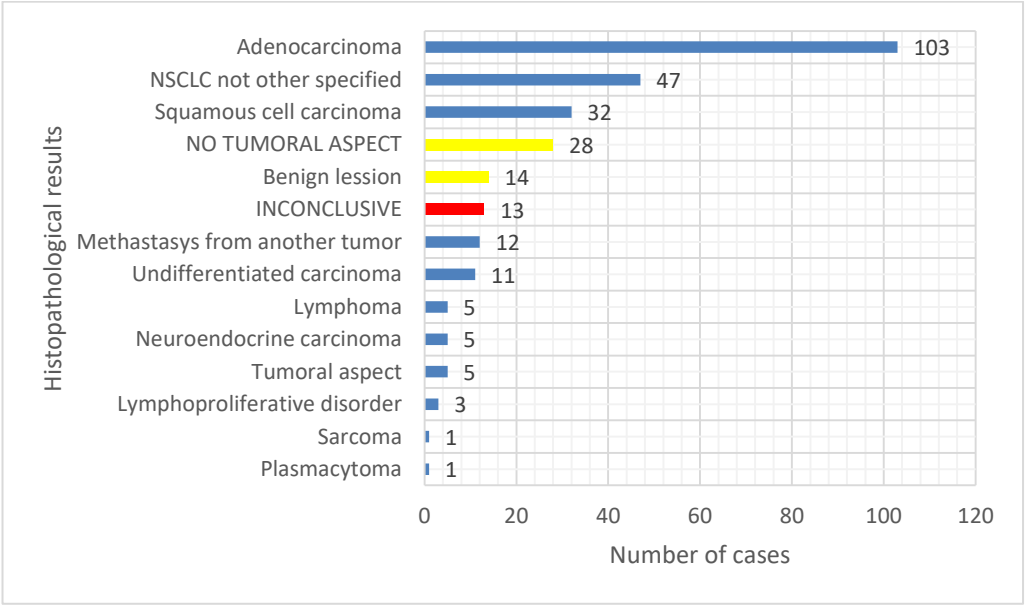

Fig. 4. Distribution of histopathological results

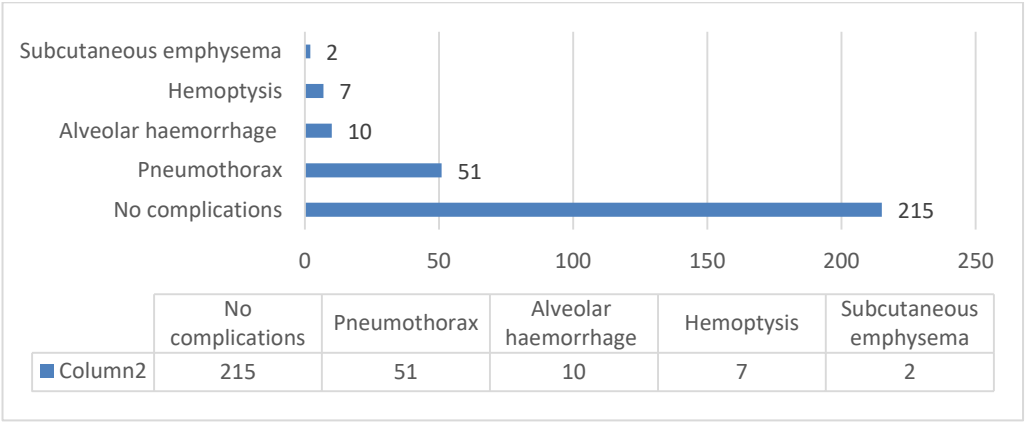

Fig. 5. Post-procedural complications

DISCUSSION

Lung cancer remains a major contributor to cancer-related morbidity and mortality worldwide. Achieving an accurate diagnosis is essential for guiding effective treatment strategies, with histopathological examination serving as a cornerstone in the diagnostic process for pulmonary tumors. Two minimally invasive techniques, bronchoscopy and CT-guided transthoracic biopsy, have proven instrumental in obtaining tissue samples for histological analysis. For centrally located tumors, bronchoscopy is the preferred method, offering direct access to the lesion. However, peripheral tumors pose a greater challenge for tissue sampling. While surgical approaches, such as classical thoracotomy or video-assisted

thoracoscopy (VATS), are highly effective for obtaining biopsies, they are invasive. A less invasive alternative is imaging-guided transthoracic needle biopsy, which uses CT or ultrasound guidance to target and sample peripheral lesions. Histopathological analysis of tissue samples obtained via these methods enables precise tumor characterization, including subtyping of non-small cell lung cancer (NSCLC) versus small cell lung cancer (SCLC), and assessing molecular biomarkers crucial for personalized therapy. This multimodal diagnostic approach plays a vital role in early detection, staging, and treatment planning of lung cancer, improving patient outcomes in this challenging disease.

The aim of this study was to determine the efficacy of imaging-guided transthoracic needle lung biopsy in a tertiary pulmonology center in Romania, and to identify periprocedural complications.

Out of 280 consecutive patients that underwent CT-guided or US-guided transthoracic needle biopsy for thoracic tumors, 267 yielded a definitive diagnosis, resulting in a diagnostic accuracy of 95.35%. This aligns with data reported in the literature, which highlights a high diagnostic success rate ranging from 78%-85% (4-6) to 92.9%-96.8% (7, 8). However, for nodules smaller than 1.5cm, the diagnostic rate is lower (9). These favorable outcomes were achieved through a multidisciplinary team approach involving pulmonologists, radiologists, and thoracic surgeons. This collaborative team carefully selected candidates for the procedure, maintaining a balance between benefits and potential risks. An important issue is to exclude extensive necrosis of the tumor, honeycombing, and large emphysema areas. High risks patients, for which the benefits would have been limited, were not accepted to undergo the procedure. For example, the case of a patient with a history of pneumonectomy due to lung cancer (10 years before the actual evaluation) and extensive emphysema with a lung nodule was rejected due to high risk of respiratory failure.

Out of 267 confirmed cases, 225 were malignant tumors, lung cancer being the most prominent (194 cases). This distribution is according to our expectations, given the hospital's focus on pulmonary diseases.

Positive results for malignant tumors were obtained in 225 cases (84.26%). Lung cancer was the most frequent (194 cases), 193 cases being Non-Small-Cell Lung Cancer (NSCLC). Pulmonary adenocarcinoma was the most prevalent (103 cases, 53.36%), which is similar to other study where 60% were adenocarcinomas (10), reinforcing the well-established trend of adenocarcinoma predominance in the last decade. This is probably due to peripheral location of the adenocarcinomas (11), that means that imaging-guided transthoracic needle biopsy is the appropriate tool to manage this situation. Other histopathological types were: NSCLC-no other specified (NOS) (47 cases, 24.35%), Squamous Cell Carcinoma (32 cases, 16,58%) and undifferentiated carcinoma (11 cases, 5,69%). For NSCLC-NOS specimens were sent to immunohistochemistry analysis to better characterize the tumor.

Statistical analysis revealed a significant correlation between COPD and malignant results (p=0.019; CI=95%), as both diseases are smoke-related diseases.

Among the non-malignant results, 28 cases (10.48%) had “no tumoral aspect”, and 14 cases (4.86%) were benign lesions: 8 chronic inflammation, 2 pulmonary tuberculosis, 2 fibrinoid pleural mass, 1 organizing pneumonia, and 1 case of thymoma. We noticed only 2 cases of pulmo-

nary tuberculosis aligned with the decreasing incidence of tuberculosis in Romania in the last decade.

In order to identify possible complications associated with the technique, a thoracic CT scan was performed immediately after the procedure, and an additional chest X-ray was done 2 hours later. These were performed to assess the presence of pneumothorax, hemothorax, or alveolar hemorrhage. Complications, such as pneumothorax, hemoptysis, alveolar hemorrhage and air embolism may occur in 25.8%, as described in one meta-analysis (12). Pneumothorax was the main complication, between 23.3 and 25.9% as in one large meta-analysis on 23,104 patients (13). Higher frequency was observed in elderly individuals with underlying diseases such as emphysema or honeycombing (14).

Our study shows a post-procedural complications rate of 23.22% with 65 cases of complications, but none of them being severe or lethal. We reported small to mild pneumothorax in 18.21% of cases, alveolar hemorrhage 3.57%, hemoptysis 2,5%, and subcutaneous emphysema in 0,71% of cases. Notably, lesions located in the lower lobes were associated with a significantly higher risk of complications (p=0.043; 95% CI).

Periprocedural complication rates appeared higher with age (p=0.046; CI=95%). Underlying COPD, diabetes mellitus, anemia, chronic heart failure, atrial fibrillation, arterial hypertension did not significantly correlate with complications (p>0,05). The lack of correlation might suggest the high safety profile of the procedure.

Additionally, the number of samples was not correlated with complications (p=0.069; CI=95%). The number of specimens collected ranged between 1 and 8 fragments, with a mean value of 3.03+/-1.15. As mentioned before, we did not determine the quantity and/or the quality of the specimens. Although this study's purpose was not to evaluate a standardized quality and quantity of the samples, we observed that a higher number of samples was not significantly correlated with a suitable, conclusive biopsy (p=0.069; CI 95%), which might suggest that the quality of specimens may be more important. Moreover, advanced lung cancer (presence of metastasis) does not correlate with higher diagnostic rate (p=0.35; CI 95%), though, regarding the technique, CT-guided technique tended to offer a higher number of fragments (p=0.01; CI 95%).

Limitations of the study: Data regarding smoking or exposure status was limited due to the patient's refusal to provide the information or the information not being tracked in the available documents. Another constraint encountered is the availability of internal immunohistochemistry infrastructure. In this situation, immunohistochemistry tests were done by external laboratories for multiple samples.

CONCLUSIONS

This study underscores the significance of a less invasive, cost-effective technique with a lower complication rate for lung biopsy. The success diagnostic rate is 95.35% and is not correlated with higher number of fragments, highlighting the importance of a quality specimen. Further studies might include a standardized qualitative and quantitative analysis of the samples in order to validate the hypothesis. Complication rate of the procedure significantly correlates with older age, and lower lobes localization.

Lung adenocarcinoma diagnosis was the main histopathological subtype of lung cancer diagnosed by this procedure.

In our center, imaging-guided lung biopsy has significantly reduced the time between detecting suspicious lung lesions and initiating appropriate oncological treatment (once confirmed). The implication of a pneumologist-radiologist-thoracic surgeon team is essential, as they proceed to select the patients in order to minimize inconclusive samples and to evaluate risk benefits ratio.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflicts of interests. The research did not receive external funding.

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ORAL HEALTH BEHAVIORS IDENTIFIED IN PATIENTS ACCESSING DENTAL EMERGENCY UNITS. A SINGLE CENTER STUDY

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ORAL HEALTH BEHAVIORS IDENTIFIED IN PATIENTS ACCESSING DENTAL EMERGENCY UNITS. A SINGLE CENTER STUDY (Abstract). **The aim** of the study was to identify the profile of patients accessing dental emergency units. **Materials and methods**: A total of 105 patients who accessed emergency units completed a questionnaire. Socio-demographic, medical, and health-related behavioral data were collected. Additionally, medical diagnostics were considered for this research. **Results**: Significant differences were identified concerning sex, indicating that men visited emergency units more frequently in the previous month (1.76 ± 0.43) than women (1.54 ± 0.50). Patients with chronic diseases were more commonly diagnosed with periodontitis and mucosal lesions, and they used less analgesic treatment for dental pain than patients with non-chronic diseases. Age correlated positively with a diagnosis of gingivitis ($r = 0.19$, $p = 0.04$), in the sense that older individuals were more likely to be diagnosed with gingivitis. The results also proved a strong positive correlation between the level of satisfaction regarding dental health and oral hygiene. The study further investigated the importance of marital status, parenthood, and oral health behaviors. **Conclusions**: Socio-demographic and medical data, along with health-related behaviors, are important factors to be considered when providing emergency dental care to patients. Such information helps dentists and policymakers to shape preventive strategies that reduce the number of unhealthy behaviors and oral health issues in patients accessing emergency dental units. **Keywords:** DENTISTRY, PUBLIC DENTAL EMERGENCY UNIT, DENTAL ANXIETY SCALE, ORAL DISORDERS, QUALITY OF ORAL HEALTH.

INTRODUCTION

According to the Global Oral Health Status Report published by the World Health Organization (WHO) in 2022, 2 billion people suffer from caries of permanent teeth, and 514 million children suffer from caries of primary teeth. A global statistical evaluation estimated that 3.5 billion people worldwide (almost 50% of the population) present oral diseases, the most common being dental caries, periodontal disease, oral cancer, oro-dental trauma, or total tooth loss.

Apart from genetic factors or the presence of comorbidities, most oral health-related problems could be prevented by

limiting alcohol consumption, eliminating the consumption of tobacco, ensuring balanced nutrition and a sugar-free diet, adopting safety measures such as using protective equipment during sports and travel, and brushing teeth with fluoride-containing toothpaste (WHO, 2022) (1). Finally, prioritizing overall quality of life, including physical, mental, and social well-being, while maintaining good health and identifying diseases that negatively impact oral health in proper time could prevent oral health-related problems. Therefore, prevention measures such as promoting good health by adopting a healthy diet, a daily dental cleaning routine, and regular dental checkups are among the most important methods to maintain good dental health and quality of life (Jain *et al.*, 2024) (2). Many factors are associated with low quality of oral health life (OHL): sex, age, level of education, financial income, oral health literacy, and oral health-related behaviors (Fisher *et al.*, 2023) (3). Hakeberg and Boman (2017) identified that the lower the socioeconomic position, the poorer the health (4). Tooth brushing less than three times a day and irregular flossing were additional outcomes associated with low OHL (Batista *et al.*, 2017) (5). Poor oral health literacy was found to be independently associated with missed dental appointments (Baskaradoss,2016) (6). These important factors have been pointed out by many other studies. For example, Ueno *et al.* (2013) (7) found that higher oral health literacy leads to more frequent brushing and better oral hygiene. Similarly, Lee *et al.* (2012) (8) demonstrated a strong positive relationship between increased oral health literacy and improved oral hygiene. This study aimed to shape the profile of patients accessing public dental emergency units in the Oradea area, focusing on dental anxiety and the impact of oral disorders on general well-being.

MATERIALS AND METHODS

Participants and Data Collection. The study was conducted in the public dental emergency unit of the Oradea Emergency Hospital. In 2021, clinical records showed that there were 2,260 patients (1,682 adults and 578 children) with a median average of 188 patients per month. A total of 197 patients, both adults and children, were registered for the period June 20th - July 20th in the dental public emergency unit. Printed questionnaires were distributed to patients waiting to undergo dental consultation. The patients were informed about the purpose of the study, about the confidentiality of the collected data, and about their right to withdraw at any time with no consequences. No incentives were offered for those who participated in the research. Completed surveys were collected in a sealed box located in the main hall. The inclusion criteria consisted of patients aged over 18, fully completed questionnaires, and questionnaires completed by the patients themselves and not by their caregivers. This last criterion was applied to select patients (being literate, assuming self-accuracy of data, and having a normal cognitive level of understanding). Ultimately, a

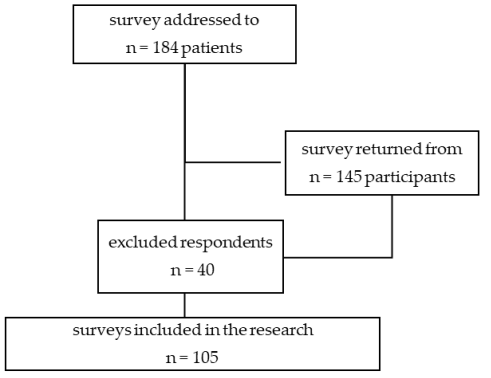

Fig. 1. Study profile.

total of 105 questionnaires were included in the research.

Instruments

A questionnaire was constructed especially for the present study, consisting of 3 parts:

- The first part collected socio-demographic and financial data such as sex, age, environment, marital and professional status, number of children, and monthly income).
- The second part contained self-rated items aimed at collecting information about health-related behaviors, the level of stress, and the quality of dental life (e.g., the presence of a chronic disease, the consumption of alcohol or tobacco, the dental hygiene routine, the consumption of pain drugs, and visits to the dentist. The patients were also required to self-rate certain questions targeting the level of pain or the satisfaction with their dental quality of life (e.g., *How do you rate your level of pain now, on a scale from 1-10,* where 1 represents "no pain")
- The third part of the questionnaire was completed by dentists, recording data about diagnostic, identified dental issues, and emergency interventions.

Statistical Analyses. All data analyses were performed using the *IBM Statistical Package for Social Sciences (SPSS) version 26* (SPSS Inc., Chicago, IL, USA). The descriptive statistics of socio-demographic and medical data were expressed as means and standard deviations (SD), frequencies, and percentages. Given that the distribution of data was normal, parametric tests were used. An independent T-test was performed for the comparative analysis among genders, and a One-Way ANOVA was performed for the comparative analysis among professional categories. Correlation analysis was performed using *Pearson* correlations. The level of statistical significance was set at $p < 0.05$.

Ethical Approval. The present study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee of the Emergency Clinical Hospital of Oradea, Romania, No. 14518.16.06.2021.

RESULTS

Sociodemographic and Medical Data

A total of 105 patients, aged 37.03 ± 14.45 (with a minimum of 18 and a maximum of 84 years old), agreed to fill in the questionnaire. The majority of the respondents were from urban areas and were female (52.4%). Almost three-quarters of them declared that they were employed, with half having a high-school level of education. Additionally, three-quarters of them stated that they were in a relationship, and most of them were parents (tab. I).

Oral health behaviors

The respondents were asked to answer some questions focusing on their oral health-related behaviors. The data analysis revealed that one-third of them consumed alcohol or cigarettes. Three-quarters stated that they used toothpaste and a brush for cleaning their teeth. Less than one-fifth reported using mouthwash, and none of the respondents used dental floss as a method for cleaning their teeth. Regarding their teeth-brushing routine, we identified that around one-third of them brushed their teeth in the morning or at night, with morning brushing being more common than evening brushing (tab. II).

The diagnostics were registered by the medical team, and all details are presented in table III.

TABLE I.
Sociodemographic Data

Sociodemographic and Medical Characteristics	n (%) M ± SD
Gender	
• male	50 (47.6)
• female	55 (52.4)
Age	37.03 ± 14.45
Area of origin	
• urban	64 (61.00)
• rural	41 (39.00)
Educational level	
• middle school	29 (27.6)
• high school	60 (57.1)
• university	16 (15.2)
Professional status	
• Student	8 (7.6)
• Employed	73 (69.5)
• Unemployed people	4 (3.8)
• Pensioner	10 (9.5)
• Social assistance recipient	10 (9.5)
Monthly income (1 EUR = 5 RON)	
• Under 500 (RON)	16 (15.2)
• Between 500 and 1,500 (RON)	23 (21.9)
• Between 1,500 and 3,000 (RON)	48 (45.7)
• Between 3,000 and 5,000 (RON)	16 (15.2)
• Between 5,000 and 7,000 (RON)	2 (1.9)
Marital status	
• Single	31 (29.5)
• In a relationship	74 (70.5)
Being a parent	
• Yes	69 (65.7)
Number of children	
• 1	22 (21.0)
• 2	34 (32.4)
• 3<	13 (12.5)

TABLE II.

Medical Data and Oral Health Routine

Medical data and oral health routine	n (%) // M (± SD)
Alcohol consumption	
• No	75 (71.4)
• Rarely	21 (20.0)
• Occasionally	9 (8.6)
Smoking	
• no	66 (62.9)
• I did, but I quit	7 (6.7)
• smoked rarely	12 (11.4)
• yes	20 (19.0)
Oral hygiene	
• only with water	7 (6.7)
• toothpaste and toothbrush	75 (71.4)
• mouthwash	19 (18.1)
• dental floss	0
• toothpicks	4 (3.8)
Do you brush your teeth in the morning?	
• never	0
• very rarely	6 (5.7)
• sometimes	21 (20.0)
• almost every morning	30 (28.6)
• every morning	48 (45.7)
Do you brush your teeth in the evening?	
• never	0
• very rarely	11 (10.5)
• sometimes	28 (26.7)
• almost every evening	32 (30.5)
• every evening	34 (32.4)
Going to the dentist	
• periodic inspection	19 (18.1)
• only when needed	80 (76.2)
• I have never been	6 (5.7)
Emergency dental office in the last 6 months	
• Yes	37 (35.2)
Number of emergency dental office visits	
• 0	60 (57.1)
• 1	23 (21.9)
• 2	14 (13.3)
• 3	5 (4.8)
• 4	2 (1.9)
• 5	1 (1.0)

Medical data and oral health routine	n (%) // M (± SD)
Dental consultation last month	
• yes	37 (35.2)
Level of dental pain (1 - *not at all* to 10 - *very much*)	M = 5.50 ± 2.88
Dental health satisfaction level (1- *Extremely dissatisfied* to 5 - E*xtremely satisfied)*	M = 2.97 ± 1.11
Level of stress in the last 6 months (1 - *not at all* to 5 - *very stressed*)	M = 2.75 ± 1.11
Chronic diseases	
• yes	8 (7.6)
Toothache medication last month	
• yes	61 (58.1)
Dental problems in childhood	
• never	14 (13.3)
• rarely	27 (25.7)
• sometimes	44 (41.9)
• often	14 (13.3)
• very often	6 (5.7)

TABLE III.

Orthodontic profile of participants

Orthodontic profile	n (%) M ± SD
Pulpitis	
• serous	33 (97.1)
• purulent	1 (1.0)
Periodontitis	
• yes	5 (4.8)
Dental abscess	
• yes	10 (9.5)
Gingivitis	
• yes	5 (4.8)
Other	
• oral thrush	1 (1.0)
• upper lip contusion	1 (1.0)
• coronary fracture	5 (4.9)
• fracture of a prosthetic restoration	1 (1.0)
• dentin hypersensitivity	1 (1.0)
• dental mobility	1 (1.0)
• acute apical periodontitis	37 (35.2)
• post-traumatic gingival injury	2 (1.9)
• root remnant	1 (1.0)
Oral hygiene	
• poor	39 (37.1)
• satisfying	54 (51.4)
• good	12 (11.4)
Dental caries	

Orthodontic profile	n (%) M ± SD
• simple	49 (47.1)
• complicated	55 (52.9)
Filling	
• compliant	39 (37.9)
• non-compliant	45 (43.7)
• none	19 (18.4)
Edentations	
• partially	61 (77.2)
• extended	18 (22.8)
Prosthetic restoration	
• compliant	20 (19.6)
• non-compliant	13 (12.7)
• none	69 (67.6)
Type of Prosthetic restoration	
• fixed	28 (84.8)
• Implant-supported	2 (6.1)
• removable/ mobilizable	3 (9.1)
Dental mobility	
• Grade I	66 (75.0)
• Grade II	13 (14.8)
• Grade III	9 (10.2)
Odontalgia	
• diffuse	18 (21.2)
• continuous	36 (42.4)
• pulsating	31 (36.5)
Periodontal pain	
• aching	18 (30.5)
• continuous	35 (59.3)
• stabbing	6 (10.2)
Gingival Bleeding	
• localized	36 (69.2)
• generalized	16 (30.8)
Tumefaction	
• localized	27 (87.1)
• diffuse	4 (12.9)
Mucosal lesions	
• yes	11 (10.8)
TMJ pain	
• aching	1 (33.3)
• continuous	2 (66.7)
• stabbing	0
Trismus	
• yes	1 (1.0)

There were significant differences in calling for dental consultation services between males and females, $t(103) = 2.336$, $p = 0.021$. Results proved that men had visited the dentist more often in the previous month (1.76 ± 0.43) compared with women (1.54 ± 0.50).

A statistically significant difference was observed in the examination of swellings between male and female patients, with women showing a higher mean number of swellings (1.22 ± 0.42) than men (1.00 ± 0.00), t(17) = -2.204, p = 0.042. An independent-sample T-test was performed to determine if there were differences in analgesic administration between patients with chronic diseases and healthy patients. Results showed that on average, patients who did not suffer from chronic diseases took more analgesics (1.44 ± 0.49) than patients suffering from chronic diseases (1.12 ± 0.35), t(9.47) = -2.360, p = 0.041.

Significant differences were observed in the examination of mucosal lesions between patients with and without chronic diseases $t(93) = 3.511$, $p = 0.001$, meaning that respondents with chronic diseases showed a higher mean number of mucosal lesions (2.00 ± 0.00) than healthy respondents (1.88 ± 0.32). Furthermore, results indicated that patients who suffered from chronic diseases were more frequently diagnosed with periodontitis (2.00 ± 0.00) than those who did not (1.94 ± 0.24), t(96) = 2.516, p = 0.014.

There were significant differences in examining swellings based on marital status, $t(23) = -2.145$, $p = 0.043$. Participants engaged in a relationship showed a higher mean number of swellings (1.17 ± 0.38) than single participants (1.00 ± 0.00). The statistical analysis of data indicated that single patients were more frequently diagnosed with periodontitis (2.00 ± 0.00) than patients who were engaged in a relationship (1.92 ± 0.27), t(73) = 2.538, p = 0.013.

A one-way ANOVA was conducted to determine if the diagnosis of dental fillings varied among groups with different levels of education. Participants were classified into four groups: *those with primary school education* ($n = 7$), *those with secondary school education* ($n = 13$), *those with high school education* ($n = 69$), and *those with higher education* ($n = 14$). The data are presented as mean ± standard deviation, and there was homogeneity of variances, as assessed by Levene's test of homogeneity of variances ($p = 0.771$). The diagnosis of fillings was statistically significantly different across education levels, $F(3, 99) = 6.837$, $p < 0.001$, showing an increase from higher education (1.43 ± 0.51) to high school (1.57 ± 0.60), to secondary school (1.92 ± 0.76), and finally to primary school (2.57 ± 0.78), in that order. Tukey post hoc analysis revealed that the increase from primary to secondary school education (1.006, 95% CI (0.36 to 1.66)), ($p = 0.001$) as well as the increase from primary school to higher education were statistically significant (1.143, 95% CI (0.38 to 1.90)), ($p = 0.001$), but no other group differences were statistically significant.

Another analysis using one-way ANOVA was conducted to determine if the number of children varied across different professional groups. The participants were classified into five groups: *students* ($n = 8$), *employees* ($n = 73$), *pensioners* ($n = 10$), *unemployed people* (n= 4) and *socially assisted people* ($n = 10$).

The results indicated that the number of children the patients had was statistically significantly different among different social categories, $F(4, 14.263) = 7.399$, $p = 0.002$. The mean number of children increased progressively from students (0.25 ± 0.46) to employees (1.29 ± 1.40), to pensioners (1.50 ± 0.97), to unemployed peo-

ple (1.50 ± 1.29), to socially assisted people (2.80 ± 2.15) groups. The Games-Howell post hoc analysis revealed that the increase in the number of children from students to employees was statistically significant (-1.03, 95% CI (-1.72 to -0.36)), (p = 0.001), the increase from students to pensioners was statistically significant (-1.25, 95% CI (-2.34 to -0.16)), (p = 0.022), and the increase from students to socially assisted people was statistically significant (-2.55, 95% CI (-4.85 to -0.25)), (p = 0.029), but no other group differences were identified. The diagnosis of fillings was statistically significant across various income levels, $F(4, 98) = 4.875$, $p < 0.001$. The rates increased from the income range of 5,000-7,000 RON (n=2)(1.00 ± 0.00), to the income range of 3,000-5,000 RON (n=15) (1.40 ± 0.50), to the income range of 1,500-3,000 RON (n = 48)(1.52 ± 0.65), to the income range of 500-1500 RON (n = 22)(1.82 ± 0.66), and finally to the under 500 RON income group (n = 16)(2.19 ± 0.65). Tukey post hoc analysis indicated that the increase from the under 500 RON group to the 1,500-3,000 RON income group (0.66, 95% CI (0.16 to 1.17)), (p = 0.004), as well as the increase from the under 500 RON group to the 3,000-5,000 RON income group were statistically significant (0.78, 95% CI (0.16 to 1.42)), (p = 0.007), while no other group differences were statistically significant.

A Pearson's correlation was conducted to assess the relationship between age and the degree of satisfaction regarding dental health. The results indicated a statistically significant negative correlation between these variables (r = -0.19, p = 0.04), suggesting that as participants aged, their satisfaction with dental health decreased. Additionally, age correlated positively with the diagnosis of gingivitis (r = 0.19, p = 0.04), in the sense that the incidence of gingivitis increases with age.

We identified that there were strong negative correlations between the level of education and the diagnosis of dental fillings (r = -0.38, p < 0.001), and respectively, between the income and the diagnosis of fillings (r = -0.39, p < 0.001). This indicated that higher levels of education and standards of living were associated with a lower frequency of this diagnosis. Additionally, the level of education correlated positively with income (r = 0.46, p < 0.001), in the sense that the higher the level of education, the higher the standard of living. The results proved negative correlations between the number of children and the number of packs of cigarettes consumed per day (r = -0.40, p = 0.008), as well as between the number of children and the prosthetic works that patients had (r = -0.37, p < 0.001). Thus, the more children the patients had, the less they smoked, and the fewer prosthetic works they had. The number of packs of cigarettes smoked per day correlated positively with dental pain (r = 0.35, p = 0.03), indicating that increased smoking leads to more pain. We identified a positive correlation between the pain felt by the patients and dental cavities (r = 0.224, p = 0.02), in the sense that more cavities result in higher pain levels. We identified a negative correlation between the frequency of visits to the dentist and periodontal pain (r = -0.26, p = 0.04), meaning that the more often patients visited the dentist, the less periodontal pain they experienced. Additionally, stress levels correlated negatively with dental pain (r = -0.35, p = 0.001), higher stress resulted in less dental pain. The results showed a strong positive correlation between the level of satisfaction regarding dental health and oral hygiene (r = 0.34, p < 0.001). Patients who took care of their teeth better were more satisfied with their dental

health. Additionally, dental health satisfaction correlated negatively with gingival bleeding ($r = -0.03$, $p = 0.029$), meaning that the more bleeding patients had, the less satisfied they were with their dental health.

DISCUSSION

Our results cover an important gap regarding the profile of patients in the emergency units in Oradea, detailing their sociodemographic characteristics and oral health-related behaviors. Most of the studies have also identified that women typically seek dental assistance in emergency units, indicating a delay in receiving dental care. However, few studies have shown that men are more likely to visit public emergency dental units. For example, Matsumoto *et al.* (2017) (9) analyzed the profile of Brazilian patients who accessed the public dental emergency units, finding that usually the patients were males, aged 30-52, with an elementary school level of education, with only one previous consultation in public dental units. Ottaviani *et al.* (2013) (10) found that male patients were more frequent beneficiaries of Italian public dental emergency services, while Verma and Chambers (2014) observed that in Australia, usually the patients were males younger than 30 years old (11). Our study found that nearly 75% of patients declared that they sought dental appointments only when necessary, while less than 20% had periodic checkups. These results are in congruency with literature, such as a study conducted by Schroeder *et al.* (2024) which indicates that lacking a regular dentist leads to higher emergency dental visits (12).

The results indicated negative correlations between the number of children and the number of packs of cigarettes consumed per day. Our study is in congruency with others identified in scientific literature. For example, Lippet (2021) (13) observed that parents, especially mothers, smoke less when having children, which improves children's oral health. Mahabee-Gittens *et al.* (2022) found that children aged 1-11 years old with less exposure to tobacco had fewer carries and less chronic difficulty with one or more oral health problems (14). Leroy *et al.* (2011) identified that previous exposure to tobacco was significantly associated with gingivitis in 5-year-old children (15). A recent meta-analysis showed a strong correlation between passive smoking and the increased prevalence of dental caries in children. Environmental tobacco smoke exposure and dental caries risk were influenced by factors such as low parental education, low socioeconomic status, poor dietary habits, low oral hygiene, and gender (Clemention *et al.*, 2025) (16).

We identified that approximately one-third of patients brushed their teeth either in the morning or at night, with morning brushing being more common than evening brushing. Teeth brushing is one of the most common behaviors that maintain oral health. Individuals from low socioeconomic (SES) backgrounds often brush less regularly (Raison *et al.*, 2021) (17). The study by Hwa-Young Lee *et al.* (2023) indicated that patients under the age of 30 years were more likely to brush after lunch, while those aged 40-79 were found to brush their teeth after dinner (18). Additionally, depending on the professional category, people engaged in services, as well as students, housewives, or unemployed people were less likely to brush their teeth after lunch compared to office workers.

Strengths and limitations of the study. The current research is important because it provides important statistical data about the environment of emergency dentistry, outlining a profile of the patients who access

public emergency medical services. These results are generalizable to the area population studied and useful for preventive campaigns. One limitation of the study is determined by the period in which the research took place, i.e., after the period of restrictions imposed by the COVID-19 pandemic, when access to dental interventions was carried out only for patients in critical dental situations and when routine checkups were avoided. It is very possible that the patients' oral health deteriorated because of these restrictions to accessing dental medical services and conventional treatment. This situation was very clearly presented by Sycinska-Dziarnowska and Paradowska-Stankiewicz (2020) (19) who, in their study, showed that during the period of restrictions imposed by the pandemic, the search for the word "toothache" had an increasing trend in all European countries and access to simple dental services was extremely limited (Nijakowski *et al.*, 2021) (20). Research conducted by Peloso *et al.* (2020) also identified that the level of anxiety among patients needing dentistry interventions was higher during pandemics (21). Patients undergoing dental treatment and orthodontics were more willing to attend appointments and were more concerned about an increase in treatment duration or expenses. The second limitation of the study is determined by the fact that the research was conducted in a single-center emergency unit, so the results should be interpreted in relationship with the socio-demographic and economic factors related to this geographic area. The third limitation of the study is caused by the number of socio-demographic factors that can force patients to access oral health services. An example would be the distance between their home and a dental clinic. A fourth limitation of the research is related to the lack of information about the profession or the level of health literacy (the latter being considered, implicitly, in this study, as being related to the patient's number of years of schooling). Finally, the medical condition-experiencing significant pain-could be also a limitation of the study, because maybe this category of patients chose not to answer the questionnaire because of their physical discomfort.

CONCLUSIONS

To prevent dental emergency problems and shape health policies in dentistry, it is essential to identify the profiles of patients seeking assistance in public dental emergency units. The results are valuable for health policymakers, as well as those responsible for health education, since health education and prevention play an important role in enhancing the quality of life in general. Given that the profile of the patients proves that most of the beneficiaries do not have more than a high-school level of instruction, it is essential to consider new strategies for delivering information through elementary school-level curricula.

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INCOME, EDUCATION, AND BARRIERS TO DENTAL CARE ACCESS IN RURAL COMMUNITIES. A CROSS-SECTIONAL STUDY

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INCOME, EDUCATION, AND BARRIERS TO DENTAL CARE ACCESS IN RURAL COMMUNITIES. A CROSS-SECTIONAL STUDY (Abstract): Access to dental services in rural areas is often limited by socio-economic and structural barriers, contributing to a high prevalence of dental caries and persistent oral health inequalities. **Aim:** This study aimed to assess access to dental services in a rural population and to analyze the associations between socio-economic factors, perceived barriers to care, and oral health status, measured using the DMFT index. **Materials and methods**: A cross-sectional observational study was conducted in 2025 among 72 adults living in rural areas of Bacău County, Romania. Participants underwent a standardized dental examination based on ICDAS criteria, and caries experience was assessed using the DMFT index. A structured questionnaire collected socio-demographic data and evaluated perceived financial, educational, and geographic barriers to dental care using a five-point Likert scale. **Results**: A very high prevalence of dental caries was identified, with DMFT values ranging from 0 to 25. Only 1.39% of participants were caries-free, while 22.22% presented DMFT ≥ 13. Higher DMFT scores were significantly associated with lower educational level ($r = -0.342$, $p = 0.003$), perceived financial constraints, and the perception that household income influences dental attendance ($r = 0.260$, $p = 0.027$). Geographic barriers also contributed, as perceived distance to the dental office was positively correlated with DMFT ($r = 0.340$, $p = 0.003$). Financial barriers were strongly interrelated and linked to delayed care and emergency-only visits ($p < 0.001$). Lower educational attainment was further associated with reduced understanding of medical advice and limited access to preventive information. **Conclusions:** Oral health disparities in rural populations are driven by interconnected socio-economic, educational, and geographic factors, underscoring the need for targeted preventive strategies, improved rural dental infrastructure, and enhanced public support. **Keywords:** RURAL POPULATION; DENTAL CARIES; ACCESS TO DENTAL CARE.

INTRODUCTION

Access to dental services in rural areas is significantly affected by household income levels. Evidence shows that individuals with low income and without dental insurance access dental care less frequently, particularly preventive services, which increases the risk of complications (1). In Romania, regional economic studies indicate a significant association between income level and the frequency of dental visits, with higher-income individuals being more engaged in preventive care and regular treatments (2). Consequently, lim-

ited financial resources in rural settings lead to a reliance on emergency treatments and delays in necessary oral healthcare.

Another essential factor is educational level and health literacy. Rural populations generally have lower levels of formal education and limited knowledge regarding oral hygiene, which negatively influences preventive attitudes. For example, a study conducted in Romania showed that only 38.8% of 12-year-old children in rural areas brush their teeth twice daily, and 11.2% have never attended a dental check-up (3). At the international level, an analysis of data from 27 countries confirmed that low educational attainment and reduced income are major predictors of infrequent dental visits and untreated oral health problems (4).

Deficient medical infrastructure and the unequal distribution of dental professionals represent additional major barriers. In the United States, more than 4,600 rural areas are classified as Dental Health Professional Shortage Areas, reflecting a chronic lack of specialized dental personnel in these regions (5). In Romania, although the overall number of dentists is relatively high, most practice in urban and private settings, while rural areas remain underserved and face difficulties in accessing care through the public system (6). This situation forces rural patients to travel long distances for dental treatment, resulting in increased time and financial costs and discouraging the utilization of dental services. Therefore, the aim of this study is to identify and analyze the socio-economic factors influencing access to dental services among the rural population, with particular emphasis on the roles of income level, education, and available healthcare infrastructure. The study seeks to highlight the main barriers contributing to inequalities in oral healthcare utilization and to propose potential intervention strategies to reduce these disparities.

The aim of the study was to assess access to dental services and to analyze associated socio- economic factors, based on clinical data and responses to a standardized questionnaire.

The study population consisted of adults aged 18 years and older who were permanent residents of rural localities in Bacău County. Participants were selected using simple random sampling, in accordance with predefined eligibility criteria, including provision of informed consent and the absence of severe systemic diseases.

For the clinical component, all participants underwent a standardized dental examination performed according to the criteria of the International Caries Detection and Assessment System (ICDAS), which was used to identify and record carious lesions (7). Caries experience was quantified using the DMFT index (Decayed, Missing, and Filled Teeth), in line with World Health Organization recommendations (8). All clinical assessments were performed in a dental clinic by a calibrated examiner.

In parallel, participants completed a structured questionnaire addressing access to dental care. The questionnaire included demographic and socio-economic variables (age, sex, educational level, occupational status, and monthly household income), as well as 15 items assessed on a five-point Likert scale (1 = strongly disagree to 5 = strongly agree). These items explored perceived barriers to accessing dental services, the influence of economic, educational, and geographic factors, and overall satisfaction with available dental care.

MATERIALS AND METHODS

This cross-sectional study was conducted as a descriptive, observational investigation between January and April 2025 among the adult population living in rural areas of Bacău County, Romania.

Collected data were processed and statistically analyzed using *SPSS software version 26.0*. Descriptive statistics included frequencies, means, and standard deviations for socio-demographic variables, questionnaire responses, the DMFT index, and ICDAS scores.

Correlation analyses

(Pearson or Spearman, depending on data distribution) were applied to examine associations between socio-economic variables (income, educational level, occupational status), caries experience (DMFT), and perceived access to dental services. Group differences were assessed using Student's *t* test or ANOVA for continuous variables (DMFT), and chi- square tests for categorical variables (perception frequencies across educational levels). Statistical significance was set at $p < 0.05$.

RESULTS

The study sample included 72 participants from rural areas, with a mean age of 39.13 ± 13.16 years. Sex distribution was balanced (52.78% men and 47.22% women). Most respondents had completed upper secondary education (62.50%), followed by those with post-secondary or higher education (31.94%), indicating a relatively high level of literacy. Regarding occupational status, 69.44% were employed, while the remainder belonged to socio- economically vulnerable groups. Monthly household income was predominantly between 2001-3000 RON (43.06%) or above 3000 RON (31.94%). With respect to dental care financing, 56.94% of participants benefited from reimbursed dental services (tab.1).

TABLE I.

Socio-demographic and economic characteristics of the study sample

Characteristic	Category	n	%
Area of residence	Rural	72	100
Age (years)	Mean ± SD	-	39.13 ± 13.16
	Dominant age groups	-	25-35; 45-50
Sex	Male	38	52.78
	Female	34	47.22
Education level	No formal education	1	1.39
	Lower secondary (gymnasium)	3	4.17
	Upper secondary (high school)	45	62.50
	Post-secondary / higher education	23	31.94
Occupational status	Employed	50	69.44
	Unemployed	10	13.89
	Retired	5	6.94
	Student	7	9.72
Monthly household income	< 1000 RON	5	6.94
	1001-2000 RON	13	18.06
	2001-3000 RON	31	43.06
	> 3000 RON	23	31.94
Payment method for dental treatments	Reimbursed by the National Health Insurance (NHI)	41	56.94
	Out-of-pocket payment	31	43.06

The analysis of DMFT scores indicates an extensive caries experience among participants, with values ranging from 0 to

25. The most frequent scores were 9 (13.89%), 6 and 12 (12.50% each), while only 1.39% of respondents presented no caries experience (DMFT = 0), highlighting a very high prevalence of the condition. Additionally, 22.22% of participants recorded DMFT values ≥ 13, suggesting advanced oral impairment and underscoring the need for analyses correlated with socioeconomic factors, as well as for preventive interventions in rural settings.

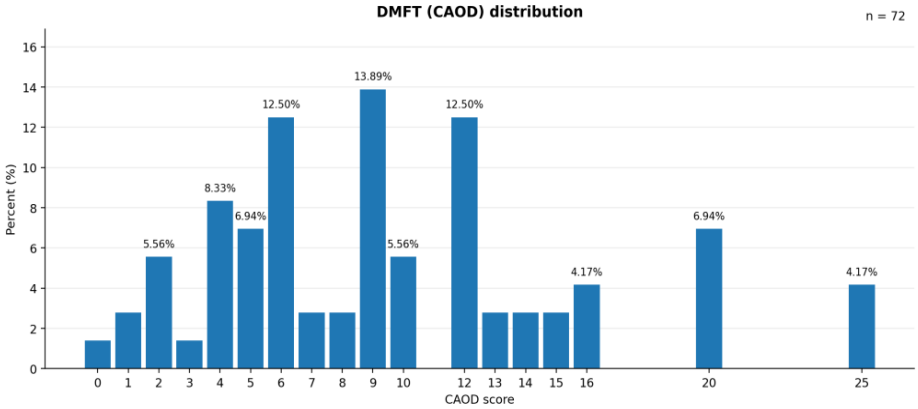

Fig. 1. Distribution of participants according to DMFT values perceptions regarding access to dental services and perceived barriers analysis

Socioeconomic factors further shaped access to care, as 86.11% of participants acknowledged that household income influences the decision to seek dental treatment. More than half of respondents (59.73%) perceived free or reimbursed dental services as insufficient, indicating limited public financial support. Preventive care was also considered inadequate: 56.94% believed that dental prevention is not a community priority, while 59.72% reported a lack of oral health information campaigns. Educational level influenced communication with healthcare providers for 59.73% of participants, emphasizing the role of health literacy. In contrast, waiting time was not viewed as a major barrier, with only 13.89% considering it problematic. Notably, all respondents (100%) agreed that greater investment by authorities in rural dental infrastructure is necessary, underscoring the need for systemic interventions to improve oral healthcare access in rural populations (tab. II).

TABLE II.

Participants' perceptions regarding access to dental services and perceived barriers (questionnaire)

Assessed statement	Strongly disagree (%)	Disagree (%)	Neutral (%)	Agree (%)	Strongly agree (%)
Distance to the dental office influences the decision to seek dental care	4.17	5.56	1.39	41.67	47.22
Cost of dental treatments represents a barrier	-	4.17	16.67	58.33	20.83

Assessed statement	Strongly disagree (%)	Disagree (%)	Neutral (%)	Agree (%)	Strongly agree (%)
Lack of adequate transportation hinders access to dental services	-	26.39	30.56	37.50	1.39
Household income influences the decision to visit the dentist	-	4.17	9.72	56.94	29.17
Information on dental prevention is insufficient in the community	-	19.44	20.83	48.61	9.72
Own education level influences the ability to understand the dentist's advice	-	19.44	18.06	55.56	4.17
Free or reimbursed dental services are insufficient	-	6.95	4.17	55.56	34.72
Local dental services are of good quality	-	29.17	41.67	27.78	1.39
Waiting time for a dental appointment is too long	13.89	37.50	34.72	11.11	2.78
Dental treatments have been postponed due to financial reasons	4.17	8.33	26.39	45.83	15.28
Visiting the dentist only in emergency situations due to financial reasons	2.78	4.17	4.17	41.67	47.22
Dental prevention is not a priority in the community	-	22.22	18.06	47.22	9.72
There is a lack of oral health information campaigns in the community	-	19.44	20.83	48.61	11.11
Access to dental services is more difficult in rural than in urban areas	-	12.50	20.83	45.83	20.83
Authorities should invest more in rural dental infrastructure	0	0	0	58.33	41.67

Correlations between socio-economic variables and clinical scores

Correlation analysis revealed several statistically significant relationships between the variables examined. Monthly household income was positively and significantly correlated with education level ($r = 0.336$; $p = 0.004$) and negatively correlated with occupational status ($r = -0.464$; $p < 0.001$), indicating that individuals with higher incomes generally have higher educational attainment and are more frequently employed, whereas lower income levels are associated with more vulnerable occupational statuses. Education level showed a significant negative correlation with the DMFT score ($r = -0.342$; $p = 0.003$), suggesting that higher educational attainment is associated with a lower caries experience. In contrast, no significant correlations were observed between the DMFT score and occupational status ($r = -0.080$; $p = 0.502$) or monthly household income ($r = -0.043$; $p = 0.721$) (fig. 2).

The statistical analysis highlighted two significant relationships between the DMFT score and perceived barriers to accessing dental services. A positive correlation was observed between DMFT and the perception that distance to the dental office influences the decision to seek care ($r = 0.340$, $p = 0.003$), indicating that individuals who perceive distance as a barrier tend to have poorer oral health status. In contrast, the perception that access to dental services is more difficult in rural areas than

in urban settings was negatively correlated with the DMFT score ($r = -0.322$, $p = 0.006$), possibly suggesting that individuals with lower DMFT scores are more aware of access inequalities. Other variables, such as lack of transportation or waiting time for appointments, did not show significant correlations (fig. 3).

The relationship between caries experience and perceived barriers to dental care was explored using Pearson correlation analysis. The results revealed a significant positive association between the DMFT score and the perception that household income influences dental attendance ($r = 0.260$, $p = 0.027$). In contrast, no significant correlations were observed between DMFT and other cost-related variables, despite the strong intercorrelations among financial barriers themselves ($p < 0.001$). Furthermore, DMFT showed a positive correlation with the perception that educational level affects the understanding of medical advice ($r = 0.389$, $p = 0.001$), indicating a higher caries experience among individuals with lower educational attainment. Although DMFT was not directly associated with other prevention- related perceptions, these factors were significantly interrelated, emphasizing the central role of education in oral health outcomes and preventive behaviors (figs. 4, 5).

To better understand the determinants of access to dental services, Pearson correlation analysis was conducted to examine the role of educational and economic factors. The results showed that lower educational level was significantly associated with perceiving distance to the dental office as a barrier ($r = -0.308$, $p = 0.009$) and with reporting longer waiting times ($r = -0.392$, $p = 0.001$). In addition, lack of transportation was significantly linked to distance, waiting time, and difficulties in accessing dental care in rural areas ($p \leq 0.032$). The negative correlation between rural access difficulties and distance ($r = -0.236$, $p = 0.046$) suggests that barriers in rural settings extend beyond simple geographic proximity (fig. 6).

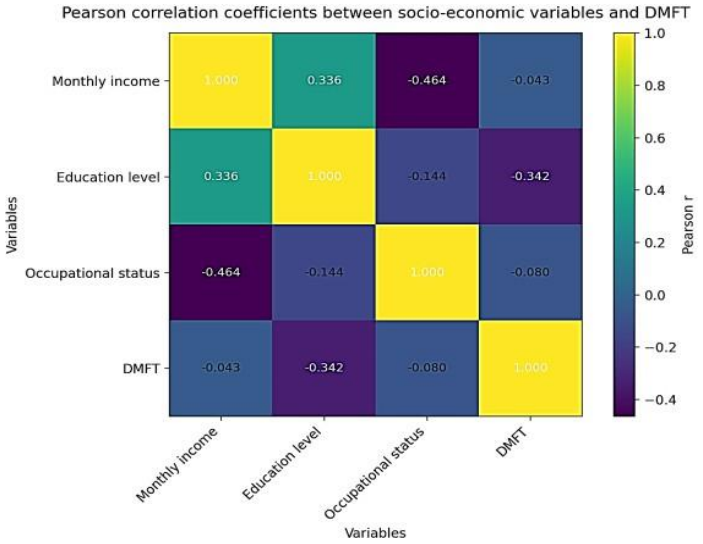

Fig. 2. Correlations between DMFT and monthly income, occupational status, and education level

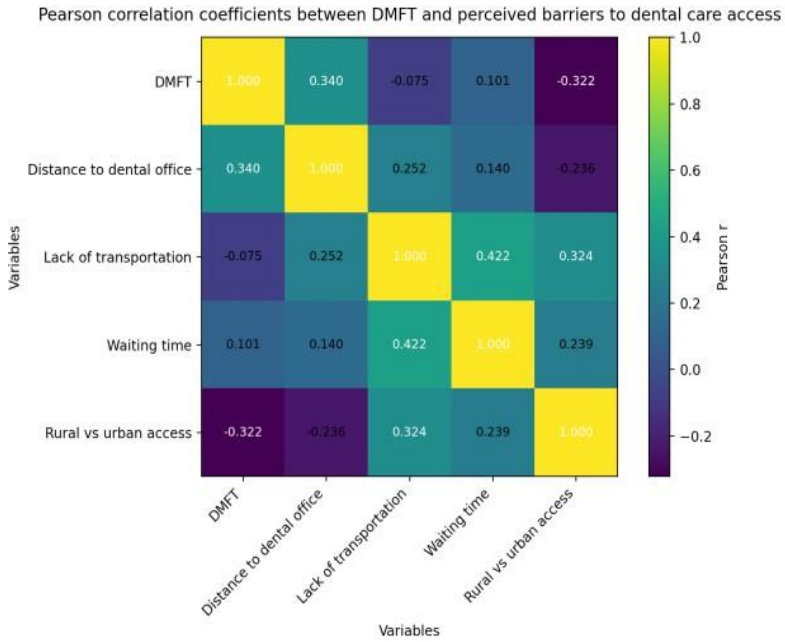

Fig. 3. Correlations between DMFT and perceived barriers to access to dental services

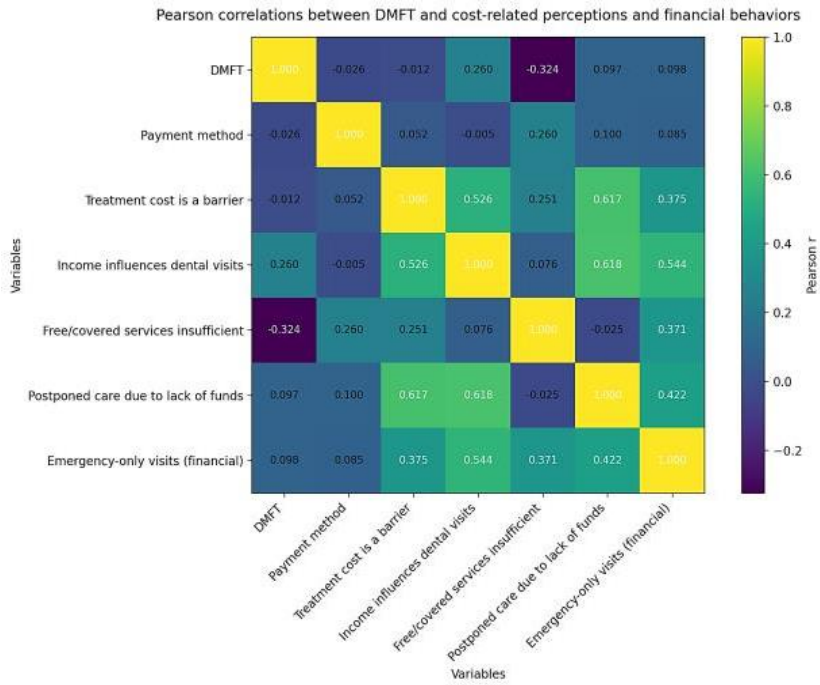

Fig. 4. Pearson correlations between the DMFT score and cost-related perceptions and financial behaviors associated with access to dental care

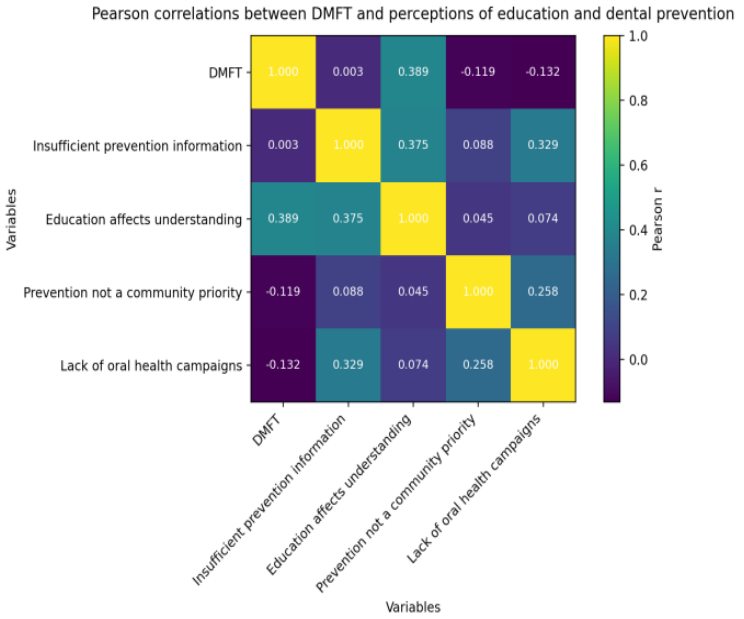

Fig. 5. Pearson correlations between the DMFT score and perceptions related to education and dental prevention

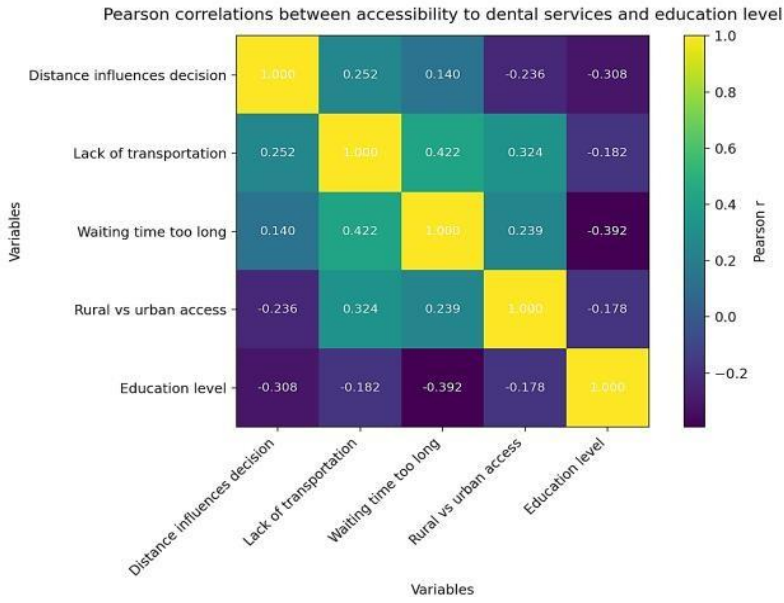

Fig. 6. Pearson correlations between accessibility to dental services and educational level

Economic constraints also played a major role: the perception that household income influences dental attendance was strongly associated with high treatment

costs (r = 0.526), postponement of dental care (r = 0.618), and reliance on emergency-only visits (r = 0.544). Conversely, higher educational attainment was negatively associated with these financial barriers, indicating a protective effect of education (fig. 7). Overall, these findings emphasize the combined and interdependent impact of education and income on access to dental care.

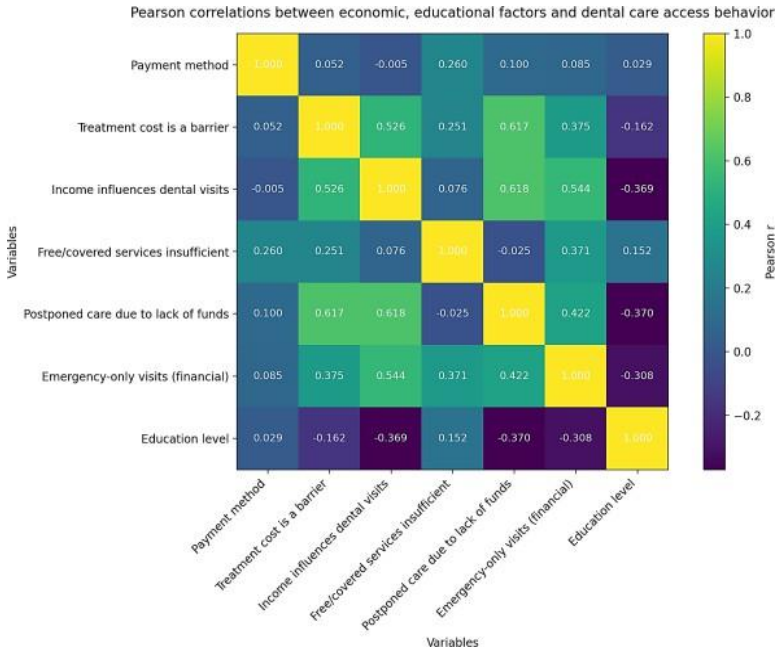

Fig. 7. Pearson correlations between economic and educational factors and dental care-seeking behavior

Lower educational level was significantly associated with difficulties in understanding dentists' advice (r = −0.557, p < 0.001) and with the perceived lack of oral health information campaigns (r = −0.268, p = 0.023), while insufficient preventive information was linked to both limited understanding and absence of campaigns (p ≤ 0.005) (fig. 8). Lower household income was associated with transportation barriers (r = −0.255, p = 0.031), rural access difficulties (r= −0.290, p = 0.014), and especially longer waiting times for appointments (r = −0.592, p < 0.001), underscoring the combined impact of education, income, and infrastructure on access to dental care (fig. 9).

Correlation analysis of socio-economic factors revealed a significant influence of income and education on dental care- seeking behavior. Household income was strongly associated with perceived financial barriers, including the decision to seek dental care (r = 0.526, p < 0.001), postponement of treatments (r = 0.618, p < 0.001), and reliance on emergency-only visits (r = 0.544, p < 0.001), while treatment costs were also perceived as a major obstacle (r = 0.617, p < 0.001). Education level was negatively correlated with these barriers (postponement of care: r =−0.370, p = 0.001), indicating greater vulnerability

among less educated individuals. Additionally, lower income was associated with the perception that dental prevention is not a community priority ($r = -0.509$, $p < 0.001$) and showed borderline negative associations with insufficient preventive information and lack of campaigns. Difficulties in understanding medical advice were significantly related to perceived educational level ($r = 0.375$, $p = 0.001$), highlighting the role of health literacy in oral health prevention (figs. 10, 11).

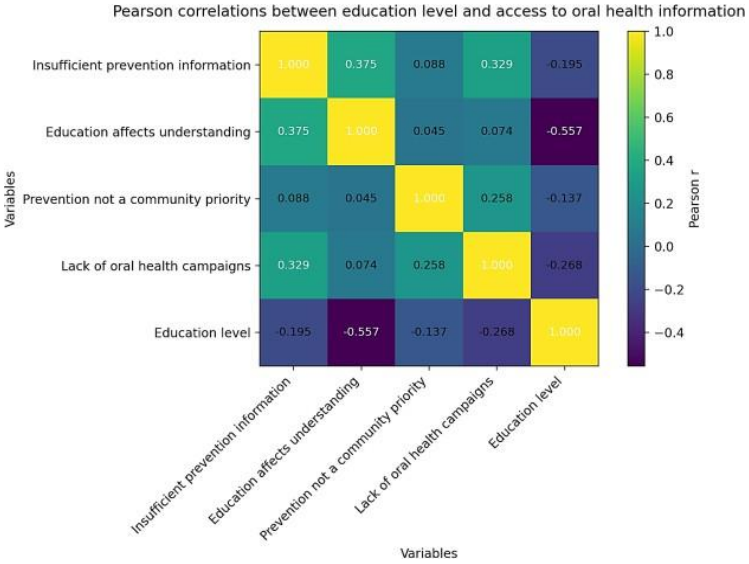

Fig. 8. Pearson correlations between educational level and access to oral health information

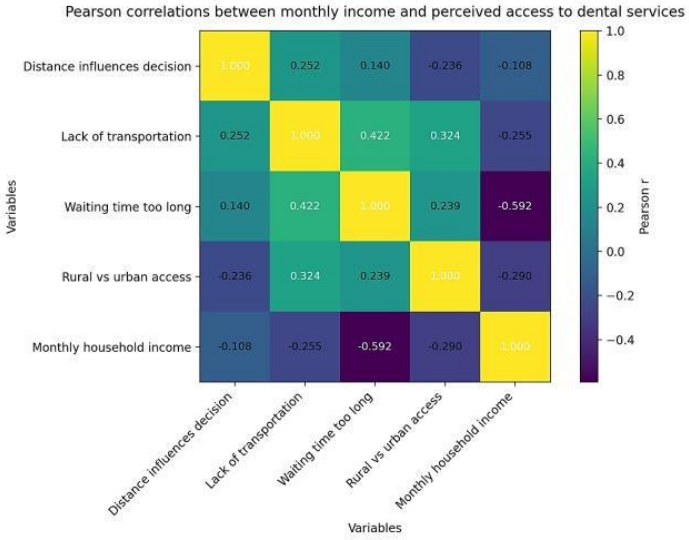

Fig. 9. Pearson correlations between monthly household income and perceived access to dental services

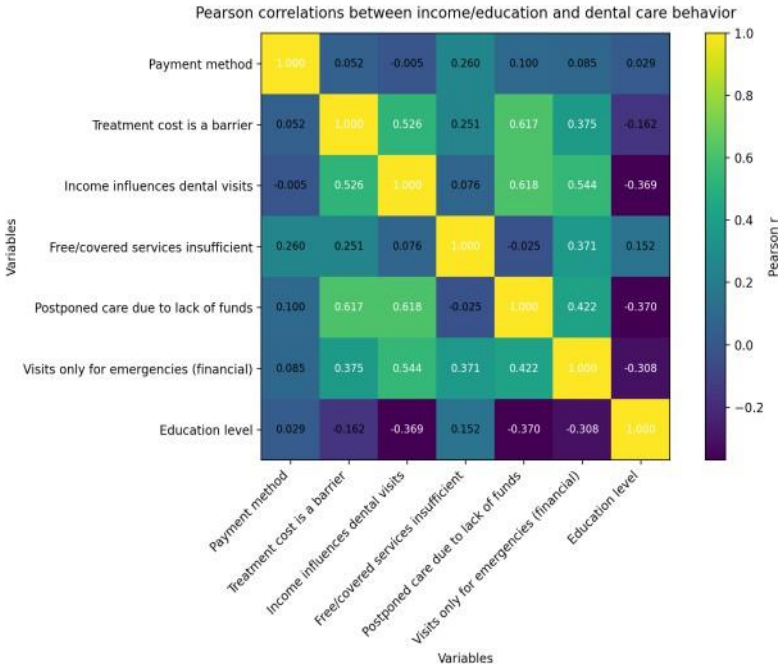

Fig. 10. Pearson correlations between income level and education in relation to dental care- seeking behavior

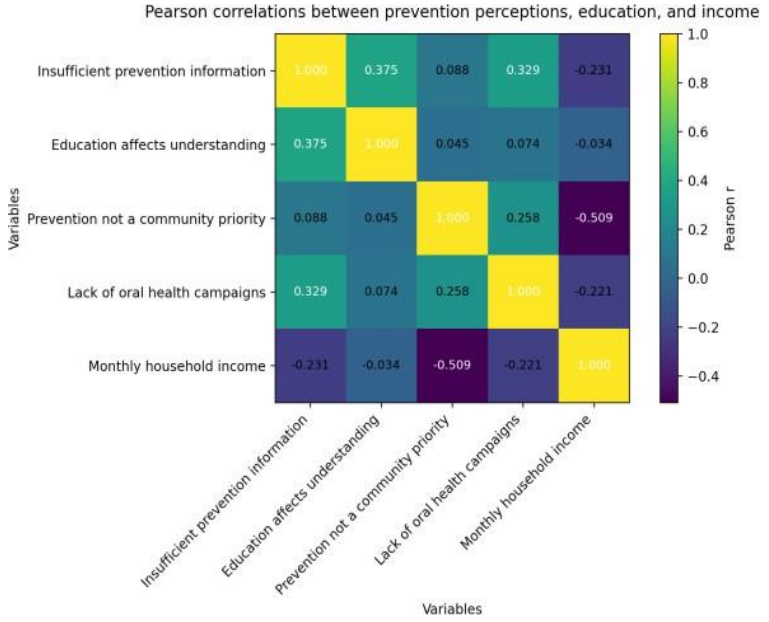

Fig. 11. Pearson correlations between monthly household income and perceptions of dental prevention

DISCUSSION

The present study provides evidence that access to dental services and oral health status in rural populations are strongly influenced by socio-economic and educational determinants. The high prevalence of dental caries observed in the study sample, reflected by elevated DMFT values, is consistent with previous research indicating that rural and socio- economically disadvantaged populations experience a disproportionate burden of oral disease (8, 9).

Educational level emerged as a key protective factor, showing a significant negative association with DMFT scores. Participants with higher educational attainment exhibited lower caries experience, likely due to improved oral health literacy, greater awareness of preventive practices, and more regular utilization of dental services. These findings are in line with international studies demonstrating that education strongly influences oral health behaviors and outcomes (10, 11).

Economic constraints also played a central role in shaping access to dental care. Although monthly household income was not directly correlated with DMFT scores, the perception that income influences the decision to seek dental care was significantly associated with higher caries experience. Financial barriers-including high treatment costs, postponement of care, and reliance on emergency-only visits- were strongly interrelated, suggesting a cumulative and systemic effect on dental care-seeking behavior. Similar patterns have been reported in both European and global studies, where cost-related barriers were identified as major determinants of delayed or avoided dental care (1, 13).

Geographic accessibility further compounded these inequalities. Perceived distance to dental clinics and lack of transportation were associated with poorer oral health outcomes, highlighting structural barriers faced by rural populations. These results align with evidence showing that rural residence is associated with reduced availability of dental professionals and increased travel distance, negatively affecting service utilization (14, 15).

Finally, the widespread perception of insufficient preventive information and lack of community-based oral health campaigns underscores the need for targeted public health interventions. Health promotion strategies focusing on oral health education, combined with improved access to publicly funded dental services, are essential to reduce inequalities and improve oral health outcomes in rural communities (16, 17).

CONCLUSIONS

The findings of this study demonstrate that oral health status in rural populations is strongly influenced by socio- economic and educational factors. Higher educational attainment was consistently associated with lower DMFT (CAOD) scores, while lower income, perceived financial barriers, and delayed care were linked to increased caries experience. Geographic accessibility, particularly perceived distance and lack of transportation, further contributed to poorer oral health outcomes. Limited health literacy and insufficient community-based preventive information were also associated with higher DMFT scores, underscoring the importance of education and preventive awareness. These results highlight a cycle of socio-economic disadvantage affecting access to dental care and oral health and support the need for targeted public investment in rural dental infrastructure, preventive programs, and health education to reduce oral health inequalities.

CONFLICTS OF INTERESTS AND FUNDING

The authors declare no conflicts of interest.

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MEANING IN LIFE AS A MEDIATOR BETWEEN CONSERVATISM AND QUALITY OF LIFE AMONG OLDER ADULTS: A CROSS-SECTIONAL STUDY

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MEANING IN LIFE AS A MEDIATOR BETWEEN CONSERVATISM AND QUALITY OF LIFE AMONG OLDER ADULTS: A CROSS-SECTIONAL STUDY (Abstract): Population aging presents growing challenges for maintaining quality of life in later adulthood. Conservative personal values and existential meaning are both associated with well-being in older adults, yet their functional relationship remains unclear. **Aim:** The present study aimed to investigate the mediating mechanism through which the presence of meaning in life influences the relationship between value conservatism and quality of life in older adults. **Materials and methods:** A sample of 210 participants (mean age = 74.5 years, SD = 6.24, 63.3% women) completed a battery of standardized questionnaires: the WHOQOL-OLD to assess quality of life, the Meaning in Life Questionnaire to assess existential meaning, and the Schwartz Value Survey to measure personal values. Mediation analysis was conducted using the *PROCESS macro* for *SPSS version 28* (Model 4), applying a bootstrap procedure with 5,000 resamples. **Results:** The mediation analysis indicated that the presence of meaning in life partially mediated the relationship between conservatism and quality of life. Conservatism significantly predicted the presence of meaning ($b = 0.072$, $p = .017$), and the presence of meaning significantly predicted quality of life ($b = 0.129$, $p = .005$), even after controlling for conservatism. The indirect effect of conservatism on quality of life through the presence of meaning was significant ($b = 0.0093$; 95% CI [0.001, 0.024]), accounting for approximately 13.8% of the total effect. **Conclusions:** The findings demonstrate that conservative values enhance quality of life in older adults both directly and indirectly by strengthening existential meaning, providing empirical support for the development of integrated interventions focused on cultivating meaning and reinforcing stable values in the service of successful aging. **Keywords:** PRESENCE OF MEANING IN LIFE; VALUE CONSERVATISM; QUALITY OF LIFE; OLDER ADULTS; MEDIATION.

INTRODUCTION

Population aging represents one of the most significant demographic transformations of the twenty-first century. The central challenge of contemporary research is to ensure high quality of life in older age through the interaction between biological resources, social context, and psychological mechanisms of adaptation (1). Quality of life is conceptualized as a multidimensional construct encompassing physical functioning, emotional well-being, social relationships, autonomy, and existential meaning (2, 3), with psychological and existential factors becoming increasingly important in advanced age (4).

Personal values represent stable motivational principles that guide behavior across the lifespan. Older adults increasingly prioritize conservation values-tradition, security, and conformity-as an adaptive strategy for maintaining identity coherence (5, 6). In parallel, meaning in life-defined as the perception that one's existence is coherent, significant, and goal-directed (7)-is associated with lower depression, better cognitive functioning, and reduced mortality in later adulthood (8, 9).

Although values and meaning have been studied as independent predictors of quality of life, their functional relationship remains insufficiently clarified. The present study proposes that value conservatism influences quality of life both directly and indirectly through the cultivation of existential meaning, grounded in continuity theory (10) and existential frameworks emphasizing meaning as a core psychological resource in later adulthood.

MATERIALS AND METHODS

Participants. The sample consisted of 210 community-dwelling older adults (mean age = 74.5 years, SD = 6.24, range = 65-91 years; 63.3% women) recruited from urban and peri-urban areas in northeastern Romania. Inclusion criteria were: (a) age >= 65 years; (b) absence of severe cognitive impairment (MMSE score >= 24); (c) ability to provide informed consent; and (d) willingness to complete self-report questionnaires. Participants were recruited through community centers, senior clubs, and religious organizations using convenience and snowball sampling.

Measurements. *Quality of life* was assessed using the World Health Organization Quality of Life Instrument for Older Adults (WHOQOL-OLD; 3), a 24-item self-report questionnaire measuring six facets: sensory abilities, autonomy, past, present and future activities, social participation, death and dying, and intimacy. Items are rated on a 5-point Likert scale (1 = not at all; 5 = completely), with higher scores indicating better quality of life. The Romanian version has demonstrated good psychometric properties (Cronbach's alpha = .91 in the current sample).

Meaning in Life. Meaning in life was measured using the Meaning in Life Questionnaire (MLQ; 11), which comprises two 5-item subscales: Presence of Meaning and Search for Meaning. Items are rated on a 7-point Likert scale (1 = absolutely untrue; 7 = absolutely true). Only the Presence subscale was used in the current analyses. The Romanian version demonstrated excellent internal consistency (Cronbach's alpha = .87).

Personal Values. Personal values were assessed using the Schwartz Value Survey (SVS; 12), which measures ten basic values organized into four higher-order dimensions. For this study, we focused on the Conservation dimension, comprising three values: security, tradition, and conformity. Participants rated the importance of each value as a guiding principle in their life on a 9-point scale (-1 = opposed to my values; 7 = of supreme importance). A composite Conservation score was computed by averaging the three subscales (Cronbach's alpha = .76).

Procedure. The study protocol was approved by the Ethics Committee of Alexandru Ioan Cuza University. Data were collected between February and September 2024. Trained research assistants administered the questionnaires in individual sessions lasting approximately 75 minutes. Participants provided written informed consent and were assured of confidentiality and the right to withdraw at any time. All procedures were conducted in accordance with the Declaration of Helsinki.

Statistical analysis Descriptive statistics and bivariate correlations were computed using *SPSS version 28.0*. To test the hypothesized mediation model, we used the PROCESS macro for SPSS (Model 4; 13), with value conservatism as the independent variable, quality of life as the dependent variable, and presence of meaning in life as the mediator. The significance of the indirect effect was assessed using bias-corrected bootstrap confidence intervals based on 5,000 resamples. Statistical significance was set at alpha = .05, two-tailed.

RESULTS

Descriptive statistics and correlations. Table I presents descriptive statistics and bivariate correlations among study variables. Value conservatism was positively correlated with both presence of meaning in life ($r = .164$, $p = .017$) and quality of life ($r = .232$, $p < .001$). Presence of meaning was also positively associated with quality of life ($r = .220$, $p < .001$). All distributions approximated normality (Skewness < |2.0|, Kurtosis < |2.0|).

TABLE I.

Means, standard deviations, and correlations among study variables

Variable	M	SD	1	2
1. Overall quality of life	3.28	0.43	---	---
2. Presence of meaning in life	3.61	0.64	.22***	---
3. Conservatism values	6.39	1.47	.23***	.16*

M = mean; SD = standard deviation. N = 210. *p < .05. **p < .01. ***p < .001 (two-tailed).

Mediation analysis. Table II presents the results of the mediation analysis. Value conservatism significantly predicted presence of meaning in life (path a: b = 0.072, SE = 0.03, t = 2.40, p = .017). Presence of meaning significantly predicted quality of life after controlling for conservatism (path b: b = 0.129, SE = 0.05, t = 2.86, p = .005). The direct effect of conservatism on quality of life remained significant after including the mediator (path c': b = 0.058, SE = 0.02, t = 2.93, p = .004).

TABLE II.

Mediation analysis examining the indirect effect of conservation values on quality of life via presence of meaning in life

Model	Outcome variable	Predictor	B	SE	t	p
1	Presence of meaning in life	Conservation values	0.07	0.03	2.40	.017
2	Quality of life	Presence of meaning	0.13	0.05	2.86	.005
2	Quality of life	Conservation values	0.06	0.02	2.93	.004
Direct effect (c')	Quality of life	Conservation values	0.06	0.02	2.93	.004
Indirect effect (a x b)	Quality of life	Conservation -> Meaning -> QoL	0.0093	0.006	---	---

N = 210. B = unstandardized regression coefficient; SE = standard error.
The indirect effect was significant, 95% bootstrap 5000, CI [0.001, 0.024]. Proportion mediated = 13.8%.

The mediation model is illustrated in first figure. The total effect of conservatism on quality of life was significant (c = 0.067, SE = 0.022, t = 3.05, p = .003). The indirect effect through presence of meaning was statistically significant (ab = 0.0093, SE = 0.0057, 95% CI [0.001, 0.024]), indicating partial mediation. The indirect effect accounted for approximately 13.8% of the total effect.

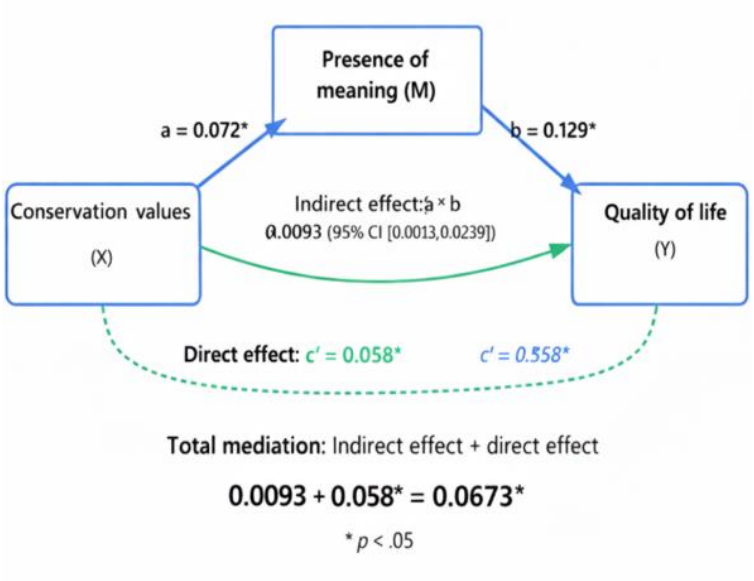

Fig. 1. Mediation model: the effect of conservation values on quality of life via presence of meaning in life

Legend for illustrations: X = independent variable; M = mediating variable; Y = dependent variable; a = 0.072* (path X → M); b = 0.129* (path M → Y); c' = 0.058* (direct path X → Y); Indirect effect: a × b = 0.0093. *p < .05

DISCUSSION

The present study examined the mediating role of presence of meaning in life in the relationship between value conservatism and quality of life among older adults. Results supported the hypothesized partial mediation model, indicating that conservative values promote quality of life both directly and indirectly through the enhancement of existential meaning.

Consistent with continuity theory (10) and socioemotional selectivity theory (14, 15), our results demonstrate that conservative values serve adaptive functions in later adulthood by providing psychological anchors amid biological and social transitions. The significant indirect effect through presence of meaning reveals that conservative values actively facilitate the construction and maintenance of existential meaning, which in turn enhances well-being. By anchoring individuals in biographical continuity and social belonging, conservative values provide interpretive frameworks through which life experienc-

es are organized into coherent narratives of purpose (16, 17).

From a practical standpoint, these findings suggest that interventions aimed at enhancing quality of life in older adults may benefit from incorporating components that strengthen both existential meaning and value clarity.

Reminiscence therapy, life review interventions, and narrative approaches that help older adults articulate their core values and connect them to life experiences may be particularly effective.

Limitations and future directions. Several limitations should be acknowledged. First, the cross-sectional design precludes causal inferences; longitudinal studies are needed to establish temporal precedence. Second, the sample was drawn from a culturally traditional context, which may limit generalizability. Third, reliance on self-report questionnaires raises concerns about common method variance. Future research should incorporate behavioral indicators and examine whether other value dimensions operate through different mediating mechanisms.

CONCLUSIONS

This study provides empirical support for the proposition that personal values influence quality of life in older adults through the cultivation of existential meaning. By demonstrating that conservative values enhance well-being both directly and indirectly via strengthened meaning in life, these findings advance understanding of the psychological mechanisms underlying successful aging and underscore the importance of integrating value-based and meaning-focused interventions in gerontological practice.

CONFLICT OF INTEREST AND FUNDING

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SOCIO-DEMOGRAPHIC CHARACTERISTICS, EDUCATIONAL MOTIVATION AND GEO-CULTURAL COMFORTABILITY RELATED TO THE PROCESS OF ADAPTATION OF FRESHMAN INTERNATIONAL STUDENTS IN A ROMANIAN UNIVERSITY

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SOCIO-DEMOGRAPHIC CHARACTERISTICS, EDUCATIONAL MOTIVATION AND GEO-CULTURAL COMFORTABILITY RELATED TO THE PROCESS OF ADAPTATION OF FRESHMAN INTERNATIONAL STUDENTS IN A ROMANIAN UNIVERSITY (Abstract): Successfully integrating international students in a university is one of the main goal of an academic institution. **Aim:** To identify the characteristics of international students enrolled in first year of study. **Material and methods:** a number of 128 freshman international students from 23 countries were included in the research. The subjects had to complete a document with socio-demographic data and to answer to several items. Data was processed using IBM SPSS Statistics, version 23. **Results**: 67 men (52.37%) and 61 women (47.66%) with an age of 19.59 ± 2.21 were included in the research. More than half of international students are fluent in three languages. 58.73% of students' fathers and 67.97% of their mothers graduated from university. A number of 13 (N = 10.48%) declared that at least one of their parents is physician and almost half of them have at least one relative studying in the university. The levels of satisfaction with the relationship with colleagues, teachers and administrative staff are analyzed. **Conclusions**: International students have criteria for choosing their academic institutions. Universities and academic staff mast take into consideration their reasons used in choosing the institution and social, cultural, religious or daily life aspects must be supervised to help them become more integrated into the community. **Keywords**: INTERNATIONAL STUDENT, UNIVERSITY, MEDICAL STUDY, DISCRIMINATION, SATISFACTION.

Pressed by the high number of students from abroad who would like to study in another country, universities had to change over the years to meet internationals students' needs and to adjust their educational offer to satisfy their professional expectations.

Both our borders and culture become more accepting towards immigrants from all over the world, and less judgmental of people who are different. The educational system in our country has been subjected to a rise in the number of students of various cultures. With that in mind, the phenomenon of acculturation stress has also become more noticeable.

With the work of psychologists, such as

Berry and Rudmin (1) certain systems and scales have been effectively put together and improved throughout the last decade, thus helping analyze and quantify the level, prevalence and importance of various stressors. It is very important to identify more efficient means of helping international people, especially students, in dealing with the pressure of a new society.

The stress related to acculturation is the phenomenon experienced by individuals or groups, on a psychological and cultural level, during the adjustment to a foreign culture (2). Berry et all helped identify vital stressors arising from the adjustment period, as well as develop the idea of the 4 different coping strategies which have been confirmed to follow 3 main steps: 1. Contact period, 2. Conflict period and 3. Resolution period (3, 4).

International students, according to research, present common stressors such as cultural shock (5) along with conflicts and psychological issues that may occur throughout the adjusting timeframe. (6, 7, 8). Among main stressors are the following: language barrier, cultural aspects, religious facilities, relationship with family, social support and academic facilities.

The present study is a preliminary one and is part of a larger study focusing on international students in a medical university from North-Eastern Romania. The aim is to identify socio-demographic characteristics of international students and to evaluate their level of satisfaction of their relationship with administrative staff, colleagues and teachers. Also, the level of comfort with some aspects of their daily life are also regarded. In the university students are enrolled to accomplish their medical studies in one of the three study languages: Romanian, English and French. This study targets international students from the first year, focusing especially on those registered in English section (from a total of approximately 220 freshman students), but English speakers from French sections were also included in the research.

MATERIAL AND METHODS

The goal of the research

The aim of the research was to identify socio-demographic characteristics and factors related to the process of integration of international students enrolled in first year of study in "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Romania. The study presents some results from a larger research obtained by a grant competition organized by University of Medicine and Pharmacy Iasi, Romania and *Agence Universitaire pour la Francophonie* (AUF) in 2017 and approved by both institutions.

Participants

A number of 150 questionnaires were distributed among freshman students from Medical Faculty and Dental Medical Faculty, studying in French and English sections. Before the completion of data, students were informed about the purpose of the study and the use of data, with the respect of confidentiality of data. The questionnaire was distributed to students after they were questioned about their level of English language, because documents were formulated in English. Students were informed that the questionnaire was optional, and they could decline filling it in, with no consequences. In total, 145 questionnaires returned to the researchers and 128 of them (85.33%) were considered for the study. The exclusion criterion was: the questionnaires were not fully filled in or they were

delivered after the requested date.

Questionnaires

The questionnaires gathered information referring to the following aspects:

- socio-demographic characteristics a family-related data (age, sex, marital status, religion, nationality, country of origin, maternal language, the number of languages spoken fluently, the level of education of their parents, if one of the parents is a physician, the number of siblings, if their parents were speaking Romanian language, if they have relatives, friends or siblings enrolled in their institution of study),
- academic data (language of study, previous experience in other universities from Romania or abroad),
- information related to their choice regarding enrollment in Faculty of Medicine in Iasi and the country where they intend to work after graduation were also gathered.

Several items were formulated to identify their level of comfort related to their living in the University City. Answered for formulated on a Lickert-like scale from 1 to 5 (1 – *not comfortable*, 2 – *somewhat comfortable*, 3 – *comfortable*, 4 – *very comfortable*, 5 – *extremely comfortable*). The items referred to their comfort of communicating in English or Romanian, climate, transportation, food or relationship with neighbors in Iasi.

Also, some questions were formulated to identify their level of satisfaction with the administrative staff, colleagues or teachers. The answers were rated on a scale from 1 (*very unsatisfied*) to 10 (*very satisfied*).

Statistical analysis of data

The statistical analysis of data was done suing IBM *SPSS Statistics, version 23.0.*

RESULTS AND DISCUSSION

Socio-demographic and family data

A number of 67 men (52.37%) and 61 women (47.66%) from 23 countries were included in the research. They are studying in two faculties of the university: General Medicine (N = 110, 85.94%) and Dental Medicine (N = 18, 14.06%). The average age is M = 19.59 ± 2.21, range 17-30 years old. Approximately 82% of participants are aged between 18-20 years old.

The majority of them are studying in English language (N = 110, 85.94%) but also 18 students from French language section (14.06% of respondents) choose to answer to the questionnaires.

Most them are single (N = 103, 81.10%), a small part sustained that they are in relationship (N = 21, 16.54%) and three of them are married.

Questioned students are from 23 nationalities from all over the world, but the highest rates are Israeli (61.60%), French (12.80%) and Greek (8%). The other self-declared nationalities are: German, Canadian, Benin, Irish, Lebanese, Polish, Cypriot, American, Morocco, Vietnam, Palestinian, Romanian, Tunisian, Turkish, Finnish, British, Italian, Philippine, Zimbabwean. Three students have double-nationality.

Most students included in the research are Muslims (N = 85, 66.4%), 26 (20.3%) are Christian-Orthodox, 3 are Protestants, 2 are Catholics and 1 students is Hindu.

More than half of freshman international students are fluently speaking three languages (N = 64, 50.39%), almost a quarter of them are speaking four languages (N = 30, 23.62%) and 26 (20.47%) are speaking two languages.

Twelve students (9.52%) have Romanian origins. They were also asked if their parents speak Romanian. Fifteen of them

declared that their mothers speak Romanian and 15 of them that their fathers speak this language.

Regarding the level of education of their parents, 74 (58.73%) of fathers are have a university degree and 87 (67.97%) of their mothers graduated from university. A number of 13 (N = 10.48%) declared that at least one of their parents is physician. Only 5 of students (4.03%) are singleton. 21 (16.94%) are having one sibling, 39 (31.45%) are having 2 siblings, 28 (22.58%) are having 3 siblings and 16 (12.90%) are having 4 siblings. A total of 12.12% of international students are coming from a family with more than 6 children.

Almost half of them are having at least one relative studying in the university (N = 60, 48%).

Academic data and information related to the educational/professional choices

A number of 36 students (28.57%) declared that they were previously enrolled in other universities, and among the most mentioned countries is France (41.18%), Israel, United Kingdom and Greece (with 5.88% each).

The main reasons for choosing to study in this university are presented in tab. 1. Among the answers related to the prestige of the university were the following: it is a good university, it offers a lot of practice, it is a European university, it was proposed by friends and former students from all over the world). Also, students mentioned their trust in the academic offer (they have or had friends enrolled in the university or they know about the country or the university from their relatives or friends). Regarding personal advantages, international students mentioned they close distance to their countries of origin that they speak the language or that studies are most cheap comparing to other universities.

TABLE I
The main reasons to choose studying in the University of Iasi

The main reason for choosing to study in Iasi	N (%)
The university prestige	52 (40.65%)
Trustful in academic offer and city life	36 (28.12%)
Personal advantages	27 (21.09%)

Life in the University City

Students were asked to answer to some questions related to their life in the university city. 63 international students (52.07%) share am apartment with a friend or a colleague. Because a large part of the students declared their religion and this aspect is an important factor for adjusting adequately to the new life as student, subjects were asked about how they succeed in being a part of their religious community. A total of 59 of them (46.1%) declared that they can practice their religion in mosque (22%), churches (22%) or at home (56%).

Also, they had to answer to five items trying to identify their level of comfort during their first months in at the faculty, related to the following aspects: climate, transportation, neighbors, communicating with the owner of the apartment or with food. The temperature and food seem to be the most uncomfortable aspects that students must deal with. The distribution of their answers is presented in Table II.

Almost a quarter of subjects (N = 29, 22.7%) declared that they feel discriminated in their first few months as student: 4.7% by neighbors, 4.7% by teachers, 6.3% by people during city transportation, 6.3% by colleagues and 10.2% by citizens.

TABLE II

The distribution of answers to items regarding students' life in the University City (N, %)

How comfortable are you with...	not comfortable	somewhat comfortable	comfortable	very comfortable	extremely comfortable	M ± st dev
...the climate in Romania?	20 (15.6%)	35 (27.3%)	45 (35.2%)	23 (18%)	5 (3.9%)	2.67±1.07
...the transportation in the city?	5 (3.9%)	41 (32%)	51 (39.8%)	26 (20.3%)	5 (3.9%)	2.88±0.91
... your neighbors?	16 (12.5%)	24 (18.8%)	52 (40.6%)	21 (16.4%)	14 (10.9%)	2.94±1.14
...communicating with the owner of apartment?	9 (7%)	15 (11.7%)	48 (37.5%)	28 (21.9%)	27 (21.1%)	1.27±1.15
...with the food?	18 (14.1%)	36 (28.1%)	40 (31.3%)	16 (12.5%)	18 (14.1%)	2.84±1.23

Educational and social aspects

Students had to rate on a scale from 1 (*very unsatisfied*) to 10 (*very satisfied*) their level of satisfaction in relationship with administrative staff, colleagues or teachers. The means and standard deviations are presented in tab. III.

TABLE III

The answer to the items targeting the level of satisfaction in relationship with colleagues, teachers and administrative staff

How satisfied are you with the relationship with ...	Mean and standard deviations
The relationship with the administrative staff	5.63 ± 2.27
The relationship with teachers	6.36 ± 2.11
The relationship with colleagues	6.99 ± 2.10

Students were also asked about the country where they would like to practice after graduation. A total of 43% of students would like to work as a physician in Israel, 9.4% in France, 5.5% in Germany, 4.7% in Romania, 3.9% in Greece or the United Kingdom and 3.1% in the United States. The other countries mentioned where: Netherlands, Italy, Canada, Maldives, Morocco, Taiwan and Australia.

The analysis of data identifies no significant difference considering the type of faculty (dental medicine or general medicine) regarding the level of comfort with neighbors, transportation or food or satisfaction with the relationship with the administrative staff, colleagues or teachers.

Considering the level of satisfaction, the results revealed that male students are more satisfied with their relationship with colleagues compared to female students. No other significant differences between men and women were identified considering the gender of students.

The present research is the first one developed in Romania. Medical universities are having a long experience in providing facilities to international students, but in the last decade the number of students coming from abroad have increased and the diversity of cultures, nationalities and religious is also larger. Providing services and facilities for all international students is an important and difficult goal but helping them to integrate in the new culture for their long period of studies must be taken into consideration by the university's administration. Some studies proved that the difficulty of integra-

tion and the stress related to acculturation are important factors related to depression and suicide ideation. The difficulty is greater in case of students who don't speak the language, with a strong relationship with family of origin, (especially when there are many siblings) or in case a great discomfort (9) but consistent teachers' support and strong parental attachment are helping to cope with the stress (10).

On the other hand, international students' motivation to study in Romania and their goals for their future profession are also important for their process of accommodation in the University City or their level of comfort. Some studies developed in European countries from northern or east parts of the continent proved that the goals of studying in these countries are divers, and not always related to the prestige of the university (low cost, European country or accepting students with low level of English language). For solving these problems, governments and educational policies provided special programs (11).

The present research has some strong point and limitation: This research is important because is the first one developed in the country and results must be guiding future measures focusing on integrating international students. The limitation of the study is because results cannot be generalized because the study is focusing on a certain university. Adding subjects enrolled in other medical universities from all over the country could add significant aspects.

CONCLUSIONS

International students have criteria of choosing their academic institutions. Universities and academic staff mast take into consideration their reasons used in choosing the institution and social, cultural, religious or daily life aspects must be facilitated to help them become more integrated into the community.

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NEWS

EVALUATION OF SERUM C-REACTIVE PROTEIN LEVELS IN SUBJECTS WITH AGGRESSIVE AND CHRONIC PERIODONTITIS IN COMPARISON WITH HEALTHY CONTROLS

The aim of a study realized by a group of indian researchers was to evaluate and to compare the serum C-reactive protein levels in subjects diagnosed with chronic periodontitis and aggressive periodontitis. Forty-five subjects were included in the study. The subjects were divided into three groups: *group I* (subjects with clinically healthy periodontium), *group II* (subjects with generalized aggressive periodontitis), and *group III* (subjects with chronic periodontitis). For the measurement of serum C-reactive protein, blood samples were collected from all subjects. Periodontal parameters (plaque index, bleeding index, gingival index, probing pocket depth, clinical attachment loss) were assessed. Serum C-reactive protein levels were assessed by means of a commercially available high sensitivity- serum C-reactive protein enzyme immunoassay kit. The results showed that serum C-reactive protein levels were increased in group III (6.0671 ± 3.15639 mg/L) and group II subjects (4.5453 ± 2.88116 mg/L) compared to the group I (1.0180 ± 0.94069 mg/L). Serum C-reactive protein levels showed a positive correlation with all clinical parameters in group I subjects. Bleeding index (r = 0.073), plaque index (r = 0.120) showed a positive correlation with serum C-reactive protein level in group II and a positive correlation was also seen for plaque index (r = 0.492), probing pocket depth (r = 0.340), clinical attachment loss (r = 0.160), and serum C-reactive protein level in group III subjects. In conclusion, the mean serum C-reactive protein levels were found to be greater in patients with chronic periodontitis compared to those with generalized aggressive periodontitis, but there was no statistically significant difference. (Bolla V, Kumari PS, Munnangi SR, Kumar DS, Durgabai Y, Koppolu P. Evaluation of Serum C-reactive Protein Levels in Subjects with Aggressive and Chronic Periodontitis in Comparison with Healthy Controls: A Clinico-biochemical Study. *Int J Appl Basic Med Res*. 2017 Apr-Jun; 7(2):121-124).

Irina Grădinaru

OPERATIVE STRATEGY IN ACUTE SURGICAL ABDOMEN OF NON-TRAUMATIC EXTRAGENITAL ORIGIN ASSOCIATED WITH PREGNANCY

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OPERATIVE STRATEGY IN ACUTE SURGICAL ABDOMEN OF NON-TRAUMATIC EXTRAGENITAL ORIGIN ASSOCIATED WITH PREGNANCY (Abstract): The association between acute surgical abdomen of non-traumatic extragenital cause and pregnancy is very rare, making assessment difficult. Consequently, a standard protocol of surgical conduct has not been established, making it necessary to often resort to intraoperative improvisations. These, however. (usually done last and poorly thought out) may generate errors. Such risks be reduced by using the experience of properly resolved cases reported in the literature. This article presents an operative strategy recommended in cases of a combination of non-traumatic acute surgical abdomen and pregnancy according to the position and etiology of the surgical condition and the age of the pregnancy. **Keywords**: NON-TRAUMATIC, ACUTE ABDOMEN, PREGNANCY.

The association between acute surgical abdomen of non-traumatic extragenital origin and pregnancy is very rare, thus making it very difficult to determine its incidence (1, 2). Due to the low incidence of this association, no standard surgical protocol was established, and intraoperative improvisation is consequently often resorted to. Such improvisation, however, adopted quickly and poorly thought out, can contain errors. This risk can be reduced by using properly resolved cases reported in the literature. The conclusions (hat can be drawn from studying such cases can be useful to other surgeons as well, from the perspective of potential encodings which would ensure an optimum assistance that would be possible for both components of the association. According to the moment in which the condition causing the surgical acute abdomen sets, we can distinguish between two categories: Treatment for acute surgical abdomen is performed in the first trimester of pregnancy, letting the pregnancy to evolve; the treatment for acute abdomen is also useful for the evolution of the pregnancy, reducing the risk of spontaneous abortion. In the second and third trimesters of pregnancy, the surgical acute abdomen can have a supra- or subumbilical cause.

When the acute abdominal surgical disease is **supraumbilical** and the pregnancy is under 32-33 weeks, abdominal sur-

gery can be performed first, leaving the pregnancy to evolve. Such cases have already been reported in the literature; the cases of Talwar *et al* (3), generalized biliary peritonitis caused by a perforation of the gallbladder and a large sub hepatic abscess caused by a perforation of the common bile duct; the case of Essen *et al*. (4) of duodenal ulcer; the case of Sajad *et al*. (5), 34 weeks of pregnancy associated with a splenic artery aneurysm rupture and hemoperitoneum, for which hemostasis and splenectomy were performed, and the pregnancy was left to evolve, etc. (3-5). A special situation is represented by the case of a fissured or broken splenomegaly when a difficult splenectomy is foreseen, and the pregnancy is older than 32-33 weeks. In this case, surgical intervention is recommended by performing a caesarean section with Pfannenstiel incision to create a comfortable surgical field for splenectomy, maintaining the fetus under the influence of general anesthetics for the shortest time possible. Even in the case in which the pregnancy comes near term, in the presence of a normal spleen, caesarean section with Pfannenstiel incision is performed for the same reasons described above. If a supraumbilical midline incision is necessary for the acute abdominal disease, Pfannenstiel incision is performed for the caesarean section to avoid a xifopubic incision (presenting a higher risk of postoperative hernia).

In the case in which the **abdominal surgical disease is located in the subumbilical space** (i.e. acute perforated appendicitis, ileo-ileal invagination through primitive tumor, perforation of Meckel's diverticulum, intestinal obstruction through clamp and adhesions located down into the abdominal cavity, in an old inflammatory focus or genital operator, perforated Crohn's disease, perforated ulcerative colitis, intraperitoneal abscess located in the subumbilical space), diagnosis and surgical-related difficulties could occur (6-8).

The pregnancy associated uterus develops anteriorly, as compared to the acute pathological surgical subumbilical situations mentioned above. Even a pregnancy of 32-33 weeks can cover and hide a surgical acute abdominal disease, creating great difficulties in its diagnostic and therapy. Therefore, we are obliged to start with the caesarean section (with median subumbilical incision) to reduce the uterine volume and to allow the approach of the surgical acute abdominal disease, for diagnostic and therapeutic purposes: MRI without oral or intravenous (gadolinium) contrast administration is a valuable adjunct to ultrasound during pregnancy; MRI and CT performed equally well in the evaluation of acute nontraumatic abdominal pain during pregnancy. Given its lack of ionizing radiation, MRI may be preferable (9); Aytekin O. *et al* (10) has used only MRI in the triage of pregnant patients with acute abdominal and pelvic pain, given its lack of ionizing radiation.

If the caesarean section is performed in a peritonitis environment (acute perforated appendicitis with peritonitis), it should be continued by subtotal hysterectomy (because it is more expedient and less bloody than the total one) to avoid a serious or even fatal postpartum infection of the uterine sectioned remnants for which treatment with antibiotics could not guarantee survival. It is after the caesarean section (and hysterectomy) that the acute surgical abdominal disease can be operated on. If the resection of an unviable intestinal loop is necessary in an occlusion by strangulation,

without installed peritonitis, post-caesarean hysterectomy is unnecessary (treatment with broad-spectrum antibiotics being available in this case), provided that the loop resection and enteroanasiomosis be executed in aseptic surgical conditions (without peritonitis) (Negură's case with ileal invagination through tumor, with intestinal loop resection and, at the same time, termino-terminal intestinal anastomosis, with a favorable evolution) (11).

In prolonger paralytic ileus, Parswa (12) affirms: "if the K seric level maintains at more 4 mmol/L, if any prolonged paralytic ileus persists more a week and if the general condition of health starts modifies, probably there is a mechanical cause which can hide a mechanical obstruction of the bowels". Thus, Parswa considers that laparotomy is necessary (eventually after a radiologic investigation).

Mechanical occlusion of the small intestine should be distinguished from prolonged paralytic ileus by two methods:

1. method of two vertical radiographs taken at 3-hour interval (13);

2. opaque index examination (water-soluble opaque substance administered orally or barium enema (14).

Radiotherapy must not exceed 5 rad (0.05 Gy). If the gaseous distension of the small bowel with air-fluid levels on the first radiograph remains unchanged on the second radiograph, and there is no change in the topography of the persistent, fixed air-fluid images and the colon is gasless (not seen), surgery is required (14). Stoppage in the progression of opaque index at a given time and at intestinal level suggests a diagnosis of mechanical obstruction and of emergency surgery (14). Prolonged paralytic ileus is associated with fever suggests peritonitis (15,16).

If the post-gangrene intestinal peritonitis has already set, post-c section hysterectomy is required. Schraut "recommends intestinal resection, bringing the intestinal ends to the abdominal wall as temporary stomas, postponing enter anastomosis to restore intestinal continuity for another time (because an enter anastomosis performed in a peritonitis environment does not hold and will break open)" (17). The surgery is very laborious (non-minimal), posing a vital risk, yet this catastrophic situation arises due to a late diagnosis of strangulated intestinal obstruction. For this reason, in case of an occlusion hidden behind a gravid uterus, difficult to diagnose, possibly with indirect signs of strangulation (hyperleukocytosis and acidosis), the surgeons arguing that early surgery is preferable even if the laparotomy is unnecessary, but not catastrophic.

In the exceedingly rare case in which the acute abdominal surgical disease is associated with a multipara pregnancy in the third trimester, with preeclampsia or eclampsia and liver hematoma revealed by ultrasound, caesarean section is began with to interrupt the physio pathological chain of preeclampsia and to prevent hepatic rupture and a related massive hemoperitoneum (8).

Another rare case is that of multipara pregnancy in the third trimester, with or without eclampsia, with spontaneous rupture of the spleen, most commonly two-stroke.

The first stroke is the painful syndrome in the upper left quadrant, which requires (if the fetus is alive and mature) caesarean section and splenectomy to prevent the second, more dangerous, stroke, i.e. hemorrhagic syndrome with hemoperitoneum and severe hemorrhagic collapse (18).

CONCLUSIONS

Our scientific paper is an up-dated for management of association between an acute surgical abdomen disease and pregnancy. It points out the most difficult hypostases, avoid dangerous improvisations, giving the most adequate solutions with a view to obtaining best results for mother and baby.

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IATROGENIC BILE DUCT INJURIES

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IATROGENIC BILE DUCT INJURIES (Abstract). Bile duct injuries may occur both in hepatopancreaticobiliary surgery and in gastrointestinal surgery, but, most commonly, during cholecystectomy (80-90%). Laparoscopic approach has increased the occurrence of these significant intraoperative accidents, although it is one of the commonly seen interventions in a surgical department. The general incidence of bile duct injuries is usually 0.4-0.8%; in classical cholecystectomy, the incidence is 0.1-0.2%, and in laparoscopic surgery, it is 0.2-0.8%. **Material and methods**: We carried out a retrospective study on a batch of 23 patients who underwent surgery, from 1997 to 2015, which shows the approach of these accidents and the proper therapeutic conduct as well. **Results:** The evolution of each case depends on how quickly the diagnostic of bile duct injury is established. Two types of injuries have been identified: 52% wounds and 48% stenosis. **Keywords:** LAPAROSCOPIC CHOLECYSTECTOMY, BILE DUCTS, IATROGENIC INJURIES

Common bile duct (CBD) injuries during classical cholecystectomy, but especially during laparoscopic cholecystectomy, are a rare case, but consistently present in worldwide surgical practice CBD injuries cause major complications with locoregional and systemic effects which, without being immediately recognised and treated, may involve vital prognosis and long-term serious sequelae.

Involving the direct responsibility of the surgeon entails psychological pressure and tendency towards precipitation, which requires co-opting an experienced colleague or referring the case to a specialised centre.

Surgical treatment should restore the CBD from a morpho-functional point of view, with immediate and especially long-term good result. The more common association of iatrogenesis in laparoscopic surgery (0.2-0.8%) indicates the involvement of side effects of technology related to visual guiding in a limited field. Approximately 77-89% of CBI causes the postoperative appearance of abdominal pain, choleperitonitis, jaundice or angiocholitis (1).

MATERIAL AND METHODS

The study represents a retrospective analysis of CBI, from 1995 to 2015, caused or treated within the clinic, following classical or laparoscopic cholecystectomy. We took 23 patients for the study, of which 19 were women (83%) and 4 were men (17%), aged between 21 and 77 years (fig. 1).

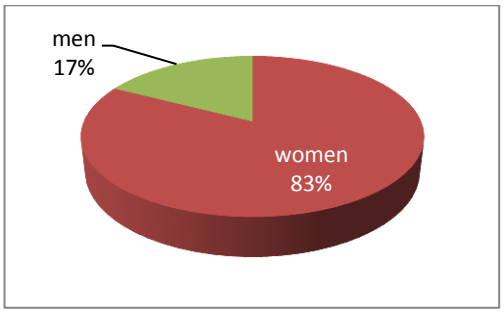

Fig. 1. Distribution depending on sex

The parameters used in the statistical analysis were represented by: initial diagnosis, intra or postoperative diagnosis of the injury, final diagnosis, surgical technique, paraclinical imaging examinations– endoscopic retrograde cholangio-pancreatography (ERCP), echography, intraoperative cholangiography, cholangio-NMR, average admission time – re-intervention, and average hospitalisation time.

The main causes of these accidents were pediculitis from the bile ducts caused by inflammation, the fact that all patients were initially diagnosed with acute cholecystitis, and biliary-vascular anatomical variations.

The identified injuries were represented by wounds of the common bile duct and stenoses of the common bile duct. The diagnosis of iatrogenic bile duct injury was established intraoperatively in 3 cases and postoperatively in 20 cases: - early (24-72 hours), in 3 cases, 2 and 3 days, respectively, after the operation; - late (>72 hours), in 17 cases, 6 cases approximately 7 days after the operation, 4 cases approximately 30 days after the operation, and the latest diagnosis of iatrogenic injury was established after 2 years.

RESULTS

Of the 23 cases, 7 cases had history of classical cholecystectomy and 16 cases had history of laparoscopic cholecystectomy, 16 cases coming from other centres. The female sex (83%) dominates the casuistry, being known the fact that biliary pathology is predominantly female. The average age of patients is 64 years.

Two types of injuries have been identified (fig. 2):

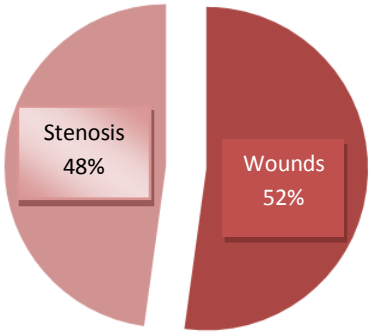

Fig. 2. Types of iatrogenic injuries

- *wounds* most commonly located at the level of the common hepatic duct, occurred as a result of a difficult dissection, due to the significant local inflammatory processes, which determined a partial or total section of the common hepatic duct; in the study, 12 wounds have been identified, of which:- 1 case with wound of the aberrant duct;- 1 case with lateral wound below the convergence + downstream stenosis;- 3 cases with lateral wound of the common hepatic duct;- 2 cases with compete section of the common hepatic duct;- 4 cases with lateral wound of the choledoch;- 1 case with section + wound of the common hepatic duct (cautery) (fig. 3).

- *stenoses* occurred because of ligature or total or partial clipping of the common hepatic duct (2, 6); the 11 identified stenoses were systematized according to the Bismuth classification:

-7 cases of Bismuth I injuries: - com-

plete choledochal ligature - 5 cases; - ligature of the common hepatic duct - 1 case;- lateral ligature of the cystic duct + common bile duct - 1 case.

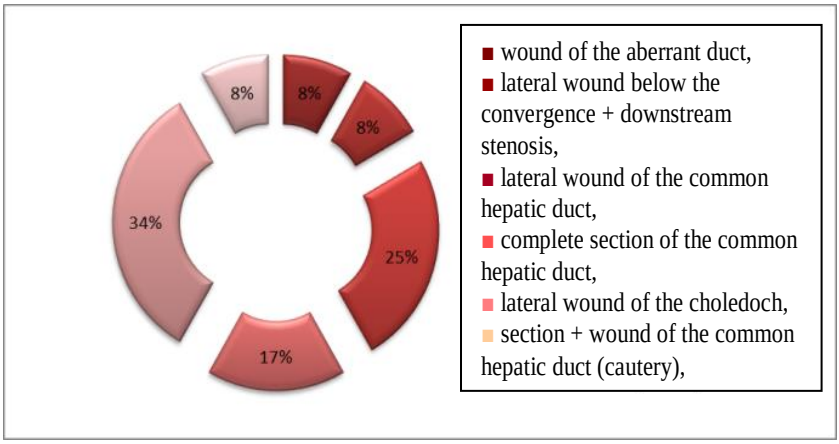

Fig. 3. Types of wounds

- 3 cases of Bismuth II injuries: - common bile duct ligatured and sectioned < 2 cm under the convergence.

- 1 case of Bismuth III injury: ligature at the level of the hilum with preserved convergence (fig. 4).

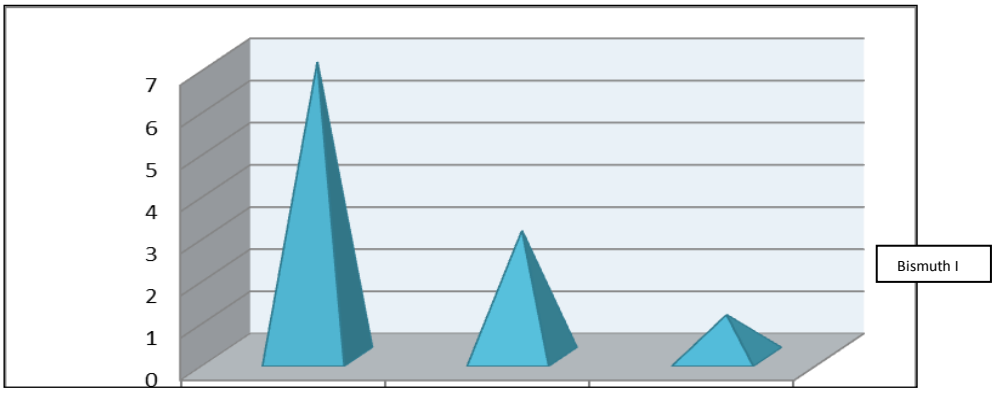

Fig. 4. Types of stenosis

According to the Strasberg classification (12), we had the following types of injuries in the studied batch: - one type C injury; - type D injuries: 8 cases;- type E1 injuries: 9 cases;- type E2 injuries: 4 cases;- type E3 injuries: 1 case (fig 5).

The main imaging explorations used for the diagnosis of the injuries were: - *abdominal ultrasound*, routine exploration, for all cases; - *intraoperative cholangiography*, which reveals leaks of contrast agent and also helps locate the injury (fig. 6, 7),

- Kehr's tube cholangiography,
- cholangio-NMR (fig. 8, 9),
- CT.

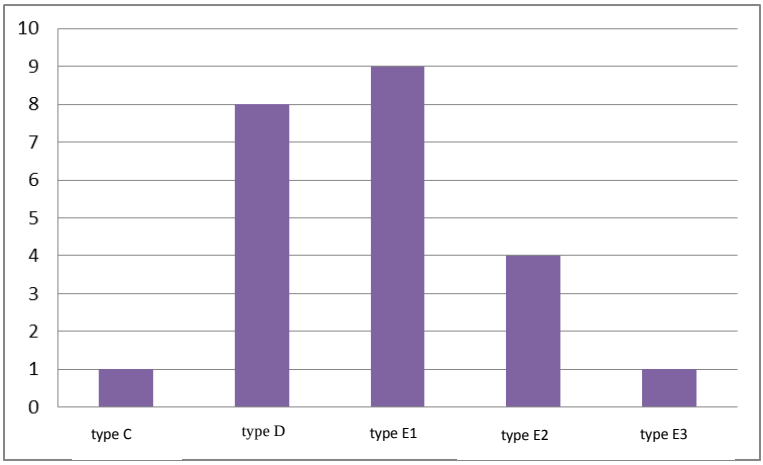

Fig. 5. Strasberg classification of iatrogenic biliary injuries

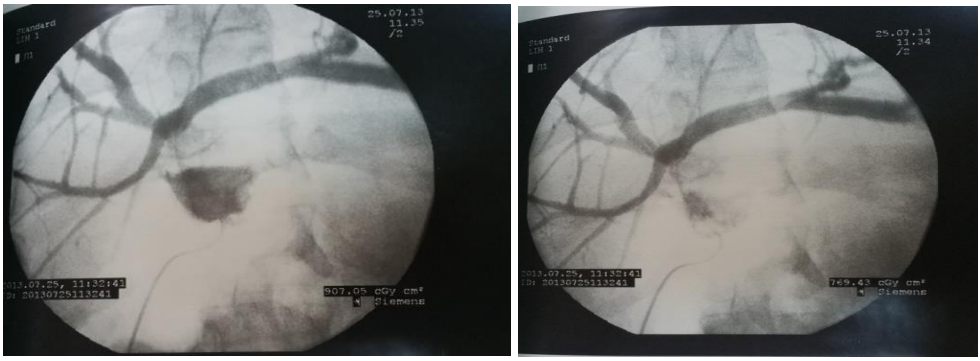

Fig. 6, 7. Intraoperative cholangiography in the patient with Bismuth III stenosis

Fig. 8. Postoperative cholangio-NMR in a patient with bile duct stenosis

Fig. 9. Cholangio-NMR in a patient with Bismuth III bile duct stenosis

The performed paraclinical explorations (blood count, biochemistry, electrolytes) reveal cholestasis syndrome in patients with iatrogenic stenosis, with high levels of total bilirubin, within the interval of 3.89-19.9 mg/dL, accompanied by phenomena of angiocholitis and sepsis, in 7 cases.

The treatment of these injuries depends on how early the injury has been recognised, and it requires experience and a technique appropriate for the type of injury. The injury was immediately identified intraoperatively in only 13% of the cases (3 patients). The approach was classical (fig. 10) and open in all cases for a better view and highlight of the injuries. The applied techniques in all 23 cases were the following:

- for stenosis:

- hepaticojejunal anastomosis with a Roux-en-Y loop - 6 cases; - hepaticojejunal anastomosis with an Ω loop - 2 cases; - T-T suture of the common hepatic duct + Kehr drainage - 1 case;- extraction of clip, suture of breach + Kehr drainage - 2 cases.

- for wounds:

- hepaticojejunal anastomosis with a Roux-en-Y loop - 3 cases; - suture of the aberrant bile duct, Kehr drainage - 1 case; - T-T suture of the common hepatic duct, transcystic drainage - 3 cases;- T-T suture of the common hepatic duct + Kehr drainage - 4 cases;- biliary drainage with two Kehr tubes in the right and left hepatic duct, one tube in the right paramedian duct - 1 case.

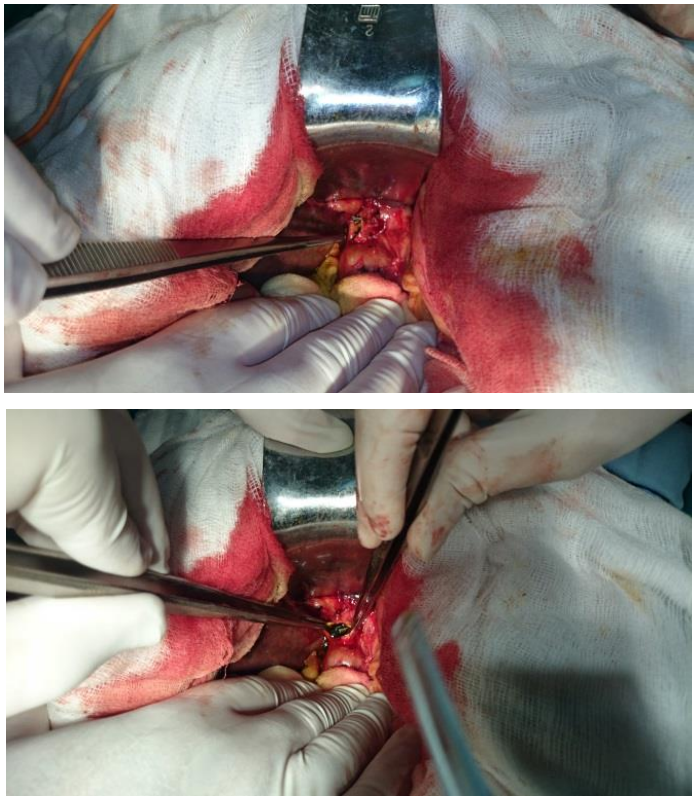

Fig. 10. Iatrogenic CBD injury - intraoperative (personal collection – C. Burcoveanu)

Morbidity was represented by the occurrence of a hepaticojejunal anastomosis fistula accompanied by choleperitoneum and septic phenomena, which cause the appearance of multiple organ failure and death, as well as the appearance of a hepatic abscess in the VIII hepatic segment, with sepsis, solved by drainage and antibiotic therapy. There were 7 cases of angiocholitis with sepsis - the association of sepsis in postoperative iatrogenic injuries, discovered late, indicate the choice of the restoration operative moment and negatively influences the results.

The mortality ratio was 4%: one patient died postoperatively due to associated personal pathologies developing septic phenomena and multiple organ failure.

DISCUSSION

Iatrogenic bile duct injuries represent a topical problem, considering the frequency of laparoscopic surgery used nowadays. They are signalled since the performance of the first cholecystectomy, in 1882, by Langenbuch.

There are multiple classifications of these injuries, depending on their location and type. The purpose of these classifications is to ease the management and choice of a proper treatment, with a surgical technique appropriate for each type of injury. Classically, the Bismuth classification is used, which is only limited to stenoses:

- I: CBD stenosis more than 2 cm from the hepatic confluence,
- II: CBD stenosis less than 2 cm from the hepatic confluence,
- III: stenosis below the confluence, but with its preservation,
- IV: stenosis at the level of the confluence, which involves both hepatic ducts, losing communication between the right and left hepatic duct,
- V: stenoses of the CBD and of a right aberrant hepatic duct (2, 3, 4).

As the Bismuth classification does not include the entire spectrum of possible injuries in the bile ducts, Strasberg *et al*. (5) have made a classification which includes that of Bismuth along with other iatrogenic injuries in the extrahepatic bile ducts:

- A: injuries of the cystic duct or the aberrant ducts from the hepatic level,
- B: stenosis of the aberrant segmental duct of the right lobe,
- C: sectioning of an aberrant segmental duct of the right lobe,
- D: lateral injuries of the CBD,
- E: stenoses or complete sections of the CBD, subdivided from E1 to E5, depending on the location of the injury according to the Bismuth classification.

Early intraoperative diagnosis is established in only 11-12% of the cases (1, 6), and, in our study, the intraoperative diagnosis ratio was around these levels, namely: 13%. Most bile duct injuries have been discovered postoperatively, late, after more than 72 hours, at variable intervals of time. For the patients in the study, the diagnosis was established with the help of imaging investigations, and they all benefited from abdominal ultrasound, while 35% of them underwent cholangio-IRM (6).

The choice of therapeutic conduct varies depending on how early the diagnosis is established, the type of injury, and the local and general physio pathological conditions (3).

In our study, most injuries were solved through reconstructive interventions (9 cases). The most used reconstructive tech-

niques to redo the biliary tree were hepaticojejunal anastomosis with a Roux-en-Y loop and hepaticojejunal anastomosis with an Ω loop, laborious techniques which require an experienced team.

Iatrogenic CBD injuries represent an undesired major complication, but possible, both in classical surgery, and especially in laparoscopic surgery.

Prevention of iatrogenesis in CBD surgery requires obsessive interest in adapting the surgical technique to the peculiarities of the case, regardless of the often-deceiving aspect of "simple gallbladder". There with, a thorough preoperative exploration and the possibility of intraoperative imaging explorations of the CBD are also required.

In our experience, along with data from the literature, the risk factors with a major impact are represented by:

- the local anatomical changes present in acute cholecystitis, but also scleral alterations from the forms of chronic cholecystitis. The choice of the operative moment in acute cholecystitis and intraoperative conversion of the technique are prevention factors of iatrogenic injuries.
- the frequency of anatomical variants at the level of the CBD, commonly accompanied by variants of hepatocystic vascularisation. Prevention of iatrogenic injuries requires preoperative and sometimes intraoperative complete exploration, and it also requires meticulous complete dissection of the anatomical structures, with no early intempestive ligatures. The approach of difficult cases is indicated in relation with the experience and level of perfection of the surgical team in classical and laparoscopic hepatobiliary surgery (7, 8, 9, 10, 11).
- If a CBD injury occurs, not complying with the specialty standard and the lack of intraoperative diagnosis technical means and of necessary materials contraindicates the resolving attempt, and the patient should be referred to a specialised centre (12,13,14).
- the perfect state of the device in laparoscopic surgery and the correct use of electrosurgery prevent possible iatrogenic injuries. The image quality in laparoscopic surgery may determine false anatomical representations, with consequences that are difficult to assess.

CONCLUSIONS

In our experience, iatrogenic bile duct injuries are serious complications that may occur both in laparoscopic and in classical surgery, associated with a high mortality, and early and long-term serious complications.

CBD injuries represent an undesired major complication, but possible. They can be prevented, but they cannot be completely avoided.

Their diagnosis is usually late, in our study, 87% of the cases being diagnosed postoperatively. Recognising the injury intraoperatively is the ideal condition of restoration intervention, with immediate and long-term favourable results. The choice of the operative moment depends on the presence or absence of sepsis and the seriousness of the inflammatory process in the CBD.

Preoperative exploration and the possibility to carry out intraoperative imaging explorations prevent the occurrence of CBD injuries.

Treatment is adapted after carrying out a complete injury report and after understanding the mechanism of how the injury occurred.

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TUBERCULOSIS IN HIV-POSITIVE PREGNANT WOMEN – TWO-YEAR EXPERIENCE OF THE IAŞI REGIONAL HIV/AIDS CENTER

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TUBERCULOSIS IN HIV-POSITIVE PREGNANT WOMEN- TWO-YEAR EXPERIENCE OF THE IAŞI REGIONAL HIV/AIDS CENTER (Abstract). **Aim:** To analyze a group of HIV-positive pregnant patients diagnosed with tuberculosis (TB) both in the intrapartum and post-partum periods and the perinatal outcomes. **Material and methods**: Over a 24 month-period (January 2015 - December 2016) we retrospectively reviewed the medical records of the patients of childbearing age actively monitored in the Iaşi Regional HIV/AIDS Center. The group included 41 pregnant women aged 17 to 39 years, three of them with confirmed tuberculosis. Data on virologic and immune parameters, level of adherence to therapy, and newborn health status were collected**. Results**: We report three cases of confirmed TB. Two of the patients were diagnosed with TB soon after delivery, and in one patient TB was diagnosed concomitantly with AIDS disease in the intrapartum period. At the time of diagnosis, all patients had suggestive symptoms, the most commonly described symptom being persistent cough lasting for a few weeks up to 4 months. Chest X-ray findings were suggestive of TB, especially in the cases with miliary tuberculosis, and in 1 patient the radiologic appearance was atypical. The diagnosis was confirmed by sputum bacteriology, positive for acid-alcohol-fast bacilli in all cases. WHO Class I anti-TB therapy along with antiretroviral therapy were initiated both during pregnancy and postpartum. In all cases the pregnancies ended in premature births at 35 weeks, the newborns being low birthweight. In immediate postpartum one patient died from sepsis of unknown etiology. **Conclusions:** Our study emphasizes the late diagnosis of TB despite the onset of symptoms several weeks earlier. We believe that the number of TB cases among HIV-positive patients of childbearing age is much higher, the implementation of a questionnaire-based screening for the early diagnosis being essential for reducing mortality. Successful treatment requires a good multidisciplinary collaboration. **Keywords:** PREGNANCY, TUBERCULOSIS, HIV, MULTIDISCIPLINARY COLLABORATION.

HIV infection is a risk factor for the progression of latent *Mycobacterium tuberculosis* infection to active TB. The risk of TB is 25-30 times higher among the HIV-positive persons compared with the HIV-negative ones. HIV-positive people are so vulnerable to primary progressive TB that the active disease may develop several

weeks after exposure to the Koch bacillus (KB). Risk factors for developing TB are: low CD4 cell count, high viral load, and non-compliance and non-adherence to antiretroviral therapy (ARV), late presentation to the doctor, anemia and decreased body mass index (1).

Tuberculosis can occur during pregnancy due to stress induced by it, especially when associated with poor nutrition, unfavorable socioeconomic background or immunodeficiency. Signs of maternal TB can range from mild cough, lasting for at least two weeks, fever and fatigue, to weight loss or hemoptysis. Pulmonary TB is the most common form of maternal TB, and of the extrapulmonary forms the most frequently reported are tuberculous meningitis and lymph node TB (2). According to literature data, TB increases the risk of prematurity and spontaneous abortion (3). Newborn infants born to both mothers suffering from TB or AIDS are more likely to be low birth weight and to have fetal distress. The exact risk of TB transmission to the newborn has not been fully elucidated. Progression from infection to disease is highest during the first year of life. Signs suggestive of active tuberculosis in children are fever, cough and poor weight gain in the context of contact with people with BK-positive sputum microbiological examination (4).

This study aimed to analyze a group of HIV-positive, pregnant or postpartum women as well as the perinatal outcomes, to determine the presence or absence of a TB diagnosis. We retrospectively reviewed the medical records of the patients of childbearing age actively monitored in the Iaşi Regional HIV/AIDS Center over a 24-month period (January 2015 - December 2016). During this period 41 pregnant women aged 17 to 39 years were admitted. In patients with confirmed TB the following data were collected and analyzed: virologic and immune parameters, level of adherence to antiretroviral as well as tuberculostatic therapy and the newborn health status. Of the study cases, only 2 were diagnosed with tuberculosis in the immediate postpartum period and 1 case in pregnancy, when a concomitant diagnosis of AIDS disease was made.

CASE REPORTS

Case 1

A 19-year-old female patient was diagnosed with stage B2 HIV infection during her first pregnancy, at 5 months' gestational age. ARV therapy with lopinavir/ritonavir and lamivudine/zidovudine was initiated. The pregnancy follow-up was not optimal, the patient skipping her follow-up visits. She delivered prematurely at 35 weeks gestational age a male baby born alive, birth weight 1930 g, Apgar score = 7. The newborn received ARV prophylaxis with zidovudine and lamivudine. In the immediate postpartum the patient presented cough, low grade fever, with patient-declared onset of symptoms 4 weeks earlier, and intrafamilial TB contact. Tuberculin skin test (TST) was negative at 72 hours, chest X-ray showed bilateral reticulomicronodular and "honeycomb" appearances, and sputum bacteriology was positive for acid-alcohol-fast bacilli (AAFB), 2+. She was diagnosed with miliary TB and received WHO Class I anti-TB therapy (isoniazid, rifampicin, pyrazinamide, ethambutol), as recommended by her pulmonologist. Shortly after the postpartum period she was transferred to the Iaşi Regional HIV/AIDS Center with a marked immunosuppressive state, CD4 count = 99 cells/mm^3 and viral load (VL) = 936.000 copies/mL (tab. I). Under these circumstances the patient developed severe sepsis with *Enterococcus faecium* isolated in

blood cultures and urine culture and *Escherichia coli* in lochia culture. HAART (highly active antiretroviral therapy) and anti-TB drugs were associated with antibiotic therapy according to the antibiogram. She developed tuberculous pleurisy and drug-induced hepatotoxicity, reason why, after pulmonology reassessment, the replacement of rifampicin by streptomycin was decided. The patient needed sustained psychological support due to recurrent depressive episodes (5). The outcome was favorable, with no relapses after nine months of anti-TB therapy, increase in CD4 count to 267 cells/ mm^3 and undetectable viral load (tab. I).

Case 2

A 27-year-old female patient belonging to the "pediatric cohort", monitored in the Iaşi Regional HIV/AIDS Center since 1999, on HAART with abacavir/lamivudine and raltegravir, stage B2 HIV infection, presented in July 2016 for virologic-immunologic assessment. As the patients stated the absence of menstrual periods, a gynecologic examination was recommended and an ongoing pregnancy, 12 weeks' gestational age, was diagnosed. In the third month of pregnancy the CD4 count = 302 cells/mm^3 and VL = 476,000 copies/mL. Her past medical history revealed one full term pregnancy, the baby being HIV-negative at the age of 18 months. She received psychological counseling to increase compliance and adherence to ARV therapy (6).

Incompletely followed-up during pregnancy, the patient delivered prematurely, at 35 weeks gestational age, a baby girl, birth weight 1750 g and Apgar score 7. The newborn baby received antiretroviral prophylaxis. In the immediate postpartum the patient presented ineffective productive cough and fever, and was transferred to our service. The patient was unable to pinpoint exactly the time of symptom onset, saying only that she had discontinued the ARV therapy for several months. Chest X-ray revealed a hyper transparent area of 10-15 cm in diameter located in the right upper lobe and homogeneous, medium intensity opacity located in the right lower lobe (fig. 1). Sputum bacteriology was positive for AAFB, 3+. The pulmonologist made the diagnosis of pulmonary TB and initiated the WHO class I anti-TB therapy.

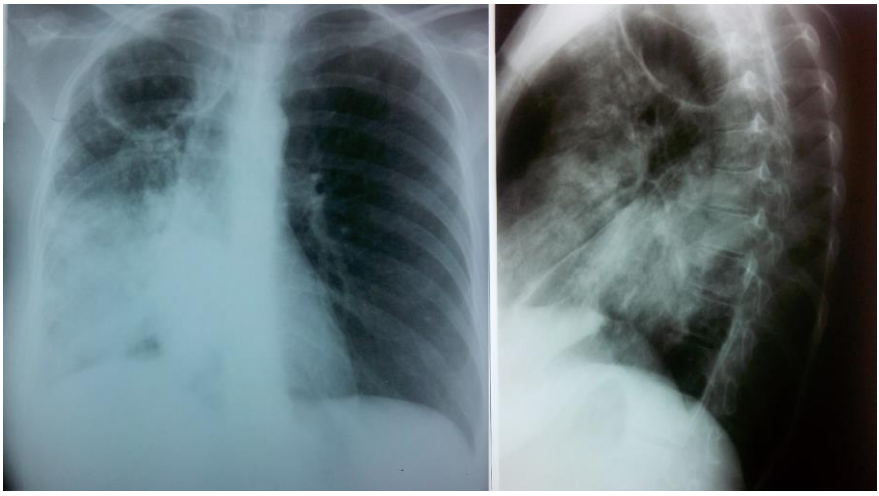

Fig. 1. Atypical radiographic appearance of pulmonary TB

CD4 count at the time of TB diagnosis was 262 cells/mm^3 and VL 150,000 copies/mL (tab. I). The outcome was unfavorable due to the occurrence of sepsis of unknown cause. Despite the complex regimens instituted, the patient died.

Case 3

A 32-year-old female patient without significant past medical history with ongoing pregnancy, 23 weeks' gestational age, presented to the Emergency Department of the Iaşi "Sf. Spiridon" County Clinical Emergency Hospital "for impaired general condition". She complained that about four months earlier she started experiencing loss of appetite, rare episodes of productive cough and difficulty swallowing, symptoms that worsened over several days before ED presentation and were accompanied by fever. The complete blood count (CBC) revealed severe leukopenia and anemia. The patient was referred for further investigations at the "Sf. Parascheva" Infectious Diseases Hospital. Serology was HIV positive, with CD4 count = 6 cells/mm^3 and VL =189,000 copies/mL being recorded (tab. I). Chest X-ray examination revealed the presence of intense micronodular opacities in both lung areas and sputum bacteriology was positive for AAFB, 2+. A diagnosis of stage C3 AIDS disease and miliary pulmonary TB was made concomitantly, and ARV therapy and WHO class I anti-TB therapy were immediately initiated.

She delivered at 35 weeks' gestation a 2,400-g viable male infant who was in distress. The newborn received ARV prophylaxis (zidovudine and lamivudine) from the first day of life. On completion of anti-TB therapy, CD4 count=316 cells/mm^3 and V=28 copies/ mL was recorded (tab. I).

TABLE I

**Virologic and immune parameters
at the time of TB diagnosis and completion of anti-TB treatment**

No.	TB diagnosis		Treatment completion	
	CD_4 count	VL	CD_4 count	VL
Case 1	99 cells/mm^3	936,000 copies/mL	267 cells/mm^3	undetectable
Case 2	262 cellsmm^3	150,000 copies/mL		deceased
Case 3	6 cells/mm^3	189,000 copies/mL	316 cells/mm^3	28 copies/mL

DISCUSSION

TB and HIV/AIDS are independent risk factors for maternal mortality; at all ages the problem is how and when they are discovered. The diagnosis of TB as well as of HIV infection has been delayed either because of the nonspecific symptoms, such as malaise, or because of seeking medical help late. WHO recommendations for the management of tuberculosis include a plan for screening using a clinical algorithm comprising four main symptoms: cough lasting at least two weeks, night sweats, fever, or weight loss. Also mentioned is the need for collaboration between medical specialties so that these symptoms to be detected and the patient referred for further investigation, regardless of the medical service where the patient was assessed (7).

It was found that pregnant women diagnosed with TB had a marked immunosuppressive state due either to non-compliance and non-adherence to ARV therapy or because of the late discovery of HIV infection. Of diagnostic relevance were both the personal history of patients - persistent

cough for several weeks up to 4 months - and household TB contact. Unless specifically asked about TB symptoms, the disease was not detected in the studied group. It was found that even the TB type depended on the CD4 count, the 2 reported cases of miliary TB having CD4 counts below 100 cells/mm^3.

The sensitivity of traditional diagnostic methods diminishes with the progression of immunosuppression. Due to the altered cellular immune response, the tuberculin skin test gave false-negative results. The diagnosis was made by sputum bacteriology positive for AAFB and radiological findings, although according to literature data the radiographic pattern is atypical when CD4 counts are below 200 cells/mm^3 (8).

In immunocompromised patients TB is difficult to diagnose as the classic diagnostic methods can give inconclusive results and the disease often remains underdiagnosed.

WHO class I anti-TB therapy along with HAART were immediately initiated, both in the immediate postpartum and during pregnancy. WHO international guidelines recommend that regardless of the CD4 count ARV therapy should be started as soon as possible, up to eight weeks, after the initiation of anti-TB therapy (7, 9).

As a side effect, drug-induced hepatotoxicity was recorded in one of the 3 reported cases, requiring replacement of rifampicin with streptomycin under the guidance of a pulmonologist. During the acute phase of tuberculosis an increase in viral load was noticed. Active tuberculosis accelerated the AIDS-induced immunological alteration, favoring the development of other opportunistic infections and death in a patient by sepsis.

TB in HIV-positive pregnant women had a negative impact on perinatal and also on infant outcomes. Deliveries were premature, at 35 weeks' gestation; newborns were low birthweight, and one of them presented fetal distress.

Infants received ARV prophylaxis from the first day of life and were also assessed for clinical and radiological signs of TB. None of them was found HIV-positive or having TB.

CONCLUSIONS

Our study emphasizes, based on this case series, the late diagnosis of TB despite the onset of symptoms several weeks earlier. We believe that the number of TB cases among HIV-positive patients of childbearing age is much higher, and the current study is merely the tip of the iceberg. Consequently, the screening for TB of HIV-positive women of childbearing age by implementing a questionnaire for the signs and symptoms of TB should be performed at the Iaşi Regional HIV/AIDS Center.

We also found that HIV/TB coinfection during pregnancy caused premature birth of low-birthweight infants, so the fetal risk should always be considered.

Treatment success depends on patient adherence and compliance to treatment, time of diagnosis, and an effective cooperation of the multidisciplinary team, including the psychologist.

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NEWS

A NEW LINK BETWEEN CARDIAC DISEASE AND CEREBRAL ISCHEMIC LESIONS

Numerous studies have shown that vessel brain disease which affect inner-brain areas are responsible for cognitive dysfunction and dementia. Identification of specific markers of cerebral ischemic lesions and their risk factors could have an important role in preventing cognitive impairment. A cohort study, published online in *JAMA Neurology*, shows that biomarkers of cardiac disease are associated with cortical cerebral microinfarcts (CMI), as marker of small-vessel brain ischemic disease. The study included 243 participants to which were carried neuropsychological evaluation, determination of plasmatic concentration of two biomarkers of subclinical and clinical cardiac disease (N-terminal pro–brain natriuretic peptide and high-sensitivity cardiac troponin T) and magnetic resonance imaging for highlight of CMI. Also, for all participants, has been investigated the history of atrial fibrillation, ischemic heart diseases, or congestive heart failure. The results showed that all 70 patients (28.8%) to which were found CMI had a significantly higher prevalence of cardiac disease, such as atrial fibrillation (rate ratio [RR], 1.62), ischemic heart disease (RR, 4.31), and congestive heart failure (RR, 2.05), compared to participants without CMI. Also, participants with CMI had significantly higher levels of plasmatic biomarkers of subclinical and clinical cardiac disease (RR, 3.16 and RR, 2.17, respectively). In researcher's opinion, these results suggested that cardiac disease may contribute to the development of CMI. Thus, they have linked cardiac disease to cerebral ischemic lesion that has been previously associated with cognitive pathology, such as dementia and suggested that controlling cardiac risk factors could reduce the progression of CMI and prevent cognitive impairment (Saima Hilal, Yuek Ling Chai, Susanne van Veluw, et al. Association between subclinical cardiac biomarkers and clinically manifest cardiac diseases with cortical cerebral microinfarcts. *JAMA Neurol*. Published online February 6, 2017).

Cătălina Luncă

PERIOPERATIVE IMMUNE RESPONSE ALTERATION. CAN IT INFLUENCE CANCER RECURRENCE?

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PERIOPERATIVE IMMUNE RESPONSE ALTERATION. CAN IT INFLUENCE CANCER RECURRENCE? (Abstract**)**: The relationship between host immune response and tumor cells is currently defined by the immunoediting concept, including three phases: elimination, equilibrium and escape. Cancer cells are initially eliminated by the immune system, but progressively can develop escape mechanisms. The balance between elimination and escape depends on the prevailing activity of cytotoxic Th1 or immunosuppressive Th2 lymphocytes. Surgery has the potential to eradicate the disease, but, along with other perioperative circumstances, can induce a variable degree and period of immunosuppression. By modifying the Th1/Th2 ratio, these perioperative circumstances may favor the escaping mechanisms and, consequently, promote cancer recurrence and metastasis altering long term prognosis of cancer patients. **Keywords**: CANCER, SURGERY, ANESTHESIA, PERIOPERATIVE PERIOD, CANCER RECCURENCE, IMMUNOSUPRESSION.

Cancer represents today a major source of morbidity and mortality worldwide. In the United States, the risk of developing any type of cancer during a lifetime is currently estimated at one in two for men and one in three for women. A report of American Society of Clinical Oncology states that, in 15 years from now, the prevalence of the disease will markedly increase and cancer will exceed heart diseases in terms of mortality, becoming the leading cause of death (1).

Facing this reality, researchers from around the world are struggling to find new early diagnostic strategies and to improve actual therapeutic armamentarium. Despite the introduction of new screening tools, cancer is diagnosed in the clear majority of patients when tumor cells compromise the organ function. However, the leading cause of death in cancer patients is not related to the complications of primary tumor, but rather with the occurrence of local recurrence and distal metastasis (2).

Risk factors for cancer recurrence are multiple, depending on the initial stage of disease, biological features of the tumor, initial therapy and immune function. In clinical practice the modifiable factors are the quality of the applied treatment and the abil-

ity to influence the patient immune system.

The current multimodal approach of cancer patients includes chemotherapy, radiotherapy, surgery and immunotherapy. Surgical resection is the primary treatment in solid tumors and has a curative intent in localized disease. By the same time, the surgical procedure, along with other perioperative interventions, can induce variable degrees and periods of immune suppression, favoring cancer cell spread.

Consequently, we may conclude that the perioperative period in cancer patients is related to the following dilemma: surgery represents the unique possibility to eradicate the tumor, but in the same time has the risk to promote the development of metastasis and alter the long-term outcome of cancer patients.

IMMUNE RESPONSE IN CANCER PATIENTS

The immune system plays an important role in the control of tumor growth. The initial theory of immune surveillance, formulated in 1957 by Burnet and postulating that lymphocytes are continuously recognizing and eliminating tumor cells (3), is now replaced by the immunoediting concept. Per this concept immunoediting has three phases: elimination, equilibrium and escape (4,5). In the *elimination phase*, tumoral cells are initially recognized by the innate immune system cells (macrophages) that are activated and migrate to lymph nodes to promote activation and clonal proliferation of cytotoxic T lymphocytes. Those cells migrate to the tumoral site and release enzymes responsible for tumor cell apoptosis (6). NK cells are also involved in the antitumoral response. The cytotoxic effect of cytokine activated cells is achieved by releasing perforins and granzymes responsible for tumor cell lysis (7). The presence of a T lymphocyte CD8+ and memory T lymphocytes (CD45RO+) infiltrate in the tumoral surrounding tissue is considered a favorable prognostic factor (8). In the *equilibrium phase* of the immunoediting concept, cancer cells, which have survived the elimination process, start to develop genetic mutation and progressively become resistant to the immune system. The *escape phase* is characterized by clinically apparent tumor, due to uncontrolled cancer cells proliferation. There are multiple mechanisms of tumor cells escape from the control of immune system - lack of recognition of tumor cell (lack of tumor antigens expression or MHC gene mutations), peritumoral inflammation that can inhibit the activation of CD8+, CD4+ T lymphocytes, tumor cells may induce the occurrence of Treg cells (9).

CD4+ T helper lymphocytes (Th1 and Th2 subpopulations) modulate the immune system response and can have both stimulatory and suppressive effects on cancer cells. Th1 lymphocytes secrete proinflammatory cytokines (IFN γ, IL-2, IL-12 and TNF-α) and promote an inflammatory response. Th2 lymphocytes synthesize cytokines with anti-inflammatory activity (IL-4, IL-5, IL-8, IL-10 and IL-13). The predominance of Th1 cell activation is associated with an anti-neoplastic immune response. Thus, IL-2, considered the prototype interleukin of Th1 cells, increases the cytotoxic activity of NK cells, induces the synthesis of IFN γ and activate macrophages. In contrast, Th2 cytokines promote the expression of Treg, matrix metalloproteinases, invasiveness and metastasis. In the elimination phase the Th1/Th2 balance favors Th1cells activity while the escape phase is characterized by a Th2 shift.

RISK FACTORS FOR IMMUNE SUPPRESSION DURING PERIOPERATIVE PERIOD

Cancer patients scheduled for surgery have already, in the preoperative period, multiple sources of immunosuppression. **Cancer** itself is frequently accompanied by an alteration of both humoral and cellular immune response (10). **Neoadjuvant therapies** (chemo/radiotherapy) produce destruction of cancer cells, but also of immune cells. **Malnutrition and cancer cachexia,** caused by systemic and local effects of the tumor depending on the size, location, histological type and stage, but also by treatment side-effects, is associated with a decreased cytotoxic activity of NK cells (11) and reduced levels of cytokines involved in the differentiation of Th1 lymphocytes (12).

Psychological stress and depression is frequently associated with cancer diagnosis and treatment. It triggers a neurohumoral response, characterized by an increased release of catecholamines and glucocorticoids, a reduction of NK cytotoxicity and a proinflammatory status and is correlated with cancer progression (13, 14).

The immunosuppressive effect of **surgery** is currently explained by the dual action of tissue trauma and surgical stress response. Surgery induces tissue damage and release of cellular debris, that will promote an inflammatory reaction. Proinflammatory cytokines (TNF alfa, IL-1, IL-6) activate hypothalamic-pituitary-adrenal axis and increase cortisol production with suppressive action on the immune system. But surgery also triggers a neurohumoral stress response, consisting of acute inflammation and hypothalamic-pituitary-adrenal axis and sympathetic nervous system activation. This complex response, proportional with the magnitude and invasiveness of surgical injury, aims initially to adapt the organ function to increased demands. When prolonged or exaggerated, this adaptive response can become harmful. The cumulative effects of released cortisol and catecholamines on immune function promote a Th2 cell dominance, with a consecutive depression of cellular immunity lasting for several days after surgery (15,16). Oncologic surgery often requires extensive tissue excision/resection, being associated with a high risk of intraoperative bleeding, **hypovolemia** and **hypotension**. The consecutive activation of the sympathetic nervous system and hypothalamic-pituitary-adrenal axis along with reduced tissue perfusion and tissue hypoxia will depressed the Th1 lymphocyte response (17). The **perioperative hyperglycemia** is the result of increased hepatic glycogenolysis and gluconeogenesis and peripheral insulin resistance. It impairs normal phagocytosis and leukocytes function and releases pro-inflammatory cytokines (18).

Perioperative anemia is a frequent finding in oncologic surgical patients due to preoperative bleeding, malnutrition, side-effects of neoadjuvant therapies and intraoperative blood loses. Immune suppressive effect of **allogeneic blood transfusion** is currently termed transfusion-associated immunomodulation (TRIM) and is characterized by the reduction of Th1 lymphocytes and NK cells count (19, 20).

Multiple techniques and drugs are available to provide appropriate anesthesia during surgery. Their influence on the long-term outcome seems to be explained by the modulation of the neuroendocrine response to stress and by the interaction with the immune system. **Anesthesia and analgesia** effects on immune function depend on the type of technique used (general anesthesia,

locoregional anesthesia or combined general anesthesia/locoregional analgesia) and the type of hypnotic and analgesic drugs. Concerning the choice of the anesthesia technique, two major retrospective analyses showed that using intraoperative locoregional analgesia instead of postoperative intravenous morphine analgesia markedly reduce cancer recurrence (21-22). This potential benefit of regional anesthesia/analgesia **techniques** can be explained by the attenuation of surgical stress response and the reduced intraoperative doses of opioids and inhalation drugs (23). **Intravenous anesthetic** drugs like ketamine, thiopental and midazolam result in a significant reduction of NK cells activity, as shown in animal studies (24-25). Conversely, propofol administration has no influence on NK cells and seems to favorably increase Th1/Th2 ratio in the postoperative period (24,26). Currently used **volatile anesthetic agents** like isoflurane and sevoflurane suppress NK cells activity and impair various lymphocyte functions in a dose and time dependent manner (23,27). **Opioids** induced immunosuppression was extensively studied in cancer patients. Morphine administration was associated with Th2 differentiation and increased glucocorticoid production (28-30). Conversely, the use of fentanyl and tramadol seems to increase the number and activity of NK cells, having protective antitumoral effects (31-32).

CONCLUSIONS

The complex interplay between immune system response and tumoral cells growth can influence cancer progression. Surgical interventions in oncological patients are focused on tumor eradication. The surgical stress response triggers a complex neurohumoral and immune reaction. Acting on an already altered immune status, the surgical procedure, anesthesia and other perioperative circumstances, induce a variable degree and period of immunosuppression, creating a window of opportunity for cancer recurrence and distal metastasis.

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BLOOD LOSS MANAGEMENT IN PRIMARY HIP SURGERY: IS REINFUSION DRAIN A FEASIBLE OPTION IN MAINTAINING HEMOGLOBIN LEVELS?

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BLOOD LOSS MANAGEMENT IN PRIMARY HIP SURGERY: IS REINFUSION DRAIN A FEASIBLE OPTION IN MAINTAINING HEMOGLOBIN LEVELS? (Abstract). The management of blood loss in primary hip arthroplasty is a controversial topic. **Aim:** To evaluate the efficacy of reinfusion drains in terms of hemoglobin levels and volume of red blood cell transfused postoperatively. **Material and Methods**: 295 patients who underwent primary hip arthroplasty were retrospectively assessed. After applying the exclusion criteria, 94 patients were included in the study and were divided into two groups: 45 patients received a reinfusion drain and 49 a suction drain. The following were analyzed: demographic characteristics of patients, preoperative hemoglobin level, 12-h and 24-h postoperative hemoglobin levels and their variations, number of transfused units of packed red blood cells, and postoperative complications. **Results:** Kruskal Wallis analysis revealed the homogeneity of the study groups (Chi-square=2.40, df=2, p=0.301). A statistically significant lower decline in mean Hb_{24} was found in suction drain group (p=0.001). Kruskal Wallis test revealed a significantly more frequent postoperative use of a higher number of packed red blood cell units in the suction drain group (Chi-square=28.70, df=2, p=0.001) compared to reinfusion drain group. **Conclusions:** We failed to demonstrate the superiority of reinfusion drains versus suction drains in maintaining hemoglobin levels. **Keywords**: ALLOGENEIC BLOOD, REINFUSION DRAIN, PRIMARY HIP ARTHROPLASTY

The development of modern society leads to an increase in life expectancy and along with of the incidence of degenerative and posttraumatic disorders of the proximal femur (1).

Hip surgery, especially hip replacement, is accompanied by significant blood loss requiring the replacement of the lost blood volume (2). Allogeneic blood transfusions pose high risks for the transmission of bacterial and viral infections, volume overload, renal failure, altered mental status, etc. (3, 4). To avoid these risks several methods have been developed: preoperative autologous blood donation, intra- and postoperative use of reinfusion drains, antifibrinolytic agents, controlled hypotension, spinal anesthesia, erythropoietin, etc. (5-7).

Reinfusion drains act on the principle of active suction of the fluid in the surgical

wound, its filtration or not, and intravenous reinfusion in order to minimize blood loss.

Although devoid of the above-mentioned disadvantages, the use of reinfusion drains remains controversial in terms of cost and efficacy in restoring the hemodynamic balance (8). Although their use has its enthusiasts (9-11), a number of studies have shown their ineffectiveness (8, 12).

The present study aims to study the efficacy of reinfusion drains in maintaining the hemoglobin levels and decreasing the postoperative need for packed red blood cells in patients with primary hip replacement.

MATERIAL AND METHODS

A total of 295 patients who underwent primary hip arthroplasty between February 2015 and March 2013 were retrospectively reviewed. After applying the exclusion criteria (tab. I), 94 patients were included in the study and were divided into two groups: 45 patients received a reinfusion drain (RD) and 49 a suction drain (SD). Data were collected from patients' clinical observation sheets and by using Unique Integrated Information System (UIIS).

TABLE I
Inclusion and Exclusion criteria

Inclusion criteria	Exclusion criteria
• Same surgeon	• Patients who refused blood transfusion
• Müller-Gay approach	• Patients with preoperative Hb levels <10 mg/dl
• lateral decubitus	• Patients with a history of thromboembolic events
• Spinal anesthesia	• Patients presenting coagulation disorders/ malignant conditions
• Age >18	• Patients presenting thrombocytopenia
• Preoperative Hb ≥ 10 mg/dl	• Patients presenting kidney disease/failure
	• Patients with cardiac insufficiency
	• Patients presenting liver failure
	• Patients on oral anticoagulants preoperatively, antifibrinolytic agents pre/intraoperatively and steroids
	• Patients presenting mental deterioration

The analyzed data included: demographics, preoperative Hb level, postoperative 12 h (Hb_{12}) and 24 h (Hb_{24}) Hb levels, their variations changes: (ΔHb_{12}), (ΔHb_{24}), number of transfused packed red blood cell units and postoperative complications.

The decision to transfuse postoperatively was made at hemoglobin levels <8 mg/dl or when symptoms of anemia were present.

A GEM Premier 3000 analyzer was used to analyze the blood samples. Statistical analysis was performed using SPSS 18.0 software. Student t, $\chi 2$ and Kruskal-Wallis tests were used. To determine the relationship between variables the multiple linear regression was used. P <0.005 was considered statistically significant.

RESULTS

Kruskal Wallis test revealed the homogeneity of the study groups by gender (Chi-square=2.40, df=2, p=0.301), mean age (p=0.059), although in group RD the mean values were slightly lower than in SD group (60.18 years and 65.90 years, respectively), and area of residence (p=0.234) (tab. II).

At the first postoperative assessment of

hemoglobin level, mean hemoglobin levels dropped significantly in both groups (p=0.001), with no significant differences between the groups (p=0.093). At the 2nd postoperative assessment, the mean hemoglobin levels decreased significantly in both groups compared to the levels recorded preoperatively (p=0.001), but in RD group the mean level was significantly lower than in SD group (p=0.001). However, hemoglobin variation was lower in the control group (tab. III).

TABLE II

Demographic characteristics of the study patients

	Group RD		Group SD		p F_{ANOVA} test
Gender	n	%	n	%	
Female	22	48.9	27	55.1	0.301
Male	23	51.1	22	44.9	
Age (years)					
30-39	3	6.7	2	4.1	0.059
40-49	4	8.9	3	6.1	
50-59	13	28.9	4	8.2	
60-69	15	33.3	17	34.7	
70-79	9	20.0	22	44.9	
80-89	1	2.2	1	2.0	
Area of residence					
Urban	26	57.8	20	40.8	0.234
Rural	19	42.2	29	59.2	

TABLE III

Mean hemoglobin level evolution by study group

Study group	N	Mean	Std. deviation	Std. error	Confidence interval		Min	Max	ΔHb	p F_{ANOVA} test
					- 95%CI	+95%CI				
Preoperative										
RD	45	13.87	1.47	.22	13.43	14.31	9.00	16.40		0.015
SD	49	13.09	1.71	.24	12.60	13.58	8.50	17.20		
Postoperative-12h										
RD	45	10.65*	1.31	.20	10.25	11.04	7.40	13.30	3.22	0.093
SD	49	10.26*	1.33	.19	9.88	10.64	7.60	12.70	2.83	
Postoperative-24h										
RD	43	10.54*	1.49	.23	10.08	10.99	6.90	13.20	3.33	0.001
SD	48	9.99*	1.05	.15	9.69	10.30	7.90	12.70	3.10	

Preoperatively, higher mean hemoglobin levels (13.87 mg/dl) were recorded in RD group compared with SD group (13.09 mg/dl) (p=0.015).

Kruskal Wallis test revealed that a higher number of packed red blood cell

units were significantly more commonly used postoperatively in SD group (Chi-square=28.70, df=2, p=0.001) compared to RD group (fig. 1).

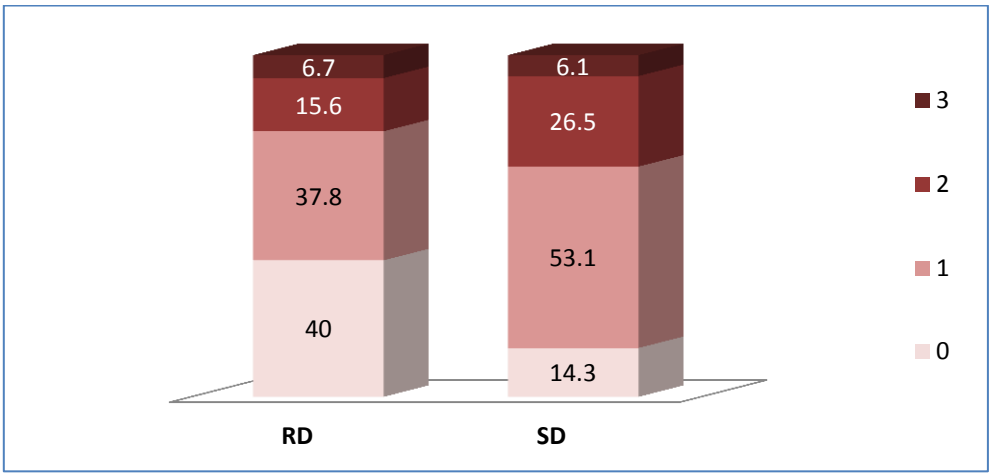

Fig. 1 Structure of groups according to the number of red blood cell units given (%)

No drain-related postoperative complications were recorded.

DISCUSSION

The number of postoperative allogeneic transfusion secondary to primary hip arthroplasty surgery is increasing (13, 14). Besides their obvious benefits, transfusions carry high risks (3) that could be reduced through the effective management of intra- and postoperative blood losses.

There are numerous studies comparing the efficacy of reinfusion drains versus the administration of antifibrinolytic substances (8, 10) but, to our knowledge, this study is the first to compare the effectiveness of two types of drains.

Our study has some limitations. Although, it is a retrospective nonrandomized study and includes a small number of patients, Kruskal Wallis test revealed homogeneity of the lots.

Our data were collected from clinical observation sheets, which involved some degree of subjective judgement. To minimize these errors a sole person collected data.

The study excluded the high-risk patients, fact that may affect the applicability of study to the general population.

The comparative analysis did not take into account patients' coagulation profile that could have influenced the postoperative bleeding and requirement for allogeneic blood transfusion.

Although the number of packed red blood cell units was higher in group SD compared to RD group (Chi-square=28.70, df=2, p=0.001), preoperative mean hemoglobin level was higher in RD group (13. 87 mg/dl *vs* 13.09 mg/dl (p=0.015).

CONCLUSIONS

Our study failed to demonstrate the superiority of reinfusion drains versus suction drains in maintaining postoperative hemoglobin levels and decrease necessary of packed red blood cells.

Because postoperative bleeding is

closely connected with prognosis, it is necessary a larger prospective double blind study to confirm our results.

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OUTCOMES OF SURGERY IN PROLIFERATIVE VITREORETINOPATHY

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OUTCOMES OF SURGERY IN PROLIFERATIVE VITREORETINOPATHY (Abstract) **Aim:** To present the anatomical and functional outcomes of surgery in cases of rhegmatogenous retinal detachment complicated (at presentation) by proliferative vitreoretinopathy (PVR).**Material and methods:** Retrospective study of the cases operated upon by a single surgeon between October 2012 and September 2014.**Results:** A total of 49 eyes of 49 patients had PVR at presentation (12 cases with PVR stage B, 21 with stage C and 16 with stage D). 82% had a VA $\leq$ 0.05 at presentation. Peripheral retinectomy was the primary surgery in 57% of cases. Reinterventions was required in 16.3% of the cases (silicon oil extraction was not considered reintervention). Although at the first postoperative examination 32% of eyes had an IOP > 21 mmHg without medication, only 8% retained a high IOP at the last visit. At the last visit 73.4% of eyes presented attached retina (an additional 6% had an attached macula under silicone oil, but sub retinal fluid in the inferior quadrants). A final VA $\geq$ 0.05 was recorded in 67.3% of eyes (of which half had VA $\geq$ 0.1) **Conclusions**: In cases presenting with retinal detachment complicated by PVR the possibility of reinterventions and the risk of intraocular hypertension should be considered. Unfortunately, even some cases with favorable anatomical outcome will not present ambulatory vision. **Keywords:** RHEGMATOGENOUS RETINAL DETACHMENT, PROLIFERATIVE VITREORETINOPATHY.

The most common cause of retinal detachment is rhegmatogenous (peripheral retinal breaks). Proliferative vitreoretinopathy (PVR) is the most important complication in retinal detachment surgery (and the main cause of surgical failure). A major issue for the retina surgeon was the late presentation of patients, often weeks or months after the detachment has occurred (although the disease has an acute onset and is associated with a significant decrease in visual acuity). Consequently, a large proportion of our cases had PVR at presentation, and the aim of this paper was to analyze the postoperative course, anatomical and functional outcomes of the PVR patients operated on in our clinic.

MATERIAL AND METHODS

We have conducted a retrospective study of the cases operated on by a single surgeon between October 2012 and September 2014. Only the cases diagnosed at presentation with rhegmatogenous retinal detachment complicated by PVR were included in the study. The classification of

PVR proposed by the Retina Society Terminology Committee was used (1). All patients underwent *pars plana* vitrectomy using the 23G sutureless system (Constellation, Alcon, USA). During surgery the following were performed: peeling of preretinal membranes in the posterior pole and in the periphery (if necessary cutting or peeling of subretinal membranes), peripheral retinectomies in anterior PVR, retinal reattachment, laser retinopexy and endotamponade with silicone oil or non-expansile mixture of C2F6 and air.

The collected data included: diagnosis, type of surgery, type of endotamponade, visual acuity (VA), intraocular pressure (IOP), use of antiglaucoma medication, crystalline lens status (at baseline and at postoperative visits). The statistical analysis was performed using MedCalc (©MedCalc Software bvba), comparing preoperative and postoperative values with paired t-test, and the threshold for statistical significance was considered $p < 0.05$.

The study conformed to the principles of the Declaration of Helsinki.

RESULTS

During the study period 315 patients underwent surgery for retinal detachment. The analysis of their medical records showed that 49 eyes of 49 patients had PVR at presentation and were subsequently included in the study. Of these, 12 eyes had stage B PVR, 21 stage C and 16 stage D. The duration of symptoms at presentation (the duration of retinal detachment) was 3.9 ± 4.3 months (range 0.5-24 months). The mean age at presentation was 63.08 ± 13.72 years (range 31-64 years). The mean visual acuity was 0.02 ± 0.04 (range 0.0001-0.2), and 82% of patients had a VA < 0.05. Mean IOP at presentation was 12.4 ± 3.6 mmHg, and 3 eyes had preoperative hypotonia (IOP < 6 mmHg). The status of the crystalline lens: 4 eyes had a transparent lens (8.1%), 26 h ad various degrees of cataract (53%), 16 eyes (32.6%) were pseudophakic and 3(6.1%) aphakic.

Relaxing peripheral retinectomy was performed in 28 eyes: 180º retinectomy in 10 eyes (20.4%), 270 º retinectomy in 2 eyes (4%) and circumferential peripheral retinectomy in 16 eyes (32.6% - all cases with stage D PVR).

The postoperative visual acuity increased to an average of 0.1 ± 0.11 (range 0.001-0.4). The difference between preoperative and postoperative VA was statistically significant (p=0.0001, paired t-test). The postoperative IOP was 15.6 ± 6.3 mmHg (range 4-32 mmHg). Not all cases of postoperative intraocular hypertension needed anti-glaucoma treatment, which was usually considered when the IOP exceeded 26 mmHg.

The first postoperative visit was scheduled at 3 weeks. The visual acuity continued to increase up to 0.13 ± 0.1 (range 0.001 – 0.4). The intraocular pressure at the first visit was 20 ± 10.3 (range 8-52 mmHg) – 14 eyes had an IOP above 21 mmHg. If the retina was found to be attached, the patient was instructed to seek monthly visits with the attending ophthalmologist, including IOP measurements (local patients returned to our clinic for monthly controls).

The second visit was at an average of 11 weeks postoperatively. Mean IOP was 17.4 ± 8.5 mmHg. An IOP > 21 mmHg was recorded in 7 eyes although 6 of them were under anti-glaucoma therapy. If the retina was considered stable under the silicone oil tamponade, usually at 3 months after the first surgery, the silicone oil was removed. In 8 eyes (16.3%) with an unsatisfactory postoperative anatomical outcome (the retina being totally or partially detached) a

reintervention was recommended (peeling of retinal membranes, supplemental retinectomies, replacing the endotamponade with high viscosity silicone oil – 5000cS).

The mean follow-up was 23 weeks. At the end of this period, 36 eyes (73.4%) had a completely attached retina. To these we may add 3 eyes (6%) that had an attached macula, but persistent subretinal fluid in the inferior quadrants. The mean final VA was 0.08 ± 0.07 (range 0.001-0.3). (fig. 1) The statistically significant difference between preoperative and postoperative VA was maintained. In the end, 67.3% of the eyes had a VA greater than 0.05, of which half greater than 0.1.

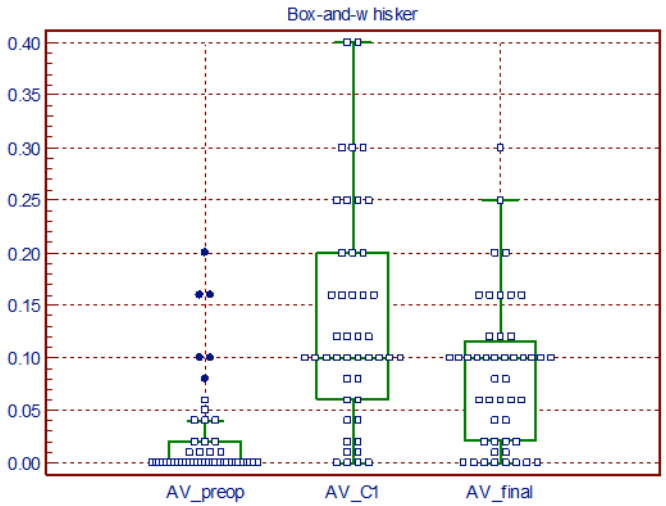

Fig. 1 The evolution of visual acuity: preoperative, at the first control visit and at the final visit

Mean final IOP was 15.6 ± 7.3 mmHg (range 2-40 mmHg). Four eyes recorded IOPs > 21 mmHg, despite the use of anti-glaucoma drops.

At the end of the follow-up period we had information on lens status in 39 cases: 10 had cataract, 16 were pseudophakic, 9 aphakic and 4 had preserved a transparent crystalline lens.

DISCUSSION

An important paper published by the European Vitreoretinal Society analyzed the surgical interventions for 7678 retinal detachments. The presence of choroidal detachment, significant hypotonia, preoperative stage C1 PVR (more advanced PVR cases were excluded), 4 detached quadrants and giant retinal breaks were identified as independent predictive factors of primary surgery failure (2). Patients with stage C1 PVR had a level 1 failure rate of 3.3%, that increased to 9.2% when a large or giant retinal break was identified (level 1 failure was defined as failed reattachment deemed inoperable by the end of the follow-up). Level 2 failure (eyes that have maintained silicone oil endotamponade by the end of the study) was present in 11.8% of the eyes with stage C1 PVR. (3)

In another multicenter trial on 555 cases that underwent *pars plana* vitrectomy and intraoperative perfluoro-n-octane tamponade

for retinal detachment complicated by PVR, the retina was attached in 78% of the eyes at the end of follow-up. The use of silicone oil or C3F8 (perfluoropropane) tamponade was considered to reduce the risk of recurrent retinal detachment (4)

Since 37 of the 49 eyes in our study had PVR stage C or higher and 79.4% had a completely attached retina at the end of follow-up, our results are comparable with those found in the literature.

A recent meta-analysis of randomized trials concluded that in retinal detachment complicated by PVR, the use of C3F8 or silicone oil tamponade is reasonable (5). In our series, most patients received a silicone oil tamponade.

In a recently published paper, 30.5% of the eyes with silicone oil tamponade had elevated IOP at 2 weeks, and 12% maintained a high IOP on medium term. (6) This tendency was also observed in our patients, at the end of the follow-up 8.16% maintaining an IOP greater than 21 mmHg with anti-glaucoma drops.

CONCLUSIONS

The outcomes of surgery for the most important complication of retinal detachment in our study are similar to those reported in the literature. In the cases complicated by PVR at presentation the patient should be informed of the high rate of reinterventions for recurrent retinal detachment and the risk of postoperative intraocular hypertension. Unfortunately, some cases with a favorable anatomical outcome do not achieve a satisfactory visual function.

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THE PATIENT-DOCTOR-PSYCHOLOGIST TRIANGLE IN A CASE OF SEVERE IMUNOSUPRESSION IN THE HIV INFECTION

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THE PATIENT-DOCTOR-PSYCHOLOGIST TRIANGLE IN A CASE OF SEVERE IMUNOSUPRESSION IN THE HIV INFECTION (Abstract). In the last two years the Romanian adult population infected with the human immunodeficiency virus (HIV) has increased due to sexual transmission, both heterosexual and homosexual. The case presented is that of a 33 year-old man, admitted to the Infectious Diseases Hospital in Iasi with acute respiratory failure and a confirmation of Kaposi's sarcoma. Tests later proved positive for HIV, the patient being included in the stage AIDS C3 (acute immunodeficiency syndrome).The respiratory failure was suspected to be caused by *Pneumocystis carinii* and cotrimoxazol therapy, oxygen therapy and anti-retroviral therapy were established. He was also referred to the oncology hospital for treatment of Kaposi's sarcoma. The patient's adherence to therapy was influenced by a strong doctor-patient relationship, as well as by psychological counseling and support. Creating a functional doctor-patient-psychologist team is key throughout the HIV-positive patient's existence, for supporting long term adherence to therapy and acceptance of the diagnosis. This case highlights the need for a strong psycho-social compartment in every medical center that deals with HIV-infected individuals. **Keywords**: HIV/AIDS, ANTIRETROVIRAL THERAPY, PSYCHOLOGICAL COUNSELING, DOCTOR-PATIENT RELATIONSHIP.

The HIV/AIDS pandemic has reached 36.9 million people globally living with HIV. At the end of 2014, two million people became newly infected with HIV, and 1.2 million people died from AIDS-related illnesses (1).

In Romania, the main characteristic of the HIV/AIDS population is the development of the "pediatric cohort", represented by HIV positive youth infected as children in the 1990's (2). This group is currently being equaled in numbers by newly infected cases. The most common means of transmission for these new cases are unprotected sexual intercourse, both heterosexual and homosexual, as well as use of injected drugs (3).

In the North-Eastern part of Romania, in October 2015 there were over 1400 people living with HIV/AIDS, this year being the first in which the number of newly diagnosed adults has equaled that of the

"pediatric cohort". The infection is transmitted mostly by heterosexual and homosexual unprotected intercourse and less through intravenous administration.

CASE PRESENTATION

We present the case of a male patient, born in 1980. This patient was transferred to the HIV/AIDS Regional Center by a local clinic, in August 2013. The reasons for hospitalization were: weight loss, fatigue, impaired general condition, acute respiratory failure, perioronasal cyanosis and of the extremities, with preserved consciousness. At admission, the weight was 53 kg, height 163 cm and 76% partial oxygen pressure.

From the patient's statements, his weight decreased progressively, 12 kg in 4 months. For the last 10 months, he developed lenticular brown spots on the skin, disseminated on the trunk, abdomen, and the upper right eyelid. There were no significant pathological aspects in the personal and family history. The biological samples from the County Hospital were near normal, except for hemoglobin 10.8 g/l, the appearance of "mat glass" of the chest X-ray, and also the positive result for HIV 1 and 2 ELISA test. Upon more in depth interrogations about the patient's personal history, he identifies the means of getting infected with HIV as unprotected homosexual intercourse.

Viro-imunologic tests show a profound immunosuppression, with CD4 2 cells/mmc (fig. 1) and a viral load (VL) of 518000 copies/ml (fig. 2). The dermatologic examination established the clinical diagnosis of Kaposi sarcoma, confirmed by skin biopsy. Serology for cytomegalovirus (CMV), toxoplasma, hepatitis B and C were negative, with also a negative VDRL. The major suspicion in the context of pulmonary failure was pulmonary pneumocystis, and therapy was initiated with cotrimoxazole 8 tablets / day, intermittent oxygen, decreasing cortisol dose. Antiretroviral therapy immediately instituted was achieved with co-formulations of lopinavir + ritonavir and lamivudine + zidovudine and enfuvirtide (T20 Fuzeon). The pulmonary phenomena remitted extremely slowly, with the possibility of transfer within 30 days in the oncology ward of the county hospital, where specific therapy for Kaposi's sarcoma was initiated.

The patient, being a university graduate, showed a high level of intelligence and seemed to understand his situation. The infectious diseases doctor managed to create a relationship with this patient based on mutual trust, which encouraged him to own his diagnosis, expressing the desire to overcome this obstacle.

Throughout the hospitalization the patient benefited from psychological counseling and support after diagnosis disclosure. This added the psychologist to his support network, alongside the infectious diseases doctor. Counseling sessions consisted in semi-structured interviews about the patient's personal and medical history, as well as free discussions regarding topics of interest for him – family, work-place, intimate relationships, social stigma. The main objective of was to explore fears and emotions linked to the HIV diagnosis, assisting the patient in dealing with them in a healthy and productive manner. The result of counseling and support were seen in a good compliance and adherence to antiretroviral (ARV) therapy, while resuming his daily routines and activities.

Three months after the first episode, the respiratory failure was resolved. The CD4

cell count increased to 92 cells/mmc (fig. 1), and the viral load decreased over 100 times, VL 5530 copies/ml (fig. 2). Cotrimoxazole prophylaxis was maintained, reducing the dose to 2 tablets every 2 days, with continued ARV treatment and specific cancer therapy.

In May 2014 the viral load remained high viral and CD4 increased slightly.

However, in October 2014 VL grew to 12.800 copies/ml (fig. 2) and CD4 cell count dropped to 65 cells/mmc (fig. 1). The patient admitted that, after a period of good general condition, and after discontinuing therapy with enfuvirtide, he began to neglect antiretroviral therapy up to 3-4 days a week.

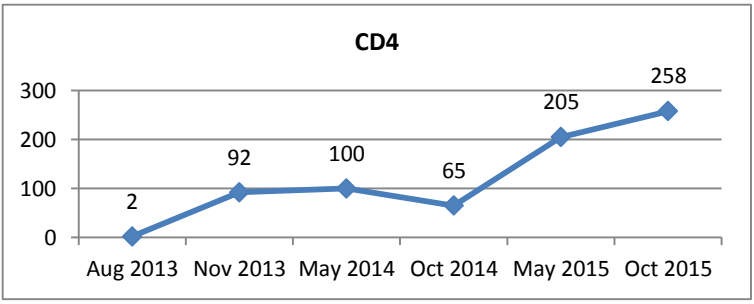

Fig. 1. CD4 cell count

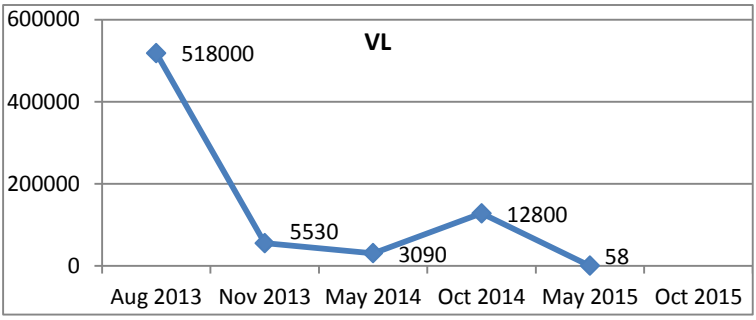

Fig. 2. Viral load

Daily psychological sessions were resumed, as well as repeated discussions with the infectious diseases doctor. These aimed to explore the patient's reasons for non-adherence and help him identify ways to prevent this in the future. The main objective was to increase long term adherence and compliance to ARV therapy. On discharge the patient seemed willing to maintain his adherence to therapy.

In the month of May 2015, biological evaluations showed a CD4 count of 205 cells/mmc (fig. 1), and VL a little over the detection limit - 58 copies/ml (fig. 1), with a weight gain of 8 kg. The oncology therapy with interferon solved the Kaposi lesion on the upper left eyelid and faded the other elements on the skin. The prophylaxis for pneumocystis with Cotrimoxazole was suppressed because CD4 cell count rose above 200 cells / mmc.

On the 29th of October 2015 the patient

had 78 kg, with a plus of 25 kg compared to the time of his first presentation. The Kaposi sarcoma faded, his viral load was undetectable, and CD4 was 258 copies / mmc.

DISCUSSIONS

The case presented is that of an intellectual patient with very severe immunosuppression caused by HIV infection. He had the chance to overcome a complex, extremely aggressive and life-threatening pathology, with acute respiratory failure and disseminated Kaposi sarcoma.

Initially, his results were good, due to the possibility of a smooth communication and understanding of diagnosis and both therapy benefits and risks of non-adherence.

The patient received psychological support throughout the hospitalization. Counseling sessions focused on internalizing information regarding the diagnosis, and especially on building a positive self-image, strengthening self-respect and shaping positive future projections.

The therapy was based on the very probable etiology of respiratory failure – that of pneumocystosis, as well as on the Kaposi's sarcoma. This specific therapy, alongside antiretroviral therapy, encouraged an increase of the immune competence over time, successfully managing the case (4,5).

However, therapeutic indications were apparently not enough. The patient's high coefficient of intelligence and education were a baseline in establishing a productive patient-doctor relationship, but were not sufficient in providing long-term support for adherence. Daily clinical practice in the HIV/AIDS Regional Center in Iasi proved that, in a chronic disease such as HIV/AIDS, with lifelong daily therapy, psychological sessions are essential in offering support and helping patients find resources to cope (6,7,8).

CONCLUSIONS

Given the complexity of hospitalized cases, the management of an HIV-positive patient should be a team effort. In our study, we highlighted the fact that trust in the infectious diseases doctor, as well as confidence in one's strength to overcome the burden of the diagnosis are as important as ARV therapy or treating opportunistic infections.

The psychologist's intake is essential throughout the life of HIV infected patients; repeated counseling sessions are important for optimal adherence and compliance to therapy, in order to achieve viro-imunologic success and social integration. The need to adapt daily life and routines to new diagnosis requires its total acceptance; the support that people diagnosed with HIV receive facilitates this process of adaptation.

Therefore the development of a psychosocial department with trained professionals in the pathology of HIV/AIDS is more than necessary in managing the success of any case of HIV infection. The triangle formed by the doctor, the patient and the psychologist, working as a team toward a common goal, is the key to a successful management of the disease and should be a model included in every center that treats HIV pathology.

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NEWS

ASPIRIN USE AND COLORECTAL CANCER

Colorectal cancer (CRC) is the second most frequent type of cancer in Europe, with higher incidence for men after 50 years of age. Aspirin has anticancer effects. The aspirin use reduced the colorectal adenomas risk and the distant metastasis for patients with CRC. A recent meta-analysis summarized the results of eleven studies and showed that aspirin therapy after CRC diagnosis improved survival particular among patients with positive prostaglandin endoperoxide synthase 2 (PTGS2) expression and mutated PIK3CA tumors. PTGS2 is an enzyme involved in prostaglandin synthesis which promote inflammation and cell proliferation. Phosphatidylinositol 3-kinase (PI3K) has an important role in carcinogenesis. Also, the meta-analysis highlighted no association between aspirin use before diagnosis and CRC mortality. Future research is needed in order to establish the optimal doses and duration of aspirin adjuvant therapy in CRC (Peiwei Li; Han Wu; Honghe Zhang; Yu Shi; Jinming Xu; Yao Ye; Dajing Xia; Jun Yang; Jianting Cai; Yihua Wu. Aspirin use after diagnosis but not prediagnosis improves established colorectal cancer survival. *Gut* 2015;64(9):1419-1425).

Cătălina Luncă

PECULIARITIES OF PHARMACOTHERAPY IN ARTERIAL HYPERTENSION AND COMORBID PATHOLOGY

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PECULIARITIES OF PHARMACOTHERAPY IN ARTERIAL HYPERTENSION AND COMORBID PATHOLOGY (Abstract): In patients with arterial hypertension (AH) pharmacotherapy often remains ineffective because of comorbid pathology. Research of haemodynamic and metabolic processes in such patients allows prescribe effective antihypertensive medicines, improve life quality and prognosis. **Material and methods:** 60 patients with AH, coronary artery disease and obesity were divided into three groups: 1st group was treated with lisinopril + atorvastatin + aspirin (regimen I); 2nd group was treated with lisinopril + atorvastatin + bisoprolol + aspirin (regimen II); 3rd group was treated with lisinopril + bisoprolol + indapamide + atorvastatin + aspirin (regimen III). Laser correlation spectrometry (LCS) was used to evaluate the homeostatic alterations. **Results:** All regimens were equally effective in normalization of systolic blood pressure and did not influence the ejection fraction. Only in the 3rd group was determined significant increase of glomerular filtration rate (92.73±2.12 vs 79.62±13.62 ml/min/1.73m^2). The LCS test showed that these patients had high level of light scattering particles IV, which may be caused by activation of anabolic processes. In other groups, spectral alterations were not so expressive. **Conclusions:** Prescription of antihypertensive therapy cause homeostatic and metabolic alterations, which may influence the efficacy of treatment. **Keywords:** PHARMACOTHERAPY, COMORBID PATHOLOGY, LASER CORRELATION SPECTROMETRY.

A considerable growth of cardiovascular diseases, especially among the elder patients, is observed all over the world and in Ukraine. Primary hypertension (PH) and coronary artery disease (CAD) are the main causes of cardiovascular morbidity (41.8% / 38.3, accordingly) [1]. PH combined with CAD make worse patient's prognosis and lead to adverse outcomes [2]. High level of systolic blood pressure is one of the main risk factors of CAD and its complications [3]. Thus, addition of CAD to PH increases cardiovascular risk to high level [4]. It was proved by PROGRESS investigation, which confirmed that every 4th death from CAD is caused by increased blood pressure [3]. In elderly patients, PH is complicated with CAD in 67.7% cases [1].

In patients with CAD combined with PH obesity is one of the most common comorbidities [5]. Our previous investiga-

tions determined negative impact of the obesity in heart failure, atrial fibrillation and decompensation of the patient state [6]. Patients with obesity and PH have high cardiac preload caused by the growth of circulating blood volume and high postload caused by high arterial stiffness [7].

In the elderly patients, choice of effective pharmacotherapy (PT) is complicated with numerous comorbidities, pharmacokinetic and pharmacodynamic peculiarities [8, 9, 10]. Nowadays there are not enough methods, which allow to determine the influence of PT on metabolic processes [8, 11]. This leads to low efficacy of antihypertensive treatment [3]. So it is important to provide complex sanogenetic monitoring in all patients with PH. Laser correlation spectrometry (LCS) is one of the most common methods, which allows quickly determine and analyze the metabolic disorders [12, 13, 14].

MATERIAL AND METHODS

In Odessa University Clinic (Ukraine) 60 patients with PH, CAD and obesity were treated according to clinical recommendations [4, 15, 17, 18]. All patients were divided into three groups: the 1st (n=20) was given lisinopril + atorvastatin + aspirin (regimen I), the 2nd (n=20) was given lisinopril + atorvastatin + bisoprolol + aspirin (regimen II); the 3rd (n=20) was given lisinopril + bisoprolol + indapamide + atorvastatin + aspirin (regimen III). Exclusion criteria: myocardial infarction and stroke in anamnesis. Body mass index (BMI) and glomerulal filtration rate (GFR) were calculated in all patients.

The special research method for estimation of plasma metabolic features was LCS [13]. It helps to measure spectral characteristics of the induced monochromatic coherent radiation in biological liquids. That makes possible to register particles with hydrodynamic radius 1 - 10000 nanometers. Results are presented as a histogram.

According to our previous studies [13], to the I zone (0-10 nanometers) are included low-molecular monomeric albumins and glycolipid free complexes; to the II zone (11-30 nanometers) are included globulin proteins and low-molecular lipoprotein complexes; to the III zone (31-70 nanometers) are included high-molecular lipoprotein complexes, RNP-, DNP molecules, the low-molecular immune complexes; to the IV zone (71-150 nanometers) are included mainly constitutive immune complexes of the average size. Huge particles ($\geq$ 150 nanometers, the V zone) are present in patients with allergization and an autoimmune sensibilization. The particle distribution and their molecular interaction characterizes different pathological states. The presence of xenobiotics (e.g. medicines) causes specific changes of the initial distribution and may characterize the treatment efficacy. Metabolic features were determined in blood plasma before and on the 10th day of PT. The investigation was carried out according to the method after Bazhora J.I. and Noskin L.A. [14].

The statistical analyze was provided with Statistica 7.0. To measure differences between values before and after the treatment we used t-test; p-value of < 0.05 was considered to be statistically significant. Values are presented as Mean ± Standard deviation (SD).

RESULTS

Mean age (MA) of the investigated patients was 67.2±5.1 years. Among them were 28 males (MA 66.2±8.9 years) and 32 females (MA 68.3±7.4 years). All of them had obesity with BMI corresponding to the

first stage (BMI: 30-35 kg / m2). Diabetes mellitus was diagnosed in 26.31% patients in the 1st group, in 15% in the 2nd group and in 25% in the 3rd group.

Before and after the prescription of antihypertensive therapy we determined changes of heart rate (HR), systolic and diastolic blood pressure (SBP, DBP), ejection fraction (EF), total cholesterol and levels (tab. I).

After the treatment in the 1st group was determined the decrease of SBP (by 35.0 mm Hg), DBP (by 12.4 mm Hg) and HR (by 14/min). EF, GFR and glucose levels remained without any changes. Total cholesterol decreased by 1.68 mcmol/L.

In the 2nd group SBP decreased by 20.91 mm Hg. DBP, HR and EF remained the same. Cholesterol reached the normal values (4.78 mmol/L) but glucose level increased by 1.14 mmol/L ($p>0.05$).

In the 3rd group SBP decreased by 29.8 mm Hg, DBP by 14 mm Hg, HR by 9.73/min. Cholesterol level reached the target borders (4.68 mmol/l). EF and glucose level remained the same. GFR rate increased to 92.73 ml/min/1.73m^2.

LCS data showed that only regimen III caused a significant growth of light scattering particles (LSP) IV (on 11.1%), which characterize anabolic reactions. Before the prescription of regimens I and II prevalence of LSP II, III and IV was observed. It is typical for catabolic, toxic and allergy-directed reactions.

These alterations remained without any significant changes after the treatment (tab. II).

Concerning spectral alterations, we determined that hydrolytic alterations remained the same after the prescription of regimen I. Normological type even decreased by 15.0% and anabolic type increased the same value due to autoimmune reactions. Regimen II caused the decrease of hydrolytic reactions by 25.0% and growth of anabolic reactions by 15.0%. Regimen III was characterised by increase of hydrolytic and anabolic type reactions (10.0% / 25.0%, accordingly) (tab. III).

TABLE I

Clinical data of all the investigated groups before and after PT

Clinical data	Regimen I		Regimen II		Regimen III	
	Before the treatment	During the treatment	Before the treatment	During the treatment	Before the treatment	During the treatment
SBP, mm Hg	163.2±16.1	128.0±8.6*	151.3±6.2	130.4±6.0*	160.7±11.5	130.9±9.1 *
DBP, mm Hg	87.3±8.5	75.0±4.6*	83.0±6.6	85.0±6.3	94.0±7.1	80.0±6.6 *
HR, bpm	79.6±13.1	65.6±9.0*	72.3±5.1	68.2±11.2	81.1±14.7	71.7±13.0 *
EF, %	64.8±6.6	65.6±6.3	59.8±8.2	60.6±8.1	59.3±9.3	58.8±8.7
Cholesterol, mcmol/L	6.3±1.8	4.6±0.4*	6.4±1.2	4.8±0.4*	5.9±0.9	4.6±0.6*
Glucose, mmol/L	6.0±1.7	6.3±1.3	5.4±0.7	6.5±1.4	6.5±1.9	6.7±0.8
GFR, ml/min/1.73m^2	81.7±13.7	78.4±12.6	79.4±14.8	76.6±12.1	79.6±13.6	92.7±12.1 *

*, $p< 0.05$ vs before treatment.

TABLE II

Subfractional redistribution before and after PT

LSP redistribution	Regimen I		Regimen II		Regimen III	
	Before PT, %	During PT, %	Before PT, %	During PT, %	Before PT, %	During PT, %
I zone	8.5	7.3	9.4	9.4	10.3	9.2
II zone	27.4	22.9	37.4	29.2	29.7	26.7
III zone	27.6	32.5	21.4	25.2	27.0	20.6
IV zone	33.3	30.9	22.6	26.2	19.3	30.4*
V zone	3.2	6.4	9.2	10.0	13.7	13.1

*, p< 0.05 vs before treatment.

TABLE III

Direction of spectral alterations before and after PT

Type of spectral alteration	Regimen I		Regimen II		Regimen III	
	Before PT, %	During PT, %	Before PT, %	During PT, %	Before PT, %	During PT, %
Normological	25.0	10.0*	0	5.0	15.0	10.0
Hydrolytic	40.0	40.0	65.0	55.0*	35.0	45.0*
Anabolic	15.0	30.0*	15.0	30.0*	5.0	30.0*
Mixed	20.0	20.0	20.0	10.0*	45.0	15.0*

*, p< 0.05 vs before treatment.

DISCUSSION.

As we observed from abovementioned data, all the three regimens equally caused stabilization of SBP, which is the main target of antihypertensive PT [19]. DBP was significantly lowered with the Ist and IIIrd regimens, but in the IInd regimen it remained without changes. Significant lowering of HR was achieved after the prescription of lisinopril. We did not determine any influence of used PT on EF rate.

In all three groups, total cholesterol level was brought to the normal values. Regimen II increased glucosa level, but not statistically significant. GFR rate didn't changed after regimens I and II, but regimen III caused growth of GFR. Thus, as we see, such a step-by-step prescription of each new drug is very convenient for observing of it's influence and helps us to optimise the treatment.

LCS data showed that regimens I and II didn't change the subfractional structure and only regimen III caused the growth of anabolic reactions. To estimate PT influence on metabolic processes we have to take into account not only subfractional structure but also directions of spectral alterations.

We also have to take into account initial alterations and compare them with alterations after the PT. Otherwise, the received data could be incorrectly interpreted.

So, regimen I caused lowering of normological type and in spite of anabolic reactions growth, hydrolytic type remained the same as before the treatment. Thus, regimen I is not effective in correction of hydrolytic reactions. Addition of bisoprolol (regimen II) caused decrease of hydrolytic reactions and simultaneous increase of

anabolic ones. So, regimen II change hydrolytic as anabolic processes. Regimen III (which included indopamide) caused increase of hydrolytic and anabolic reactions. So, as we see, we have to compare both LCS data: LSP redistribution and spectral alterations in order to estimate PT influence on metabolic processes. In our investigation spectral alterations appeared to be more informative because of proved statistical significance.

CONCLUSIONS.

All the prescribed regimens are optimal for SPB stabilization but they do not influence EF. In elderly patients with PH and comorbid pathologies PT should be individualized. LCS is very sensitive method, which allows determine homeostatic alterations and metabolic transformations in the process of treatment. Addition of LCS to basic clinical, laboratory, and instrumental data helps to choose an appropriate PT.

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NEWS

EVALUATION OF CLINICAL OUTCOMES IN PATIENTS UNDERGOING DUAL VESSEL PERCUTANEOUS CORONARY INTERVENTION USING SIROLIMUS-ELUTING CORONARY STENT SYSTEM IN INDIA

The safety and efficacy of percutaneous transluminal coronary angioplasty (PTCA) has been demonstrated in selected patients with symptomatic coronary artery disease. Application of PTCA in patients with multiple vessels or in more extensive coronary artery disease has been limited, and the safety and short- and long-term efficacy are less clear. Stent implantation has added an important dimension to percutaneous re-vascularization strategies and has been shown to be an effective rescue device after acute or threatened vessel closure after failed PTCA. However, some studies with multivessel disease reported higher restenosis and repeat revascularization rates in patients treated with bare metal stents (BMS) than in those after surgical treatment. The introduction of drug-eluting stents (DES) indicating advantage over bare-metal stents in reducing the restenosis incidence and has narrowed the re-intervention gap between percutaneous coronary intervention (PCI) and coronary artery bypass graft (CABG) surgery in multivessel coronary artery disease (CAD). Additionally, performing multivessel PCI in a single index procedure has potential economic and social advantages. Here, we present our experience of the use of the Supralimus-Core sirolimus-eluting stent (SES) in patients with dual vessel CAD in an unselected real-world population. No study was especially designed to evaluate the safety and effectiveness of SES in patients with dual vessel disease. The study shows that, dual vessel Supralimus-Core SES implantation allows safe and effective treatment with low rates of TLF at one year follow-up in Indian population (Chandwani P, Prajapati J, Porwal S,et al. Evaluation of Clinical Outcomes in Patients Undergoing Dual Vessel Percutaneous Coronary Intervention Using Sirolimus-Eluting Coronary Stent System in India. Journal of Clinical and Diagnostic Research. 2015, Vol-9(2): 5-9).

Doina Butcovan

BURKITT LYMPHOMA IN ANADULT AIDS C2 FEMALE PATIENT

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BURKITT LYMPHOMA IN AN ADULT AIDS C2 FEMALE PATIENT (Abstract): We report the case of a 24- year-old female patient with stage C2 HIV infection presenting with complaints of fatigue and altered general status. She reported having amenorrhea for 4 months and pelvic pain. Her medical history was characteristic to lack of compliance with treatment, the viral load remaining high despite the prescribed intensive antiretroviral treatment. Intraoperatively we found: hypoplastic uterus of 30/20/20 mm in size and complete cystic transformation of the right ovary, the cyst size being of about 60/50 mm and the capsule intact. A right adnexectomy was performed. Immunohistological assessment about one month after surgery made the final diagnosis of Burkitt lymphoma. Studies show that an old HIV infection is associated with a higher risk for non-Hodgkin lymphoma (NHL) compared to patients with quite recent HIV infection. Conclusions: The reported case had high CD4 levels at the time of diagnosis and also a particular extranodal involvement, the right adnexa. The CD4 level was 198/mmc at the time the adnexal tumor was discovered, level reached after a long period of time of low CD4 levels and high viral load. **Keywords:** BURKITT LYMPHOMA, HIV INFECTION, OVARIAN TUMOR.

Burkitt lymphoma is a high-grade B-cell lymphoma classified as a non-Hodgkin lymphoma (NHL) (1). There have been described 3 main clinical types of Burkitt lymphoma: endemic, sporadic and immunodeficiency-associated (2). Immunodeficiency-associated Burkitt lymphoma is usually associated with HIV (human immunodeficiency virus) infection or occurs in post-transplant patients taking immunosuppressive drugs (3). Burkitt lymphoma can be one of the diseases associated with the initial manifestation of AIDS (acquired immune deficiency syndrome) (4).

As the other HIV-related non-Hodgkin lymphomas, Burkitt lymphoma has a poor prognosis, mainly due to the advanced stage of disease at the time of diagnosis, extranodal involvement and B symptoms (weight loss >10% of original body weight, night sweats and fever) (5). Patients are often included into the intermediate or high-risk group, based on the International Prognostic Index (IPI), which scores patients according to age (> 35 years), stage III or IV of disease, increased serum lactate

dehydrogenase (LDH), and extranodal involvement (6).

Burkitt lymphoma is the second commonest histological subtype of HIV-related lymphoma, after diffuse large B-cell non-Hodgkin's lymphoma (7). There is a clear correlation between the levels of CD4 cell count and the risk of developing NHL (5). It is thought that immune stimulation by HIV and reactivation of previous Epstein–Barr virus (EBV) infection due to defective T-cell surveillance, results in long-term stimulation and proliferation of B lymphocytes, ultimately leading to the development of HIV-related NHL (5).

CASE REPORT

A 24-year-old Caucasian female patient, diagnosed HIV-positive at the age of 11 and currently in C2 stage of HIV infection due to recurrent pneumonia, presented to our clinic in June 2013 complaining of abdominal pain, edema of the upper and lower limbs, fatigue and altered general status. The patient reported having amenorrhea for 4 months and the occurrence 3 month earlier of pelvic pain which tended to get worse.

Her *medical history* was characteristic for noncompliance to treatment (8), the viral loads remaining high despite the prescribed intensive antiretroviral treatment. Until August 2006, the CD4 counts were between 250-400 cells/mm3, but the viral load had never decreased enough to get close to undetectable levels, the lowest viral load that was ever recorded for the patient being 42.100 copies /ml. After Indinavir was withdrawn from the Romanian pharmaceutical market, she was prescribed another type of protease inhibitor, Saquinavir (SQV), associated with Ritonavir (RTV), Abacavir (ABC) and Didanosine (ddI). Despite the aggressive treatment the results were far from optimal, the viral load reaching a level of 2.500.000 copies/ml. In September 2009, the patient was prescribed Kaletra (Lopinavir and Ritonavir) together with Combivir, the combination offering a comfortable administration of only 6 cps/day, association known as highly active antiretroviral therapy (HAART), which covers the sanctuaries of HIV infection such as the ovarian stroma. Unfortunately the patient was noncompliant. At that time also appeared the idea of a possible virus resistance to therapy due to the high viral loads difficult to control therapeutically. As CD4 levels were low (123/mm3) while the viral load continued to be high (128.000 copies/ml), an antiretroviral drug-resistance test was performed in February 201.As the test showed Darunavir (DRV) to be the most effective the therapeutic scheme was changed, but until December 2011 the CD4 levels continued to decrease while the viral load increased. The patient admitted that sometimes she was not taking her medication for 10-14 days in a row.

Physical examination revealed: afebrility, slightly pale teguments, micropolyadenopathy in the inguinal, cervical, supraclavicular and axillary groups and normal respiratory tract and cardiovascular system evaluation. The patient complained of pain in the right iliac fossa. Gynecological examination showed a white vaginal discharge, confirmed a hypoplastic uterus, an enlarged right ovary of approximately 60/50 mm in diameter, with regular contour, sensitive to bimanual palpation.

Relevant *laboratory tests* included: anemia (Hb-8.6 g/dl), normal white blood count (WBC-5.6x10³/μL), low GR (2.7x10³/μL), low APTT (20.1/sec), normal

inflammatory markers (CRP and ESR), high uric acid (6.61 mg/dl), ALAT, ASAT (32 U/L and 46 U/L), Coli/ESBL in vaginal culture.

On the first night in hospital the patient was hyperpyretic (39ºC) and shivering; before the antibiogram for Escherichia Coli vaginal infection was available ampicillin was used as empiric treatment to which the fever having no response it was replaced with a 3rd generation cephalosporin that also proved to be ineffective. The next day the patient was referred to the Infectious Disease Hospital for further investigations and specific treatment.

After one month the patient was rescheduled for surgery. During this time the physical status had improved, amenorrhea, fatigue and the slight abdominal pain remaining the only complaints. Anemia was corrected with oral iron supplements (Hb-10.2g/dl), the patient presenting normal white blood cell count and moderate increase of inflammatory markers (CRP and ESR).

At surgery, after opening the peritoneal cavity the following were found: hypoplastic uterus (30/20/20 mm); the left adnexa appeared normal; the right adnexa: right ovary - complete cystic transformation, size of the cyst about 60/50 mm in diameter, intact capsule, with a regular contour and the right tube impossible to differentiate from the cystic tumor. Through the transparent cyst capsule a solid component of the tumor was noticeable. No lymphadenopathy was visible macroscopically in the pelvic area. Right adnexectomy was performed without damaging the cyst capsule. The next day the patient was transferred to the Infectious Disease Hospital for further care.

The macroscopic examination of the ovarian tumor made the diagnosis of solid ovarian tumor and cut sections of tumor revealed necrotic areas (Fig. 1, 2).

Fig. 1 Ovarian tumor - macroscopic aspect

Fig. 2 Ovarian tumor - macroscopic aspect

The histopathological examination revealed: proliferation of small tumor cells containing solid deposits, moderate pleomorphism, 1-3 macronucleoli, reduced or moderate cytoplasm, intensive mitotic activity and necrosis (Fig. 3, 4). The histopathological diagnosis was suggestive of small cell neoplasia or non-Hodgkin lymphoma (p T1a Nx Mx G3), the immunohistological examination performed about one month after surgery establishing the final diagnosis - Burkitt lymphoma (CD20, BCL6 and KI67 positive and CD3, Cytokeratin AE1/3 negative).

The patient was referred to the Regional Cancer Institute for further treatment. The protocol for Burkitt lymphoma required a CT-scan which was performed in Septem-

ber and showed supraclavicular, inguinal, and laterocervical micropolyadenopathies and a connective tissue mass located in the thymic compartment.

The routine follow-up 2 months postoperatively revealed: absence of pelvic pain, persistence of amenorrhea for 6 months, absence of digestive or urinary complaints, fully healed surgical scar. She was prescribed Duphaston 10 mg (2 tablets/day) for 7 days, followed by combined oral contraception starting on the first day of menstruation, in order to control the ovarian function.

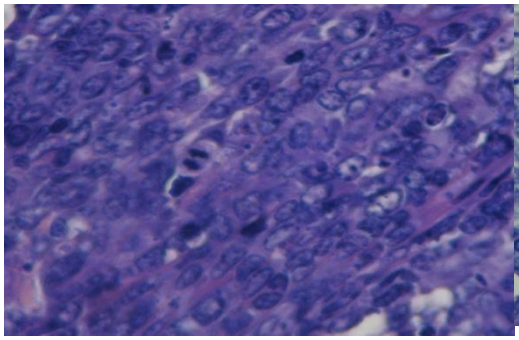

Fig. 3. Tumoral necrosis and intensive mitotic activity (HE x 40)

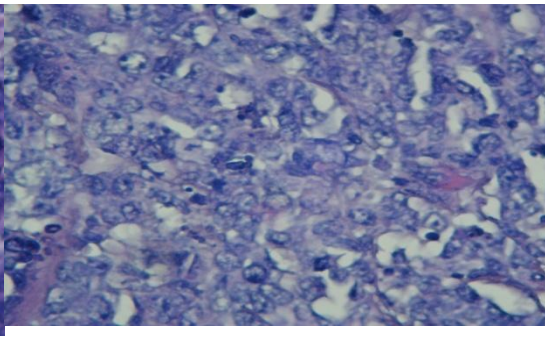

Fig. 4. Intense proliferation of small cells (1-3 macronucleols), reduced or moderate cytoplasm, containing solid deposits, moderate pleomorphism (HE x 20)

In October chemotherapy was initiated, using the fractioned protocol: course A consisting in the administration of cyclophosphamide, vincristine, adriamycin and dexamethasone, followed by course B including the administration of methotrexate and cytarabine, very well tolerated by the patient. The next scheduled appointment for chemotherapy is October 30. Until then the patient was prescribed Neupogen-1 (granulocyte colony-stimulating factor) subcutaneously for 10 days.

DISCUSSION

AIDS and immunosuppression create an adequate environment for the development of malignant tumors that are highly aggressive and generally have a poor prognosis (2). Studies show that an old HIV infection (≥8 years) is associated with a higher risk for NHL compared to patients with quite recent HIV infection (<4 years), our patient being diagnosed HIV positive since she was 11 years old (4). Prolonged immunosuppression is correlated with high CD4 count that is associated to a higher risk for NHL (CD4 >150 cells/μL), in our case the viral load was always high due to noncompliance with treatment (6).

The most common symptoms in AIDS-related lymphomas are the B type symptoms: fever, weight loss and night sweats (9), of which our patient presented only fever (5). Extranodal involvement is quite common, the most frequently affected being the gastrointestinal tract, liver or central nervous system, bone marrow included (3). Adnexal involvement is considered a rare extranodal site of the Burkitt lymphoma, especially in the case of a primary

extranodal involvement (3). Most patients are diagnosed with advanced-stage disease (1).

The treatment of Burkitt lymphoma in AIDS patients involves complex chemotherapy regimens (10). The HAART therapy, also administered to our patient, has been proven to determine a decline in CD4 cells count and associated with Neupogen determines less myelotoxicity and a better tolerance to therapy (10).

The prognosis in AIDS-related Burkitt lymphoma is poor and survival rates are of 2 to 24 months, evaluated according to CD4 T-helper lymphocyte count (<100 cells/μL), age (> 35 years), intravenous drug use and stage III or IV disease at the time of initial diagnosis (4).

CONCLUSIONS

The case we have reported had high CD4 levels at the time of diagnosis and a particular extranodal involvement. The CD4 level was 198/mmc at the time the adnexal tumor was discovered, level reached after a very long period of time of low CD4 levels associated with high viral load. The patient presented fever, but no weight loss or night sweats. She was diagnosed with stage I disease.

In our patient the prognosis was relatively favorable on specific treatment for AIDS and complex chemotherapy regimen administered for Burkitt lymphoma.

The particularity of this case is the primary extranodal involvement of the right adnexa, uncommon in Burkitt lymphoma.

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PALLIATIVE TREATMENT OF MALIGNANT ESOPHAGOPULMONARY FISTULAS WITH COVERED SELF - EXPANDABLE METALLIC STENTS (SEMSS). A SINGLE CENTER EXPERIENCE

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PALLIATIVE TREATMENT OF MALIGNANT ESOPHAGOPULMONARY FISTULAS WITH COVERED SELF - EXPANDABLE METALLIC STENTS (SEMSS). A SINGLE CENTER EXPERIENCE(Abstract): **Aim:** The aim of our study was to determine the efficiency of SEMSs in patients with esophagopulmonary fistulas, regarding fistula closure,, enhancement of dysphagia scores and survival rates. **Materials and Methods**: Between January 2004 and June 2014, from a total of 133 patients who underwent stent placement procedures, 26 were diagnosed with esophagopulmonary fistulas. In 19 cases the fistulas were caused by esophageal carcinomas and in 7 cases by bronchogenic ones. 16 patients developed aspiration pneumonia, 3 lung abscess and 7 subclinical fistulas. **Results**: Complete fistula sealing occurred in 26 patients (100%). There were no immediate procedural complications except chest pain in 5 cases. After sealing of the fistulas and antibiotic treatment, pneumonia has regressed. After stent insertion, the dysphagic syndrome improved significantly (mean dysphagia scores decrease from 3.28 to 1.3 after stent insertion). The main goal of palliative therapy in patients with unresectable cancer and esophago-pulmonary fistulas is to close the fistulas, thus preventing the aspiration of saliva and food into the bronchus. Other goals include amelioration of dysphagia symptoms, maintenance of oral intake and improvement of quality of life. Ultimately covered expandable metal stents may increase survival rate as compared with other therapies. **Conclusions**: The endoscopic placement of covered SEMSs is the treatment of choice for malignant esophago-pulmonary fistulas. **Keywords**: ESOPHAGO-PULMONARY FISTULAS, SELF-EXPANDABLE METALLIC STENTS (SEMSs).

Patients with esophageal cancers often do not recognize any symptoms until late when the dysphagia is present and more than 75% of the luminal diameter is obstructed. At this time the tumor is often unresectable, either due to invasion of vital adjacent structures, or due to distant metastasis in liver/lungs. Esophago-pulmonary fistulas develop in patients with advanced esophageal and lung cancer and lead to continuous aspiration of saliva or food in the bronchial tree. Their development can be related to tumor invasion, laser treatment, radiation therapy or pressure necrosis caused by a previously placed stent (1).

The goals of palliative therapy in pa-

tients with unresectable cancer aim to ameliorate symptoms of dysphagia, treat complications such as esophago-pulmonary fistula, maintain oral intake, minimize hospitalization, relieve pain, eliminate reflux/regurgitation, prevent aspiration, and of course to improve the quality of life and to increase survival rates (2). There are multiple prospective case series using SEMSs for esophago-pulmonary fistulas reporting occlusion rates of 70-100% and complication rates between 10 and 30% (3).

Self-expanding metal stents (SEMSs) were first introduced for the treatment of esophageal stenosis in the early 1980 and nowadays are gold standard for palliation therapy (4). Esophago-pulmonary fistula is the only condition in which covered expandable metal stents certainly increase survival rates compared with other therapies (5). For persistent fistulas or recurrent fistula, placement of an airway stent may be additional palliative option. Multiple studies evaluate closure rates of fistulas and complications after stent placement. Most of them report complications between 10-30% and occlusion rates between 70-100% (6). Shin et al had a 80 % fistulas closure and a mean survival of 3 months (1-56 weeks) significantly longer in patients with complete fistula sealing compared with incomplete fistula closure (15.1 *vs.* 6.2 weeks, $p < 0.05$) (7).

MATERIAL AND METHODS

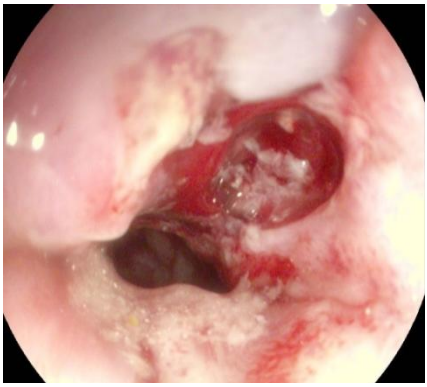
Between January 2004 and June 2014 a consecutive series of 133 patients with malignant dysphagia (86.5% men, 13.5% women), aged 46-86 years (mean 61.74 yrs) underwent stent placement under general anesthesia, using fluoroscopic control. Malignancy was established using upper endoscopic examination and biopsy in all patients.

In 84.3% of cases, the etiology of malignant dysphagia was intrinsic, due to esophageal cancer, while in 63.7% of the cases the etiology of malignant dysphagia was extrinsic due compression by bronchial cancer. A total of 26 of the patients with aero-digestive fistulas were identified, ages between 48 and 85 yrs. (mean 60.24 yrs.). In our group 73% of the fistulas were caused by esophageal primary lesion (6 located in upper third, 11 located in middle third, and 1 in lower third of the esophagus) and 27% by bronchogenic carcinomas. At the time of stent placement, 16 patients had aspiration pneumonia and 3 had lung abscesses visible on thoraco-abdominal scan. Seven patients had subclinical fistulas identified during stents placement, not visible on previous CT scan and not manifested clinically. From the 3 patients with lung abscesses, two had esophageal cancer and one had bronchogenic cancer. Only 4 women were diagnosed with fistulas, with a large majority of men. In one case at least 2 fistulas were observed. Only one case was associated with a preinstalled stent, a fistula being noticed close to the proximal opening.

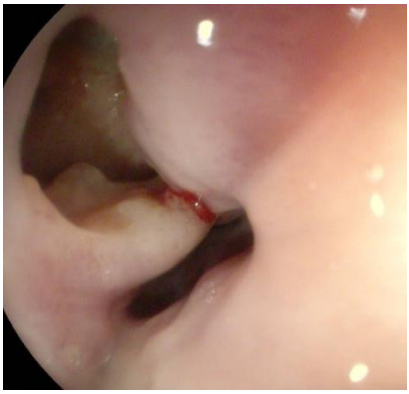
All patients were treated with successfully with a single covered expandable metallic esophageal stents. We used Self-expandable metallic stent (SEMSs) made of a nickel-titanium alloy (Nitinol) with partial polyurethane coverage (20 Ultraflex and 6 Wallflex). We used SEMSs with length between 70-150mm and diameters from 10 mm to 23 mm according to stenotic lesion and position of stricture.

While majority of patients were treated under general anesthesia with tracheal intubation, whenever a major fistula was suspected, we choose in favor of light sedation with topical anesthesia, in order to avoid

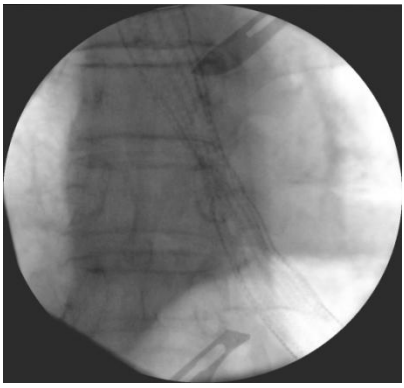
ventilation problems generated by the abnormal communication. An upper gastrointestinal endoscopy was performed to establish the upper limit of the esophageal stricture, and a radio-opaque skin marker was placed. Using endoscopic and fluoroscopic help (fig. 1), the guide wire was gently inserted through the esophageal stricture into the stomach. The stent introducer was inserted over the guide wire under fluoroscopic guidance and the stent deployed from the delivery system. In relatively large stenosis the endoscope may be passed and the lower limit marked. Stents are generally selected at least 4 cm longer than the stricture (2 cm above and 2 cm below the stricture). The uncovered portion of the partially covered stents allows for embedding and better anchoring of the stent. Fistula identification relative to stricture was essential, so that the covered part of the stent will cover it and seal the fistula tract.



Fistula caused by esophageal cancer



Fistula caused by bronchogenic cancer



Complete SEMSs deployment between radio-opaque markers

Fig. 1. Endoscopic and fluoroscopic aspects of fistulas

RESULTS

Stent placement was technically successful in 26 cases (100%), and there were no immediate postprocedural complications, with the exception of moderate chest pain in 5 cases. Complete fistula sealing resulting in resolution of aspiration symptoms (clinical success) was achieved in 26

patients (100%). Before stent placement all patients reported significant dysphagia, with a mean dysphagia score before stent insertion of 3.28. All patients were able to swallow well postprocedural with an overall dysphagia score of 1.3 (tab. I).

TABLE I

Dysphagia score was adapted from the Mellow-Pinkas dysphagia score

Dysphagia scoring scale
0 Able to consume a normal diet
1 Dysphagia with certain solid foods
2 Able to swallow semi-solid soft foods
3 Able to swallow liquids only
4 Unable to swallow saliva (complete dysphagia)

All patients died during the follow-up period (median survival 62.28 days; range 12-137 days) with earlier deaths for those with septic complications (pneumonia or pulmonary abscess). It is very difficult to estimate a benefit in survival, as the life expectancy with an untreated fistula and symptomatic aspiration can be days or weeks. We assume that our patients survived probably longer, but certainly with a better quality of live.

DISCUSSION

Esophageal stenting using SEMSs is nowadays the gold standard in the palliation of malignant dysphagia (10). SEMSs are clearly superior to rigid plastic prostheses in the management of unresectable obstructive esophageal cancers, and covered SEMSs are preferred to uncovered SEMS mainly because of lower rates of tumor ingrowth. Multiple complications caused by stent placement in esophageal malignancies have been described, and their frequency range from 10 to 30 % in most series (11). They are related to tumor location and tumor diameter, the presence or absence of the fistula or tumor shelf, use of concomitant chemo radiation (especially radiation which can minimize the tumor diameter), tumor vascularity, and, of course, diameter, length and design of the prosthesis itself. The most typical complications are described in the table below (tab. II).

TABLE II

Possible complications of esophageal self-expandable metal stents

Procedural Complications	Post-stent Placement Complications
Stent misplacement	Halitosis
Chest pain	Food impaction
Procedural infection	Over - growth/ In - Growth
Aspiration	Stent migration
Tracheobronchial perforation and pneumothorax	Tracheoesophageal fistula
Bleeding	Bleeding
	Gastroesophageal reflux disease / aspiration
	Infection and septic shock

Chest pain is typically observed following stent insertion, in all patients, with an intensity related to the degree of stricture (11). We reported only 5 cases with major

chest pain requiring antalgic medication for more the 24 hours, but we assume thoracic discomfort as a norm in all patients. We do not consider pain a complication unless it prolongs admission and may raise the possibility of subclinical perforation.

Stent migration is the single most important problem related to SEMS. While in some patients it is associated with food impaction due to lack of dietary compliance, in most cases a single factor cannot be pinpointed. Strong esophageal contraction can dislodge a stent, while chemo-radiation induced shrinking will make the stent loose. We did not encounter major procedure related complications in our series. While migration is not unusual this was not a significant event in the series. There was one stent migration into the stomach with recurrent dysphagia requiring another stent placement. Endoscopy identified the first stent in the stomach and decided to abandon it as removal can be very difficult and the typical life expectancy of these patients does not warrant a risky retrieval procedure (12). In one case, the tracheo-esophageal fistula was secondary to pressure necrosis by a previously placed stent, so a second stent was deployed inside the first one with excellent result.

The overall results show that SEMSs are very useful for symptom palliation in patients with unresectable or metastatic esophageal cancer and much more so in patients with aero-digestive fistula.

Survival in these cases is very short and without a method for fistula sealing one can expect to survive days or weeks. SEMS are excellent for aero-digestive fistula sealing which is achieved in all patients immediately and the major benefit is in the quality of life.

CONCLUSIONS

Esophagopulmonary fistulas are life-threatening complications, so complete closure of fistulas is the main aim of palliative treatment. The risk of septic shock is higher in patients with lung abscesses. The median survival in our series (~ 62 days) is within the range reported by others (40 to 110 days) but the benefit should not be evaluated in survival per se, but in the quality of life, reflected in the ability to swallow and feed.

Recurrent dysphagia due to overgrowth / in growth tumor is frequently observed in uncovered SEMSs and almost never in partially covered stents. However we recommend to our patients a semi solid diet, followed up by carbonated beverages to avoid food impaction. Usually, recurrent dysphagia is secondary to stent migration in noncompliant patients to regime restrictions.

The endoscopic placement of covered SEMSs is the treatment of choice for malignant esophago-pulmonary fistulas.

ACKNOWLEDGEMENTS

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NEWS

A NEW COMPOUND FROM *CASSIA AURICULATA* WITH ANTI-PROLIFERATIVE EFFECT AGAINST COLON ADENOCARCINOMA

Cassia auriculata (*Caesalpinaceae* family) is one of the most common Asian medicinal plants. It is used in traditional medicine for the treatment of diabetes, skin problems or eye infections. Extracts from flowers, seeds and leaves possess anti-diabetic, hepatoprotective and emollient effects. A new compound, 4-(4-chlorobenzyl)-2,3,4,5,6,7 hexahydro-7-(2-thoxyphenyl) benzo[h][1,4,7] triazecin-8(1H)-one, was isolated from the leaf ethanolic extract. After structure elucidation, the compound was tested for its anti-proliferative effect against human colon cancer HCT15 cell line. The MTT assay showed that the cell viability was inhibited by 50% at a dose of 25 µg/mL after 48 h treatment. In addition, the new compound induced apoptosis at 25 and 100 µg/mL as revealed by propidium iodide staining protocol. Lactate dehydrogenase assay confirmed the cytotoxic potential of this compound. The results proved that the new isolated compound from *Cassia auriculata* leaves possess anti-proliferative effects and it is a promising compound for the treatment of colon adenocarcinoma (Esakkirajan M, Prahbu NM, Arulvasu C, Beulaja M *et al*. Anti-proliferative effect of a compound isolated from *Cassia auriculata* against human colon cancer cell line HCT 15. *Spectrochim Acta A* 2014; 120: 462-466).

Cristina Lungu

RETROMOLAR TRIGONE – OROPHARYNX JUNCTION MALIGNS TUMOR SURGERY: TRANSMANDIBULAR VERSUS ORAL APPROACH

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RETROMOLAR TRIGONE – OROPHARYNX JUNCTION MALIGN TUMOR SURGERY: TRANSMANDIBULAR VERSUS ORAL APPROACH (Abstract) **Aim**: This study proposes a new approach to a borderline pathology between Otorhinolaryngology (E.N.T.) and Oral and Maxillofacial Surgery (O.M.F.), the malignant tumors of the oropharyngeal and retromolar trigone junction. **Material and Methods**: 52 cases of retromolar trigone and oropharynx malign tumors were solved in the ENT department of "St. Spiridon" Universitary Hospital Iasi between 2012 and 2014. All patients were males, 35-64 years old, in different TNM stages. The novelty stands in the multidisciplinary approach, with an operating team consisting of both E.N.T. and O.M.F. surgeons, which joined their knowledge and expertise in order to offer a better treatment for the patient. *Human Papilloma Virus* (HPV) infection has been known as a trigger factor in head and neck cancers. The connection between HPV infection and malignant tumors of the oropharyngeal – retromolar trigone junction, together with the other traditional risk factors (smoking, alcohol, stress and sexual behavior) are involved in the therapeutic protocols, improving the life quality, the survival rate and reducing the treatment costs. **Results and discussion**: Excision of the malignant tumors at the level of the junction between the oropharynx and retromolar trigone often requires repairing the tissular defects that remain using different flaps. Postsurgical mecanotherapy (physiotherapy) under the surveillance of an experienced physiotherapist is also needed for a complete recovery. **Conclusions**: This therapeutical protocol aims to assure a better life quality for the patients, with a faster postsurgical recovery and social reinsertion by reducing the healing time of the areas affected by inflammation and necrosis generated by the neoplastic process.
Keywords: MALIGNANT TUMORS OF THE OROPHARYNGEAL AND RETROMOLAR TRIGONE JUNCTION; ORAL APPROACH; TRANSMANDIBULAR APPROACH.

Access to bulky tumors of the oral cavity and most tumors of the oropharynx via the open mouth is often difficult and inadequate (1, 2, 3).

In the past, segmental mandibulectomy was routinely performed for resection of large primary tumors of the oral cavity or oropharynx. In many instances the mandible was neither clinically, nor radiographically involved in the tumor but was

sacrificed to gain access to the oral cavity and oropharynx.

Division of the mandible instead of resection provides equally good exposure of the interior of the oral cavity and permits a satisfactory monobloc resection of the primary tumor, often in continuity with neck dissection if is indicated (3, 4, 5, 6).

A paramedian mandibulotomy would be ideal to gain access to the oral cavity as it offers all the advantages of a median mandibulotomy and avoids disruption of the genial muscles, the only muscle requiring division being the mylohyoid on one side. Covering the defects after the excision with different skin flaps is indicated for stage III tumors (6, 7, 8).

Establishing a direct connection between Human Papilloma Virus (HPV) infection associated with other external factors (alcohol, smoking, emotional stress) and oropharyngeal malignant tumors is important in setting the most suitable therapeutical plan for the patient. Finding the exact type of HPV that colonizes the oropharynx for our region could lead to a vaccine similar to that for the cervix cancer (10).

An early diagnosis and prophylactic measures (proper hygiene, advising against smoking and alcohol consumption, sexual education) can improve the survival rate and life quality of these patients and will reduce the treatment costs (11).

MATERIAL AND METHODS

Fifty-two cases of retromolar trigone and oropharynx malign tumors were solved in the Ear, Nose and Throat (E.N.T.) department of "St. Spiridon" Universitary Hospital Iasi between 2012 and 2014. All of them were male patients between 35 and 64 years old with carcinomas in different TNM stages. The main surgical approaches were: 1. Transmandibular mandibulotomy, mandibulectomy; 2. Oral approach: classical surgery or CO_2 laser. In all cases we management of the neck lymph nodules and submandibular gland was considered.

The choice of approach depends on the size/location of the tumor, the chosen method of reconstruction and whether a concomitant neck dissection is being planned. Oral approach was performed in 46 cases and is indicated when the tumor is limited to the tonsilar pillars, one half of the soft palate, uvula, palatine tonsil, retromolar trigone (stage I–II tumors) and also for tumors located only at the base of the tongue. Oral approach is indicated when the tumor is limited to the tonsilar pillars, one half of the soft palate, uvula, palatine tonsil, retromolar trigone (stage I–II tumors) and also for tumors located only at the base of the tongue. Transmandibular approach is used if both anatomical segments are affected. It is indicated for stage III tumors that need ganglia extirpation and, if necessary, covering of the defect with skin flaps (6, 13, 14, 15).

Transmandibular approach was performed in 6 cases and is used if both anatomical segments are affected. It is indicated for stage III tumors that need lymph nodules extirpation and, if necessary, the defect is covered with skin flaps. Most surgeons agree that resection should be reserved for those oropharyngeal lesions with well-defined borders. When the margins of the primary tumor blend imperceptibly with the adjacent mucosa or when multifocal disease is present, radiotherapy is usually preferable. We also check the presence of HPV infection which has been known as a trigger factor in head and neck cancers. Establishing whether the patient is

infected with HPV and has other risk factors like smoking, alcohol, stress and abnormal sexual behavior is necessary for deciding the surgical protocol, improving the quality of life and the survival rate (10).

Patient selection for a mandibulotomy approach requires accurate assessment of the proximity of the tumor to the mandible. In some patients who had painful lesions or trismus, examination under general anesthesia was necessary. Ideally, there should be a rim of normal tissue between the tumor and the mandible, but the line of excision was occasionally carried to the alveolus or the anterior border of the ramus if the tumor involved the anterior tonsillar pillar and approached the periosteum (6, 8, 12, 16).

CASE 1

Male, 50 years old, hospitalized in the E.N.T. department of "Sf. Spiridon" Hospital Iasi for disphagia, trimus, pain and lymph nodules at the level of the neck which appeared a couple of months prior to the examination.

Local examination: well delimited right laterocervical tumefaction of about 5/4 cm, of hard consistency, mobile on the deep and superficial fascias, covered by normally colored skin. Oral cavity exam: infiltroproliferative process set at the level of the right tonsil with infiltration of the anterior tonsilar fold and inferior extension to the right one third of the tongue and the right glossotonsilar sulcus and retromolar trigone (fig. 1).

The CT exam shows tumoral appearance at the level of the right palatine tonsil of 33/24/33 mm with the following limits: 1. Lateral - the pterigoidian muscle; 2. Anteromedial - the base of the tongue; 3. Posterior - the pharyngeal constrictor muscle; 4. Superior – plane through the soft palate; 5. Inferior – plane through the epiglottis (fig. 2), right superior jugular adenopatic block of 38/32/47 mm with mass effect over the internal jugular vein and the common carotid artery and multiple small laterocervial adenopathies (fig. 3).

We performed right extended tonsillectomy and excision of the retromolar trigone by endoral approach, together with right radical lymph node dissection (fig.4, 5, 6).

Anatomopathological exam: moderately differentiated non-keratinizing squamous cell carcinoma, with infiltration of the striated muscle fascicles.

Fig. 1. Tumor at the level of retromolar trigone and right tonsil

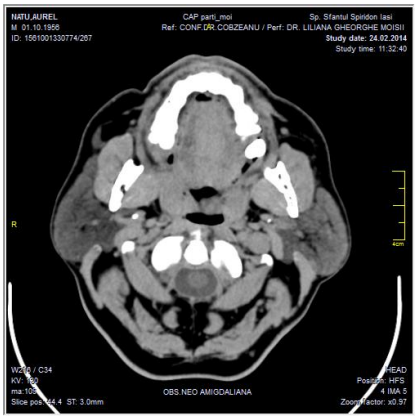

Fig. 2. The CT exam shows a tumor at the level of the right palatine tonsil.

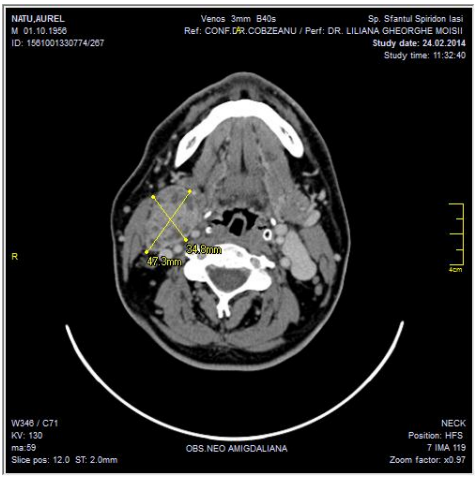

Fig. 3. Right superior jugular adenopatic block

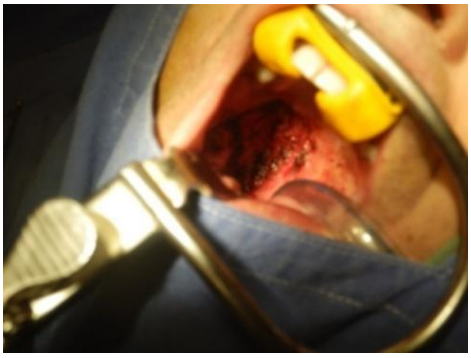

Fig. 4. Palatine tonsillectomy by endoral approach

Fig. 5. Lateral functional neck dissection

Fig. 6. The excised specimens

The surgical treatment was followed by chemo and radiotherapy. The 1 year follow-up showed quasi-normal deglutition and phonation, with no signs of local recurrence.

CASE 2

Male, 53 years old, hospitalized in our department for a tumor at the level of left retromolar trigone and palatine tonsils, with extension to the anterior palatine fold and left cervical lymph nodules in groups II and III.

Local exam: well delimited left latero-cervical tumefaction, of 5/3 cm, of hard consistency, mobile on the deep and superficial fascias, covered by normally colored skin. Oral cavity exam: infiltro-proliferative process set at the level of the left tonsil, with infiltration of the anterior and posterior tonsilar fold and inferior extension to the left one third of the tongue and the left glosso-tonsilar sulcus (fig. 7). CT exam: oropharynx asymmetry with the thickening of the left lateral wall and left jugulo-carotidian adenopathy that has mass effect on the internal jugular vein (fig. 8).

Transmandibular approach was used, with inferior labial middle line incision which continued to the mental foramen form the opposite side of the osteotomy.

The left palatine tonsil and the retromolar trigone were excised and the defect was covered with a temporo-parietal fascial flap. Neck dissection was performed with resection of lymph nodules and submandibular gland. Anatomopathological exam: poorly differentiated non-keratinizing squamous cell carcinoma. The 6 months follow-up showed quasi-normal deglutition and local aspect, with no signs of local recurrence (fig. 9, 10, 11).

Fig. 7. Left palatin tonsil and retromolar trigone tumor.

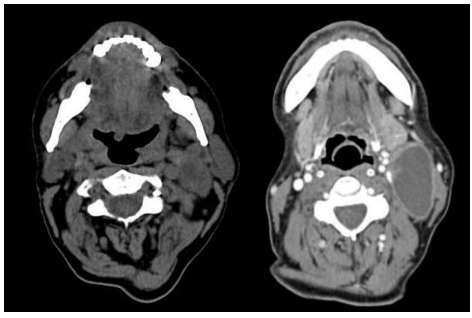

Fig. 8. CT exam aspect.

Fig. 9. Temporo-parietal flap

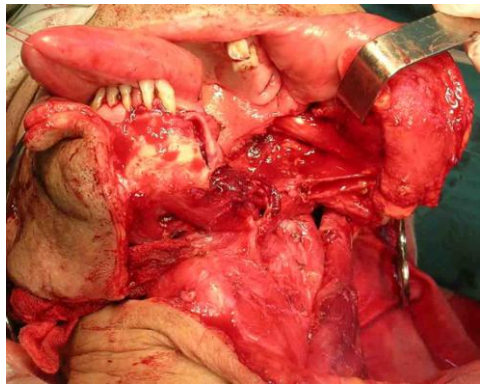

Fig. 11. Tissue defect after resection

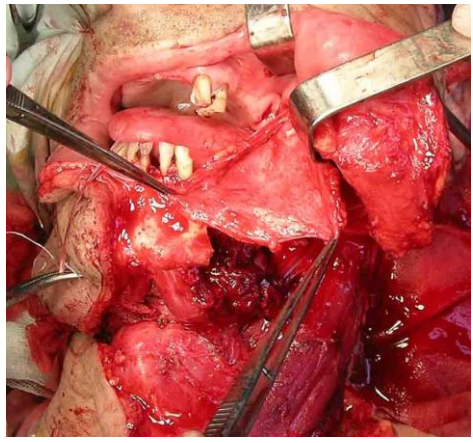

Fig. 12. Translation of temporo-parietal flap

DISCUSSION

If the mandible is resected, the surgical approach is done through the lower lip, on the midline, all the way down to the labiomental groove and then curbing in the opposite direction of the osteotomy.

The osteotomy is done paramedially on the side of the lesion, between the canine and the premolar, in front of the mental foramen in order not to damage the inferior alveolar nerve. Patient selection for a mandibulotomy approach requires accurate assessment of the proximity of the tumor to the mandible. In some patients who had

painful lesions or trismus, examination under general anesthesia was necessary.

Ideally, there should be a rim of normal tissue between the tumor and the mandible, but the line of excision was occasionally carried to the alveolus or the anterior border of the ramus if the tumor that involved the anterior tonsillar pillar approached the periosteum

The gingival and lingual mucosa is then dissected from the premolar all the way to the third molar tooth, followed by detaching the mylohyoid muscle from the bone. The mandibulotomy is never made in the middle because it produces functional and statical dysfunctions at the level of the tongue by denervation and displacement of the structures. This ensures the preservation of the inferior alveolar neurovascular bundle. The periosteal vasculature remains viable on the external side of the mandible. Osteosyntesis with plates is preferred.

For esthetical reasons, the inferior medial labial incision must be arched through the labiomental groove, opposite the mandibulotomy, so that the bone incision does not overlap with the soft tissue incision. In general, these defects are very complex, affecting both the aesthetics and the functionality of the area (mastication, deglutition, phonation).

Different methods are available that range from primary closure and healing by secondary intention for the smaller defects to the use of local, myocutaneous and free microvascular tissue transfer flaps. The flaps must be thin and wide because the area that needs to be covered is large. Temporoparietal fascial flaps, Bichat's lobule pedicle flaps and radial free flaps are preferred for covering the defects (6, 13, 14).

Neck dissection has been previously performed. Conventional unmodified radical neck dissection was employed in patients who were staged N1 on admission. At patients who had no evidence of cervical metastasis, we perform elective neck surgery, with conventional radical neck dissection, modified radical neck dissection, supraomohyoidian neck dissection or suprahyoidan neck dissection. We believe that paramedian mandibulotomy in combination with postoperative radiotherapy, when appropriate, offers an attractive alternative to high dose radiotherapy alone in patients with oropharyngeal and retromolar trigone junction carcinoma. Transmandibular approach gives the possibility of excising the lesion and covering the tissue defect with a temporoparietal flap (6).

Three main issues that result from curative surgical treatment appeared in our statistics: 1. Sequelae or complications of the treatment in 3 cases; 2. Functional deficits or interference of physiological functions in 5 cases; 3. Recurrent/persistent disease in 2 cases.

All of the patients were solved by surgical methods and were seen back 12-14 days after discharge and instructed on diet, swallowing therapy, physical therapy of the neck and shoulders and voice therapy.

CONCLUSIONS

All the patients included in our study, with tumours at the level of the oropharynx and retromolar trigone were early diagnosed. Otherwise the survival rate decreased significantly.

If the tumoral process is extended into the retromolar trigone, the middle of the tongue, the base of the tongue or valeculas we highly recommend to perform a computed tomography (CT scan) to precisely evaluate the extension of the lesions. A

mixed operating team formed by ENT and OMF surgeons should perform the surgery.

For stages I-II, either surgical treatment or radiotherapy can be applied, with similar results.

Antibiotic therapy prior surgery is essential for stopping tumoral infection, a problem in the pharyngeal area.

Oral approach is indicated when the tumor is limited to the tonsilar pillars, one half of the soft palate, uvula, palatine tonsil, retromolar trigone (stage I–II tumors) and also for tumors located only at the base of the tongue, the transmandibular approach being indicated in advanced stages (stage III-IV tumors).

Patients infected with a high-risk type of HPV have better prognosis after surgery than those with different phenotypes or without HPV.

ACKNOWLEDGEMENTS

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NEWS

STUDY OF AUTOPHAGY-RELATED PROTEIN LIGHT CHAIN 3 (LC3)-II EXPRESSION LEVELS IN THYROID DISEASES

Thyroid cancers are the most common malignant tumors of the endocrine system. The survival-promoting role of autophagy in tumor cells has been received universally. The aim of the study was to explore autophagy-related protein light chain 3 (LC3)-II expression levels in thyroid diseases including papillary thyroid cancer. A total of 45 thyroid samples, including 19 samples of papillary thyroid cancer, 7 samples of nodular goiter, 8 samples of Hashimoto thyroiditis and 11 samples of normal thyroid tissue resected during surgery, were selected and divided into pathological groups using light microscope. Levels of autophagy-related protein LC3-II in four different types of thyroid tissue were tested through Western blot. SPSS19 software was utilized to analyze the research data statistically. LC3-II protein levels in papillary thyroid cancer tissues were lower than that in normal thyroidtissues significantly ($P<0.05$). Compared with normal thyroid tissue, expression levels of LC3-II protein were higher in samples of Hashimoto thyroiditis and nodular goiter ($P<0.05$). Multi-factor analysis of variance showed that there was no significant correlation between LC3-II protein levels and patients' gender of thyroid cancer, while there was significant variation between patients with and without lymph node metastasis. Compared with patients of thyroid cancer without lymph node metastasis, the level of LC3-II protein was lower in patients of thyroid cancer with lymph node metastasis ($P<0.05$). Detection of LC3-II protein expression levels in thyroid diseases may contribute to the clinical diagnosis and provide theoretic basis for the therapy. (Zhang N, Li L, Wang J, Cao M, Liu G, Xie G, Yang Z, Li Y. Study of autophagy-related protein light chain 3 (LC3)-II expression levels in thyroid diseases. *Biomed Pharmacother* 2015; 69:306-10. doi: 10.1016/j.biopha. 2014.12.021).

Radu Dănilă

IMMUNE FETAL HYDROPS - A SEVERE FORM OF RH ISOIMMUNIZATION

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IMMUNE FETAL HYDROPS-A SEVERE FORM OF RH ISOIMMUNIZATION (Abstract). The prevalence of pregnancies with Rh isoimmunization decreased to about 0.6% of all cases due to anti-D immune globulin prophylaxis. Rh incompatibility or Rh isoimmunization pregnancies must be monitored by serial measurements of titers of maternal Rh antibodies and, if possible, by serial Doppler evaluation of peak systolic velocity in the middle cerebral artery. Nowadays, for the detection of fetal blood group and Rh we can use techniques which identify free fetal DNA in maternal plasma. We report the clinical case of a 35 years old woman, admitted to our clinic after not being able to perceive active fetal movement. She proved to be Rhesus negative, with a pathological obstetrical history and through ultrasound examination, the diagnosis of fetal hydrops was established. The presence of fetal hydrops determines the viability of a small percentage of about 11%, and the long-term prognosis of these children is burdened by the need of repeated transfusions to combat the inhibition of erythropoiesis. Our report highlights the importance of early diagnose and precise antibody monitoring, to provide proper management and prophylaxis. **Keywords:** IMMUNE FETAL HYDROPS, RH ISSOIMMUNISATION, DOPPLER ULTRASOUND.

Fetal hydrops is defined as an abnormal fluid collection in two or more areas of the fetal body, such as ascites, pleural effusion, pericardial effusion and skin edema (1, 2). The etiologies of fetal hydrops are classified as immune or non-immune hydrops. Immune hydrops develops due to fetal hemolysis mediated by circulating maternal antibodies to fetal red blood cell antigens. Recent advances in obstetric and neonatal medicine achieved significant improvements in diagnosis, prevention and management of fetal hydrops. Specifically, immune hydrops has been decreased by routine screening and prophylaxis of Rhesus iso-immunization (1,3). Non-immune hydrops can result from many causes including cardiac abnormalities (structural anomalies, cardiomyopathies and arrhythmias) and non-cardiac anomaly, aneuploidy, congenital infection, twin-to-twin transfusion syndrome, chorioangioma and other conditions. Different types of infections, such as toxoplasmosis, cytomegalovirus, herpes simplex virus, syphilis, and Parvovirus B19 are the most common congenital infections that cause fetal hydrops. But, the precise cause of non-immune hy-

drops remains unknown in 15% to 25% of cases (3, 4), with an unchanged incidence (6) and a high mortality rate (4, 6, 8).

Fetal hydrops is easily recognized by ultrasound examination, during which we should try to figure out the etiology and nature of the hydrops, including the location, number and amount of fluid collections, amniotic fluid index, placental thickness, fetal echocardiography and Doppler velocimetry. However, there is limited information regarding the association between the ultrasound findings and perinatal outcomes of fetal hydrops, specifically neonatal mortality (10, 12, 13). Therefore, the aim of this case report is to show the clinical characteristics of fetal hydrops and to find out whether the antenatal ultrasound findings, especially the location or number of fluid collection sites, are associated with adverse perinatal outcomes.

CASE REPORT

We report the case of a pregnant woman 35 years old from the rural environment that presents to the hospital in emergency for the lack of perceiving of the active fetal movements.

The anamnesis highlights the fact that she didn't followed the prenatal follow-up, she was Rhesus negative (her partner was Rhesus positive) and she had a titer of serum anti D antibodies of 1/32.

Her obstetric history consists of 2 normal birth of two healthy children of 3000 and 3500g and 2 spontaneous abortion at gestational age of 2 months for which she didn't followed the anti-D immune globulin prophylaxis.

At admission to the hospital her blood pressure was 160/100 mm Hg, and she complained of epigastric pain that irradiates to her right shoulder (tab. I).

TABLE I

Patient biochemical profile

Hb= 9.9g/dl Ht = 29.6% PLT = 200000/ mm³ WBC = 12100/mm^3	Glucose = 70mg/dl FIBRINOGEN: 528mg/dl ESR = 12 mm/h
INR = 0.97 Ind Q = 102% TIMP Q = 12.7/s MARTOR = 13/s APTT = 40.6/S PDF = 1600-3200 ng/ml LDH = 658 U/L	Creatinine = 0.85mg/dl Urea = 19mg/dl Uric acid = 7.67mg/dl TGP = 64 U/L TGO = 106 U/L Group Rh: AII negative.
Direct urinary exam: Albumin 100 mg / dl; ketones: traces; bilirubin ++; nitrites present; microbial flora present; leukocyte cylinders present.	

Ultrasound reveals the diagnosis of hydrops fetal: a single living fetus of 29 weeks three days with an abdominal circumference (AC) of 39 weeks 3 days; Oligoamnios; Placental thickness 100mm gr I (fig. 1a); Scalp edema 17mm (fig. 1b); IR ACM-0.70; IR AO-0.61; ICP> 1.14; severe fetal ascites (fig. 1c); bilateral pleural effusions (fig. 1d); cardiomegaly.

At approximate 24 h after the admission, we decided to perform caesarean section for the diagnosis: VG IIIP 28 weeks of pregnancy, single living fetus, cranial presentation, intact membranes, Imminent premature birth,

Rh isoimmunization, Immune fetal hydrops, Preeclampsia, Acute Fetal Suffering.

We extracted a newborn who didn't respond to resuscitation (Apgar score = 0), female, weight 2,040g (fig. 2). The placenta weighed ~ 1,700g.

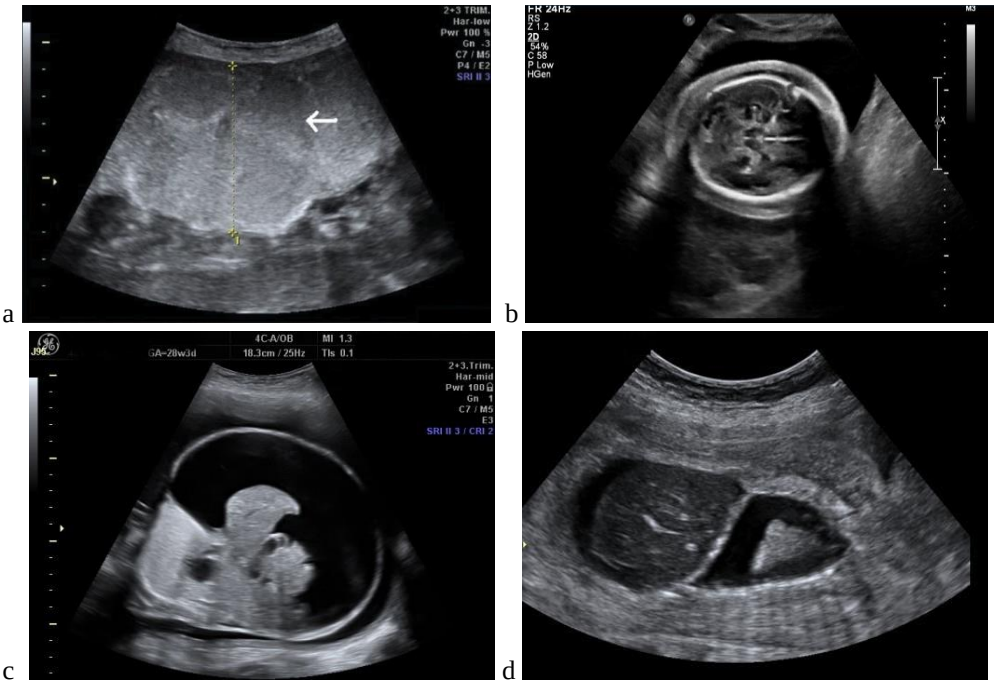

Fig. 1. Ultrasound at admission
(a. Placental thickness, b. Scalp edema, c. Fetal ascites, d. Pleural effusion)

Fig. 2. Severe fetal ascites – clinical aspects: a. Edema and distention of abdomen due to ascites; b. coarse facial features, hypertelorism, depressed nasal bridge, anteverted nostrils, long philtrum, brachycephaly.

DISCUSSION

The diagnosis and management of fetal hydrops have improved in recent years with advances in prenatal diagnostic and thera-

peutic interventions, together with the advances in neonatal intensive care. However, fetal hydrops is still associated with a high mortality rate.

In recent years, most newborn infants with this pathology were classified as non-immune fetal hydrops as the proportion of infants with immune fetal hydrops decreased to 10% due to the use of anti-D immunoglobulin prophylaxis, intrauterine transfusions, and close follow-up of the pregnancies with Rh-isoimmunization in developed countries. The etiology of fetal hydrops is known to be significantly associated with prognosis (4, 10).

With the recent advances in prenatal ultrasound and the increase acceptability of the method, fetal hydrops is almost always diagnosed by antenatal ultrasound. Therefore, we presented a case of immune fetal hydrops due to the Rh incompatibility, which was diagnosed incidentally by an antenatal ultrasound, presenting fetal ascites, pleural effusion, scalp edema and placental thickness, with a poor perinatal outcome.

According to various literature sources, the presence of pleural effusion was a poor prognostic factor of fetal hydrops (11). The most important findings in different studies was that the number of fluid collection sites was highly correlated with neonatal outcome including low Apgar score and neonatal death, as it was in our case. A similar result was found in a recent study by Kim et al. who studied 43 women with non-immune fetal hydrops. They developed an 'ultrasonographic severity scoring of non-immune hydrops (USNIH) defined as a total number of abnormal fluid collections. Perinatal mortality rate, defined as stillbirth or neonatal death ≤28 completed days after birth, was significantly higher in cases with USNIH of ≥3 than in those with USNIH of 2 (13,14).

Usually, in early stages, the doppler velocimetry is one of the most important predictor factors for fetal or neonatal mortality in various fetal compromise conditions, including fetal hydrops (11, 12, 13). The pattern of abnormal Doppler velocimetry indices may depend on different cause of fetal hydrops. For example, abnormal umbilical artery Doppler or MCA pulsatility index reflects fetal hypoxia, abnormal MCA peak systolic velocity may reflects fetal anemia, and abnormal ductus venosus Doppler occurs as a result of fetal cardiac decompensation (15). In our case, Doppler velocimetry wasn't required, considering the advanced form of the disease, with multiple effusion sites.

CONCLUSIONS

These results from different studies, taken together with our clinical case, may suggest that the number of fluid collection sites is the strongest antenatal ultrasound risk factor of prediction the poor outcome in fetal hydrops, as we illustrated in this presentation

This case underlines the importance of enforcing anti-D immune globulin prophylaxis when necessary, mainly to avoid the consequences of isoimmunization in Rhesus negative mothers that can result in unfavorable outcomes for both the mother and the fetus.

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NEWS

TREATMENT ALGORITHM FOR THE MANAGEMENT OF COMPLICATIONS CAUSED BY PERMANENT FILLERS IN THE FACE

Despite recent developments in the usage of resorbable substances such as hyaluronic acid, non-resorbable substances are still used to enhance soft-tissue volumes. Inflammatory reactions are frequent and disabling. Therapeutic options are nonspecific. A study was conducted as a review of 219 consecutive patients referred between 2006 and 2013. Screening was made with an ultrasound soft-tissue examination and the lesions were classified as either cystic or infiltrating, infiltrating patterns were treated intralesionaly withan 808-nm diode laser laser treatment alone, cystic lesions were also drained through stabwound incisions. In 62 percent the lesions disappeared completely, Partial improvement wasobtained in 30 percent, whereas 8 percent discontinued the treatment. A systematic approachis proposed, based on 7 years of experience, with an overall improvement rate of 92 percent (Cassuto D, Pignatti M, Pacchioni L, Boscaini G, Spaggiari A, De Santis G. Management of Complications Caused by Permanent Fillers in the Face: A Treatment Algorithm. *Plastic and Reconstructive Surgery* 2016; 138(2): 215-227).

Mircea-Dinu Bordiniuc

METASTATIC CARCINOID TUMOR- ATYPICAL PRESENTATION

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METASTATIC CARCINOID TUMOR - ATYPICAL PRESENTATION (Abstract): Carcinoid tumor is a slow-growing type of neuroendocrine tumor, originating in the enterochromaffin cells and secreting mainly serotonin. Neuroendocrine tumors (NETs) are found throughout the intestinal tract, the appendix and terminal ileum being the most common locations, and are classified by site of origin and by degree of differentiation, with well-differentiated lesions representing those tumors formerly referred to as carcinoid tumors. The clinical symptoms are characterized by flushing, diarrhea, abdominal pain, and/or bronchial constriction and occur almost exclusively in patients with liver metastases due to the release of bioactive peptides and amines directly into the systemic circulation. We report the clinical, serological and histological diagnosis of a 67-years-old male patient with congestive heart failure secondary to carcinoid heart disease in the context of liver metastases of an ileum carcinoid tumor. **Keywords**: CARCINOID SYNDROME, HEART DISEASE, METASTATIC LIVER DISEASE, SEROTONIN.

Neuroendocrine tumors (NETs) are a very heterogeneous group arising from the neuroendocrine cells, and include carcinoid, non-carcinoid gastroenteropancreatic tumors, catecholamine-secreting tumors (phaeochromocytomas, ganglioneuroblastomas, paragangliomas, and neuro-blastoma), medullary carcinoma of the thyroid, chromophobe pituitary tumors, small cell lung cancer and Merkel cell tumors. The different NETs may be divided into functioning and non-functioning tumors (1).

The carcinoid syndrome occurs in approximately 10% of NETs and becomes manifest when vasoactive substances from the tumors, such as serotonin as well as several other chemicals, enter the systemic circulation escaping hepatic degradation (2, 3).

This particular type of syndrome is often encountered when metastatic lesions are found spread throughout the liver or they develop from the bronchi. The clinical properties are characterized by flushing (63%–94%), diarrhea (68%–84%), abdominal pain (10%–55%), telangiectasia (25%) and bronchial constriction (3%–19%) (4, 5). High levels of vasoactive substances released from hepatic metastases can cause, in 10-20% of patients with carcinoid syndrome, the appearance of lesions that compromise the integrity and functionality of the endothelium and cardiac valves causing heart failure (6, 7).

CASE PRESENTATION

A 67-year-old man was admitted for

watery diarrhea, abdominal discomfort and shortness of breath. He had been having loose stools for 5 years, with 3 to 4 bowel movements per day, progressing over the previous 2 months to 4-5 large volume watery stools daily, including nocturnal stools. Abdominal cramps preceded these episodes and were partially relieved by bowel movements. He noted no blood or oil in the stool.

On examination, the patient was alert and oriented. His body mass index was 17. The temperature was 37.2°C, blood pressure 95/65 mm Hg, pulse up to 100 beats per minute and regular, respiratory rate 19 breaths per minute, and oxygen saturation 99% while he was breathing ambient air. The scleras were anicteric, and the oropharynx had no ulcers or lesions. The neck was supple and without jugular venous distention.

Further clinical examination revealed a diffuse rash characterized by fixed pink patches on his scalp, trunk and limbs that became red and pruritic when scratched or exposed to sun and heat and facial flushing, brought on by exertion or strong emotion.

The abdomen was slightly distended, depressible and diffusely painful with a painless, firm, nodular hepatomegaly of 6 finger-widths. Splenomegaly was not noted. Brown stool was negative for occult blood. The neurologic examination was unremarkable.

Apparently, he was diagnosed 20 years ago with idiopathic liver disease and multiple hepatic nodular areas spread throughout the liver which were recorded and measured periodically to see any signs of growth. They maintained relatively stable in size over the last few years.

Six months prior to admission the patient underwent major cardiac surgery to replace the aortic and mitral valve, because of severe aortic stenosis and mitral regurgitation with favorable postsurgical outcome. After surgery, he was encouraged to seek gastroenterological advice for liver distress and intestinal discomfort.

On admission, the patient recalls he had had multiple similar episodes during the previous 5 years, with flushing, hypotension and mild diarrhea, but he managed to avoid seeking medical care. The symptoms, which were usually provoked by physical exertion, mental stress, or intense emotion, lasted up to 2 hours.

The ultrasound performed in our clinic confirmed the presence of numerous nodular areas spread throughout the liver which were suggestive of metastatic liver disease (fig. 1).

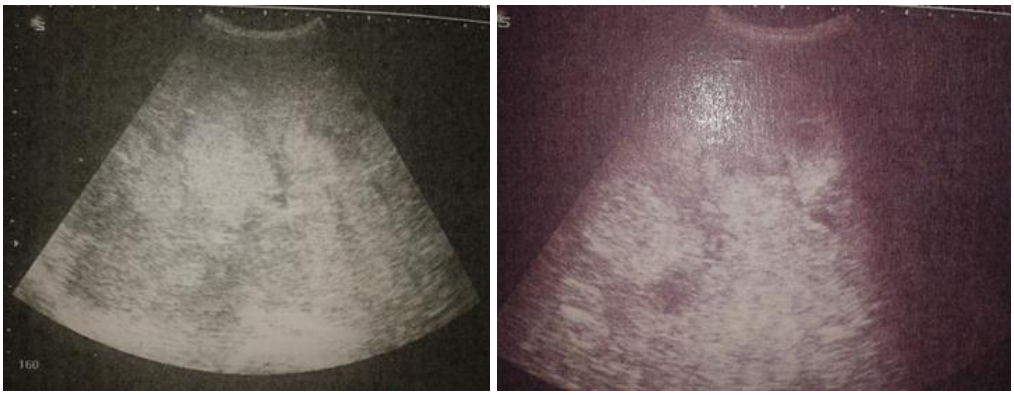

Fig. 1. Liver ultrasound: multiple hyperechoic nodules

Abdominal CT scan showed multiple hepatic masses, the largest was 10 cm in diameter and another mass located in the mesenteric region approximately 40 mm in diameter relatively well defined which needed additional tests to establish its nature.

Endoscopic video capsule was performed and it showed multiple irregular masses located in the proximal jejunum and ileum protrusive in the lumen and covered by normal looking mucosa, with visible vascular pattern but with no obstructive potential (fig. 2).

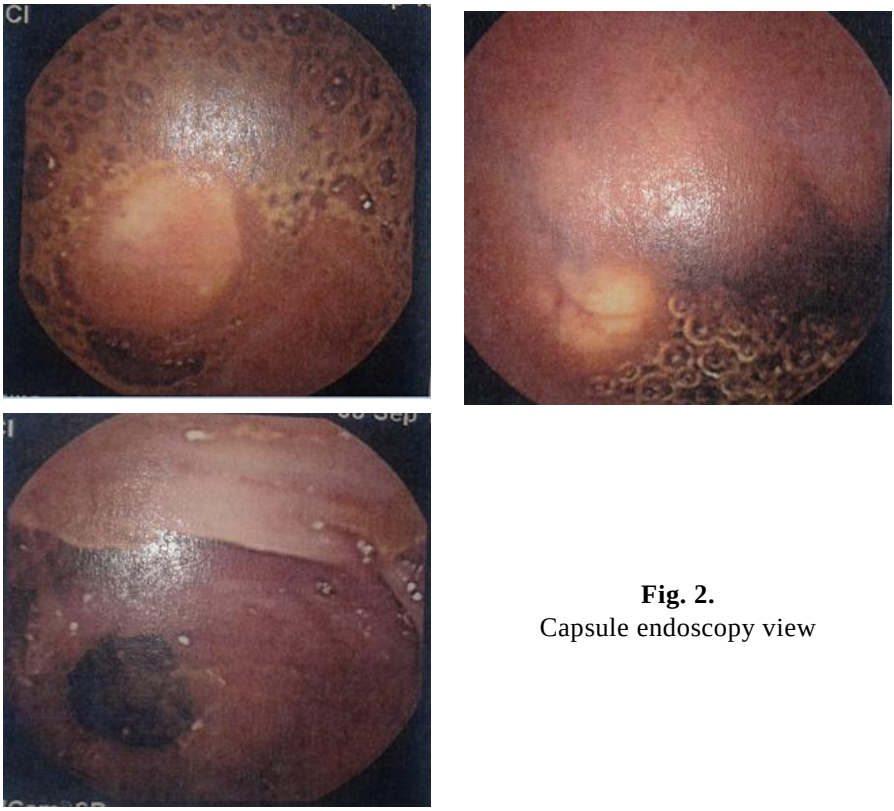

Fig. 2.
Capsule endoscopy view

Adding all the pieces of the puzzle, the clinical presentation (flush, diarrhea, low blood pressure), metastatic liver disease, intestinal tumor and heart disease the diagnosis of carcinoid tumor became obvious.

Serological tests including urinary levels of 5-hydroxyindoleacetic acid (5HIAA), serotonin (Ser) levels and chromogranin A concentration (Cg A) were performed and high levels of these parameters confirmed the diagnosis (tab. I).

TABLE I
Laboratory findings

Parameter	Value
Chromogranin A	22064µg/L
Urinary excretion of 5-HIAA.	148.5 mg/24h
Serotonin	450 µg/L

The patient was discharged on his own free will and returned to Modena, Italy. There he performed additional investigations.

Whole-body scintigraphy and single photon emission computed tomography (SPECT) after intravenous injection of indium-111-octreotide showed hepatomegaly highlighting a large and high uptake halo that occupied almost the entire right hepatic lobe with other several liver focal accumulations. Also, a focal uptake localized in the small intestine, femoral neck, sternum and right shoulder were detected.

Liver biopsy was performed and the histology report showed a well differentiated neuroendocrine tumor with no necrosis or mitotic activity. Chemotherapy was initiated. Chemotherapy sessions were marked by severe granulocytopenia which needed growth factor correction and temporization of chemotherapeutic drugs.

A recent assessment (December 2013) showed clinical and biological improvement, with a stationary size of the hepatic lesions and decrease in size of the intestinal and bone tumors. In June 2014 liver ultrasound showed neoplastic hepatomegaly with multiple large nodular areas which increased in size compared to the previous examination, but with relative good clinical tolerance (fig. 3).

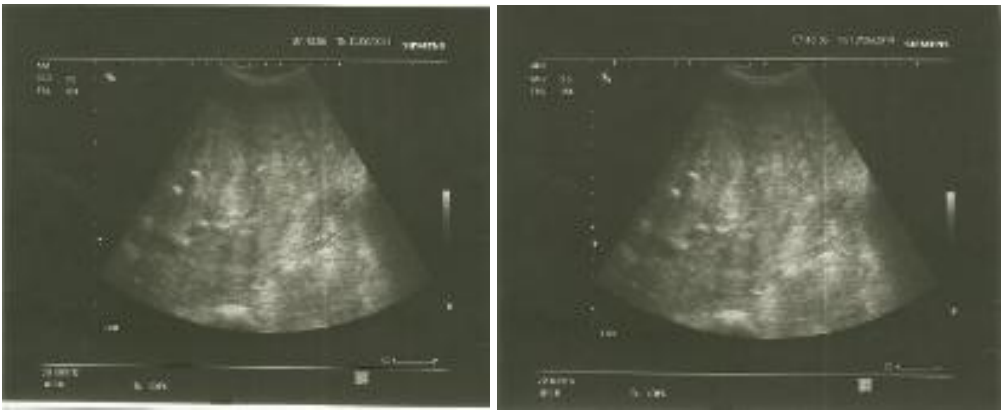

Fig. 3. Current liver ultrasound: multiple hepatic metastatic lesions

DISCUSSION

When liver metastasis occurs, the heart is exposed to intermittently high levels of vasoactive substances, which is believed to result in damage to the endocardium.

Carcinoid heart disease occurs in up to 60% of patients with carcinoid syndrome (8). Left-sided cardiac involvement can also occur in <10% of patients (2, 3, 10). The left side of the heart is relatively protected as the lungs filter the vasoactive peptides, inactivating them in the pulmonary circulation before they reach the left atrium (11). Therefore left sided disease is seen only in patients with bronchial carcinoid or patent *foramen ovale* or in those with poorly controlled severe carcinoid syndrome that overwhelms the degradation capacity of the lung (12, 13, 14).

Tumor size is an unreliable predictor of metastatic disease, and metastases have been reported even from tumors measuring less than 0.5 cm in diameter. Long-term survival correlates closely with the stage of

the disease at presentation (1). With regard to the clinical course of cardiac involvement, high urinary 5HIAA levels are a predictor of progression of carcinoid heart disease (10). This finding was corroborated by Bhattacharyya *et al* (11) who demonstrated that a urinary 5HIAA level greater than 300μmol/24 h and more than 3 flushing episodes per day are independent predictors of the development or progression of carcinoid heart disease.

The case presented is unique because the disease had a long and mostly mild progression, with the onset of left cardiac complications and liver metastasis prior to carcinoid syndrome manifestation. In our patient, carcinoid heart disease involved all 4 cardiac valves: the aortic valve to a higher extent than the pulmonary valve and the mitral valve to a worse extent than the tricuspid valve. It is hard to estimate if the severe left cardiac involvement is the soul result of carcinoid stress or the combined outcome of both degenerative and carcinoid disease.

CONCLUSIONS

The case presented had a peculiar development with silent liver metastases and left cardiac involvement, long disease progression and relatively favorable outcome. The primary goal of treatment in patients with NETs is curative, with symptom control and the limitation of tumor progression. However, as most patients with NETs are diagnosed once metastases have occurred, curative surgery is generally not possible. Octreotide decreases the secretion of serotonin by the tumor and can improve the symptoms of carcinoid syndrome and stabilize tumor growth in many patients. Unfortunately, there are no specific guidelines and no opportunity to accurately assess prognosis. Disease progression and overall survival are case dependent, as illustrated by the case here presented.

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NEWS

EVALUATION OF ADJUNCTIVE SYSTEMIC DOXYCYCLINE WITH NON-SURGICAL PERIODONTAL THERAPY WITHIN TYPE 2 DIABETIC PATIENTS

The aim of a study realized by a group of researchers from King Abdulaziz University, King Saud University, Riyadh, Kingdom of Saudi Arabia was to evaluate the effects of systemic doxycycline on clinical and microbiological parameters of diabetic subjects with chronic periodontitis. The 9-month multi-center, randomized, parallel, single-blinded study was conducted from different hospitals in Riyadh, Saudi Arabia between april 2010 - december 2010. A total of 76 diabetic subjects with chronic periodontitis were randomized into 2 groups: control group (CG) received only scaling and root planing (SRP), and the treatment group (TG) receiving systemic doxycycline during the reevaluation visit 45 days after the completion of SRP. Probing pocket depth, clinical attachment level, gingival index, plaque index, and bleeding on probing were collected at baseline, 45 days after SRP, and one, 3, and 6 months after the use of systemic doxycycline. Microbiological analysis comprised the detection of *Tannerella forsythia* (Tf), *Aggregatibacter actinomycetemcomitans* (Aa), *Porphyromonas gingivalis* (Pg), and *Prevotella intermedia* (Pi) by polymerase chain reaction method. Sixty-eight (33 CG and 35 TG) subjects completed the study. Greater reduction in the population of Tf, Pg, and Pi were observed in TG compared with CG in the first month after the administration of systemic doxycycline. The TG showed a significant improvement in gingival index scores compared with the CG by the end of the first and 6 months after the administration of doxycycline. The conclusion of the study was that the adjunct systemic doxycycline can be associated with a reduction of Tf, Pg, and Pi in the first month after the administration of doxycycline with an improvement in the GI. (Al-Nowaiser AM, Al-Zoman H, Baskaradoss JK, Robert AA, Al-Zoman KH, Al-Sohail AM, Al-Suwyed AS, Ciancio SG, Al-Mubarak SA. Evaluation of adjunctive systemic doxycycline with non-surgical periodontal therapy within type 2 diabetic patients. *Saudi Med J* 2014 ; 35(10):1203-1209)

Irina Grădinaru

NUTRITIONAL FACTORS IN TRANSDIFFERENTIATION OF SCHELETAL MUSCLES TO ADIPOCYTES

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NUTRITIONAL FACTORS IN TRANSDIFFERENTIATION OF SCHELETAL MUSCLES INTO ADIPOCYTES (Abstract): A current area of interest is the determination of factors able to promote the transition from muscle to adipose tissue. The current review has highlighted that treatment of myoblasts with fatty acids (especially oleic acid) and thiazolidindiones causes conversion to adipocytes. The molecular mechanisms mediating the adipogenic action of thiazolidinediones and fatty acids in myoblasts could involve peroxisome proliferators-activated receptor-γ (PPARγ and CCAAT-enhancer-binding protein C/EBP . The role of 1,25-D_3 in adipogenesis is mediated at the molecular level through VDR-dependent inhibition of C/EBP and PPARγ expression and a decrease in PPARγ transactivation activity. Vitamin D supplementation increases muscle strength and ultimately reduces the incidence of falls. Additional research is needed to fully clarify the role of nutritional factors in adipogenesis. **Keywords**: TRANSDIFFERENTIATION, MYOGENESIS, ADIPOGENESIS, PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS, FATTY ACIDS, VITAMIN D.

Transdifferentiation is defined as the irreversible switch of one type of differentiated cell to another (1). Transdifferentiation is associated with a discrete change in the program of gene expression and there is a direct ancestor-descendant relationship between the two cell types. At molecular level, the cause of transdifferentiation is presumably a change in the expression of a master switch gene (selector or homeotic gene), whose normal function is to distinguish the two cell types in normal development (1).

TRANSDIFFERENTIATION OF SKELETAL MUSCLES TO ADIPOCYTES

Determination of the muscle and adipocyte lineages is controlled by a small number of tissue-specific transcription factors. In muscle, proteins MyoD and myf-5 play an important role in lineage determination, while the related factors myogenin and MRF4 function to execute the differentiation program (2). In adipocytes, differentiation appears to be controlled by two major factors or groups of factors: PPARs and the C/EBPs. However, a number of diverse lipids and lipid-like compounds can activate the PPARs, including fatty acids and synthetic arachidonate leukotrienes as well as drugs known to induce peroxisome proliferation.

Several pathologies of adult skeletal muscle are associated with increased in-

tramuscular adipose tissue. In the muscles of frail elderly people, muscle loss occurs and quality declines, with the accumulation of intramuscular fat and fibrosis impairing both contractile and metabolic function. Furthermore, there are also numerous diseases such as obesity and type 2 diabetes, as well as a range of muscular diseases where fibro-fatty accumulation occurs.

The essential role of satellite cells in muscle regeneration is well known, but it has been suggested that satellite cells, which are adult skeletal muscle stem cells can be diverted from the myogenic lineage and transformed into adipogenic cells under appropriate culture conditions *in vitro* and also *in vivo* (3).

Recently, a population of undifferentiated 'fibro-adipogenic' precursors (FAPs) isolated from mouse skeletal muscle was reported to differentiate into fibroblasts or adipocytes (4). Fatty acids (FAs) and adipocyte-inducing medium (AIM) treatment of human muscle fibroblasts induced a strong upregulation of PPARγ and C/EBP expression, and an extensive accumulation of lipid droplets (3). By contrast, when myogenic cells were exposed to AIM they failed to upregulate expression of either PPARγ or C/EBP , and did not accumulate lipid droplets. In another experiment, the human muscle-derived stem cells exposed to FAs and AIM accumulated some lipid, but showed no evidence of adipogenic differentiation (3).

BIOLOGIC IMPLICATION OF TRANSDIFFERENTIATION OF SCHELETAL MUSCLES TO ADIPOCYTES

The formation of muscle, bone, and adipose tissue are complex processes involving the determination of common precursor cells towards specific differentiation pathways. A current area of interest is the determination of factors that are able to promote the transition from one differentiated lineage to another, in particular, the transdifferentiation of muscle to adipose tissue. The imbalance between adipose and muscle mass is a hallmark of many disorders, including type 2 diabetes, obesity, and sarcopenia. Skeletal muscle and adipose tissue are key players in the development of insulin resistance, manifested by decreased insulin-stimulated glucose transport and metabolism in these tissues. It is known that myogenic cell lines and muscle satellite cells are capable of transdifferentiating to adipocytes, among other cell types (2). Thus, the study of transdifferentiation of muscle tissue to fat is highly applicable to the study of disorders where muscle atrophy and increase in adipose tissue mass are correlated.

Increased amounts of intramuscular adipose tissue are desirable in meat animals and are of interest to meat scientists. Raising lipid synthesis through feed is not economical (5). Therefore, assuming that myoblasts and adipoblasts have similar germinal origins (6), the possibility of transdifferentiation was hypothesized and tested to generate marbling or intramuscular adipogenesis.

PATHWAYS OF TRANSDIFFERENTATION OF MYOBLST TO ADIPOCYTE

The development of skeletal muscle is controlled by the muscle regulatory factors, such as myf-5, myogenin, and MyoD (7). Fusion of myocytes into multinucleated myotubes is the end step of muscle differentiation. In vitro, the major steps in muscle differentiation can be reproduced with myoblastic cell lines, such as C2C12 murine myoblast cells (8). Differentiation of

adipocytes appears to be controlled PPARγ and the C/EBP families of transcription factors (9). PPARγ, a nuclear hormone receptor, is expressed primarily in adipose tissue, and is induced early in the process of adipose differentiation. Fatty acids, natural and synthetic agonists of PPARγ, and vitamin D deficiency are known to promote adipogenesis (Fig. 1). These factors activate PPARγ resulting in the upregulation of adipocyte-specific genes, such as adipocyte lipid-binding protein 2 (aP2), Glut4, and fatty acid transporter. The mitogen-activated protein (MAP) kinase signaling pathways are crucial for the processes of myogenesis and adipogenesis (7).

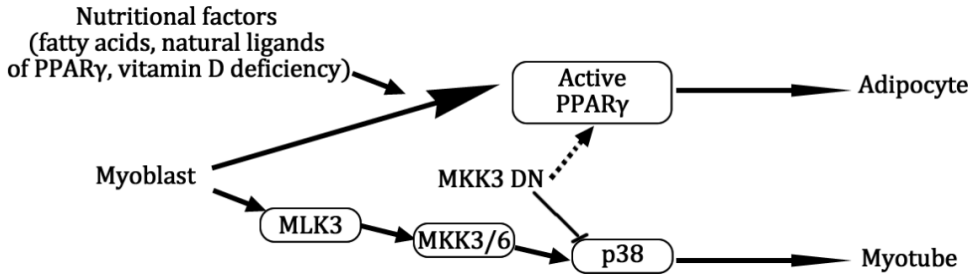

Fig. 1. This diagram summarizes the nutritional factors involved in transdifferentiation of myoblast to adipocyte [adapted from (7)]

Myoblasts and adipocytes arise from the same germ layer of the embryo, the mesoderm. Therefore, Li and collaborators hypothesized that it is possible to induce a direct conversion of myoblasts to adipocytes (6). Using a tissue model of myogenesis (G8 myoblasts), they demonstrated that these cells can differentiate spontaneously to myotubes when cultured in medium containing fetal calf serum. Subsequently, studies revealed that the expression of transcription factors such C/EBPα and PPARγ during the differentiation of G8 myoblasts can suppress the muscle- specific transcription factors (2). In addition, upregulation of PPARγ and subsequent activation of C/EBPα, β, and γ can stimulate adipogenesis in fibroblasts in the presence of PPARγ ligand (10). Thiazolidinediones (TZD), synthetic activators of PPARγ, are considered potent stimulators of adipogenesis and are presently used to reduce hyperglycemia in type 2 diabetes.

FACTORS IN TRANSDIFFERENTIATION OF SKELETAL MUSCLES TO ADIPOCYTES

Fatty acids. Inclusion of food rich in saturated fats or linoleic acid (LA) and n−6 polyunsaturated fatty acids (n−6 PUFAs) in the daily diet leads to insulin resistance (11). Many studies have also demonstrated that cis-unsaturated, nonesterified fatty acids, such as oleic acid (OA) and LA, exert a proliferative effect in cultured vascular smooth muscle (12, 13). OA induces proliferation of rat aortic smooth muscle cells by activating protein kinase C (13). These findings imply that fatty acids may play an important role in regulating skeletal muscle growth.

Hurley *et al.* have reported the pro-differentiation effects of fatty acids on

skeletal cell differentiation *in vitro* (14). On myoblast cell lines (rat cell line L6), the fatty acids showed different effects on muscle differentiation. The experiments indicated that inhibition of differentiation by the trans10, cis12 (t10, c12) isomer of conjugated LA (CLA) was dose-dependent (up to 200 mM) and may be via increased cell proliferation. LA and c9, t11 CLA stimulated differentiation at low concentrations (up to 50 mM), but inhibited differentiation at high concentrations (200 mM). In contrast, OA stimulated differentiation at all concentrations, whereas the saturated fatty acid, palmitic acid, had no effect. The mechanism appeared not to involve either PPAR or γ. The data suggest that only unsaturated fatty acids have an effect and the presence or absence of a cis-9 double bond may be important.

Natural agonist of PPARγ and thiazolidinediones. PPARs are members of the nuclear receptor superfamily that regulate the gene expression of proteins involved in energy, glucose and lipid metabolism, adipocyte proliferation and differentiation, and insulin sensitivity (15). PPARγ regulates fatty acid storage and glucose metabolism. The genes activated by PPARγ stimulate lipid uptake and adipogenesis by fat cells. The study of the natural agonist of PPARγ is in its infancy. The potential natural ligands of PPARγ are several unsaturated fatty acids, such as 15-hydroxiecosateraenoic acid, 9- and 13-hydroxy-octadecadienoic acid , 15-deoxy delta 12,14 prostaglandin J2, and PGJ2 (16).

Thiazolidinediones (TZDs) are an important class of synthetic agonists of PPARγ. TZDs are antidiabetic agents that target adipose tissue and improve insulin sensitivity, and they are currently used in the treatment of type 2 diabetes.

TZDs promote the transition from myogenic lineage to adipogenic lineage. The transition of porcine satellite cells from myogenic lineage to adipogenic lineage under the influence of ciglitizone or pioglitazone (TZDs), the adipogenic mixture, or both was observed (17). Similarly, the transition of C2C12 myoblasts to adipoblasts upon rosiglitazone (TZD) treatment (7) has also been reported. TZDs have been reported to have physio-logically relevant effects on semi-tendinosus muscle of postnatal pigs in potentiating marbling or intramuscular adipogenesis (18).

1,25 dihydroxy vitamin D_3. 1,25-Dihydroxyvitamin D (1,25-D_3), the active form of vitamin D, is widely recognized for its regulation of calcium and phosphate homeostasis in relation to bone development and maintenance and for its calcemic effects on target organs, such as intestine, kidney, and parathyroid glands. Emerging evidence has shown that vitamin D administration improves muscle performance and reduces falls in vitamin D-deficient older adults. However, little is known of the underlying mechanism or the role 1,25-D_3 plays in promoting myogenic differentiation at the cellular and/or molecular level.

Vitamin D, a fat-soluble secosteroid prohormone, is obtained from sun exposure or from dietary sources. 25-Hydroxivitamin D_3 is biologically inactive and is converted primarily in the kidney by the 25-hydroxyvitamin D-1-hydroxylase to its biologically active form 1,25-D_3 or calcitriol. Activation then enables 1,25-D_3 to bind to the vitamin D receptor (VDR), a type of nuclear receptor, and thereby regulates transcription of a number of vitamin D target genes, which is thought to be the

main mechanism of action of vitamin D.

VDR is expressed in human muscle tissue, which provides a rationale for a direct role of vitamin D in muscle function. Muscle biopsies in adults with severe vitamin D deficiency showed predominantly type II (fast-twitch) muscle, which may help explain the falling tendency of vitamin D-deficient elderly individuals (19). It has been reported that 1,25-D_3 induces genomic effects, leading to the synthesis of new proteins that affect muscle cell contractility, proliferation, and differentiation (20). Furthermore, mice lacking VDR show a skeletal muscle phenotype with smaller and variable muscle fibers and persistence of immature muscle gene expression during adult life, suggesting a role of vitamin D in muscle development (21).

Recent findings suggest that low (deficient) levels of 1,25-D_3 (i.e. 10^{-13} M) may actually enhance adipogenic trans differentiation (on murine C2C12 muscle cell line), whereas high (sufficient) levels (i.e. 10^{-9} M) inhibit adipogenesis, thereby potentially impacting on fat infiltration and muscle function (22). This increased accumulation of fat with low physiological concentrations (10^{-13} and 10^{-11} M) was associated with a sequential up-regulation of PPAR□2 (Pparg) and Fabp4 mRNA, indicating formation of adipocytes, whereas higher concentrations ($\geq 10^{-9}$ M) reduced all these effects. The nanomolar concentrations of 1,25-D_3 could inhibit adipogenesis and reduce the accumulation of triacylglycerol by 50% compared to fully differentiated control cells (23). In addition, treatment of preadipocytes with other vitamin metabolites, such as 24,25-dihydroxyvitamin D, could also inhibit preadipocyte differentiation, but at higher concentrations than 1,25-D_3 that paralleled their reduced affinity for the vitamin D receptor. This finding is consistent with the idea that any influence of vitamin D on adipogenesis would likely be exerted early in the preadipocyte to adipocyte transition when more VDR was available.

Recent studies, predominantly using the mouse 3T3-L 1preadipocyte cell culture model, have shown that the role of vitamin D in inhibiting adipogenesis is mediated at the molecular level through a VDR-dependent inhibition of C/EBP and PPARγ expression and a decrease in PPARγ transactivating activity in the preadipocyte (24). Also, in the presence of 1,25-D_3, VDR blocks adipogenesis by down-regulating both C/EBP mRNA expression and C/EBP nuclear protein levels at a critical stage of differentiation. In contrast, in the absence of 1,25-D_3, the unliganded VDR appears necessary for lipid accumulation, since knock-down of the VDR using siRNA both delays and prevents this process (25).

CONCLUSIONS

A current area of interest is the determination of factors that are able to promote the transition from muscle to adipose tissue. The change of skeletal muscle to adipocyte could be associated with certain diseases and may also be useful in the early diagnosis (e.g., diagnosis of insulin resistance), in the treatment of the disease itself (reversing the transformation of muscle to adipocyte in the development of sarcopenia), or in improving the meat quality by generating marbling or intramuscular adipogenesis in cattle and pigs. Nutritional factors, such as fatty acids or vitamin D deficiency, could modulate the expression of the genes involved in adipogenesis.

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NEWS

CLINICAL EFFICACY OF POLYSPECIFIC INTRAVENOUS IMMUNOGLOBULIN THERAPY IN PATIENS WITH STREPTOCOCCAL TOXIC SHOCK SYNDROME

Streptococcal toxic shock syndrome (STSS) and necrotizing fasciitis are the 2 most severe invasive manifestations caused by group A *Streptococcus* (GAS). Intravenous immunoglobulin (IVIG) therapy has been suggested as adjunctive treatment with a beneficial effect on mortality. A study made by Linner et al aimed to document the clinical efficacy of administered IVIG therapy in a comparative observational study of well-defined patients with STSS. The study involved patients with STSS prospectively identified in a nationwide Swedish surveillance study conducted between April 2002 and December 2004. Detailed data on symptoms, severity of disease, treatment, and outcome were obtained from 67 patients. Twenty-three patients received IVIG therapy compared with 44 who did not. No significant difference in comorbidities, severity of disease, organ failures, or sex was seen, but the IVIG group was slightly younger and had a higher degree of necrotizing fasciitis (56% vs 14%). The primary endpoint was 28-day survival. Adjusted analysis revealed that factors influencing survival in STSS were Simplified Acute Physiology Score II (odds ratio [OR], 1.1; $P = .007$), clindamycin (OR, 8.6; $P = .007$), and IVIG (OR, 5.6; $P = .030$). This comparative observational study of prospectively identified STSS patients demonstrates that both IVIG and clindamycin therapy contribute to a significantly improved survival in STSS. (Linnér A, DarenbergJ, Jan SjölinJ, Normark BH et al.Clinical efficacy of polyspecific intravenous immunoglobulin therapy in patients with streptococcal toxic shock syndrome: A comparative observational study. *Clin Infect Dis*. (2014) 59 (6):851-857).

Ecaterina Enache

MALIGNANCY AND MORTALITY OF COLORECTAL POLYPS

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MALIGNANCY AND MORTALITY OF COLORECTAL POLYPS (Abstract) **Objectives**: To evaluate the rate of morbidity and mortality associated with colorectal polyps after the next 8-years period of endoscopic polypectomy, in a high risk managed care population. **Material and method:** Cohorts of 77 subjects with benign neoplasms were identified with a colonoscopy in 1999. Three groups of subjects: benign neoplasms with polypectomy, benign neoplasms without polypectomy, and no neoplasms were evaluated. Five years recurrence rates (1999-2004) of benign or new malignant colorectal neoplasms were identified: for the benign determined for the baseline benign neoplasms with polypectomy and no neoplasm groups neoplasm without polypectomy, only rates for malignancy were observed. Malignancy was evaluated with immunohistochemical p53 (tumor protein 53) and PCNA (Proliferating Cell Nuclear Antigen) staining pattern. Over the next 8 years 2004-2012 were evaluated the mortality and the recurrence rate of the benign polyps. **Results**: 77 subjects were enrolled in our study; 71.4% were diagnosed with benign and 2.5% with malignant neoplasms. The 5-years cumulative incidence rates of malignant colorectal neoplasms in the no neoplasm (n=20) and benign neoplasm groups (n=55) were (n=1) 5% and (n=10) 18.1%, respectively ($p < 0.005$). A lower 5-years malignancy rate was observed in benign neoplasms group with polypectomy (12%) compared to the benign neoplasm group without polypectomy (33.3%) ($p < 0.05$). The 8-years mortality rate was compared into benign recurrent polyps group and into malign group: the lower 8-years mortality rate was observed into benign polyp no neoplasm group (0%) and into benign recurrent polyps group (40%); the highest rate was observed into neoplasm group (100%). **Conclusions**: The high recurrence rate of benign colorectal neoplasms and a higher incidence of colorectal cancer in subjects at high risk-history of benign colorectal neoplasm-highlight a healthcare opportunity for surveillance and/or interventions to reduce the morbidity associated with colorectal neoplasms. **Keywords**: COLORECTAL POLYPS, POSTPOLYPECTOMY RECURRENCE, BENIGN POLYPS, PCNA, p53.

Over one million new cases of colorectal cancer (CRC) are diagnosed worldwide each year, and incidence seems set to rise with the progressive westernization of lifestyles in Asian and African populations.

Many colorectal cancers are thought to arise from adenomatous polyps in the co-

lon, defined as circumscribed lumps of epithelial dysplasia with uncontrolled crypt cell division. Single transformed cells may generate clones through new mutations, with the possibility of an enhanced growth rate. The present study was specifically designed to determine the recurrence rate of benign colorectal neoplasms in a managed care population over a 5-years period of follow-up. By using this defined population, the study also provided us the opportunity to estimate and compare the rate of development of malignant neoplasms in subjects' postpolypectomy and in subjects without polypectomy over 8 years following period postpolypectomy.

MATERIAL AND METHODS

77 subjects diagnosed with colonoscopy were categorized into three groups: benign neoplasms, malignant neoplasms, and no neoplasms. Subjects with a malignant neoplasm at baseline were excluded from the study. Thus, the remaining subjects had either benign neoplasms or no neoplasms detected based on the baseline colonoscopy. We further divided subjects with benign neoplasms at baseline into two groups: colonoscopy with polypectomy and colonoscopy without polypectomy (fig. 1). The subjects in the three diagnostic groups: no neoplasm, benign neoplasm with polypectomy, and benign neoplasm without polypectomy, were subsequently evaluated for up to 5 years. If at any time during the follow-up period a malignant neoplasm diagnosis was recorded, then that subject was excluded from subsequent follow-up. If at any time during the follow-up period a benign neoplasm was detected, then the subject was only evaluated further for estimating the rate of subsequent malignant neoplasm development.

For subjects who had no baseline neoplasms, we evaluated the subsequent incidence of benign and malignant neoplasms over the 5 years follow-up period. For subjects who had benign neoplasms at baseline and underwent polypectomy, we assessed the recurrence rates of benign neoplasms and the incidence of malignant neoplasms. For subjects who had benign neoplasms at baseline and did not undergo polypectomy, we evaluated the incidence of malignant neoplasms only; for this group the recurrence rate of benign neoplasms was not estimated. The 8 years malignancy rate was observed into benign recurrent neoplasm group *vs.* malign group. Malignancy was evaluated with immunohistochemical staining pattern of p53 and Proliferating Cell Nuclear Antigen (PCNA).

Chi-square analysis was conducted to check for differences in rates of benign and malignant colorectal neoplasms based on age and gender, malignancy in benign neoplasm subjects (with and without polypectomy) and, recurrence and incidence of benign neoplasms in subjects with benign neoplasm diagnosis and no neoplasm diagnosis at baseline, respectively. *EpiInfo* and *Statgraphics Plus 6.0* programs were used for statistical analyses; qualitative variables were compared by χ^2 test and Fischer's Exact Test (2-tailed, p-level < 0.05).

RESULTS

The percentage of subjects with benign and malignant neoplasms at baseline were 71.4 (n=55) and 2.5 (n=2), respectively. Males appeared to have a higher rate of benign (46% *vs.* 33%) ($p<0.001$) and malignant neoplasms (5.7% *vs.* 5.2%) ($p > 0.05$) than females. In general and as expected (7, 8) the rate of benign or malignant neoplasms increased with increasing

age ($p < 0.001$). Subjects without neoplasms (n=20) and benign neoplasms (n=55) at baseline colonoscopy were included in subsequent analyses.

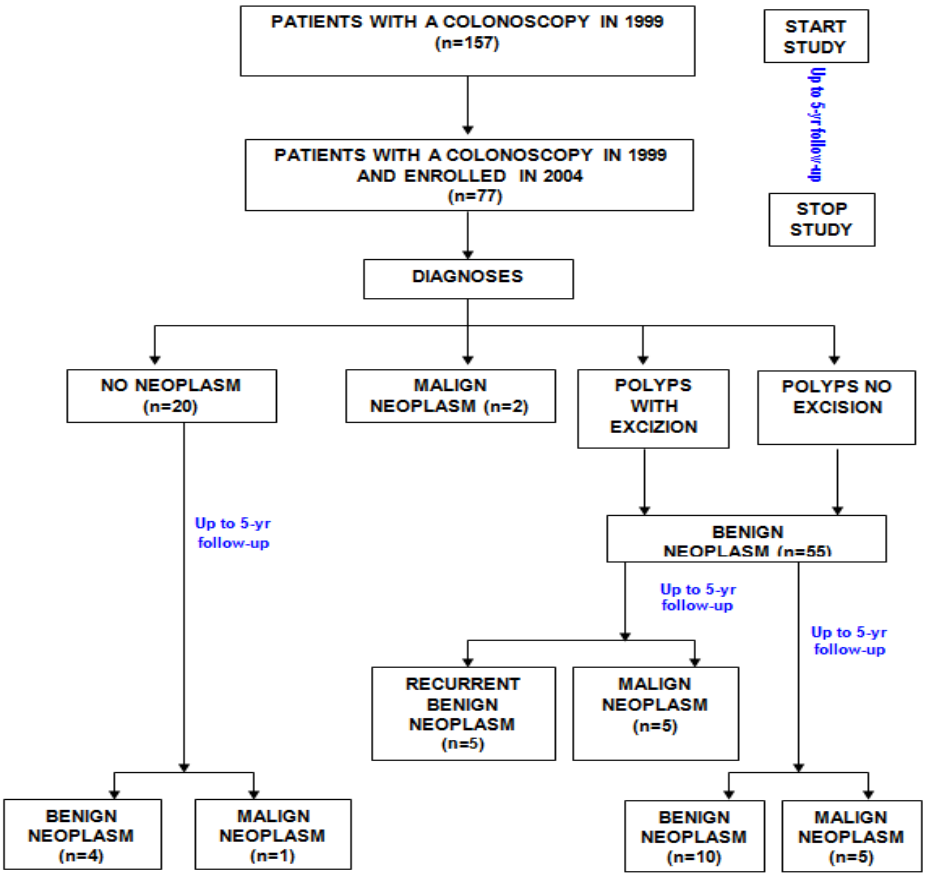

Fig. 1. Follow-up of Subject Cohort

Five-year diagnostic outcomes of these subjects are presented in table I. Among 20 subjects without neoplasm at baseline, the incidence rate of benign neoplasms ranged from 0 to 10 % for each year with a 5-year cumulative rate of 20%. In the same cohort, the incidence rate of malignant neoplasm ranged from 0 to 5 % for each year with a cumulative rate of 5%. It is interesting to note that of the 55 subjects with benign neoplasms at baseline (with and without baseline polypectomy), 10 subjects (18.1%) develop malignant neoplasms over the subsequent five years, which was significantly higher ($p<0.05$) than the rate observed in the subjects without neoplasms at baseline (5%). The highest rates of malign neoplasm follow-up from no neoplasm at baseline were observed during the 4th year of study (5%); in the 4th year of study was observed the maximum of benign neoplasm at follow-up from no neoplasm at baseline

(10%). The adenomatous polypoidal character and carcinomatous polypoidal character were established by immunohisto chemical staining for p53 and PCNA.

TABLE I

Five-year diagnostic outcomes of patients with no neoplasms baseline

Diagnosis at Colonoscopy	Years										Cumulative rate	
	1st		2nd		3rd		4th		5th			
	No	%	No	%	No	%	No	%	No	%	No	%
No neoplasm at baseline	20	100	20	100	19	95	17	85	16	80	20	100
Benign neoplasm at follow-up FROM No neoplasm at baseline	0	0	0	0	1	5	2	10	1	5	4	20
Malign neoplasm at follow-up FROM No neoplasm at baseline	0	0	0	0	0	0	1	5	0	0	1	5

Table II presents results for subjects entering the study with a baseline benign neoplasm (n=55 patients). For those subjects who had a baseline polypectomy (40 patients) the rates of recurrent benign neoplasms and the incidence of malignant neoplasms are reported. For subjects without baseline polypectomy (15 patients) only the rate of malignant neoplasms is reported. As can be seen, the yearly rates of benign neoplasm recurrence for the post-polypectomy group ranged widely from 5% to 12.5%; the highest rates were observed during the last two years. To assess the effect of polypectomy on the rate of subsequent malignancies, the rates of malignant neoplasms at follow-up were compared for the two groups: with and without polypectomy. As expected polypectomy was associated with a lower five year cumulative rate (20%) as compared to no polypectomy (73.3%) ($p < 0.05$). The 5-years cumulative incidence rates of malignant colorectal neoplasms in the no neoplasm (n=20) and benign neoplasm groups (n=55) were (n=1) 5% and (n=10) 18%, respectively ($p < 0.005$). A lower 5-years malignancy rate was observed in benign neoplasms group with polypectomy (12%) compared to the benign neoplasm group without polypectomy (33.3%) ($p < 0.05$).

In patients without polypectomy, the malignancy was evaluated by immunohistochemical staining pattern of p53 and PCNA; to patients with initial polypectomy (1999) immunohistochemical pattern of p53 and PCNA confirmed the malign neoplastic lesions.

Comparing the five year cumulative incidence of benign neoplasms in subjects without a baseline neoplasm (n=4; tab. I) to those with a baseline benign neoplasm with

polypectomy (n=15; tab. II) it is obvious that the 5-years cumulative recurrence rate of 40% was significantly greater ($p < 0.005$) than the 5% rate in subjects without baseline neoplasm. These data demonstrate the risk associated with a past history of benign tumors (high risk).

The 8 years (2004-2012) mortality rate was compared into benign recurrent polyps group, into benign group and into malign group: the lower 8-years mortality rate was observed into benign polyp no neoplasm group (0%) and into benign recurrent polyps group (40%); as expected the highest rate was observed into neoplasm group (100%) ($p < 0.005$) (fig. 2).

TABLE II

Five-year diagnostic outcomes of patients undergoing colonoscopy and with the diagnosis of benign neoplasms at baseline (with *v*. without polypectomy)

Benign Neoplasm at Baseline	Years									
	1st		2nd		3rd		4th		5th	
	No.	%	No.	%	No.	%	No.	%	No.	%
Colonoscopy with polypectomy	40	100	40	100	40	100	40	100	40	100
→ Recurrent benign neoplasm:	0	0	2	5	4	10	5	12.5	5	12.5
Colonoscopy with polypectomy	40	100	40	100	40	100	40	100	40	100
→ Malign neoplasm:	0	0	0	0	2	5	1	2.5	5	12.5
Colonoscopy without polypectomy	15	100	15	100	15	100	15	100	15	100
→ Malign neoplasm:	0	0	0	0	3	20	3	20.0	5	33.3

DISCUSSION AND CONCLUSIONS

In present, there are reported several studies with estimated rate of benign polyp recurrence with widely varying results. So, National Polyp Study (9), a prospective clinical trial, the reported cumulative rate of adenomatous polyp recurrence was 41.7% when subjects were examined with colonoscopy at one and three years and 32% when examined at only three years. Our results indicate a similar polyp recurrence rate (40%) (tab. II) over a 5 years' time frame; the estimate of the three-year polyp recurrence rate (15%) in our study was Whereas our results are numerically similar, it must be noted that the study populations and conditions for follow-up varied greatly between our study and the National Polyp Study. In National Polyp Study were included the subjects with adenomatous polyps only. Additionally, the National Polyp Study was a prospective trial with scheduled colonoscopies. In contrast, no such procedures were required in our analysis. Despite these very significant differences, our naturalistic retrospective study still was numerically similar in its findings. Our study did not explore the reasons for performing colonoscopy. Different reasons for colonoscopy (for "cause" *vs.* surveillance *vs.* screening) could influence and lead to different rates of colorectal neoplasm detection. In light of this limitation, the conclusions of this study are

limited to recurrence rates in subjects with a baseline neoplasm, without consideration to the clinical indication associated with the baseline colonoscopy.

To assess the effect of polypectomy on the rate of subsequent malignancies, the rates of malignant neoplasms at follow-up were compared for the two groups: with and without polypectomy. In our study (tab. II), as expected, polypectomy was associated with a lower five year cumulative rate (20%) as compared to no polypectomy (73.3%) ($p < 0.05$). Most of colorectal cancers that occur within 5 years after colonoscopic polypectomy develop because of failure to identify or completely remove high-risk neoplasms (advanced adenomas or cancers) at the time of the initial colonoscopy (10, 11). The adequacy of a polypectomy is assessed according to the endoscopic appearance of the polypectomy site and by a review of the pathological specimen to determine whether its margins are free of neoplastic tissue. The adenoma "miss rate" which can vary by a factor of 2 to 3 among examiners, is about 6 to 12% for adenomas that are 1 cm or larger and up to 25% for smaller adenomas (12, 13, 14). Thus, missed adenomas or cancers may contribute to the occurrence of colorectal cancer despite colonoscopic surveillance and may underlie many instances of "metachronous" neoplasia reported during surveillance. To be most effective, colonoscopy should be performed by well-trained, certified endoscopists who meticulously examine the entire colon during withdrawal of the instrument.

There is a higher rate of recurrence of benign and malignant neoplasm detected in our study population in the last two years. This may reflect early recurrence of symptoms, repeated examinations and the detection of neoplasms that may have been missed on the baseline evaluation. Additionally, and despite national guidelines to the contrary, these examinations may have been scheduled early for subjects thought to be at high risk. With increased surveillance a higher rate of recurrences may have been estimated in these shorter time frames.

Our study (fig. 2) indicated a higher rate of lesional prevalence to men of benign (46% *vs.* 33%) ($p < 0.001$) and malignant neoplasms (5.7% *vs.* 5.2%) ($p > 0.05$) than females. In general and as expected (7, 8) the rate of benign or malignant neoplasms increased with increasing age ($p < 0.001$). Similar results were reported from Jemal, Siegel and Ward that appreciated colonic adenomas, the precursors of almost all sporadic colorectal cancers, are found in up to 40% of persons by 60 years of age (1).

Although controlled trials have not compared surveillance intervals that are longer than 3 to 4 years, the low rate of colorectal cancer after 5 to 6 years of follow-up in patients with only one or two small, tubular adenomas on initial colonoscopy (15, 16, and 17) suggests that a follow-up interval of 5 or more years is safe for these patients.

The adenoma–carcinoma sequence the progression from normal colonic mucosa to small tubular adenomas to larger adenomas and those with more advanced histological features (villous features, high-grade dysplasia, or both) to cancer is a central tenet of our understanding and management of colonic adenomas. Although not all colonic polyps are adenomas (hyperplasic polyps account for about half of small, rectosigmoidian polyps) and more than 90% of adenomas do not progress to cancer, it is currently not possible to reliably identify those that will progress. Thus, colonic polyps identified at colonoscopy should be

removed if it is technically feasible to do so.

Complete removal of a colonic adenoma eliminates the risk of cancer from that adenoma, but the finding of a colonic adenoma may indicate an increased risk of metachronous adenomas and colorectal cancer for both the patient and his or her first-degree relatives (i.e., parents, siblings, and children). Colonic adenomas are typically asymptomatic and are most commonly found by means of colonoscopic or radiological imaging studies performed because of unrelated symptoms or for colorectal cancer screening. Since at least 25% of men and 15% of women who undergo colonoscopic screening by experienced endoscopists are found to have one or more adenomas, the cumulative burden of subsequent surveillance colonoscopy on the health care system is substantial.

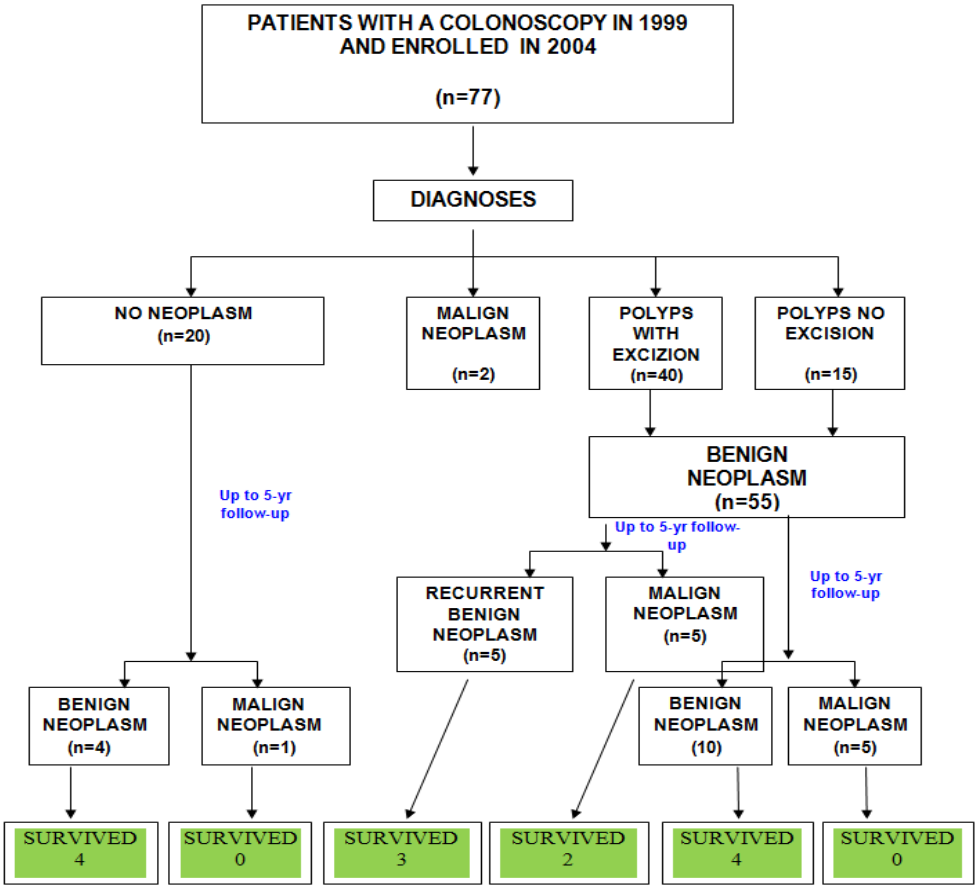

Fig. 2. 8-Year Follow-up of Subject Cohort

The high recurrence rate of benign colorectal neoplasms and a higher incidence of colorectal cancer in subjects at high risk-history of benign colorectal neoplasm-highlight a healthcare opportunity for surveillance and/ or interventions to reduce the morbidity associated with colorectal neoplasms. The active interventions for health

care providers can include adherence to colonoscopic guidelines for surveillance in high-risk populations and possibility the use of secondary chemoprevention to prevent the morbidity and mortality associated with colorectal neoplasms.

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THE EFFECTS OF TWO POLYMERIC MATRICES FOR INDOMETHACIN IN CUTANEOUS NOCICEPTIVE REACTIVITY IN MICE

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THE EFFECTS OF TWO POLYMERIC MATRICES FOR INDOMETHACIN IN CUTANEOUS NOCICEPTIVE REACTIVITY IN MICE (Abstract): **Aim**. This paper is focused on the investigation the effects of two polymeric matrices for indomethacin in cutaneous pain models in mice. There were used two different co-polymers polyhydroxyethyl methacrylate and undecan, polymerization reactions being conducted under nitrogen, at 80^0C. **Material and methods:** The experiments were carried out on white Swiss mice (20-25 g), divided into 6 groups of 7 animals each, treated orally. Biocompatibility properties of indomethacin-loaded co-polymeric matrices ware evaluated by assessing the effects on the blood parameters, the serum biochemical tests of animals treated. The nociceptive somatic testing was performed using hot plate assay and tail immersion test. The latency (second) response to paw, respectively tail thermal noxious stimulation, was measured before the experiment and 15, 30, 60, 90, 120 minutes, 4, 6, 8, 10, 12 hours after the substances administration. **Results:** Laboratory analysis did not show significant differences of blood parameters, serum biochemical tests between control mice group (IND) and groups treated with 1 M, 1 IND, 3 M, 3 IND. In our experimental conditions IND determined a significant increasing of the latency period response, in hot plate and also in tail immersion tests. Using two different co-polymers for indomethacin incorporation we obtained an increasing of the latency time pain reaction in hot plate assay, respectively in tail immersion test, statistically significant (*$p< 0.05$) compared with the simple co-polymers administration. **Conclusions:** We demonstrated that indomethacin co-polymeric matrices 1 IND and 3 IND determined similar immune responses with indomethacin and simple co-polymers after oral administration in mice, indicative of good *in vivo* biocompatibility. Oral administration of both 1 IND and 3 IND resulted in prolonged antinociceptive effects in hot plate assay and also in tail immersion test in mice. **Keywords:** INDOMETHACIN, POLYMERIC MATRICES, HOT PLATE, TAIL IMMERSION.

According to its colossal potential in medicine and medical technologies, the actual directions of nanomedicine are represented by the development of the basic sci-

ences experiments according to the multidisciplinary vision of the nanoscience, and training programs particularly for young researchers in this field. The importance of nanomedicine is represented by the opportunity of understanding the molecular and genetic level of pathophysiological mechanisms of the diseases, the improvement of pharmacotherapeutic management due to targeted action of drug loaded nanoparticulate systems, and reducing the side effects of substances. Nowadays the use of nanoparticulate formulations is under research in experimental studies and in clinical trials (1, 2). Nanoparticle drug carriers consists of solid biodegradable particles in size ranging from 10 to 1000 nm in which the substances is dissolved, entrapped or encapsulated, and/or to which the active principle is absorbed or attached (3). The development of analgesic drugs loaded nanoparticulate systems may represent a future challenge to realize promising agents for local drug delivery in inflammatory and painful conditions (4, 5).

The supposition that the utilization of various materials at the nanometer scale, will determine the improvement of different pharmaco-dynamic actions of active substances entrapped in them is based on different experimental reports indicating the possibility of reaching otherwise less accessible sites in the body and also the sustained release of various drugs (3, 4).

To directly prove this hypothesis we investigated the effects of two polymeric matrices for indomethacin in hot plate and tail immersion tests. The performed studies are significant because they offer a fundamental perspective on the self-assemblage processes from the bio-molecular systems and also represent a basis for controlled release applications which can be valuable for the biomedical and pharmaceutical fields.

We aimed to investigate the effect of two polymeric matrices for indomethacin in two different experimental cutaneous pain models in mice.

MATERIAL AND METHODS

Materials: 2 - Hydroxyethyl Methacrylate (HEMA) (from Fluka, purity>96%) was purified by passing it through an inhibitor removal column. The inhibitor - remover replacement packing (for removing hydroquinone (HQ) and hydroquinone monomethyl ether (MEHQ) was purchased from Aldrich. 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane (U) (Aldrich, 98%), sodium lauryl sulfate ($C_{12}H_{25}O_4SNa$) – (SLS) from Sigma (c > 95 wt %), PAV (polyvinyl alcohol) and the radical initiator 4,4'-azobis(cyanopentanoic acid) (ACPA) (Fluka, 98%), were used without further purification. In all experiments it was used twice distilled water which contained no foreign ions. The water used in all experiments was purified using an Ultra Clear TWF UV System.

Male white Swiss mice (20-25g) were used. Lighting was on a 12-h light/dark cycle (lights on at 6:00 a.m.), with standard laboratory food and tap water freely available, except during the time of the experiments. Before the experiment, mice were placed on a raised wire mesh, under a clear plastic box and allowed 2 hours to acclimate to the testing room.

Methods: The drug powder (about 10 wt% of the copolymers weight) was dissolved in ethanol/phosphate buffer solution (pH=7.2) 50:50. The obtained drug concentration in the initial loading solution was $c=1.9005\times10^{-5}$ mol/ml. The co-polymers (approximately of the same weight) were immersed in this solution and swollen to

equilibrium. Polymerization reactions were conducted under nitrogen atmosphere (nitrogen purged to remove oxygen), at 80^0C in a constant temperature bath, with a mechanical stirring rate of 180 rpm (6). The swollen drug-loaded samples were then dried at ambient temperature for several days to constant mass and used for the release experiments. After synthesis the polymeric particles were precipitated three times with methanol from water solution and finally freeze-dried by lyophilization during 24 hours (7).

The experiments were carried out on white Swiss mice (20-25 g), divided into 6 groups of 7 animals each, treated orally (using an eso-gastric device) as follows groups: I (Control): 0,1ml/10g weight; II (IND): indomethacin 5mg/kbw (IND); III (1 M): polyhydroxyethyl methacrylate (HEMA) 10% + polyvinyl alcohol (APV); IV (1 IND): HEMA 10% + APV + IND 5mg/kbw; V (3 M): HEMA 10% + undecan 3,5% + APV; VI (3 IND): HEMA 10% + undecan 3,5% + APV + IND 5mg/kbw. The experiment was performed according to the "Grigore T. Popa" University of Medicine and Pharmacy guidelines, for handling of experimental animals, also to the recommendations and policies of the International Association for the Study of Pain. Each animal was used once only and sacrificed immediately after the experiment (8).

RESULTS AND DISCUSSION

Biocompatibility evaluation. We evaluate the acute toxicity of indomethacin-loaded co-polymeric matrices, by assessing the effects on the blood count, the serum biochemical tests (glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT) and lactic dehydrogenase (LDH) activity) and on some immune system parameters (serum opsonic capacity (OC), phagocytic capacity (PC) and bactericidal capacity (BC) of peritoneal macrophages). Data were expressed as mean ± S.E.M. and statistically analyzed using t-student of Windows EXCEL program test.

Hot plate test. The nociceptive somatic testing was performed using hot plate assay to determine the latency of nocifensive reactions evoked by thermal heat stimuli applied on the paws. In this test, animals were individually placed on a hot plate maintained at a constant temperature ($55+0.3^o$C). The latency to first sign of hind paw licking or jump response to avoid heat nociception was taken as an index of nociceptive threshold (9). Pre-dosing latencies (seconds) were measured before administration of any drug or vehicle. The baseline latency (before drug injection) in hot plate test was 4.2±0.2 seconds (mean ± standard error of mean - SEM) (10). The recommended cut-off time of 12 seconds was used to prevent tissue damage. Latency time response is measured at 15, 30, 60, 90, 120 minutes, 4, 6, 8 hours after the substances administration. Differences between the experimental and baseline latencies are interpreted as an index of analgesia. Increases in the latency time response for the mouse to avoid thermal stimulus are indicative of analgesia, while decreases in the latency reaction are indicative of hyperalgesia (11). Response latency data from paw reaction latency were converted to per cent of maximum possible effect (%MPE) according to the formula: %MPE = [(observed latency-baseline latency)/(cut off time–baseline latency)] × 100 (12).

Tail immersion test. The effects of these two polymeric matrices for indomethacin were assessed in tail immersion test. The apparatus consists of a water bath heated to

a nociceptive temperature of approximately 54°C. The animal is placed into individual restraining devices leaving the tail hanging out freely. The lower 5 cm segment of the tail is immersed in the bath hanging out freely. The lower 5 cm segment of the tail is marked. This part of the tail is immersed in the water bath. Within a few seconds the animal reacts by flicking its tail and the latency to withdrawal the tail as a response to noxious thermal stimulation is measured by stop watch (9, 12). The baseline latency (before drug injection) in the tail immersion test was 5.5 ± 0.3 seconds (mean ± standard error of mean - SEM). The recommended cut-off time of 15 seconds was used to prevent tissue damage. Differences between the experimental and baseline latencies are considered as an index of analgesia. Prolongation of the reaction time to the typical tail-withdrawal reflex is an indicative of analgesia, while reduction in tail-flick latency corresponds to a hyperalgesic effect. The tail-withdrawal reactivity (seconds) was recorded before administration of any drug or vehicle. Latency time response was determined 15, 30, 60, 90, 120 minutes, 4, 6, 8, 10, 12 hours after substances administration. Response latency data from tail withdrawal measurements were converted to per cent of maximum possible effect (%MPE) according to the formula: % MPE = [(observed latency-baseline latency) / (cut off time – baseline latency)] × 100 (11).

In both experiments, results of time response from each group were calculated as mean ± standard error of mean (SEM) and significance was tested by *SPSS 13.0* Statistics and ANOVA method, followed by Neumann Keuls test as *post hoc*. *p*-values less than 0.05 are considered statistically significant comparing with those of control group. Laboratory analysis did not show significant differences of leucocyte formula elements, GOT, GPT and LDH levels, nor immune parameters (OC, PC, BC), between control mice group (IND) and groups treated with 1 M, 1 IND, 3 M, 3 IND relevant elements for a good *in vivo* biocompatibility. The behavioral reflex evoked by noxious heat stimuli is a relatively good predictor of pain sensitivity and for the study of the antinociceptive actions of different substances.

In our experimental condition in models of hot plate and tail-immersion in mice, which are measures of thermally-induced pain, the two polymeric matrices for indomethacin increased nociceptive pain threshold, actions that are prolonged comparing with the effects of simple drug administration. Statistical analysis of the results obtained in hot plate test shows that indomethacin (IND) determined a rapidly and statistically significant ($p<0.05$) increasing of the latency time period of the response, effect prolonged 4 hours after oral administration of the substance. Then the effect of indomethacin was gradually decreased ($p<0.05$) to 8 hours after its administration. The results of our experimental study are congruent with the literature communicated data, regarding the effects of the nonsteroidal antiinflammatory drug indomethacin, in this experimental cutaneous pain model in mice (13). The administration of co-polymers 1 M and 3 M did not influence the latency time response to thermal noxious stimulus in this cutaneous pain model. Using two different copolymers for indomethacin incorporation we obtained an increasing of the latency time pain reaction in hot plate test, statistically significant compared with simple copolymers (*$p< 0.05$) (fig. 1).

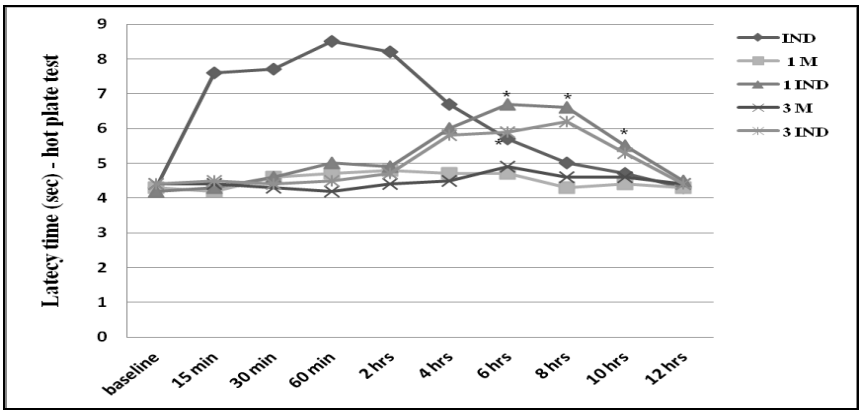

Fig 1. The latency time of IND, 1 M, 1 IND, 3 M, 3 IND response to thermal noxious paw stimulation, in hot plate test. Each point is the mean ± SEM of latency time (seconds) for seven mice. *p<0.05, **p<0.01 *vs.* control. Both 1 IND (6.7±0.1 after 6 hours, 6.6±0.7 after 8 hours, 5.5±0.3 after 10 hours) and 3 IND (5.9±0.7 after 6 hours, 6.2±0.9 after 8 hours, 5.3±0.5 after 10 hours) increases the latency time period, statistically (*p<0.05) significant *vs.* control.

Both 1 IND and 3 IND proved the retard release of the non-steroidal anti-inflammatory indomethacin from the synthesized polymer networks, 1 IND eliciting a higher effect than 3 IND in the somatic pain model used.

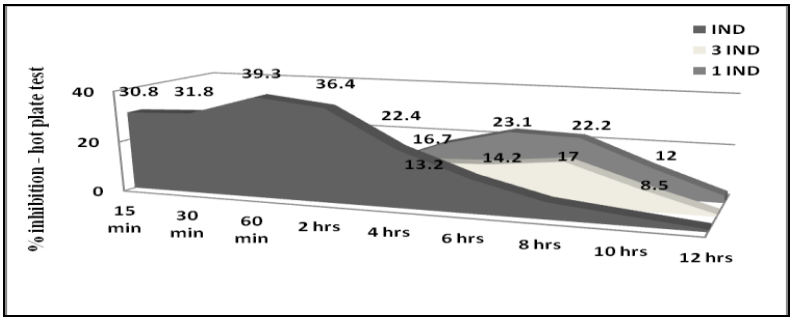

Fig. 2. Time course of the maximum possible effect (%MPE) of IND, 1 M, 1 IND, 3 M, 3 IND on the paw latency response in hot plate test. Each point is the mean ± SEM of percentage of maximum possible effect (%MPE) for seven mice.

Moreover, we used the percentage of maximum possible effect (%MPE), to quantified the intensity of antinociception which is important to corroborate and confirm these previous results. Indomethacin administration resulted in an increase in %MPE in hot plate assay, statistically significant in a first 2 hours after substance administration. The higher percent of inhibition was obtained in the interval between 60 minutes (39.3%±1.7) and 90 minutes (36.4%±2.3) in the experiment. Oral administration of 1 IND determined an increase of response latency in the hot plate

test, after 2 hours, effects prolonged around 8 hours, with a higher values in the interval between 6 hours (23.1%±1.3) and 8 hours (22.2%±2.5), after substance administration. 3 IND produced a significant increase of response latencies between 4 hours (12.2%±2.1) and 8 hours (17%±0.9) after substance administration, but less than submaximal level in the hot plate test (< 60% of MPE) (fig. 2).

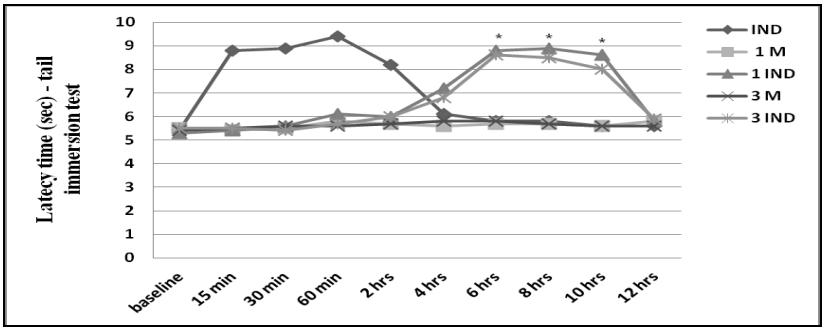

Fig 3. The latency time of IND, 1 M, 1 IND, 3 M, 3 IND response to thermal noxious tail stimulation, in tail immersion test. Each point is the mean ± SEM of latency time (seconds) for seven mice. *p<0.05, **p<0.01 vs control. Both 1 IND (7.2±0.7 after 4 hours, 8.8±0.3 after 6 hours, 8.9±0.5 after 8 hours, 8.6±0.9 after 10 hours) and 3 IND (6.8±0.3 after 4 hours, 8.6±0.5 after 6 hours, 8.5±0.1 after 8 hours, 8.0±0.7 after 10 hours) increases the latency time period, statistically (*p<0.05) significant vs control.

Indomethacin oral administration produced a rapidly and statistically significant (p<0.05) increasing of the latency time period of the response, effect prolonged 2 hours in the experiment. After this time its effect was gradually decreased (p<0.05) to 6 hours after its administration. These results are in concordance with data existing in literature, regarding the effects of indomethacin, in this experimental somatic pain model in mice (14). The treatment with 1 M and 3 M co-polymers did not influence the latency time response to thermal noxious stimulus in tail immersion test. The administration of co-polymers including indomethacin resulted in an increasing of the latency time response, statistically significant (*p< 0.05) compared with simple co-polymers (fig. 3).

Both 1 IND and 3 IND used demonstrated the prolonged release of the indomethacin from the polymer matrices. The effect of 1 IND was most intense than the effect of 3 IND in tail immersion model.

In these two experimental pain models used the retard release of the drug from the polymer network may be explicated as it follows: 2 - Hydroxyethyl Methacrylate is coupling indomethacin by hydrogen bonds through hydroxyl groups and release the active substance after bursting the physical links. The association of 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5]undecane facilitated the formation of both intra and intermolecular physical bonds and at the same time induced steric hindrances generated by the spiroacetal groups. Much more it functioned as cages which coupled and retained of the drug into the network meshes, slowing down of the bioactive substance release from them (15).

The treatment with indomethacin de-

termined an increase in %MPE statistically significant in a first 2 hours after substance administration, in tail immersion test. The higher percents of inhibition were obtained in the interval between 30 minutes (36.5%±0.9) and 60 minutes (41.7%±1.3) after substance administration. 1 IND administration resulted in a significant increase in inhibition percent between 6 hours (36.1%±1.2) and 10 hours (34%±0.7), but with a peak value 8 hours (37.1%±1.3) in the experiment. 3 IND produced an increase in inhibition percent after 2 hours, effects prolonged around 10 hours, with a higher values in the interval between 6 hours (32.6%±0.7) and 8 hours (31.6%±1.1), after substance administration (fig. 4).

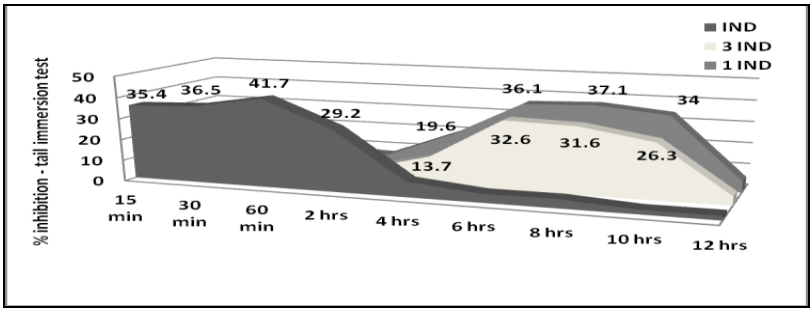

Fig. 4. Time course of the maximum possible effect (%MPE) of IND, 1 M, 1 IND, 3 M, 3 IND on the tail withdrawal response in tail immersion test. Each point is the mean ± SEM of percentage of maximum possible effect (%MPE) for seven mice.

CONCLUSIONS

We evaluated the *in vivo* biocompatibility and investigated the effects of different copolymeric metworks based on 2- Hydroxyethyl Methacrylate in association with 3,9-divinyl-2,4,8,10-tetraoxaspiro[5.5] undecane, using polyvinyl alcohol as protective colloid for indomethacin incorporation in somatic nociceptive reactivity in mice.

Indomethacin co-polymeric matrices 1 IND and 3 IND determined similar immune responses with indomethacin and co-polymers after oral administration in mice, indicative of good *in vivo* biocompatibility. The administration of both indomethacin co-polymeric matrices 1 IND and 3 IND proved antinociceptive effects in hot plate test in mice. Oral administration of these two co-polymeric networks including indomethacin 1 IND and 3 IND resulted in a significant and prolonged analgesic effect in tail immersion test.

The good *in vivo* biocompatibility and the antinociceptive evident effects of different copolymeric systems including indomethacin attests the possibility for using these compounds as innovative drug-carriers with applicability in biological and medical fields.

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NEWS

NEW APPROACHES AGAINST ANTIFUNGAL DRUG RESISTANCE

Fluconazole (FLC) is widely used in the treatment of candidiasis because of its efficacy and low toxicity. The emergence of FLC-resistant *Candida albicans* isolates and biofilm-associated infections pose a great challenge to antifungal treatment. According to Liu *et al.*, in order to increase the susceptibility of *C. albicans* to FLC different combinations of FLC with non-antifungal agents have been proposed, such as: calcineurin inhibitors, antibacterials, calcium homeostasis regulators, heat shock protein 90 inhibitors and traditional Chinese medicine drugs. The modes of action of these compounds and the mechanisms of their synergistic effects against resistant strains of *C. albicans* are: increased membrane permeability, reduced efflux of antifungal drugs, interference with intracellular ion homeostasis, inhibition of enzymes required for fungal survival, and inhibition of biofilm formation. The study concludes that the reversal of fungal resistance can be achieved through various mechanisms (Liu S1, Hou Y2, Chen X3.et al. Combination of fluconazole with non-antifungal agents: A promising approach to cope with resistant Candida albicans infections and insight into new antifungal agent discovery. *Int J Antimicrob Agents*. 2014 pii: S0924-8579(14)00002-8. doi: 10.1016/j.ijantimicag.)

Teodora Vremeră

THE BODY MASS INDEX IN RELATION WITH THE SELF-PERCEPTION OF WEIGHT AND THE BODY COMPOSITION FROM DIFFERENT POPULATIONAL GROUPS

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THE BODY MASS INDEX IN RELATION WITH THE SELF-PERCEPTION OF WEIGHT AND THE BODY COMPOSITION FROM DIFFERENT POPULATIONAL GROUPS (Abstract): The purpose of this paper is to analyze the self-perception of weight in relation with the body mass index and the body composition of three working groups composed of physically untrained young students, young athletes and patients diagnosed with chronic diseases. The working groups consisted of 110 participants: untrained students, athletes and dialysed patients; 50.9% women and 49.1% men; with an average age of 26.03. The BMI of the patients is significantly higher than the BMI of the athletes and untrained students. No significant differences were found between groups in terms of self-perception of weight. Compared to the physically untrained students and patients, the athletes have a significantly higher level of muscle tissue. The athletes also have a significantly lower adipose tissue than that of the physically untrained students and patients. **Keywords:** THE BODY MASS INDEX, SELF-PERCEPTION OF WEIGHT, BODY COMPOSITION

The contemporary world is experiencing a marked increase in obesity and obesity-related diseases while the history of obesity, analyzed in terms of healing, represents a history of failure (1-4). WHO recommends as a screening modality for overweight and obesity, the body mass index method (5-7). The subjective self-perception of weight, more or less overlapping the actual nutritional state, can trigger a genuine psychopathology at all age groups (8). The body composition is used by health, fitness, clinical and school medicine experts. Regarding weight management, the estimation of the total amount of fat and fat-free mass can be used to determine the actual weight, to help formulate recommendations for diet and physical activity, to monitor the changes in body composition of those who participate in weight reduction or body remodeling programs.

The purpose of this paper is to analyze the body mass index in relation to the self-perception of weight and the body composition of three working groups composed of physically untrained young students, young athletes and patients diagnosed with chronic diseases.

MATERIAL AND METHODS

Material: The working groups were composed of 110 participants, divided into 3 groups: a group of untrained students 70.9% (78), a group of young athletes

15.5% (17) and a group of dialyzed patients 13.6% (15). In the sample, the gender distribution was: 50.9% (56) women and 49.1% (54) men. The participants' age ranges from a minimum of 19 and a maximum of 72 years, with an average of 26.03 years and a SD equal to 12.95.

Methods:

1. Determining the BMI (the body mass index)

The body mass index is calculated using other anthropometric measures, i.e. the weight (Silver Crest electronic scale) and height (stadiometer), using the following formula: BMI = weight / height^2.

The standard BMI classification and the degree of risk for co morbidities are presented in table I.

TABLE I
The standard BMI classification

Classification	BMI (kg/m^3)	The risk for co-morbities
Underweight	< 18.5	Moderate
Normal	18.5 – 24.9	Medium
Overweight	25 – 29.9	Slightly increased
Obesity class I	30 – 34.9	Moderate
Obesity class II	35 – 39.9	Severe
Obesity class III	>40	Extreme

2. The self-perception of weight. It was conducted within a population-based survey using a CORT 2004 questionnaire concerning health risk behaviors.

3. Evaluation of certain body composition elements. In order to have a complete picture of the body composition, of insufficient or fat excess and/or muscle deficiency on different segments of the body, we conducted a comprehensive evaluation of body composition using a multi-frequency bioimpedance method (InBody 720).

4. Processing advanced medical statistics. We used version 18 (2010) of the *SPSS* programme and the Kruskal-Wallis and Mann-Whitney tests for nonparametric data.

RESULTS AND DISCUSSION

1. The body mass index (BMI). The BMI from the group of untrained students varies between 16.43 and 37.77, with an average of 22.76 and a SD of 3.95. The BMI from the group of student athletes varies between 17.82 and 28.12, with an average of 22.22 and a SD of 2.89. The BMI from the group of patients varies between 18.42 and 35.11, with an average of 28.32 and a SD of 4.21 (tab. II).

The participants in this study can be divided according to the BMI as follows. A 9% (7) of untrained students are underweight, 66.7% (52) are normal weight and 24.4% (19) are overweight or obese. In the student athletes' group, 5.9% (1) are underweight, 76.5% (13) are normal weight and 17.6% (3) are overweight or obese. In the patients' group, 6.7% (1) are underweight, 13.3% (2) are normal weight and 80.0% (12) are overweight or obese (fig. 1).

TABLE II

The characteristics of the BMI descriptive analysis according to each group

BMI		Untrained students	Student athletes	Patients
N	Valid	78	17	15
	Missing data	0	0	0
Average		22.76	22.22	28.32
Medium		22.09	21.36	29.84
Standard deviation		3.95	2.89	4.21
Minimum		16.43	17.82	18.42
Maximum		37.77	28.12	35.11

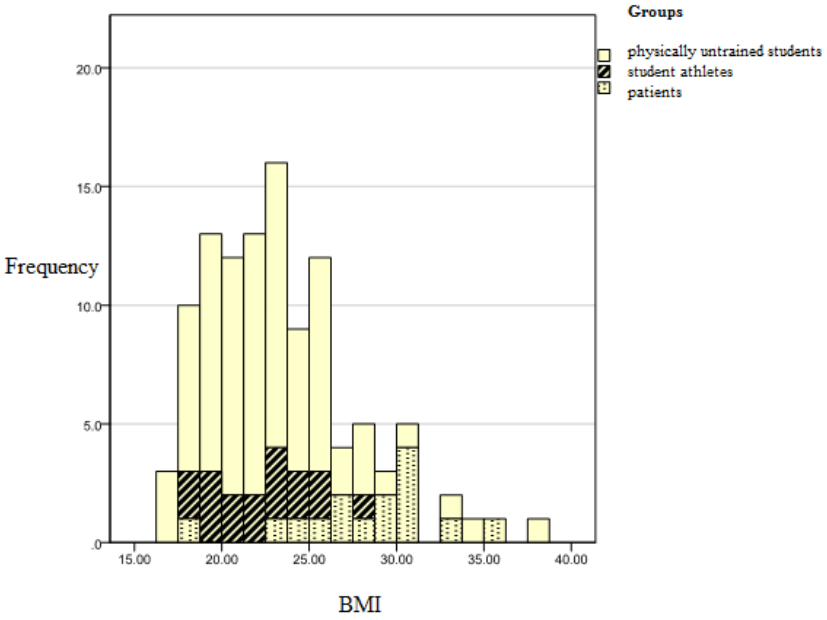

Fig. 1. The distribution of participants' histogram according to the BMI, for each group

2. The self-perception of weight. From the untrained students' group, 42.3% (33) consider themselves to be normal weight, 14.1% (11) think they are underweight and 43.6% (34) say they are overweight. In the student athletes' group, 58.8% (10) state they are normal weight, 17.6 (3) believe they are underweight and 23.5% (4) think they are overweight. In the patients' group, 46.7% (7) say they are normal weight, 6.7% (1) are underweight and 46.7% (7) believe they are overweight (fig. 2).

3. No significant differences were found between groups regarding the self-perception of weight, p>0.05. We found out that 59% of the untrained students, 58.9% of the athletes and only 33.3% of the patients correctly perceive their weight.

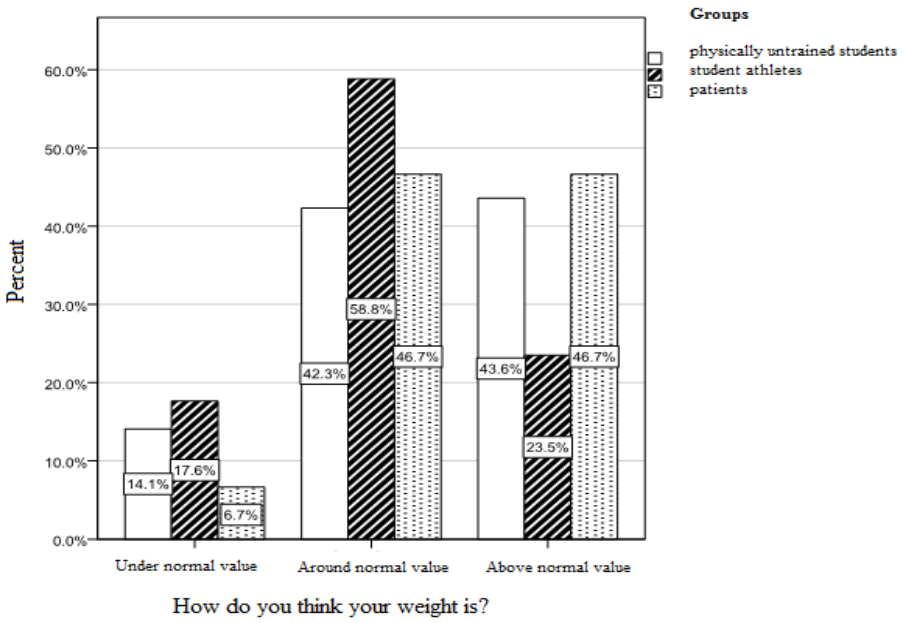

Fig. 2. The percentage distribution of participants according to their perception of weight, for each group

4. Body composition elements. ***The muscle tissue level*** is significantly different according to the perception of weight H (2) = 13.69, p<0.001. We have further investigated this difference and the level of statistical significance was reduced to 0,016 due to the Bonferroni correction. The muscle tissue level is significantly reduced at those who perceive themselves as overweight compared to those who consider their weight to be normal U=715, z=-3.05, p<0.01, respectively those who believe they are underweight U=173, z =-2.80, p<0.01. Among those who believe they are under normal weight and those who perceive themselves as normal weight, the differences in muscle tissue percentage are not statistically significant, p>0.016 (fig. 3).

The adipose tissue level is significantly different according to the perception of weight H(2)=25.43, p<0.001. We have further investigated this difference and the level of statistical significance was reduced to 0,016 due to the Bonferroni correction. The adipose tissue level is significantly higher at those who perceive themselves as overweight compared to those who consider their weight as normal U=610, z=-3.83, p <0.001, respectively those who perceive themselves as underweight U=99.5, z=-4.06, p<0.001. Those who perceive themselves as being under normal weight have a significantly lower adipose tissue level than those who perceive themselves as normal weight U=209.5, z=-2.57, p<0.01 (fig. 4).

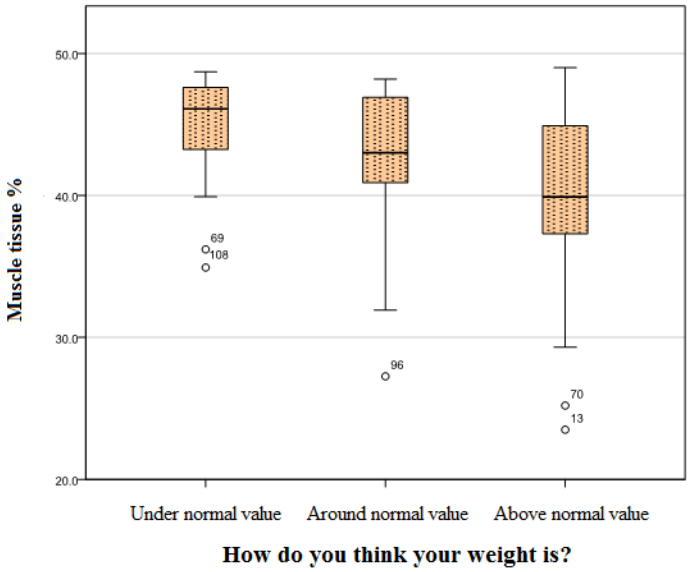

Fig. 3. The muscle tissue percentage distribution of the participants according to the perception of weight

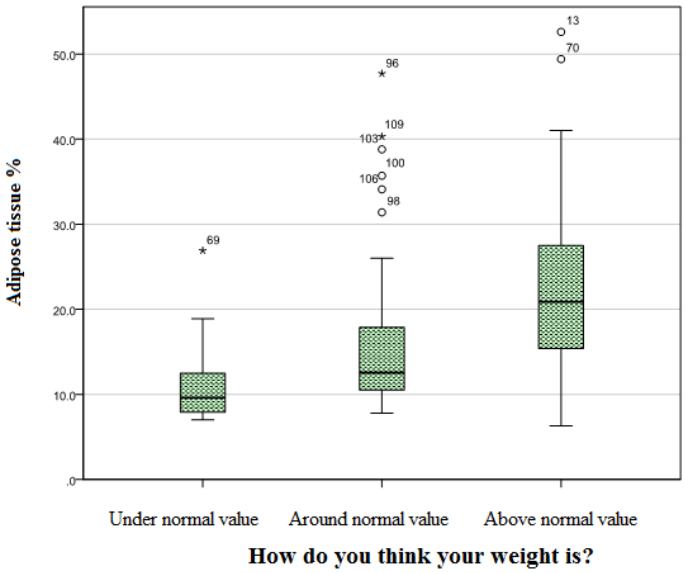

Fig. 4. The adipose tissue percentage distribution of the participants according to the perception of weight

Previous studies have shown that there is an association between the perception of weight and the obesity control methods, often independent from the actual weight

(9, 10).

In a recent study, Darlow et al. (11) have discovered that patients who perceived themselves as overweight and believed that being overweight is a health problem and who also had a family history of diabetes, had a higher risk of developing this disease. Patients with the same type of association, with obesity and a family history of cardiovascular diseases had higher chances of perceiving themselves as exposed to a higher development risk of various cardiovascular diseases.

CONCLUSIONS

The BMI of the patients is significantly higher than the BMI of the student athletes with a large size difference and with an average size difference than that of the untrained students. The differences between the group of untrained students and the student athletes group are not statistically significant, $p>0.05$. No significant differences between groups were found regarding the self-perception of weight.

Compared to the physically untrained students and patients, the student athletes have a significantly higher level of muscle tissue. The muscle tissue percentage of the patients is significantly lower than that of the physically untrained students. The muscle tissue percentage is positively correlated with the BMI in the student athletes' group and negatively correlated regarding the physically untrained students' group.

The student athletes have a significantly lower level of adipose tissue than that of the physically untrained students and patients. The adipose tissue percentage of the patients is significantly higher than that of the physically untrained students. The adipose tissue percentage is correlated with the BMI in the patients' group and the physically untrained athletes' group.

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NEWS

BORIC ACID IRRIGATION AS AN ADJUNCT TO MECHANICAL PERIODONTAL THERAPY IN PATIENTS WITH CHRONIC PERIODONTITIS

A group of researchers from the Department of Periodontology of Izmir University,Turkey realized a single-masked, randomized, controlled clinical trial whose purpose was to evaluate the effects of boric acid irrigation as an adjunct to scaling and root planing (SRP) on clinical and microbiologic parameters and compare this method with chlorhexidine irrigation and scaling and root planing alone in patients with chronic periodontitis. In the study were included forty-five systemically healthy patients with chronic periodontitis, divided into three groups: the first group *(C)* - scaling and root planing + saline irrigation; the second group *(CHX)* - scaling and root planing + chlorhexidine irrigation; the third group *(B)* - scaling and root planning + boric acid irrigation. A preclinical analysis was realized to determine an ideal concentration of boric acid. Clinical measurements (plaque index – PI , gingival index –GI , probing depth - PD, clinical attachment level - CAL, and bleeding on probing – BOP) were performed at baseline, 1 month, and 3 months after treatment. Subgingival plaque samples were also taken. Quantitative analysis of *Porphyromonas gingivalis, Tannerella forsythia,* and *Treponema denticola* was performed using real-time polymerase chain reaction (PCR) procedures. The concentration of boric acid was 0.75% in this study. All clinical parameters showed statistically significant reduction at all time points compared to baseline in all groups ($P < 0.001$). Whole-mouth PD and CAL reduction was similar in all groups at all time points after treatment ($P > 0.05$). The PD and CAL reductions for moderately deep pockets ($PD \geq 5$ and < 7) were greater in the B group compared to other groups between baseline and 1 month ($P < 0.05$). For deep pockets ($PD \geq 7$), reductions were similar in the B and CHX groups ($P > 0.05$). BOP (percentage) was significantly lower in the B group compared with the CHX and C groups in the first month after treatment ($P < 0.001$). GI and PI scores were significantly lower in the B and CHX groups compared with the C group at all time points after treatment ($P < 0.05$). The amounts of *Porphyromonas gingivalis, Tannerella forsythia* and *Treponema denticola* were significantly reduced in all treatment groups after 1 month ($P < 0.05$). No statistically significant differences were detected among the groups for microbiologic parameters at any time points after treatment ($P > 0.05$). The obtained data suggest that boric acid could be an alternative to chlorhexidine, and it might be more favorable because boric acid was superior in whole-mouth BOP as well as PD and CAL reduction for moderate pockets in early time periods. (Sağlam M, Arslan U, Buket Bozkurt S, Hakki SS. Boric Acid irrigation as an adjunct to mechanical periodontal therapy in patients with chronic periodontitis: a randomized clinical trial. *J Periodontol*, 2013; 84 (9):1297 – 1308).

Irina Grădinaru

INVERTED DUPLICATION DELETION OF 8P: CHARACTERIZATION BY STANDARD CYTOGENETIC AND SNP ARRAY ANALYSES

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INVERTED DUPLICATION DELETION OF 8P: CHARACTERIZATION BY STANDARD CYTOGENETIC AND SNP ARRAY ANALYSES (Abstract): Inverted 8p duplication deletions are recurrent chromosomal rearrangements that most often arise through non-allelic homologous recombination (NAHR) during maternal meiosis between segmental duplications made up of the olfactory receptor (OR) gene clusters. The presence of a paracentric inversion polymorphism in 8p23.1, found in ~26% of European population, may trigger meiotic misalignment and NAHR between the OR gene repeats. We report clinical, cytogenetic, and molecular findings in a 4 year 8 month-old female with concomitant inverted duplication and terminal deletion of chromosome 8p. The girl, the first child of unrelated parents, was born at term, by normal delivery, after an uneventful pregnancy. Clinical examination revealed dysmorphic features, pectus excavatum, hypertonia, severe developmental delay. Brain ultrasound and MRI showed agenesis of the corpus callosum without other abnormalities. Conventional cytogenetic analysis identified additional material on chromosome 8 at band p21. SNP array analysis further characterized the abnormality as a duplication of about 31.3 Mb, from 8p23.1 to 8p11.1, and additionally revealed a terminal deletion of about 6.8 Mb, from 8p23.3 to 8p23.1. Genomic microarray also identified a region of disomy between deletion and duplication. Chromosome analysis of both parents revealed normal results. Based on clinical examination, conventional cytogenetics and SNP array, we established the diagnosis of inverted duplication deletion of 8p. SNP array analysis precisely defined the breakpoints of rearrangement and, by identifying a region of disomy between the duplication and deletion, indicated that NAHR between segmental duplications was the most likely mechanism for this type of abnormality. **Keywords**: INVERTED DUPLICATION DELETION OF 8P, SNP ARRAY, AGENESIS OF CORPUS CALLOSUM.

Inverted duplication deletion of 8p [inv dup del(8p)] is a recurrent chromosomal rearrangement first described in 1976 by Weleber et al. (1). It has an estimated prevalence of about 1:20,000 newborns (2), around 50 cases being reported worldwide.

Clinical manifestations include: intellectual disability (ID), agenesis of the corpus callosum, minor facial abnormalities, congenital heart defects, hypotonia with a tendency to develop progressive hypertonia and severe orthopedic problems (3, 4).

Inverted duplications with terminal deletions on 8p most often arise through NAHR during maternal meiosis between segmental duplications made up of OR

gene clusters (OR-REPD and OR-REPP). The presence of a paracentric inversion polymorphism in 8p23.1, found in ~26% of the European population, may trigger meiotic misalignment and NAHR between the inverted OR gene repeats (5).

We report the clinical, cytogenetic, and molecular findings in a girl with concomitant inverted duplication and terminal deletion of chromosome 8p and compare the phenotype of the patient with the cases reported in the literature, highlighting the importance of microarray technique in the identification of genomic disorders and their underlying mechanisms.

CASE PRESENTATION

The patient, a 4 year 8 month-old female, was the first child of non-consanguineous, healthy parents (mother - 31 years old, father - 37 years old at birth of the proband). There was no family history of ID, congenital anomalies or psychiatric disorders. Pregnancy was uneventful. She was born at term, by normal delivery, with a birth weight of 3,150 g, length of 50 cm and head circumference of 33 cm; Apgar score was 9 both at 1 and 5 minutes; physical examination revealed left parietal cephalohematoma and hypotonia. She presented neonatal seizures and cyanotic crises. Echocardiography showed a patent foramen ovale. Abdominal ultrasound, routine biochemical and hematological tests, as well as ophthalmologic examination and TORCH test were normal. No hearing impairment was identified. Brain ultrasound and MRI revealed agenesis of the corpus callosum without other abnormalities.

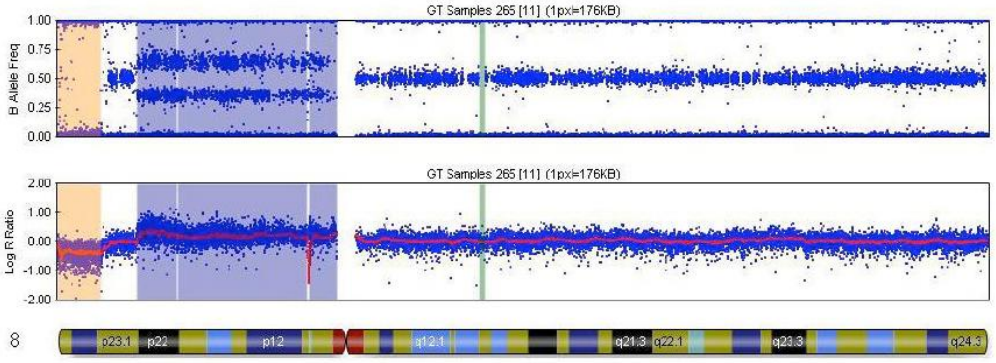

Fig. 1. SNP array results of chromosome 8 in the patient, showing a 6.8 Mb deletion in 8p23.3-p23.1 and a 31.3 Mb duplication in 8p23.1-p11.1

She achieved head control at 9 months and spoke her first word at the age of 12 months. Clinical examination at the age of 4 years and 8 months showed height of 106 cm (+0.43 SD), weight of 13.5 kg (-2.64 SD), and head circumference of 47 cm (-2.58 SD). She had facial dysmorphic features including square face, high forehead, hypoplastic zygomatic bones, large mouth, thin upper lip and everted lower lip, high palate and abnormal dental development, large ears, and dry, curly hair that receded from the temples. Other features were a short neck, pectus excavatum, slender arms and legs with long, tapering fingers, joint contractures in the legs and hips. She showed severe developmental and speech delay: she could not sit alone and she only said few single words.

Standard G banding chromosomal analysis identified an additional material on chromosome 8 at band p21: 46,XX,add(8)(p23.1). Parental karyotypes were normal. A SNP array was performed in University Medical Center Groningen using HumanCytoSNP-12 v 2.1 BeadChip platform (Illumina Inc., San Diego, CA) containing approximately 300.000 SNPs per sample. The data were processed using Genome Studio V 2010.1 software (Illumina). Genomic positions were defined using GRCh37/hg19. SNP array analysis detected a terminal deletion of approximately 6.8 Mb, from 8p23.3 to 8p23.1 (52,041-6,928,066), and a duplication of approximately 31.3 Mb, from 8p23.1 to 8p11.1 (12,599,114-43,951,038) (fig. 1). The deleted and duplicated regions contained 15 and 144 OMIM genes, respectively.

Based on clinical examination, conventional cytogenetics and SNP array, we established the diagnosis of inverted duplication deletion of 8p.

DISCUSSION

Our patient is severely affected compared with patients reported in the literature. The concomitant presence of deletion and duplication makes genotype/phenotype correlations difficult. Considering the size of the duplicated region and the large number of OMIM genes involved, the phenotypic findings in our patient are mainly due to duplication.

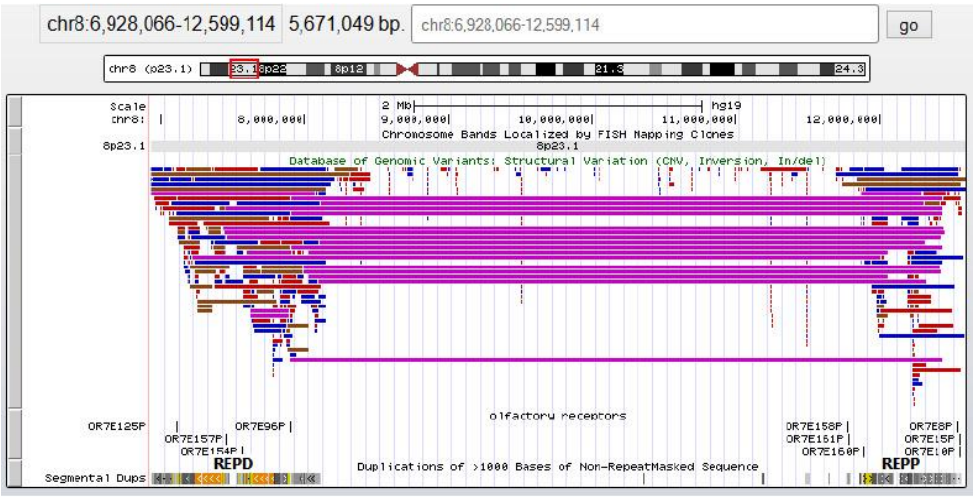

Fig. 2. Annotated screenshot of 5.6 Mb of band 8p23.1 (UCSC Genome Browser on Human Feb. 2009 Assembly (hg19), http://genome.ucsc.edu/). From bottom to top:
- segmental duplications that contain the OR repeats (labelled REPD and REPP);
- olfactory receptor genes based on The Human Olfactory Data Explorer (HORDE);
- multiple copy number variations of REPD and REPP in the Database of Genome Variants (sideways) and common polymorphic inversion between REPD and REPP (middle lines)

Three mechanisms may explain the formation of this abnormality: recombination within a paracentric inversion, recombination between inverted low copy repeats, or U-type exchange following a double strand break. All three mechanisms involve the formation of a dicentric chromosome 8 which subsequently breaks ei-

ther during a meiotic division or during early stages of embryonic development to produce an inv dup del(8p). Observation of a region of disomy between duplication and deletion can distinguish the U-type exchange from the other two mechanisms. The inv dup del(8p) in our patient can be explained by the second mentioned mechanism because the duplication was separated from the 8pter deletion by a ~5.6 Mb single copy region flanked by OR-REPD and OR-REPP regions (fig. 2). Stabilization of the broken chromosome ends can be achieved by: direct addition of telomeric repeats (''telomere healing'') (6); ''telomere capture'', in which broken chromosomes obtain the telomeric end of another chromosome (7); or by formation of a ring chromosome (8). Using high-resolution microarray no additional duplications could be identified, therefore in our patient the broken chromosome end of 8p has been stabilized by telomere healing.

The rearrangement is *de novo* as both parents had normal karyotypes. Dual colored FISH can be performed in the parents to investigate whether the meiotic recombination event among the OR gene clusters could have been stimulated by the presence of an inversion heterozygosity at 8p23.1. Even if inv dup del(8p) is a recombinant product of a parental 8p23.1 heterozygous inversion, there is no recurrence risk for this rearrangement and no invasive prenatal investigations are indicated for the next pregnancy.

CONCLUSIONS

In conclusion, microarray analysis permited precise molecular characterization of the rearrangement and indicated that NAHR between segmental duplications is the most likely mechanism for this abnormality. Also, it indicated that the terminal deletion was stabilized by telomere healing.

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COMPLEX UTRASOUND STUDY OF THE ATHEROSCLEROTIC PLAQUE

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COMPLEX UTRASOUND STUDY OF THE ATHEROSCLEROTIC PLAQUE (Abstract)**:** Carotid surgery concept is wining ground both among neurologists who recommend and vascular surgeons who perform an increased number of interventions. Regardless of the technique, we are interested in the tendency of the plaque to grow and determine stenosis. Ultrasound (US) is 91-94% sensitive and 85-99% specific in detecting a significant stenosis of the internal carotid artery. **Aim:** To establish a correlation between the preoperative US and intraoperative plaque characteristics in order to determine the restenosis rate. **Material and methods:** From January 1, 2012 to December 31, 2012, 70 consecutive patients were included in this study. Two groups were formed: 35 patients with stenotic ICA and 35 patients presenting stenosis at the femoral artery bifurcation (control group). The comparison between these two groups started from the premise of a similar pattern for internal carotid artery – deep femoral artery and external carotid artery- superficial femoral artery. US scans were performed on admission in all patients. All images were normalized and Gray Scale Median (GSM) was calculated. **Results:** Femoral plaque GSM values were higher in relation with hyperechogenicity highlighting the intensely calcified structure. Unstable plaques were more heterogenic, with higher transparency and lower GSM than stable plaques. This was the case of carotid plaques. During follow-up US revealed no restenosis. **Conclusions:** Femoral bifurcation plaques are calcified and determine chronic ischemic symptoms, while carotid bifurcation plaques are unstable and determine cerebral symptoms. US remain the gold standard non-invasive technique both for screening and diagnosis and set the therapeutic coordinates. **Keywords:** CAROTID PLAQUE, CAROTID ARTERY ULTRASONOGRAPHY, GRAY SCALE MEDIAN.

Carotid surgery concept is wining ground both among neurologists who recommend and vascular and cardiovascular surgeons who perform an increased number of interventions. Regardless of the technique used (open surgery, stenting) we were interested in the tendency of the plaque to grow and determine stenosis.

The prevalence of significant internal carotid artery (ICA) stenosis (more than 50%) is 0.5% among the age range 50 - 59 years, 10% in elderly over 80 years and 20% in patients presenting lower limb peripheral arterial disease (1).

Worldwide stroke is the third common cause of death. The prevalence of stroke after a transient ischemic attack (TIA) is 7% per year and the prevalence of stroke in an asymptomatic patient with a carotid stenosis larger than 80% is 3% per year (1).

The main cause of stroke is atheroembolism followed by the low blood flow through the stenosis.

The benefit from surgery in terms of stroke prevention in patients with symptomatic 70 - 99% stenosis (NASCET criteria) is higher than the one obtained from medical treatment (1). The available level I evidence suggests that in symptomatic patients surgery is currently the best option. The recommendation was based on a meta-analysis by The Cochrane Collaboration of eight randomized trials comparing carotid artery stenting with carotid endarterectomy (2). Patients with recent symptomatic stenosis (70-99%) are at highest risk of subsequent stroke. The efficacy of CEA falls with time (2).

Duplex ultrasound is the gold standard technique for carotid arteries evaluation. Ultrasound (US) uses B-mode US imaging and Doppler US to detect focal increases in blood flow velocity indicative of high grade carotid stenosis. The peak systolic velocity is the most frequently used to measure the severity of stenosis. Color Doppler technique increases the efficiency of the test, but it has not been shown to improve accuracy. US is 91-94% sensitive and 85-99% specific in detecting a significant stenosis of the ICA (3, 4, 5). Doppler US is also useful in obtaining information about plaque composition. Intraplaque hemorrhage, which may increase the risk of embolism and impact on prognosis, can be visualized on high resolution B-mode (3, 4, 5, 6, 7, 8). The ratio of ICA peak systolic velocity to the common carotid artery (CCA) velocity predicting a high grade stenosis sets the indication for surgery (9).

MATERIAL AND METHODS

The patients entering this study were selected from: patients referred by neurology departments with a high probability of advanced ICA lesion, patients with peripheral vascular disease already hospitalized in the vascular surgery department screened positive for significant carotid or femoral stenotic lesions, patients with arteriopathy hospitalized in cardiology and internal medicine departments diagnosed with significant femoral artery stenosis. 98% of the patients were diagnosed and selected in the Vascular Surgery Department. Before surgery all patients benefited from a new Doppler US (performed on Siemens Echo-Doppler XP 300 Premium - 2010).

All patients gave their informed consent for the study.

Two groups were formed: patients with stenotic ICA and patients presenting stenosis at the femoral artery bifurcation. The inclusion criteria for the first group were: asymptomatic ICA stenosis greater than 70%, history of TIA, history of minor stroke with self-sufficiency and 50-95% ICA stenosis. The patients in the second group meet the following inclusion criteria: arteriopatathy stage III and IV with atherosclerotic lesions at the femoral bifurcation, regardless of the chosen surgical procedure.

The exclusion criteria were: autoimmune diseases, hypothyroidism, osteoporosis, hepatic insufficiency (GOT higher than 2.5 U / l), chronic kidney disease (creatinine above 2.0 mg / dl), ischemic heart disease class III, IV NYHA, atrial fibrillation and malignancies.

The comparison between these two groups started from the premise of the existence of a similar pattern for ICA – deep femoral artery (DFA) and external carotid artery (ECA) - superficial femoral artery (SFA). Thus, the bifurcation of the

CCA into the ICA and ECA anatomically resembles the bifurcation of common femoral artery (CFA) into DFA and SFA at each bifurcation there is an artery that will face increased resistance (ECA and SFA) and an artery that faces low resistance in the periphery (the ICA and DFA). Because of this, plaques at the two bifurcations are supposed to have a similar structure. Although clinically the manifestations of obstruction/stenosis of the ICA are much more severe than in obstruction/stenosis of DFA, the two models can be studied together in terms of atheromatous plaque progression.

US scans were performed on admission in all patients in both groups. B-mode settings were adjusted and then saved to ensure uniformity throughout the study (fig. 1). The stenosis grade was measured both in transversal and longitudinal section (fig. 2, 3).

Fig. 1. ICA and CCA B - mode US

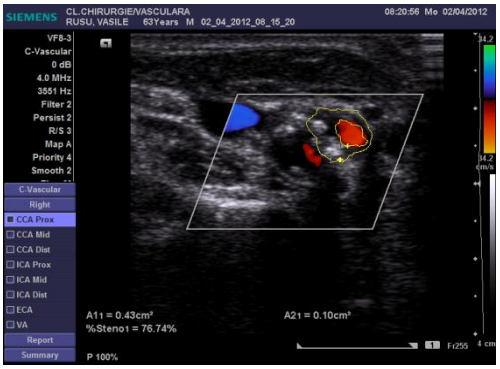

Fig. 2. Stenosis measurement on transversal section

Fig. 3. Stenosis measurement on longitudinal section

Two reference points were used for normalization of B-mode images, blood (black), and adventitia (white). Patients were examined supine with the neck-rotated 45 degrees to the opposite side. A peripheral vascular transducer was used. The image was "frozen", stored and transferred to a computer in order to calculate the Gray Scale Median (GSM). All images were normalized according to the method proposed by El-Atrozy et al. eliminating sonographic gain-induced variability of echogenicity in which the entire image is changed by a process of linear scaling to two reference points (blood and adventitia) with computer software (Adobe Photoshop 7.0). The GSM of the blood was set to 0, and the GSM of adventitia was set to 255. To determine the GSM for each stenotic plaque we acquired the histogram (10, 11, 12, 13, 14) (fig. 4, 5).

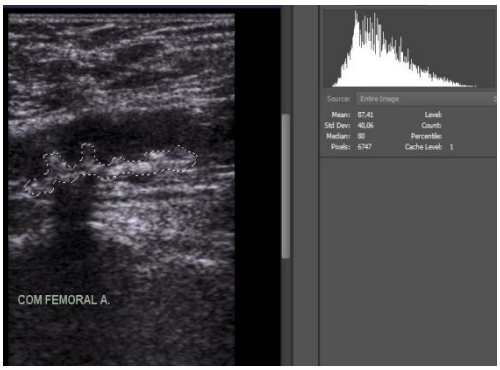

Fig. 4. Selected atherom plaque and prenormalized histogram

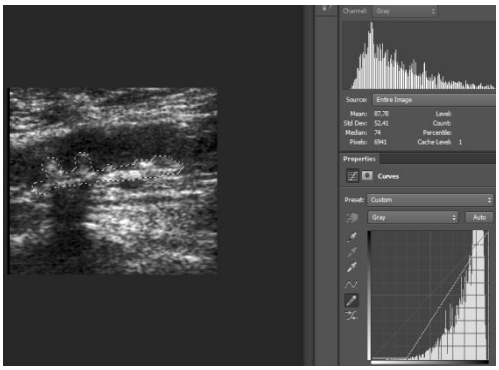

Fig. 5. Selected atherom plaque and normalized histogram

The results were used to compare the groups with one another and to draw conclusions from each group.

All patients underwent surgical interventions (endarterectomy and angioplasty in the patients with carotid artery stenosis or femoral artery stenosis and endarterectomy associated with bypasses in the patients with femoral artery stenosis)

US was performed postoperatively and as follow-up at 1, 3, 6, 9 and 12 months (fig. 6,7).

Fig. 6. Follow-up US - carotid angioplasty

Fig. 7. Follow-up: carotid angioplasty Dopper US

RESULTS

From January 1, 2012 to December 31, 2012 we included in the study 70 consecutive patients meeting all criteria, 35 patients in each group.

Twelve patients with carotid lesions showed ICA stenosis greater than 90% stenosis. Patients with femoral lesions had CFA stenosis greater than 50% associating in 13 cases DFA stenosis greater than 70%.

No statistically significant differences in demographic characteristics, vascular risk factors or therapy were identified.

Post-Hoc analysis (paired analysis) comparing the femoral or carotid plaque area before and after the normalization

revealed the absence of statistical difference. The difference between prenormalization and normalization GSM values in each group were statistically significant (tab. I, II).

The average carotid plaque area was twice as big as the average femoral plaque area (tab. III).

TABLE I

Post-Hoc GSM analysis (paired analysis)

Newman-Keuls test	P	Statistics
prenormalized ICA vs. normalized ICA	0.014924	ss
prenormalized ICA vs. prenormalized DFA	0.142735	ns
normalized ICA vs. normalized DFA	0.036642	ss
prenormalized DFA vs. normalized DFA	0.001911	ss

*P - 95% confidence interval, ss= statistically significant, ns= not statistically significant

TABLE II

Post-Hoc aria analysis (paired analysis)

Newman-Keuls aria test	P	Statistics
prenormalized ICA vs. normalized ICA	0.690022	ns
prenormalized ICA vs. prenormalized DFA	0.000028	ss
normalized ICA vs. normalized DFA	0.000036	ss
prenormalized DFA vs. normalized DFA	0.580894	ns

*P - 95% confidence interval, ss= statistically significant, ns= not statistically significant

TABLE III

Statistical area values [mm] pre-normalized vs. normalized

		Avr	Average		SD	SE	Min	Max	Md
			-95%	+95%					
ICA	pre- N	2625.3	2136.1	3114.6	1424.3	240.7	615.9	5520.0	2322.2
	N	2729.9	2235.4	3224.4	1439.5	243.3	671.5	5800.5	2747.7
DFA	pre- N	1390.9	1196.4	1585.4	566.2	95.7	610.4	2487.9	1188.5
	N	1535.6	1321.7	1749.6	622.9	105.3	456.7	2906.7	1422.9
All		2070.4	1862.3	2278.6	1245.7	105.3	456.7	5800.5	1875.8

*pre-N prenormalized, N normalized, SD standard deviation, SE standard error, Md median

Femoral plaque GSM values were higher in relation with hyperechogenity, highlighting the intensely calcified structure.

Unstable plaques were more heterogenic, with higher transparency and lower GSM than stable plaques.

Carotid plaques average GSM was lower than the femoral one, meaning the carotid plaques are less stable.

Minimum-maxim differences reveal a high grade of heterogenity in both groups (tab. IV).

Patients showed no carotid or femoral restenosis during the 1, 3, 6 and 12 months of US follow-up. Patients remained under surveillance.

TABLE IV

Statistical GSM values [mm] pre-normalized vs. normalized

		Avg	Average		SD	SE	Min	Max	Md
			-95%	+95%					
ICA	pre- N	41.17	34.17	48.17	20.37	3.44	13	86	40
	N	55.06	46.26	63.85	25.60	4.33	20	112	49
DFA	pre- N	48.49	42.91	54.06	16.23	2.74	23	78	44
	N	65.49	58.53	72.44	20.24	3.42	35	142	69
All		52.55	48.79	56.31	22.51	1.90	13	142	48

*pre-N prenormalized, N normalized, SD standard deviation, SE standard error, Md median

DISCUSSION

Our study showed higher GSM values than those reported in the literature (15, 16). This is a consequence of the patients' tendency to present to the vascular surgeon in advanced disease stages.

The correlation of a lower GSM with plaque instability needs to be proved. During the US follow-up we aim at detecting the occurrence of restenosis.

CONCLUSIONS

Bifurcation plaques occurring in peripheral atherosclerotic disease are calcified and determine chronic ischemic symptoms (intermittent claudications, rest pain), while carotid bifurcation plaques are unstable and determine cerebral symptoms either by TIA or ischemic stroke. US remain the gold standard non-invasive technique both for screening and diagnosis and set the therapeutic coordinates. The GSM analysis is a new and versatile tool used to establish plaque characteristics and to assess the risk for acute events.

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NEWS

NEW GENE INVOLVED IN ANEMIA

Some forms of congenital anemia are caused by the deficiency in heme biosynthesis. Heme is a constitutive element of hemoglobin that is a protein in red blood cells responsible for transporting oxygen. Americans researchers have discovered a new gene that regulates heme biosynthesis. This gene named mitochondrial ATPase inhibitory factor 1 (Atpif1) is involved in the enzymatic activity of ferrochelatase, the terminal enzyme in heme biosynthesis. Using an animal model, the researchers found that loss of Atpif1 reduces ferrochelatase activity and increases mitochondrial pH which leads to deficiency in heme biosynthesis and causes anemia. Understanding of the mechanisms regulating heme biosynthesis may contribute to development of improved therapies for certain type of anemia such as congenital sideroblastic anemia and mitochondrial disease (Shah DI, Takahashi-Makise N, Cooney JD *et all*. Mitochondrial Atpif1 regulates haem synthesis in developing erythroblasts. *Nature*. 2012; 491(7425): 608-12).

Cătălina Luncă

EARLY RETROPANCREATIC LAMINA DISSECTION DURING PANCREATICODUODENECTOMY: HOW, WHEN AND WHY?

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EARLY RETROPANCREATIC LAMINA DISSECTION DURING PANCREATICO-DUODENECTOMY: HOW, WHEN AND WHY? (Abstract):Pancreaticoduodenectomy is the procedure of choice for tumors of the pancreatic head and periampullary region. During pancreaticoduodenectomy, early neck division may be inadequate in case of hepatic artery anatomic variants, suspected involvement of the superior mesenteric vessels or intraductal papillary mucinous neoplasms. **Material and methods:** We describe our early approach to the superior mesenteric vessels during pancreaticoduodenectomy, by retroportal lamina dissection before pancreatic transection. **Results:** We used this approach in 41 patients. There were 28 patients with abnormal hepatic artery, which was spared in 26 cases. Hepatic artery reconstruction was required in 2 cases. Nine patients with intraductal papillary mucinous neoplasms underwent 6 pancreaticoduodenectomiesextented to the body and 3 total pancreaticoduodenectomies. Four patients with adenocarcinoma involving the portomesenteric vein required pancreaticoduodenectomy with venous resection and reconstruction. **Conclusions:** Early retropancreatic lamina dissection is useful way to tailor a pancreaticoduodenectomy which is recommended in selective indications. **Keywords**: PANCREATIC SURGERY, TECHNIQUE, HEPATIC ARTERY, SUPERIOR MESENTERIC ARTERY.

Pancreaticoduodenectomy (PD) is the treatment of choice for tumors of the pancreatic head and periampullary region. Since the first PD performed by Whipple in 1937, more than 65 improvements of the technique were made, concerning mainly pylorus preservation or reconstruction of pancreatico-digestive continuity. Standard PD is usually performed backward, with transection of the pancreatic neck before the superior mesenteric artery (SMA) dissection. Recently, indications of PD have extended to intraductal papillary mucinous neoplasms (IPMN) and periampullary tumors invading the mesentericoportal vein. In these last two conditions, division of the pancreatic neck may be inappropriate so division of the body can be preferred. We perform in such cases, as well as in hepatic artery (HA) variants, a PD with early retropancreatic lamina dissection (RPLd), close to the origin of the SMA, to readily assess

variant pattern of the arterial blood supply to the liver and radicality of resection, to perform mobilization of the specimen before pancreatic division, and, if necessary, to safely perform venous clamping. RPLd was first suggested by Pessaux (1). The purpose of the current study is to describe how, when and why we perform PD with RPLd.

MATERIAL AND METHODS

One hundred thirty six consecutive patients underwent PD for periampullary and pancreatic head tumors between January 1, 2007 and December 31, 2011. Among them, 41 (22 males and 19 females, median age 59 years, range 44-78), underwent PD with early RPLd. Twenty eight patients have been preoperatively assessed by multidetector computed tomography with HA anomalies and 4 with adenocarcinoma involving the portomesenteric axis. IPMN have been preoperatively assessed by abdominal multidetector computed tomography and endoscopic ultrasound with guided fine needle aspiration. Twenty cases had RHA arising from SMA (18, replaced=11 or accessory=7) or Ct (2 cases of replaced RHA). Eight cases had a RCHA originating in SMA.

TECHNIQUE

Surgical exploration must be completed by intraoperative ultrasonography. The pancreas head exposure is obtained by an extended Kocher maneuver, incision of the attachment of the transverse mesocolon to the right perinephric area, and opening of the lesser sac by separating the greater omentum from the transverse colon. The superior mesenteric vein (SMV) is exposed where it lies to the anterior third duodenum (fig. 1).

Dorsal to the pancreas, the dissection must pass beyond the aorta, to get full posterior mobilization of the duodenopancreas to the left, and to distinguish the plane between the SMV and the SMA. Liver and peritoneal exploration is performed as well as the palpation of the mesenteric root and biopsy examination of aortocaval nodes with frozen section.

The retropancreatic dissection is carried on downwards from the inferior border of the Winslow foramen along the Treitz fascia, exposing the inferior vena cava on its left side, the left renal vein with its upper margin and in between, the origin of the SMA (fig. 2, 3).

The SMA origin is isolated in this angle, and then, along the plane of its adventitia, the RPL, which is inserted on its right aspect, is dissected and removed “step-by-step” in a frontal plane behind the pancreas (fig 4). This dissection is achieved along 3-4 cm, from the SMA origin until its entrance into the mesentery, after progressive exposure and gentle medial retraction of the portal vein (PV), which is also set free from the RPL (fig 3, 4).

RPL is retracted rightwards and the lymphatic tissue between SMA and SMV is removed. The possible SMA invasion is early detected, due to RPL involvement, avoiding the risk for nonradical resection. Such exposure allows easier dissection of a right HA (RHA) originating from SMA or celiac trunk (Ct) or a replaced common HA (RCHA) coming from the SMA. This vessel, which arises 1-2 cm beneath the SMA origin, is looped and freed from the RPL, upwards to the hepatic pedicle. To facilitate the SMA and aberrant RHA or RCHA dissection, the doudenopancreas is retracted en bloc upwards, ventrally and to the left (Fig 3). Limited dissection along the right

side of the SMA (fig. 4) is advocated, to avoid an extensive removal of the perivascular nervous plexus, resulting in postoperative intestinal motility troubles.

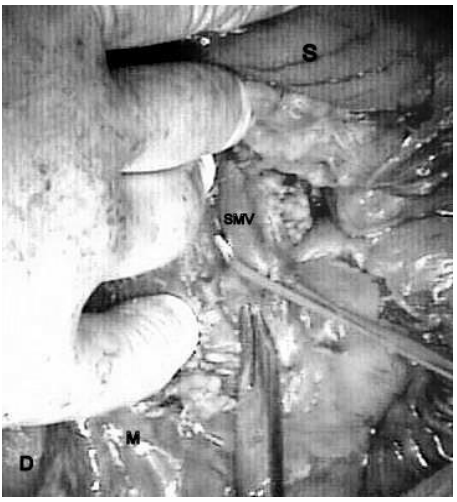

Fig. 1. Exposure of the SMV on the anterior third duodenum

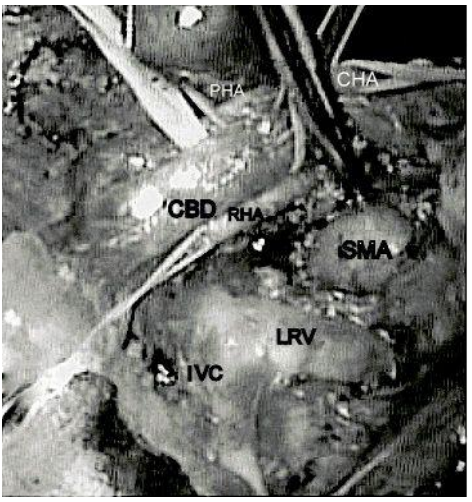

Fig. 2. Retropancreatic dissection with a replaced RHA originating in SMA before RPLd and common bile duct division

Fig. 3. Retropancreatic dissection: the duodenopancreas with the tumor is retracted medially and to the left

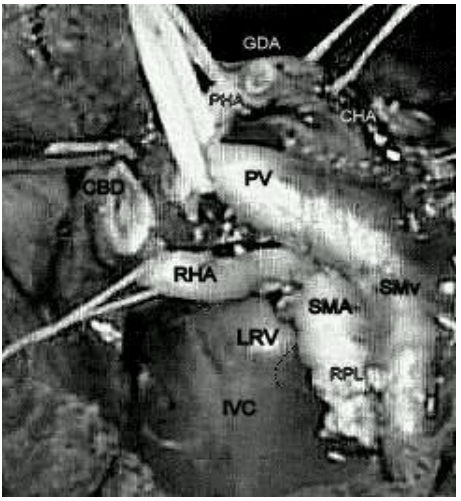

Fig. 4. Intraoperative image just before pancreatic transection; the SMA was set free from the RPL along 3-4 cm

At this point the hepatic pedicle is dissected. After cholecystectomy, the common and proper HA are isolated, when they exist as such. The right gastric vessels are divided, the gastroduodenal artery is identified and clamped to ensure that the arterial

flow either in hepatic and gastric arteries remains normal and there is no unrecognized Ct stenosis. The gastroduodenal artery is divided, as well as the common bile duct above the entry of the cystic duct. This improves the exposure of the supra-pancreatic PV. Care must be taken during lymphadenectomy around the PV to an accessory or replaced RHA originating from the SMA or Ct, but also to a RCHA from the SMA. This vessel runs upwards behind the PV and we loop it. The SMV is entirely dissected at the inferior pancreatic margin with ligation of the right gastroepiploic vein and of all veins draining the uncinate, which is exposed up to the right side of the SMA. At this stage the posterior wall of the portomesenteric vein is entirely exposed. The Treitz ligament is divided, equally by a posterior approach, allowing mobilization of the duodeno-jejunaljonction and its retraction beneath the superior mesenteric vessels, so the specimen to be removed reaches the right side of the mesenteric root. The inferior pancreaticoduodenal artery is identified and ligated. Once the radicality of PD is established jejunal and distal gastric division is performed according to Whipple procedure. The last step of the resection phase is the pancreatic neck transection, just in front of the PV. When pancreatic division must be performed on the body, we divide the dorsal pancreatic artery and collaterals of both the SMA and the SMV (from the inferior edge of the pancreas). In case of involvement of the portomesenteric confluence, we control the splenic vein behind the body. Adequate mobilization of the mesentery and right colon is necessary to perform safely *,"en bloc"* resection and venous reconstruction. We estimate this mobilization as useful in case of limited portomesenteric invasion, in order to avoid vein grafting during venous reconstruction. In case of IPMN extending from the head to the body, we carry on the retro-pancreatic mobilization towards the left and dissect the splenic vessels with successive ligation of their collaterals. When the pancreatic body is mobilized enough from the splenic vessels, we can divide the pancreas at any level, or entirely remove it if necessary. Frozen section analysis is performed on the cut surfaces, to assess the malignant status of the remnant pancreas. The reconstruction phase, drains placement and postoperative care are similar to those from standard PD.

RESULTS

The aberrant vessel was preserved in 26 (63.41%) cases. A RCHA originating from the SMA was involved by an enlarged lymph nodes mass behind the pancreatic head in 2 patients. A segmental resection of the involved RCHA had to be performed with arterial reconstruction, using the reversed splenic artery. Four patients with ductal adenocarcinoma involving the portomesenteric confluence required en bloc resection, mobilization of the right colon and mesentery root followed by mesentericoportalveno-venous suture. We reimplanted the splenic vein into the PV in all cases. When vascular reconstruction was required, clamping time did not exceed 22 minutes. Anastomotic patency and normal blood flow were confirmed by Doppler ultrasound at the end of the procedure.

The same approach was used in 9 patients with IPMN (6 PD extented to the body- IPMN in the head, neck or uncinate, and 3 total PD - IPMN diffusely involving the pancreatic duct). Mean operative time over all the 41 patients was 315 minutes

and mean blood loss 430 ml. The mean hospital stay was 16.5 days. There were no early complications particularly related to this approach. For the malignant tumors, a R0 resection was achieved in 24 (58.54%) patients and a R1 in 2 (involvement of the RPL). The median survival time was 25.8 months. The follow-up lasted until patient death or until the cut-off date of January 31, 2012. At the time of the last follow-up, 29 (70.73%) patients were still alive.

DISCUSSION

Because of recent decrease in mortality rate, PD is now routinely performed for tumors of the pancreatic head and periampullary region, but also for IPMN or periampullary tumors invading the mesentericoportal vein. We have described herein our technique of early RPLd during PD on the right side of the SMA, before the digestive and pancreatic continuity should be interrupted. We consider this technique particularly suitable in case of HA abnormality, with RHA originating from SMA or Ct, or RCHA from the SMA, suspected involvement of the SMA, IPMN extended from the pancreatic head to the body and involvement of the portomesenteric vein.

Standard PD includes the creation of a tunnel between the pancreatic neck and the PV, followed by neck transection. The pancreatic continuity is interrupted before radicality of the resection could be assessed close to the SMA. Even in some recent series, nonradical PD represents 9 to 25 % cases (2). Moreover, in the standard PD, dissection of a RHA or RCHA is usually performed late, when bleeding from the resection specimen decreases the exposure of the SMA and of an aberrant RHA. Early neck transection is not suitable when the neck is involved, as in tumors involving the portomesenteric vein or in IPMN extended to the body. One of the difficulties of PD is variability of peripancreatic vessel anatomy (3). Assessment of variant pattern of the arterial blood supply to the liver in patients who are about to undergo a PD avoids unnecessary complications, as fatal hepatic injury. Accidental ligation of aberrant HA may result in hepatic necrosis, ischemic biliary injury or anastomotic complications (4). The importance of sparing this artery during PD lies not so much in preventing hepatic ischemia, but in preventing a breakdown of bilioenteric anastomosis, because the blood supply to the cranial part of the common bile duct is entirely dependent on the RHA after PD. Preoperative assessment of coelio–mesenteric vascular pattern (variants, strictures) is of the utmost importance for the surgeon. RHA or RCHA from SMA may course behind, within the pancreas head, or along its ventral side (5).

Ductal carcinoma with venous limited involvement can be safely resected with a long-term survival similar to that observed after radical resection without venous involvement (3). In this situation, venous resection is best performed *"en bloc"* to obtain disease-free margins. This approach results in the tumor being attached only to the involved veins so clamping of the portomesenteric confluence is easier and shorter. Mobilization of the right colon and mesentery root are useful to avoid vein grafting during reconstruction of the PV (6). It is expected that, because pancreatic transection is performed at the end, congestion and bleeding are less likely whereas the venous drainage of both the specimen and bowel are compromised minimally during most of the procedure (7).

In case of IPMN, the most frequent localization is the pancreatic head, but in-

volvement of the body may occur to some patients (8). In this setting and particularly in malignancies, en bloc resection requires pancreatic division of the body. The final transection of the pancreas can be performed at the desired place if it is enough mobilized from the splenic vessels, preventing tumor opening, which might disseminate cancer into the abdomen. Furthermore, dissection along the splenic vessels can be extended up to the splenic hilum allowing splenic preservation if the whole pancreas must be resected (8).

In conclusion, early RPLd is useful technique to expose the retropancreatic-mesenteric vasculature during PD. We advocate this approach in selective situations, such HA anatomic variants, suspected SMA involvement, limited invasion of the mesentericoportal axis and IPMN. RPLd also standardizes the radicality of PD and allows earlier vascular control. Further prospective studies are required to assess its advantages over standard PD, since there is no consensus worldwide.

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CERVICAL INCOMPETENCE ASSOCIATED WITH CONGENITAL UTERINE MALFORMATIONS

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CERVICAL INCOMPETENCE ASSOCIATED WITH CONGENITAL UTERINE MALFORMATIONS (Abstract): Cervical incompetence is basically a cervix that is too weak to stay closed during a pregnancy. It is generally categorized as premature opening of the cervix without labor or contractions. **Aim:** To estimate the incidence of incompetence of the uterine cervix at patients with congenital uterine malformations. **Material and methods**: Our study was a retrospective and prospective one between 2002 and 2009. We evaluate the length of the cervix by transvaginal ultrasonography during the second trimester of pregnancy**. Results:** We had 316 cases with congenital uterine malformations. From these we found 49 (15.3%) women with incompetence of the cervix: 8 (2.5%) with unicornuate uterus, 11 (3.4%) with bicornuate uterus and 30 (9.5%) cases uterus with septum. For all these wad made cerclage when we found funneling of the cervix or the cervix was open less than 4 cm. The use of ultrasonography has been very helpful with the diagnosis, and is made when the cervical os (opening) is greater than 2.5 cm, or the length has shortened to less than 20 mm. All patients had preterm delivery. **Conclusions:** When we have cases with congenital uterine malformations we must think that incompetence of the cervix could be associated, so we must assess the status of the cervix for these patients by serial ultrasound examinations starting between 16 and 20 weeks of gestation. Our patients had preterm delivery caused not only the incompetent cervix, but the malformation of the uterus is implicated also. **Keywords**: CERVICAL INCOMPETENCE, CONGENITAL ULTRASONOGRAPHY, GESTATION.

Cervical incompetence is related with 15 - 20% of abortions appeared in the second quarter of pregnancy. The incidence of congenital uterine malformations (Cg.UM) in the general population and among women with recurrent pregnancy loss is not clearly known. Although the reported incidence is 0.16 to 10%, recent data suggest that it is 1% in the general population and 3% in women with recurrent pregnancy loss and adverse reproductive outcome (1, 2).

Cervical incompetence (CI) due to Cg.UM has as a consequence premature birth which in turn is associated with 75% of perinatal morbidity and mortality. Two thirds of premature infants are the result of labors between 26 and 32 weeks of pregnancy and are marked by the highest rate of mortality, respiratory distress, intraventricular hemorrhage, necrotizing enterocolitis, developmental delay, visual and

hearing deficit and chronic lung diseases (1).

Effective methods for detecting preterm birth can avoid unnecessary interventions and treatments in women without risk (2). It is well-known the necessity of a suitable cervical length in order to maintain a normal pregnancy, and length and diameter of the cervix must remain steadily as pregnancy progresses, both in primiparous and multiparous.

In women with low risk, cervical length (CL) is 35-40 mm at 40 weeks of pregnancy; the lower limit is 25 mm and 50 mm the higher. Hibbard (cit. 2) detected the presence of a CL of 38.5 + 8 mm at 20 weeks of pregnancy. The progressive shortening of CL occurs naturally after 30 weeks in normal full term pregnancy patients (3).

A length less than 25 mm is abnormal before 24 weeks, with a risk of premature birth under 35 weeks of 4% in low-risk population and a positive predictive value (PPV) of 18%, but for pregnant women at high risk, the incidence of premature birth increases to 26% and PPV to 55% (4).

MATERIAL AND METHODS

The study consisted of 49 cases with a lot cervical incompetence (CI) selected from 316 patients with congenital uterine malformations (Cg. UM) and assisted Municipal Hospital, Radauti, Suceava County, during 2002-2009. This subset analysis showed a rural origin for 31 (63.3%) cases as compared with urban, 18 (37.7%); with occupational relationship represented by 41 (83.6%) patients employees, 7 (14.3%) households and 1 (2.1%) other categories. Age group 25-33 years represented 31 (63.3%) of total cases (fig. 1).

Distribution by age groups in relation to birth type indicated a significantly higher frequency ($p < 0.05$) for the natural way: 36 (73.4%) *vs.* 13 (26.6%) cases by caesarean section (fig. 2).

By the age of 33, prevailed full term births (75.6%), when 93.7% were primiparous and by 2.1% - bi, tri and multiparous (fig. 3).

Referral to marital status of the entire group of 49 patients with CI, 39 (79.5%) were married, 14.3% divorced, 4.1% in concubinage, and 2.1% other (fig. 4).

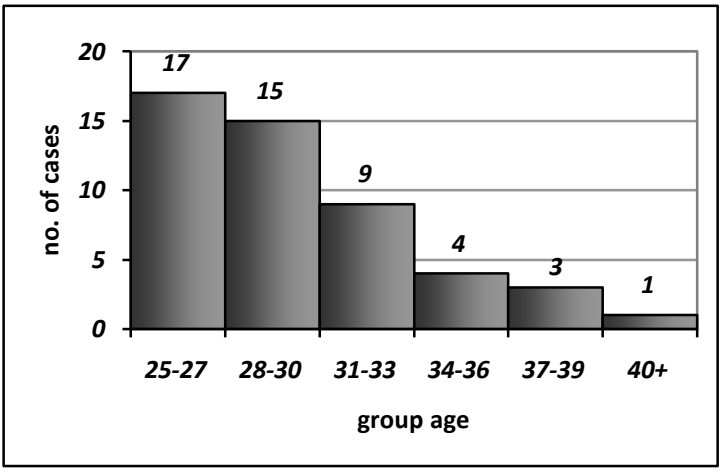

Fig. 1. Age histogram

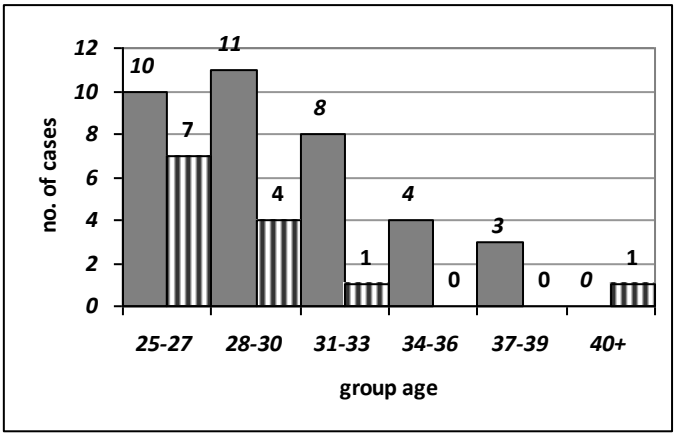

Fig. 2. Age-related delivery type

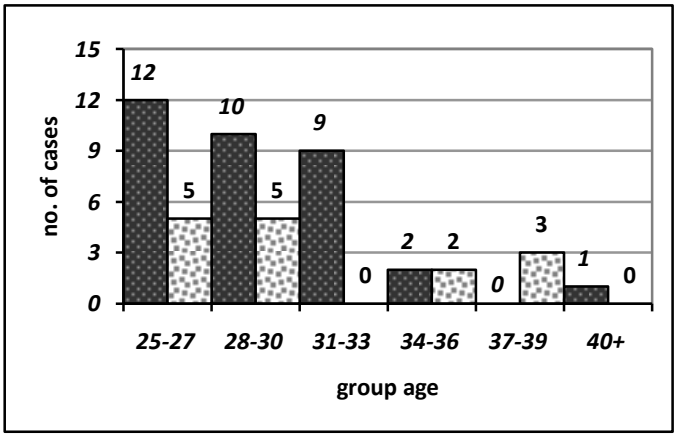

Fig. 3. Age-related full and preterm delivery

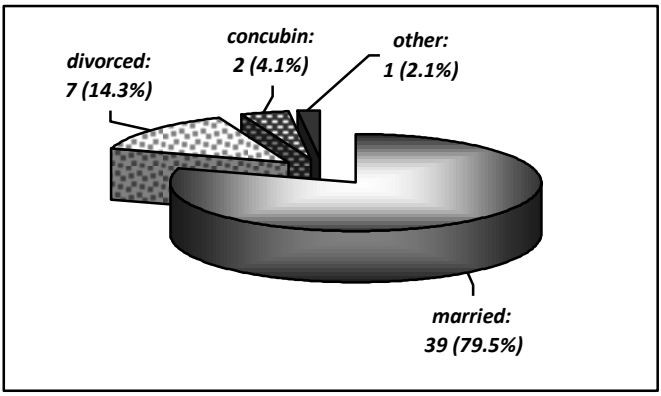

Fig. 4. Patients' marital status

Gynecological medical history was recorded in 47 (95.8%) of cases and other categories had low values (2.1%).

Patients received abdominal and vaginal ultrasound, ensuring a good view of the internal cervical os (ICO) and measurement of the cervical length (CL).

Incompetent cervix diagnosis was achieved by highlighting the funneling, expansion and protrusion of membranes, the ICO aspects of *Z*, *V* and *U*, respectively. If funneling highlighted, its width and length was measured, a closed LC, as well as the proportion of funneling and cervical index, calculated by the formula: funneling length +1 / cervix length. Incompetent cervix risk assessment in patients with Cg.UM was performed between 16-20 weeks (fig. 5).

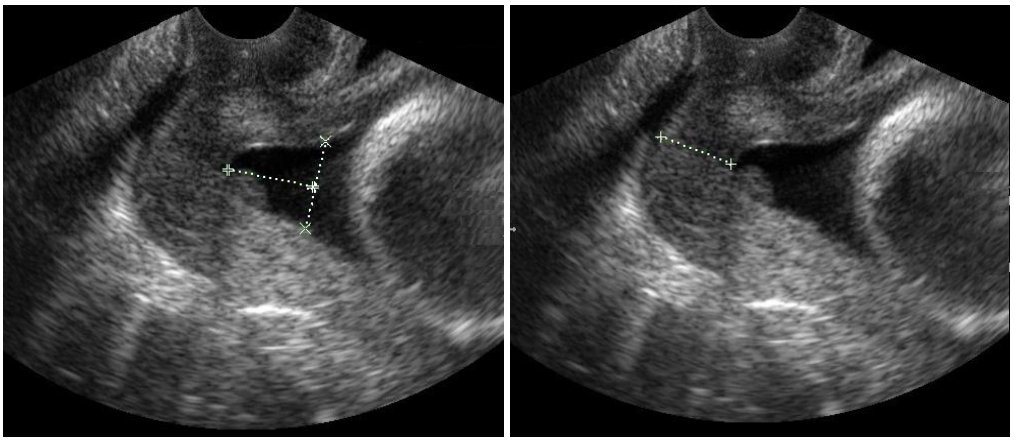

Fig. 5. Funneling measurement - diagnosis at 18 weeks

RESULTS

The ultrasound evaluation of the 49 women with CI showed that 15.5% were diagnosed with incompetent cervix, 30 (61.2%) had septated uterus, 11 (22.4%) bicornuate, and 8 (16.3%) unicornuate uterus (fig. 6).

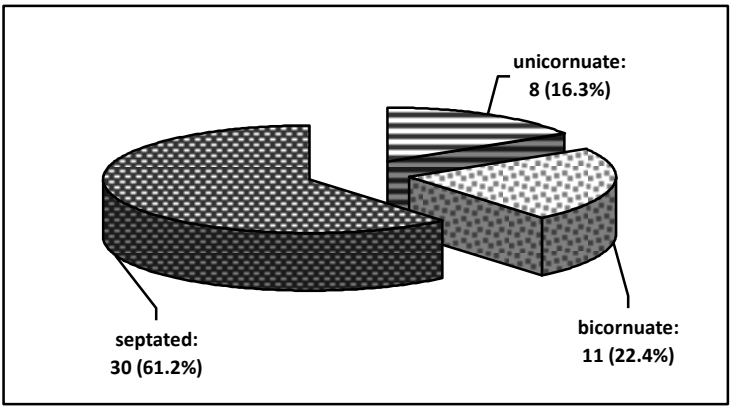

Fig. 6. Types of malformed uterus diagnosed with cervical incompetence

When the diagnosis of CI was established for all studied pregnant women, cervical cerclage was performed by Mc Donald technique (7). After performing cerclage, the patient remained under surveillance in order to detect any occurrence of uterine contractions and after discharge was advised bed rest to reduce pressure on the cervix thereby increasing the chances of maintaining the pregnancy until the fetus becomes viable. In the study group, no funneling appeared after cerclage and all patients delivered prematurely at a gestational age between 28 and 35 weeks (tab. I). The largest number of patients who delivered after 32 weeks had septated uterus - 22 (45%) pregnant women. It was noticed that CL should be correlated with other risk factors such as vaginal infections because a shortened cervix is less resistant to infection ascension, with the risk of amniotitis.

TABLE I

Gestational age at birth correlate with the type of uterine malformation

UTERINE MALFORMATION					
SEPTATED		**BICORNUATE**		**UNICORNUATE**	
no. of cases	*pregnancy age**	*no. of cases*	*pregnancy age**	*no. of cases*	*pregnancy age**
1	28 weeks, 2 days	1	28 weeks, 4 days	1	29
1	29	1	29	2	30
1	30	1	30	1	31
4	34	4	31	2	32
1	31	2	32	1	33
12	32	1	33	1	34
4	33	1	34		
3	34				
3	35				
30		**11**		**8**	

*PA – weeks of gestation

From the group of patients with Cg.UM and incompetent cervix, 35 cases were natural delivery, proving that the malformed uterus and incompetent cervix are not indications of cesarean section (fig. 7).

The risk of premature birth was assessed based on clinical criteria, a score including maternal and social risk factors, and especially on the digital vaginal examination. These elements can be associated with biochemical factors, including the presence of fetal fibronectin in the cervix, its absence being an indicator with negative predictive value of premature birth in the next 7-14 days.

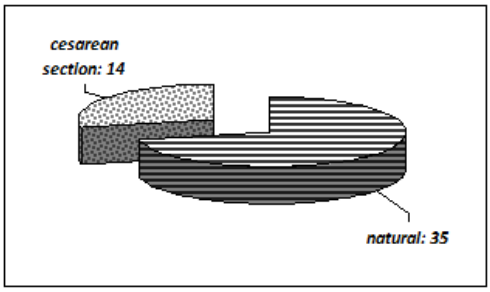

Fig. 7. Type of delivery (number of cases)

DISCUSSION

On endovaginal ultrasound in patients at risk of premature birth, NP-risk, CL is only 50% of <25 mm between 10-14 weeks. Ultrasound is performed at 18-22 weeks, in order to notice the presence of cervical shortening or funneling, with the ability to relieve the risk of premature birth in 70% of cases, as compared with 40% when screening is done at 19-24 weeks (5).

For the studied group, ultrasound was performed between 16-20 weeks. CL measurement has proved useful in deciding cerclage necessity, improving the pregnancy evolution only if previous 3 or 4 premature births are in antecedents. The ultrasound follow-up indicates that 25-30% of patients develop funneling after cerclage (6). In our study, none of the patients showed funneling after cerclage.

Iams (7) followed pregnancy patients with malformed uterus (septated, unicornuate, bicornuate), between 24 and 28 weeks and found that less a CL than 25 mm was two times more frequent than pregnancy of normal uterus.

Endovaginal ultrasound represented the most effective way for assessing the condition of cervical status, because it allows better visualization of the ICO. A high pressure may pseudo increase the cervical length, hiding thus funneling (dilation of the ICO).

CONCLUSIONS

Our study enabled the assessment of premature birth risk in patients with cervical incompetence due to congenital uterine malformations, highlighting that the endovaginal ultrasound, performed in the second trimester of pregnancy is the most effective method. Thus, a cervical length <25 mm and funneling over 50% of the total length of the cervix were associated with an increased risk of premature birth.

On the other hand, determining the diagnosis of incompetent cervix in optimal time, allowed the cervical cerclage intervention and the progress of pregnancy until the fetus was viable.

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IMPORTANCE OF POSTURE ASSESSMENT IN ANKYLOSING SPONDYLITIS. PRELIMINARY STUDY

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IMPORTANCE OF POSTURE ASSESSMENT IN ANKYLOSING SPONDYLITIS. PRELIMINARY STUDY (Abstract): **Aim:** The aim of this study is to perform a screening of patients diagnosed with ankylosing spondylitis (AS) in order to evaluate the static spinal disorders and correlate the results with the main clinical and functional parameters that characterize this disease. **Material and methods:** Ninety-five patients diagnosed with AS according to the 1994 New York criteria were screened, and 68 of them (all males) presenting static spinal disorders and on physical therapy programs in different outpatient physical therapy units throughout 2011 entered the study. **Results:** The distribution of the patients according to static spinal disorders was almost even. There were no statistically significant differences in mean age and disease duration ($p>0.05$). The assessment of lumbosacral pain in the morning (VAS1) and daytime lumbosacral pain (VAS2) showed a higher scores in patients suffering from kyphoscoliosis than in those with scoliosis ($p=0.020$), ($p=0.000$), or kyphosis**.** Ott and modified Schöber index, and chest expansion, had higher mean values in patients with scoliosis compared with the other postural disorders ($p<0.001$). Statistically higher mean BASFI values were recorded in patients with kyphoscoliosis ($p=0.038$), while the mean BASMI values were lower in scoliosis patients ($p<0.001$). As to the quality of life of AS patients, HAQ-DI index recorded significantly lower mean values for kyphoscoliosis compared with other postural disorders ($p<0.001$). **Conclusions**: Our study suggests that posture assessment and implicitly the correction of possible misalignments should be part of the kinetic physical therapy program. Rigorous observing of postural recommendations can prevent the respiratory system complications. **Key words**: POSTURE, KYPHOSIS, SCOLIOSIS, KYPHOSCOLIOSIS.

Ankylosing spondylitis (AS) is a chronic, progressive inflammatory disease, seriously affecting the musculo-skeletal disorders, especially in younger patients, and causing serious disabilities (1-3). Mobility and deformity are parameters which sometimes evolve independently (4).

Changes in the spine start early in the first 10 years of disease (5), the patient adopting such inappropriate positions as poking head neck, thoracic hyperkyphosis, lumbar lordosis deletion with hip flexion and internal rotation and knee flexion (6). Depending on patient activity, such changes

can cause various degrees of disability, significantly affecting the quality of life (7).

In AS patients posture is often affected early after the onset or as the disease progresses (8-10), and this should be taken into account in the rehabilitation treatment.

A poor body physical structure should be detected, assessed both with respect to its magnitude and consequences, and eventually corrected. Posture, determined by the reports of musculoskeletal components, is the main objective of the assessment. It was believed that a perfect body posture and a good relation between its segments are synonymous with 'physical beauty' (11).

The aim of this study is to perform a screening of patients diagnosed with AS in order to evaluate the static spinal disorders and correlate the results with the main clinical and functional parameters that characterize this disease.

MATERIAL AND METHODS

Ninety-five patients with AS were screened (New York criteria, modified for AS), of which only 68 male patients which followed outpatient physiokinetic treatment in various medical rehabilitation units in Iasi town in 2011 entered the study. Study exclusion criteria were: patients presenting sindesmofites in the cervical-dorsolumbar spine, were under biologic drug treatment, and presented other conditons with axial involvement (Scheuermann disease, sacroiliac osteochondrosis, spinal stenosis, herniated discs, and congenital abnormalities)

Patients were assessed according to a standard protocol that included: (i) general data (age, disease duration), (ii) clinical and functional parameters (visual analogue scale for morning lumbosacral pain (VAS1) and daytime lumbosacrate pain (VAS2), Bath Ankylosing Spondylitis Functional Index (BASFI), Bath Ankylosing Spondylitis Metrology Index (BASMI), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), values recorded on a scale of 0-10 (0 -normal, 10 -severe); lumbar spine mobility (Schöber's modified test and finger-to-floor distance (FFD), dorsal spine mobillity (Ott index), thoracic spine flexibility (chest expansion) and quality of life Health Assessment Questionnaire (HAQ-DI, score that ranges from 0 -impossible and 30 -normal); (iii) static spinal disorders assessed by "lead wire" test and anthropometric data (Table I).

For statistical analysis we used SPSS 13.0 software. Descriptive data were presented as mean ± standard deviation (SD). Distribution of all variables was assessed using the nonparametric Kolmorov-Smirnov test. Student t-test and χ^2 test (chi-square) were used to assess homogeneity of subgroups of patients with vertebral static disorder. A $p<0.05$ value was considered statistically significant.

RESULTS

Patient distribution in relation to static spinal disorders was almost even. Patients with AS, were aged between 18 and 31 years; mean age 24.32 ± 3.36 years. In relation to the type of static spinal disorders, patients'mean age did not differ significantly (p=0.417), the same being true for disease duration (p=0.913) (tab. I, fig. 1).

Morning lumbosacral pain (VAS1) and daytime lumbosacral pain assessment (VAS2) gave significantly higher scores in patients with kyphoscoliosis than in those with scoliosis (p=0.020) or kyphosis (p=0.000).

As to FFD and BASDAI indices, no significant differences between the types of spinal disorders were found (p=0.108), (p=0.069) respectively.

TABLE I

Demografic, clinical, functional, and quality of life data in AS patients

Characteristics	Mean ± SD	Lowest	Highest
Age (years)	24.32 ±3.36	18	31
Disease duration (years)	4.79 ± 2.70	1	10
VAS1 (range 0-10)	3.91±1.218	2	5
VAS2 (range 0-10)	2.66±1.087	0	4
BASFI (range 0-10)	3.37±1.97	0.4	6
BASMI (range 0-10)	3.49±723	2	4
BASDAI (range 0-10)	5.22±1.95	2.1	7.6
Ott index (cm)	2.52±1.167	1	5
Modified Schöber (cm)	2.46±85	1	4.5
FFD (cm)	-17.24±9.841	-33	-5
Chest expansion (cm	2.56±79	1.5	5
HAQ-DI (range 0-30)	25.88±2.209	23	29

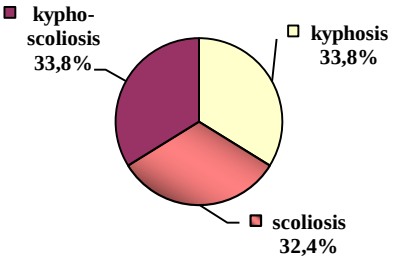

Fig. 1. Distribution of vertebral static disorders

Spinal mobility, assessed by Ott and modified Schöber indices, and chest expansion index showed higher mean values in patients with scoliosis compared with other types of vertebral static disturbance ($p<0.001$).

The functional BASFI index had significantly higher mean values in patients with kyphoscoliosis as compared with those with scoliosis or kyphosis ($p=0.038$), and mean BASMI was significantly lower in patients with scoliosis ($p<0.001$).

As to the quality of life of AS patients, HAQ-DI index had significantly lower mean values in patients with kypho-scoliosis as compared with the other types of static spinal disorders ($p<0.001$) (tab. II).

TABLE II

Correlation between vertebral static disorders and clinical and functional parameters in AS patients

Parameters	Kyphosis (mean ±DS)	Scoliosis (mean ±DS)	Kyphoscoliosis (mean ±DS)	p
VAS1	3.35±1.229	4.14±1.125	4.26±1.137	0.020
VAS2	1.74±810	2.64±658	3.61±839	0.000
BASFI	3.12±2.12	2.78±1.87	4.20±1.69	0.038
BASMI	3.91±288	2.59±503	3.91±288	0.000
BASDAI	4.83±2.06	4.82±1.93	5.98±1.69	0.069
Ott index	2.00±71	3.82±91	1.80±60	0.000
Modified Schöber	2.74±58	3.00±79	1.70±56	0.000
FFD	-15.65±9.890	-15.23±8.530	-20.74±11.128	0.108
Chest expansion	2.22±56	3.20±77	2.24±60	0.000
HAQ-DI	26.39±2.105	26.86±2.054	24.43±1.727	0.000

DISCUSSION

In this study, posture assessment revealed what are the onset static spinal disorders (preankylosing spondilitis), which, with disease progression, lead to severe clinical outcomes that aggravate the disease-generated general dysfunctional process (12).

The main feature of AS is its progression to ankylosis by the replacement of inflammation with bone formation (13). Given this, the unbalanced use of muscles, ligaments and joint will faster lead to poor posture by the inflammation of overused tissues (4).

It is known that the treatment of AS substantially contributes to preventing postural deformities (14, 15). In AS, pain and stiffness are symptoms are inflammation-related that can last as long as 10 years (5). In our study, the patients with kyphoscoliosis experienced more pain (VAS1 and VAS2).

One characteristic of AS is the loss of spinal mobility and less frequently of peripheral joint mobility (5). In this study the patients were thus selected as to allow differentiation of spinal mobility according to the spinal static disorders. Thus, modified Schöber index showed higher mean values in patients with scoliosis as compared to those with kyphosis and kyphoscoliosis, demonstrating the fact that first it is noticed the dissapearance of physiologic lumbar lordosis (by reduced mobility of the lumbar spine) and then kyphotic deformation (4).

Chest impairment in AS is due to changes in the spine and inflammatory process in small joints of the rib cage (thoracodynia) (4). Thus, in our study the lowest Ott index values, and implicitly a lower chest expansion, were found in patients with kyphosis and kyphoscoliosis with repercussions on the quality of life (HAQ-DI), especially in the patients presenting kyphoscoliosis.

BASDAI index was accepted as a standard tool for assessing disease activity in recent years (16, 17). In our assessments, no significant differences in BASDAI score between the different types of spinal disorders were recorded ($p=0.069$). However, this supports the suggestion that poor posture in AS equally affects the clinical disease activity in the different types of static spinal disorders.

Some aspects of functional capacity are often affected in patients with AS (18, 19). In our study, BASFI had significantly higher mean values in patients with kyphoscoliosis than in patients with scoliosis or kyphosis ($p=0.038$), BASMI showed significantly lower mean values in patients with scoliosis ($p<0.001$).

CONCLUSIONS

Posture assessment as part of the initial or check-up consultation of AS patient revealed a tendency towards antalgic or inadequate postures. This has allowed us a better understanding of the clinical and functional aspects of the patient with onset AS.

Based on these results, our study suggests that posture assessment and implicitly the correction of possible misalignments should be part of the kinetic physical therapy program. Rigorous observing of postural recommendations can prevent the respiratory system complications.

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KNEE OSTEOARTHRITIS, DYSLIPIDEMIA SYNDROME AND EXERCISE

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KNEE OSTEOARTHRITIS, DYSLIPIDEMIA SYNDROME AND EXERCISE (Abstract). The **aim** of our study was to evaluate the influence of aerobic training on the dyslipedemia in patients with knee osteoarthritis (KOA)**. Material and methods:** Prospective observational six-month study performed on 40 patients with KOA, fulfilling the inclusion criteria, classified according to their participation in specific aerobic training program (30 minutes/day, 5 days/ week) in two subgroups. A standard evaluation protocol was followed assessing lipid parameters (total cholesterol, triglycerides, LDL-cholesterol, HDL-cholesterol levels) at baseline, three and six months. Statistical analysis was performed in SPSS 16.0, $p<0.05$. **Results:** Subgroup analysis has demonstrated a statistical significant improvement in plasma lipids levels in all patients performing regular aerobic training (cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol) ($p<0.05$). Although the difference reported for total cholesterol, triglycerides and LDL-cholesterol after six months between subgroups was not significant ($p>0.05$), the mean level of HDL-cholesterol was significantly higher in patients performing aerobic training, reaching the cardio-vascular protective levels. **Conclusions:** Regular aerobic exercise has a positive effect on plasma lipoprotein concentrations; further research is needed for the assessment of long-term effects of physical exercises for both KOA and lipid pattern. **Key words:** KNEE OSTEOARTHRITIS, DYSLIPIDEMIA SYNDROME, EXERCISE.

Knee osteoarthritis (KOA) is recognized as a degenerative joint disease, characterized clinical by chronic pain, enlargement and deformity of joints leading to muscle atrophy, decreased mobility, gait abnormalities, physical disability and impaired quality of life. It is widely accepted that the complex therapeutically algorithm is actually based on pharmacologic, surgical and kinetic approach. Strengthening, stretching/range of motion and aerobic training are commonly involved in improving symptoms and physical functioning (1,2). As the confirmed risk factors associated with osteoarthritis are female gender, obesity, age, speculation continues about the role of other reported risk factors such as hypercholesterolemia, high levels of glucose and hypertension, the protective effect of current use of estrogens in postmenopausal women (3,4).

Dyslipidemia defined as abnormal blood lipids levels, has a major role in the devel-

opment and progression of atherosclerotic plaque conducting to major cardiovascular risk. Exercise and diet are used in the prevention and management of dyslipidemia (5,6).

It is well known as hypercholesterolemia, hypertriglyceridemia and low HDL-cholesterol are some of the major cardiovascular risk factors. Those parameters seem to be in association with osteoarthritis, moreover having a role in their progression as revealed by many studies. Significant radiological changes were found in unilateral knee osteoarthritis with elevated blood glucose levels and moderately elevated serum cholesterol levels while bilateral knee osteoarthritis is associated with hypertension and elevated cholesterol (7).

In muscle occurs increase the activity of lipoprotein lipase and oxidize non-esterified fatty acids in response to training exercise. Physically active individuals have decreased levels of total plasmatic cholesterol with higher levels of HDL1- and HDL2-cholesterol, lower levels of VLDL- and LDL-cholesterol and reduced triglycerides (8).

The aim of our study was to evaluate the influence of aerobic training on lipid profile in patients with knee osteoarthritis**.**

MATERIAL AND METHODS

We have performed a prospective observational study on 40 patients diagnosed with KOA who were admitted in the Rheumatology Department, Clinical Rehabilitation Hospital of Iasi, Romania, between 2010 and 2011. All patients fulfilled the inclusion criteria as follows: (i) the American College of Rheumatology diagnostic criteria for clinical knee osteoarthritis, (ii) radiographic evidence of grade I to III tibio-femoral or patello-femoral KOA (Kellgren-Lawrence grading system), (iii) sedentary activity pattern and (iv) willingness to undergo a kinetic program. Patients were divided into two groups (20 patients each group), equally distributed by age and gender, according to their participation in the aerobic exercise training comprising 30 minutes of walking per day, five days per week for a period of six months.

Patients with recent knee surgery/ arthroscopy and other conditions that contraindicate physical training, as well as undergoing lipid-lowering drugs were excluded from our study.

A standard evaluation was performed in all cases, including the following parameters at baseline (visit 1), after three months (visit 2) and six months (visit 3): total cholesterol levels (reference values 110-200mg / dl), serum triglycerides (reference values 50-150mg/dl), LDL-cholesterol (reference values 70-130mg/dl) and HDL-cholesterol (38-75mg/dl reference values).

Statistical data processing was performed in SPSS 16.0 software, both descriptive (mean $\pm$ standard deviation) and analytical tests (t-student, Mann-Whitney, Wilcoxon, Kolmogorov-Smirnov) being used in subgroup analysis. The level of statistical significance was set at $p<0.05$ for all tests.

RESULTS

Baseline characteristics of all patients are shown in table I. Although both groups were comparable for demographic data (sex ratio, age), weight, body mass index, a statistically significant difference was demonstrated for all parameters of lipidic metabolism ($p <0.05$). Therefore we selected the first group (group A) to perform the kinetic program as they presented higher abnormalities in lipidic pattern.

*Total cholesterol***.** Statistically **s**ignificant improvement of cholesterol level was de-

tected after three and six months of aerobic training as compared to baseline in all patients with KOA undertaking regular exercises (mean cholesterol level: 224.88 mg/dl at visit 1, 218.45mg/dl at three months, 213.05 mg/dl at six months) ($p<0.05$).

TABLE I
Baseline characteristics of patients with knee osteoarthritis

	Group A	Group B	p
Age* (years)	58.45	57.15	0.531
Cholesterol (mg/dl)	224.88	206.07	0.048
Tryglicerides (mg/dl)	134.88	127.30	0.014
LDL-chol (mg/dl)	136.15	126.02	0.025
HDL-chol (mg/dl)	43.94	46.50	0.048
Weight (kg)	89	82.4	0.186

*mean

On the other hand, we have demonstrated a significant increase in the mean cholesterol level in patients from group B in whom aerobic program was prohibited ($p<0.05$), the upward trend being reflected by the following average values of total cholesterol: 206.07 mg/dl at baseline, 217.40 mg/dl at visit 2 and 210.45mg/dl at the last visit (fig.1).

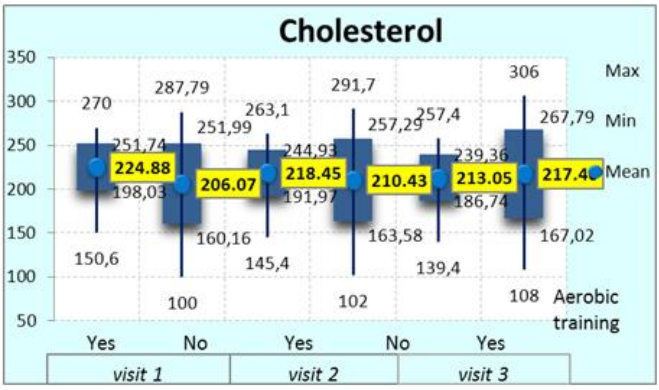

Fig.1. Monitoring of cholesterol in knee osteoarthritis subgroups

*Triglycerides***.** Furthermore, we analyzed triglycerides levels in both groups. A statistically significant decrease has been demonstrated in patients performing aerobic training (mean triglycerides: 134.89 mg/dl at visit 1, 124.42 mg/dl at visit 2 and 116.99 mg/dl at visit 3), while in group B mean triglycerides had a significant upward slope (127.30 mg/dl at baseline, 130.39 mg/dl at three months, 133.39 mg/dl at six months) (fig. 2).

LDL-cholesterol. A progressive decrease in the mean LDL-cholesterol level was demonstrated during the all visits in patients with KOA performing exercises (136.15 mg/dl at visit 1, 127.80 mg/dl at visit 2 and 123.87 mg/dl visit 3). Moreover, the difference in LDL-cholesterol between visits was

also statistically significant (p=0.0001). Compared with patients completing regular aerobic exercises, the mean LDL-cholesterol in patients with KOA in which the aerobic training was not allowed showed a slight increase (126.02 mg/dl at baseline, 128.90 mg/dl at visit 2, and 130.92 mg/dl at visit 3) (fig. 3) .

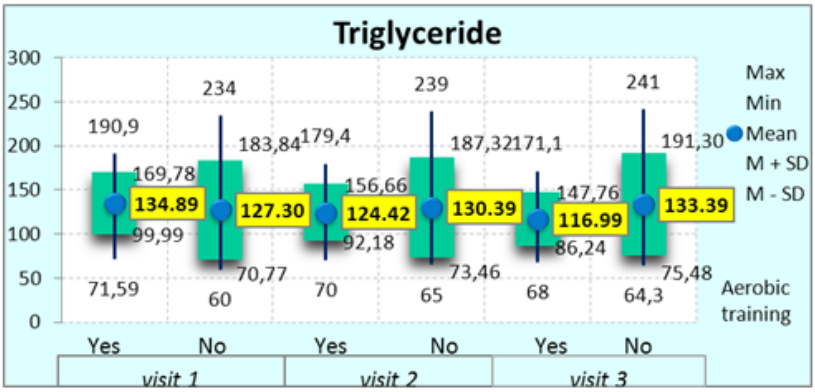

Fig.2. Monitoring of triglycerides in knee osteoarthritis subgroups

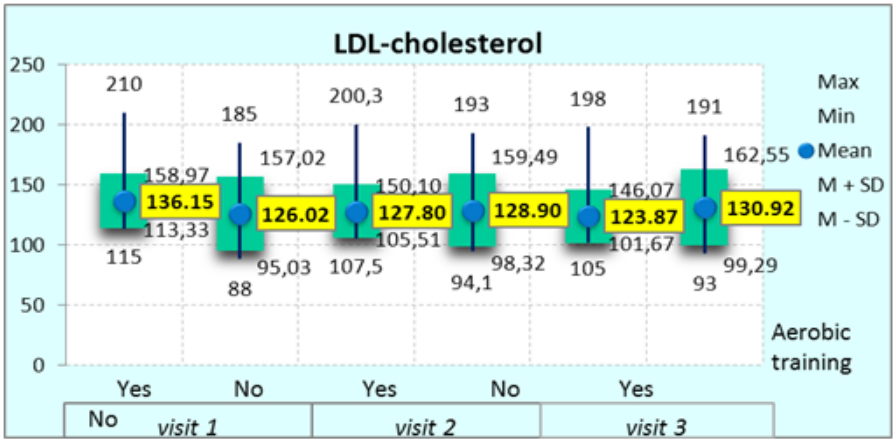

Fig. 3. Monitoring of LDL-cholesterol in knee osteoarthritis subgroups

HDL-cholesterol. **A** progressive increase in the HDL-cholesterol level was reported in patients with performing training over the course of all three visits (43.94 mg/dl at visit 1, 50.03 mg/dl at visit 2 and 57.68 mg/dl at visit 3). The difference between visits was statistically significant (t-Student, p= 0.0001).

Conversely, a progressive decrease in HDL-cholesterol in the second subgroup was observed from baseline (45.1 mg/dl at visit 1, 46.50 mg/dl at visit 2 and 44.56 mg/dl at visit 3).

Figure 4 shows the mean HDL-cholesterol levels in different time points for both groups. A statistical significant difference was registered between groups after six months (p=0.0001).

Weight. We have registered a progressive

decrease in KOA patients completing regular aerobic exercises (89 kg at visit 1, 87.1 kg at visit 2 and 84.7 kg at visit 3), with significant difference in mean weight values at visit 2 and visit 3 (p<0.0001). In contrast, for KOA patient without training weight levels have a upward trend (82.4 kg at baseline, 83.9 kg at visit 2, 85.9 kg at visit 3) (fig 5).

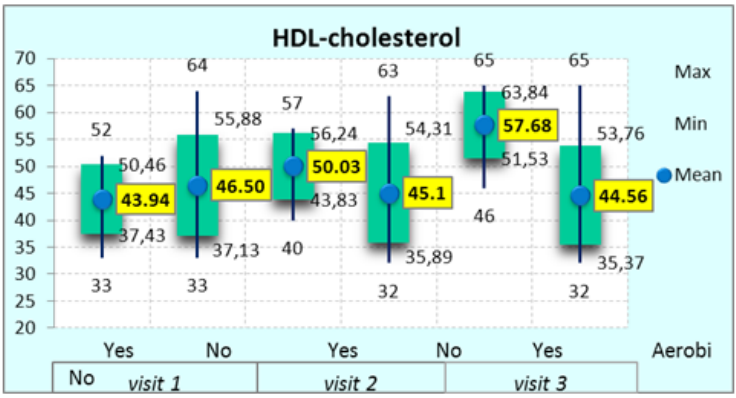

Fig. 4. Monitoring of HDL-cholesterol in knee osteoarthritis subgroups

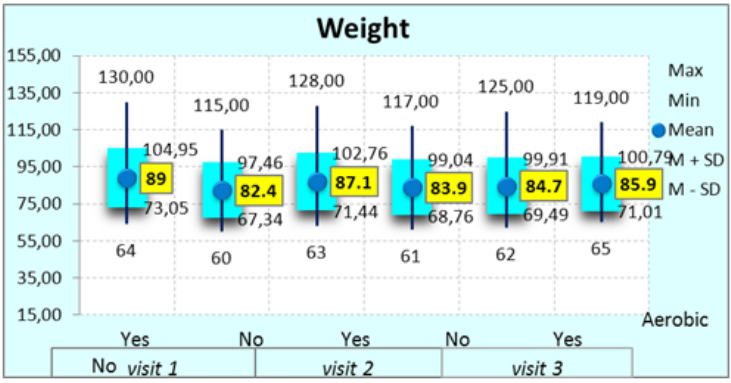

Fig. 5. Monitoring of cholesterol in knee osteoarthritis subgroups

DISCUSSION

Results of the present study suggest that regular aerobic exercise training lowers all the classic lipidic metabolism parameters including: cholesterol, triglycerides, LDL-cholesterol and weight, increasing in the mean time HDL-cholesterol values.

Regarding the intimate mechanism of aerobic training on lipidic metabolism data is lacking in current literature. Some studies proved a connection between weight loss and variation of all lipidic profile parameters (triglycerides, cholesterol, LDL-cholesterol and in HDL-cholesterol concentration) (9,10, 11).

Various studies report changes in serum lipids in different percentages. In general, it was observed that high levels of aerobic activity are accompanied by high levels of HDL-cholesterol. Thus, it was observed that in overweight people with low serum levels of HDL-cholesterol, physical activity in-

creases HDL-cholesterol (12,13).

It is known that exercise has a greater impact on lipid spectrum for women than for men. In our case we encountered no difference, both sexes presenting the same changes in serum lipids.

Effects of exercise on lipids are directly proportional to the duration and intensity of the performed effort. It is widely recognized that aerobic activity such as brisk walking, jogging, swimming, bike riding, or cross-country skiing, loss weight by decreases excess fat and fat-positive change (14,15).

CONCLUSIONS

Regular aerobic exercise appears to have a positive and comparable effect on plasma lipoprotein concentrations. Statistical significant difference was found for HDL fraction reflecting the benefit of aerobic training on cardio vascular risk factors profile. Exercise may be the most efficient way to achieve optimum results in knee osteoarthritis treatment, improving general health status. Further research is needed for the assessment of long-term effects of physical exercises for both KOA and lipid pattern.

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UNILATERAL OPTIC NEURITIS ASSOCIATED WITH INFLUENZA IN ADULT. CASE REPORT

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UNILATERAL OPTIC NEURITIS ASSOCIATED WITH INFLUENZA IN ADULT. CASE REPORT (Abstract): The term optic neuritis refers to inflammation of the optic nerve due to many causes, characterized by subacute unilateral loss of vision, periocular pain and impaired color vision. Optic neuritis can be caused by idiopathic inflammatory demyelination, autoimmune diseases, infectious causes (bacterial, viral, fungal), tumors or ischemic diseases. A few cases of optic neuritis caused by influenza infection have been reported. We report the case of a 51-year-old woman that came to our clinic complaining of a sudden decrease in visual acuity following an episode of flu. The fundus of her left eye showed optic nerve edema. Based on the medical history, fundus appearance, optical coherence tomography, visual field and laboratory investigations (magnetic resonance imaging, blood tests), the diagnosis of optic neuritis associated with influenza was made. **Keywords**: OPTIC NEURITIS, INFLUENZA, CORTICOTHERAPY.

The term of optic neuritis (ON) refers to inflammation of the optic nerve due to many causes, characterized by unilateral painful vision loss. Patients with ON typically complain of the triad of subacute unilateral loss of vision, periocular pain and impaired color vision (1). The pathogenesis of ON is not well understood. It is likely due to some inflammatory processes which lead to delayed type-IV hypersensitivity reaction induced by the released cytokines and other inflammatory mediators from activated peripheral T-cells which can cross the blood-brain barrier and cause destruction of myelin, neural cell death and axonal degeneration (2). In most individuals, ON is caused by idiopathic inflammatory demyelination. This may occur as an isolated syndrome or in association with multiple sclerosis (MS). Approximately 50% of patients with isolated ON develop definite MS within 15 years (3). Nonetheless, inflammatory ON may also be caused by other autoimmune diseases such as sarcoidosis, systemic lupus erythematosus, Sjogren's syndrome or Behcet's disease. Although infectious causes of ON such as herpes Zoster, Lyme disease, syphilis, tuberculosis or toxoplasmosis are rare, they should be kept in mind because a specific treatment is required rapidly (4). Many other conditions such as tumors or ischemic diseases can also cause ON. Based on the location of involvement, ON can be categorized as: 1. retrobulbar neuritis with

normal optic disc appearance; 2. papillitis with swollen disc; 3. perineuritis, which involves the optic nerve sheath while the optic disc may or may not be swollen;4. neuroretinitis with optic disc edema and macular star exudates.

CASE REPORT

We report the case of a 51-year-old woman who came to our clinic complaining of sudden decrease in visual acuity (VA). The patient had a history of flu, with fever, cough and discrete myalgia 2 weeks prior to the ophthalmological problem. She received a treatment with high dose acetaminophen and ibuprofen.

On examination, the best corrected VA was 10/10 for the right eye (RE) and 1/10 for the left eye (LE). Slit lamp examination of the anterior segment of the eye was within normal limits. The fundus of each eye was examined after tropicamide-induced mydriasis and revealed a normal fundus in the RE and optic nerve edema in the LE. In the LE we noticed a protruding and congested optic nerve head, with peripapillary hemorrhage, narrowed arteries and dilated veins and in the lower part of the macula a yellowish nodule (fig. 1).

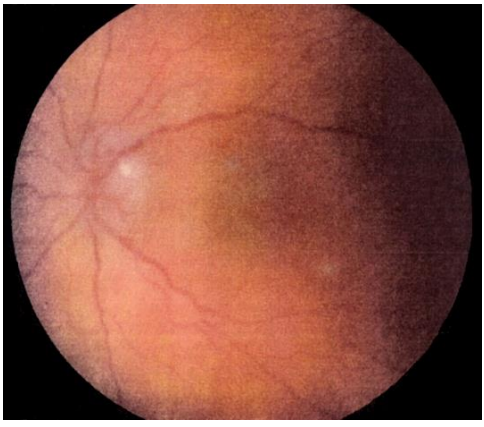

Fig. 1. Left eye fundus - optic nerve edema

Fluorescein angiography revealed normal arterial and venous filling, no delay in disc filling, but late optic disk leakage (fig. 2, 3). The macular nodule showed delayed filling and discrete late phase leakage.

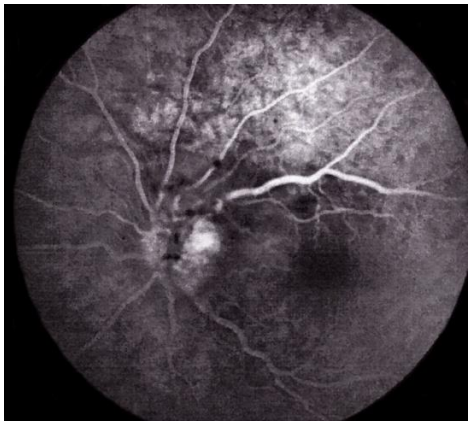

Fig. 2. Angiofluorography-normal vascular filling

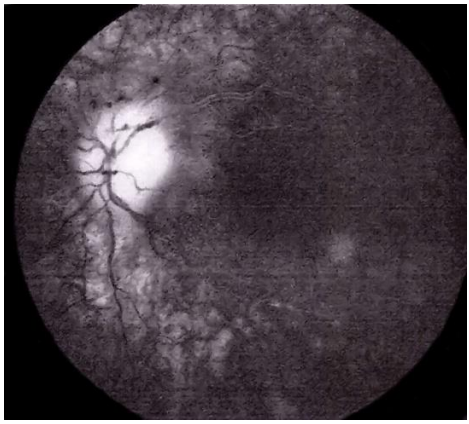

Fig. 3. Angiofluorography – optic disc leakage

Optical coherence tomography (OCT) revealed swollen optic nerve, thickening of the peripapillary retinal nerve fiber layer (RNFL) in the superior, nasal and inferior quadrants (fig. 4), and normal macular thickness.

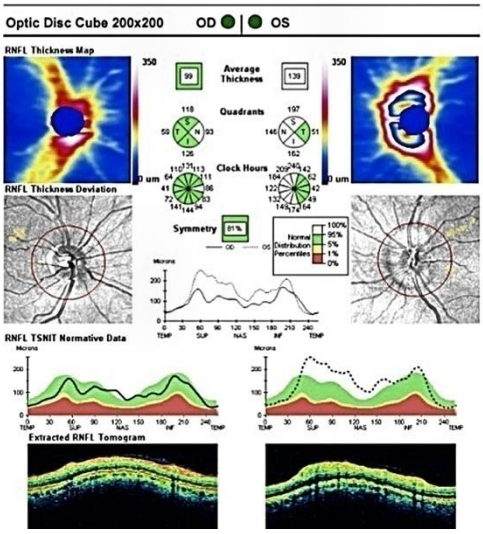

Fig. 4. OCT –RNFL normal in the RE and thickened in the LE

The Humphrey visual field analyzer full field 120 points showed a central scotoma (fig. 5) and Roth 28 Hue test revealed dyschromatopsia in both axes, blue/yellow and red/green (fig. 6).

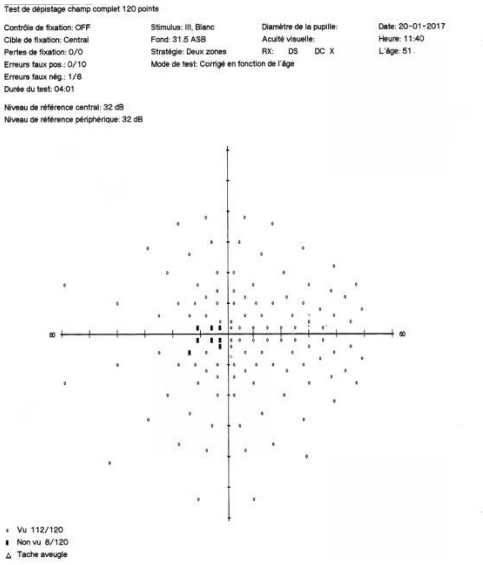

Fig. 5. Visual field LE central scotoma

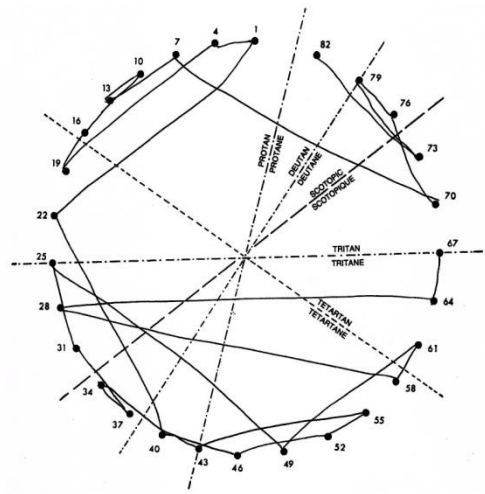

Fig. 6. Roth 28 Hue Test dyschromatopsia

Our first diagnosis was of LE optic neuritis of unknown cause. Initial differential diagnosis included infectious causes (bacterial-Lyme disease, syphilis, tuberculosis, toxoplasmosis; viral - influenza, herpes simplex, herpes zoster, cytomegalovirus), demyelinating causes such as MS, autoimmune diseases (sarcoidosis, systemic lupus erythematosus), compressive or infiltrative diseases.

First, we wanted to exclude a neurosurgical problem, elevated intracranial pressure, as the cause of optic nerve swelling, so we used magnetic resonance imaging (MRI). The MRI revealed unilateral optic nerve swelling, with high T2 signal and contrast enhancement, with no other pathological findings such as expansive intracranial mass or demyelinating MS lesions (fig. 7).

Following exclusion of elevated intracranial pressure, we carried out complete blood tests, including complete blood count, specific antibodies for autoimmune and infectious diseases. The blood count revealed lymphocytosis (55%) and neutropenia (37%). A rapid influenza diagnostic

test was positive for type A influenza. Chest radiography showed no pathological features. Antibodies against Borrelia, Toxoplasma, Treponema pallidum (TPHA), Bartonella henselae, Rickettsia were absent. Tuberculin skin test was negative. As for viruses, we found borderline IgM antibody against herpes simplex virus and positive varicella zoster virus (1.505 mIU/mL) and cytomegalovirus IgG antibodies. The angiotensin converting enzyme was negative (35 U/L), the rheumatoid factor IgM borderline (22 UI/mL), and the antinuclear antibodies (ANA), anti-DNA antibodies and perinuclear anti-neutrophil cytoplasmic antibodies (pANCA) were negative.

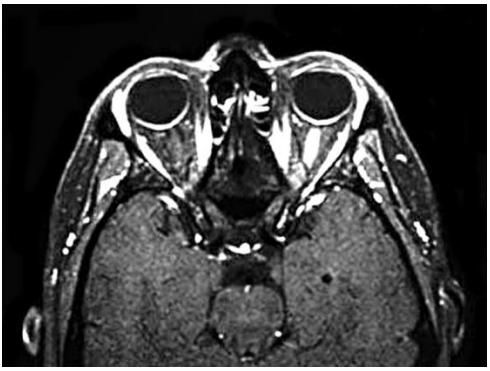

Fig. 7. MRI - LE optic nerve contrast enhancement

Based on medical history, fundus appearance, OCT, VF and laboratory investigations (MRI, blood tests), the diagnosis of LE viral optic neuritis associated with influenza was made. High dose corticotherapy with methylprednisolone (1 g/day for 3 days) was initiated and then decreased to 1mg/body weight. An oral antiviral agent, oseltamivir phosphate 75 mg, was administered twice a day for 5 days. One week later, the disc edema and the hemorrhage were resolved, with slow VA improvement over the first month. Two months later, the VA in the LE was 8/10.

DISCUSSION

Optic nerve involvement with variable visual impairment has been associated with a wide variety of infectious disorders (5). The pathogenesis of infectious optic neuropathies remains speculative. Direct involvement of the optic nerve by a pathogen and indirect involvement with inflammatory, degenerative or vascular mechanisms might contribute to the development of optic nerve involvement (6). Appropriate clinical diagnosis and laboratory work-up of a patient with infectious optic neuropathy are based on history, systemic symptoms and signs, epidemiological data. A few cases of optic neuritis in the setting of influenza infection have been reported. The visual outcome was good after corticosteroid therapy. Neuroretinitis and neuromyelitis optica with a self-limited course were also reported (7). However, there are few reports on ocular complications of influenza A infection. These reports described symptoms such as impaired ocular movement, keratitis, macular lesion, angiitis (8). More often, optic neuritis associated with influenza occurs in children and affects both eyes. We described the case of a 51-year-old woman diagnosed with optic neuritis associated with influenza, which responded well to pulse steroid therapy for 3 days, with smooth recovery from blurred vision and ocular inflammation. Lai reported the first case of acute anterior uveitis and optic neuritis after influenza A infection in a 11-year-old boy. This patient achieved VA improvement with high-dose intravenous pulse steroid therapy for 3 days (9). Nakagawa reported a rare case of acute

anterior uveitis and optic neuritis associated with influenza A infection, which also responded well to corticosteroid therapy (10). Optical coherence tomography suggests involvement of axons (gray matter) in addition to myelin sheath (white matter) in this process. Recent studies have shown that OCT-measured RNFL values may represent a marker for axonal damage in the anterior visual pathway of optic neuritis (11).

CONCLUSIONS

Optic neuritis has been associated with a wide variety of infectious disorders. It is very important to make a thorough examination of a patient with optic nerve involvement to establish the correct diagnosis.

We described a rare case of unilateral optic neuritis associated with influenza A infection in an adult patient. This case calls our attention to the unusual association between systemic infection and optic nerve inflammation. Despite the important decrease in visual acuity at presentation and the optic disc edema, the patient responded well to corticosteroid therapy, with almost full recovery of the visual acuity. Studying the literature, we found very few reports on ocular complications of influenza A infection in adults.

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CARDIOMIOPATIA TAKOTSUBO

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TAKOTSUBO CARDIOMYOPATHY (Abstract): Takotsubo cardiomyopathy or “heart broken syndrome” or transient apical ballooning syndrome is an increasingly reported syndrome characterized by the fact that most patients are women aged over 65 years. The most common electrocardiographic changes are ST-segment elevation and negative T waves in precordial leads. Symptoms at onset are similar to those of acute myocardial infarction; the ventricular dysfunction is shaped like a *takotsubo* (a Japanese pot for fishing octopus), echocardiography and ventriculography show akinesia, hipokinesia or diskinesia(ballooning) of apical segments of left ventricle and hyperkinesia of the basal; the coronary arteries are normal angiographic; the dysfunction improves rapidly within a few weeks. Its origins are unclear, there are several pathophysiological theories, most sustained is a myocardial stunning secondary catecholamine-induced excess of intense physical or mental stress. There is no clear standard of treatment. In cases evolving with hemodynamic instability the use of beta agonist agents must be avoided, aortic balloon counterpulsation is preferable. The patient prognosis is usually good, reaching maximum mortality of 8%. In rare situations were reported complications such as acute pulmonary oedema, cardiogenic shock, acute mitral regurgitation, potentially fatal arrythmias ventricular fibrillation or torsades de pointes. In Cardiology Clinic of Cardiovascular Diseases Institute “Prof. Dr. George I.M. Georgescu” Iasi were hospitalized 4 cases with Takotsubo cardiomyopathy from 2008 to 2011. Their features are presented in this paper. The difference of prognosis and treatment between Takotsubo cardiomyopathy and acute myocardial infarction require an accurate diagnosis, the clinical hypothesis result only after knowledge the clinical and paraclinical peculiarities of this pathological entity. **Key words:** TAKOTSUBO, CARDIOMYOPATHY, APICAL BALLOONING SYNDROME.

Cardiomiopatia Takotsubo este un sindrom caracterizat prin manifestări clinice compatibile cu cele din infarctul miocardic acut(IMA), supradenivelare de segment ST şi unde T negative în derivațiile precordiale, creştere uşoară sau moderată a enzimelor de citoliză miocardică, tulburări tipice de kinetică regională de ventricul stâng (VS), complet reversibile, în prezența unor artere coronare normale angiografic sau fără leziuni semnificative.

În paralel cu datele teoretice vom prezenta particularitățile a patru cazuri de cardiomiopatie Takotsubo, diagnosticate în Institutul de Boli Cardiovasculare Iaşi, în perioada 2008 – 2011.

ETIOLOGIE

Cauza acestui sindrom rămâne necunoscută (1). S-a constatat că afectează predominant femeile în postmenopauză (apare într-un procent de 70 – 80% la femei de peste 65 ani), cel mai frecvent după un stres emoţional sau fizic (1, 2). Factorul stresor anterior este o caracteristică importantă, în literatură citându-se foarte rare cazuri când acesta nu a putut fi anamnestic decelat.

În clinica noastră, toate cele 4 cazuri de cardiomiopatie Takotsubo au fost femei, anamneza punând în evidenţă un stres psihic intens anterior declanşării simptomatologiei.

EPIDEMIOLOGIE

Datorită caracteristicelor sale, în camera de gardă este adesea confundat cu un sindrom coronarian acut prin obstrucţie coronariană, motiv pentru care este dificil de estimat prevalenţa reală a acestei afecţiuni. Datele din literatură indică o prevalenţă de 2% din totalul cazurilor de sindroame coronariene acute, cu o creştere în ultimii ani probabil prin scăderea cazurilor de diagnostic fals negativ (2).

PATOGENIE

S-au elaborat mai multe teorii cu privire la fiziopatologia acestui sindrom, fără a exista un concept unitar. Cea mai susţinută teorie este cea a unui *stunning* miocardic indus de descărcarea în exces de catecolamine, consecinţă a unui stres puternic, psihic sau fizic. Catecolaminele, în cantitate crescută, pot determina prin efect direct injurie miocardică (toxicitate catecolaminică). Modificările histologice tipice de toxicitate catecolaminică sunt observate în cardiomiopatia Takotsubo precum şi în alte condiţii clinice asociate cu producţie crescută de catecolamine - feocromocitom, hemoragie subarahnoidiană. De remarcat faptul că anomaliile de kinetică întâlnite în cardiomiopatia Takotsubo nu sunt similare cu cele din hemoragia subarahnoidiană sau intracraniană când sunt afectate segmentele bazale ale VS (deşi conform acestui concept au acelaşi mecanism fiziopatologic) (1).

Alte teorii propuse pentru fiziopatologia acestui sindrom sunt cea a unui spasm miocardic multivascular (nu a fost demonstrat decât la un număr mic de pacienţi) şi cea a unei disfuncţii endoteliale generalizate la nivelul microcirculaţiei (este neclar dacă reprezintă mecanismul patogenic primar sau este un fenomen secundar; în plus, cauza acestei disfuncţii endoteliale rămâne necunoscută) (1).

CLINIC

Cele mai frecvente simptome de debut sunt durerea toracică anterioară similară celei din IMA, dispnee şi uneori sincopă(3). Au fost semnalate cazuri cu degradare hemodinamică – edem pulmonar acut, şoc cardiogen(4). Au fost citate aritmii cu potenţial letal – fibrilaţie ventriculară, torsada vârfurilor (1,2,5,6).

Un caz de cardiomiopatie Takotsubo diagnosticat în clinica noastră s-a manifestat prin şoc cardiogen, ceea ce a impus măsuri suplimentare şi speciale de tratament (inclusiv contrapulsaţie aortică cu balon). Nu s-au înregistrat aritmii maligne în nici unul din cele 4 cazuri, dar la pacienta cu şoc cardiogen s-a iniţiat încărcarea cu amiodaronă în contextul instalării fibrilaţiei atriale.

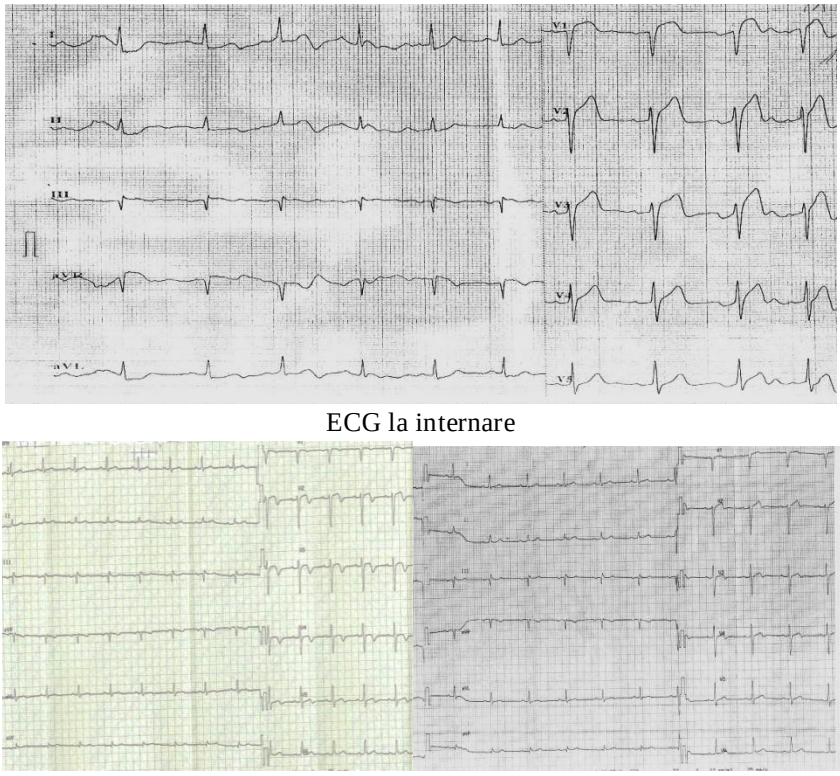
ELECTROCARDIOGRAFIC

Cele mai frecvente modificări sunt supradenivelarea de segment ST şi unde T negative în derivaţiile precordiale, ce au fost descrise de unii autori ca evoluând în stadii succesive (1,5,7).

În evoluţie apar unde T negative, adânci, largi, cu alungirea intervalului QT

– anomalie ce poate explica apariția uneori a tulburărilor de ritm maligne. Într-o proporție mai scăzută pot apare şi unde Q patologice (2,8).

Modificările electrice înregistrate în cele patru cazuri din clinica noastră sunt comparabile cu cele descrise în literatura de specialitate (fig. 1).



ECG la internare

ECG în ziua a treia de evoluție ECG la externare

Fig. 1. Evoluția modificărilor electrice într-un caz de Cardiomiopatie Takotsubo spitalizat în IBCV Iaşi

BIOCHIMIC

Enzimele de citoliză miocardică sunt uşor - moderat crescute, dar pot fi şi în limite normale după unii autori; de asemenea, s-au descris concentrații serice crescute de BNP la pacienții cu cardiomiopatie Takotsubo; valori serice scăzute ale NT pro-BNP s-au corelat cu evoluția favorabilă a acestui sindrom (1).

În clinica noastră, s-a determinat CK-MB, valorile maxim înregistrate fiind modeste şi în concordanță, de asemenea, cu datele din literatură (33 U/L - 68U/L).

ECOCARDIOGRAFIC

În secțiunea apicală a 4 camere se evidențiază modificările tipice de kinetică regională ale cardiomiopatiei Takotsubo: akinezia, hipokinezia sau diskinezia segmentelor apicale ale ventriculului stâng şi hiperkinezia celor bazale, aspect morfologic care a dus la denumirea de "takotsubo". Fracția de ejecție(FE) a VS este deprimată, cu valori ce variază între 20% - 49%(2). În

foarte puține cazuri s-au descris trombi apicali (9,10). În unele situații, în faza acută, s-a evidențiat obstrucție la nivelul tractului de ejecție a VS, după unii autori cu un gradient maxim de până la 60 mmHg. De asemenea, a fost observată balonizarea medioventriculară ce separă segmentele apicale de cele bazale (11,12) sau insuficiența mitrală acută (1,13).

Au fost raportate cazuri care au evoluat cu implicarea ventriculului drept(VD), situații în care s-a semnalat o deprimare mai accentuată a FE a VS. Segmentele cele mai frecvent afectate ale VD au fost: lateroapical, anterolateral şi cel inferior. Reacția pleurală a fost descrisă ca fiind adesea asociată acestor cazuri (14). Au fost raportate cazuri de Takotsubo inversat, caracterizate printr-un stunning al segmentelor bazale şi hiperkinezie a celor apicale (15). Tulburările de kinetică descrise în cardiomiopatia Takotsubo sunt complet reversibile într-un interval de timp de zile – săptămâni, ceea ce constituie una dintre cele mai importante caracteristici ale acestui sindrom.

CORONAROGRAFIA ŞI VENTRICULOGRAFIA

În toate cazurile raportate, coronarografia a exclus prezența leziunilor coronariene semnificative, caracteristică importantă a acestui sindrom. Ventriculografia evidențiază anomaliile de kinetică regională (fig. 2).

Fig.2. Coronarografie, ventriculografie (efectuată de I. Nedelciuc) şi secțiune ecocardiografică în sistolă la o pacientă spitalizată în IBCV Iaşi pentru Cardiomiopatie Takotsubo

Într-unul din cazurile din clinica noastră, suplimentar față de tulburările tipice de kinetică regională, s-a obiectivat insuficiența mitrală acută prin ischemia muşchiului papilar, ceea ce explică şi starea clinică gravă de la internare (şoc cardiogen) (fig. 2).

Pacientele diagnosticate cu cardiomiopatie Takotsubo în clinica noastră nu au prezentat leziuni coronariene semnificative, criteriu fundamental pentru susținerea diagnosticului (fig. 2).

ALTE INVESTIGAȚII

Rezonanța magnetică nucleară oferă informații privind morfologia şi funcția VS şi exclude infarctul miocardic sau existența unui proces inflamator. În numeroase cazuri s-a evidențiat un defect de perfuzie în regiunile apicale în timpul fazei acute, în pofida absenței leziunilor coronariene semnificative, modificări ce se ameliorează progresiv în timpul fazei de recuperare. S-au realizat biopsii endomiocardice atât de la nivelul VS, cât şi a VD ce nu au evidențiat semne de miocardită (1).

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TRATAMENT

Nu există o standardizare clară a tratamentului. Se consideră totuşi necesar abordarea inițială a acestor pacienți similar celor cu IMA, până la obținerea unui diagnostic diferențial invaziv. În cazurile ce evoluează cu instabilitate hemodinamică, trebuie evitată utilizarea de agenți ß-agonişti, în aceste situații fiind preferabil suportul mecanic – balon de contrapulsație aortică. În clinica noastră acesta a fost montat la pacienta care a evoluat cu şoc cardiogen.

Studiile efectuate pe animale au arătat că evoluția a fost îmbunătățită de administrarea de agenți ß-blocanți care ar trebui utilizați atât în faza acută, cât şi cea cronică în vederea prevenției recurenței, care poate apare până la 8% din cazuri (1).

Având în vedere cazurile citate în literatură care au evoluat cu formare de trombi în VS, unii autori consideră prudent instituirea unui tratament anticoagulant cu heparină până la recuperarea funcției sistolice a VS. Dintre agenții terapeutici utilizați în cazurile citate în literatură mai fac parte inhibitorii enzimei de conversie a angiotensinei şi antiagregantele plachetare.

PROGNOSTIC

Prognosticul pacienților cu cardiomiopatie Takotsubo este favorabil, rata mortalității intraspitaliceşti raportată fiind cuprinsă între 0–8% (1). Simptomatologia, modificările electrocardiografice, enzimatice şi eco-cardiografice dispar în majoritatea cazurilor într-un interval de timp variabil (zile – săptămâni) (1).

Evoluția intraspitalicească a cazurilor din clinica noastră a fost favorabilă. Cel mai precoce, tulburările de kinetică au dispărut într-unul din cazuri după 20 ore de evoluție. Pacienta cea mai gravă, care a prezentat inițial şoc cardiogen şi insuficiență mitrală acută severă, a avut o evoluție lent favorabilă, cu dispariția tulburărilor de

kinetică segmentară şi reducerea regurgitării mitrale până la gradul I, după trei luni de tratament.

CONCLUZII

Deşi relativ rară**,** cardiomiopatia Takotsubo este o afecțiune cu care trebuie făcut diagnosticul diferențial al unui infarct miocardic acut în anumite situații particulare. Diferențele terapeutice şi prognostice dintre aceasta şi IMA, fac necesar un diagnostic corect cât mai precoce, ipoteza clinică de boală Takotsubo putând rezulta doar în urma cunoaşterii şi aprofundării particularităților clinice şi paraclinice a acestei entități patogenice.

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HIDATIDOZA MALIGNĂ – CAZ CLINIC

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MALIGNANT HYDATID DISEASE – CASE PRESENTATION (Abstract): We report the case of a 79-year-old woman admitted for sepsis from a hepatic hydatid cyst with multiple disseminations diagnosed one month earlier who refused the surgical treatment. Her family history was irrelevant with regard to the circumstances leading to *Taenia Echinococcus* infestation, and given her poor health status and absence of previous medical documents no useful information was obtained from her medical history. The patient also presented chronic ischemic heart disease and anemia secondary to a bleeding duodenal ulcer, found at necropsy, which worsened the already unfavorable course by the acute hepatic and renal failure secondary to toxic and septic shock. Despite the complex treatment, death occurred 6 days later, following a second episode of upper digestive hemorrhage. Particular to this case is the absence in patient’s history of the anaphylactic/allergic reactions suggestive of hepatic hydatid cyst dissemination. **Key words:** DISEMINATED HEPATIC CYST, MALIGNANT HYDATID DISEASE, TAENIA ECHINOCOCCUS.

Maladia hidatică este determinată de agentul patogen Taenia echinococcus care poate infesta organismul uman la orice vârstă exprimându-se prin suferința organului afectat atunci când determină fenomene compresive sau prin complicații. Având în vedere posibilitatea infestării la nivelul oricărui organ cu caracter primar sau secundar precum şi severitatea evolutivă în absența tratamentului adecvat această boală a fost cunoscută şi sub denumirea de “hidatidoză malignă”. Prezentarea cazului urmăre şte caracterul imprevizibil şi incurabil al acestei boli ajunsă în stadiul de complicații.

PREZENTAREA CAZULUI

D.M , 79 ani, sex feminin din localitatea Ia şi, jud. Ia şi, a fost internată în clinică pentru dureri abdominale difuze, astenie fizica marcată, stare septică, simptomatologie progresiv agravată în ultima lună. Dintre antecedentele personale patologice s-a remarcat diagnosticarea cu o luna anterior la vârsta de 79 ani a cardiomiopatiei ischemice complicate cu fibrilație atrială permanentă şi insuficiență cardiacă cronică globală ocazie cu care s-a menționat prezenta unui chist hidatic hepatic complicat, pentru care s-a recomandat tratament chirurgical refuzat de pacientă şi de familie. Din anamneza efectuată cu ajutorul familiei nu s-a putut semnala în antecedente existența unor circumstanțe care să explice infestarea cu *T. echinococcus* iar condițiile de viață şi muncă nu au obiectivat circumstanțe particulare cu implicații patologice.

Istoricul pacientei nu s-a putut face corect, anamneza fiind dificilă atât prin starea generală influențată cât și prin lipsa documentelor medicale antecedente. Cu o lună anterior a fost internată într-o clinică medicală pentru investigarea simptomelor cardiovasculare cronice, concomitent diagnosticându-se chist hidatic hepatic cu posibilă diseminare peritoneală precum și un sindrom anemic de etiologie neprecizată. Sub medicația necesară patologiei cardiace, la domiciliu, starea generală a pacientei s-a degradat rapid și progresiv, apărând dureri abdominale difuze, anorexie și astenie fizică.

Examenul clinic a relevat tegumente și mucoase palide, afebrilitate, semne de deshidratare (limba prăjită, pliu cutanat persistent), elemente de decompensare cardiacă globală exprimată prin edeme membre inferioare, raluri crepitante bazal bilateral, jugulare turgide gr. 3, TA la internare 100/60 mmHg, zgomote cardiace tahiaritmice 100/min, hepatomegalie din sp.VI medioclavicular drept la 10 cm sub rebordul costal cu contur neregulat, dura, dureroasă la palpare superficială, cu apărare musculară la palparea profundă, formațiuni tumorale multiple, de dimensiuni diferite, palpabile pe suprafata abdominală, oligoanurie (aprox. 400ml/24h), confuzie.

Datele de laborator au arătat sindrom de retenție azotată cu accentuare progresivă - creatinina de la 2,03 mg/dl la internare la 3,20 mg/dl, ureea de la 159 mg/dl la 308 mg/dl in urmatoarele 5 zile, anemie hipocromă microcitară feriprivă cu evoluție de la 10 g/dl la 8,4 g/dl, fier seric 12 µg/dl, sindrom inflamator nespecific (leucocitoză - de la 18300/mm^3 la 22800/mm^3 cu neutrofilie 85% și eozinofilie 7%, VSH crescut - 99mm/1h, 131mm/2h), hipopotasemie – 2,8 mEq/l (la internare, ulterior corectată).

Pe EKG s-a obiectivat fibrilație atrială cu frecvența ventriculară medie de 80/min, elemente de impregnare digitalică.

Radiografia toracică - ICT=0,58, cord cu dimensiuni crescute, aorta ateromatoasă, desen pulmonar accentuat. Ecocardiografic - aspect de cardiomiopatie ischemică și hipertensivă, calcificări de valvă mitrală și aortică cu regurgitare aortică moderată, regurgitare mitrală severă, regurgitare tricuspidiană severă, revărsat lichidian pericardic în cantitate moderată.

La ecografia abdominală s-au evidențiat chist hidatic hepatic în segmentul VI cu dimensiuni de 95/67/76 mm (fig. 1), un alt chist hidatic în segmentul III de 90/112/56 mm cu mici vezicule fiice în periferie cu tendința la decolarea membranei (fig. 2) și un alt chist hidatic cu vezicule fiice localizat subhepatic în dreptul lobului stâng hepatic cu dimensiuni de 53/34/52 mm (fig. 3); rinichi drept si stang normali, splină normală.

Datele obținute clinic și paraclinic au pus în discuție prezența unei hidatidoze complicate cu supurație și diseminări multiple, cu elemente de insuficiență hepatică și renală acută secundare șocului toxicoseptic, fără a se putea explica tipul anemiei (în aceste circumstanțe – de tip inflamator)

Evoluția a fost nefavorabilă în ciuda tratamentului complex administrat și în a 5-a zi de la internare pacienta a prezentat un episod de melenă pentru care s-a instituit tratament medicamentos, starea generală gravă nepermițând endoscopia digestivă superioară. Decesul a survenit in a 6-a zi de evoluție după al doilea episod de hemoragie digestivă superioară (hematemeza), prin stop cardiorespirator ireversibil la manevrele de resuscitare. Anamneza antecedentelor personale patologice, reluata cu ajutorul familiei, nu a semnalat prezența unor leziuni digestive cunoscute cu potențial hemoragic.

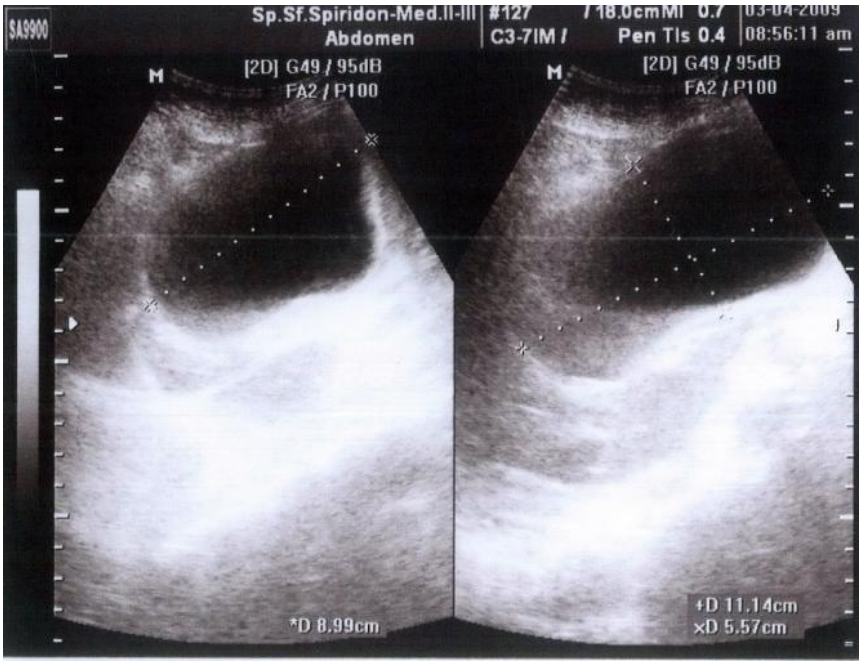

Fig. 1. Chist hidatic hepatic

Fig. 2. Chist hidatic cu decolarea membranei

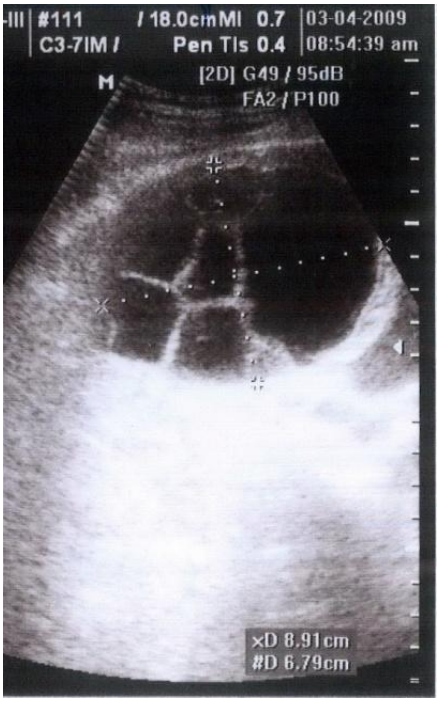

Fig. 3. Chist hidatic cu vezicule fiice

Examenul necroptic efectuat a arătat multiple chisturi hidatice hepatice (lob stâng şi drept), peritoneul aderent şi calcificat la marginea anterioară a ficatului, aderenţe strânse ale unghiului hepatic al colonului la unul din chisturile hidatice din lobul hepatic drept şi inflamaţie purulentă de tip flegmonos subhepatic şi perirenal drept, ulcer duodenal hemoragic (2,5/1,5 cm) însoţit de multiple ulceraţii hemoragice de vecinătate, tub digestiv cu conţinut hemoragic parţial digerat, cardiomegalie cu hipertrofie ventriculară stîngă concentrică si dilatarea cavităţilor drepte, aterosclerozã generalizată cu plăci calcificate aortice, unele ramolite, stază şi edem pulmonar.

DISCUŢII

Boala chistică hidatică se datorează în special *Echinococcus granulosus*. Infestarea cu *E. vogeli* este foarte rară şi este raportată în special în zonele sudice ale Americii de Sud (3). Infestarea cu *E. granulosus* este endemică în regiunile crescătoare de oi şi vite din Orientul Mijlociu, ţările din bazinul Mediteranei, Europa Centrală şi de Est, America de Sud, Australia, Noua Zeelandă şi Africa de Sud (1, 2, 3).

La om hidatidoza este cel mai frecvent hepatică (55-70%), apoi pulmonară (18-35%) iar aceste două organe pot fi afectate simultan în 5-13% din cazuri (4, 5). Incidenţa echinococcozei cistice în regiunile endemice este de 1-220 cazuri la 100.000 locuitori, pe când incidenţa echinococcozei alveolare este de 0,03-1,2 cazuri la 100.000 locuitori (3).

La fel ca şi în alte infecţii date de cestode, speciile de echinococcus prezintă atât gazde definitive cât şi gazde intermediare. Gazdele definitive sunt câinii care elimină ouăle în materiile fecale. Atunci cînd gazdele intermediare – omul, bovinele, oile, caprele, caii (pentru *E. granulosus*) şoarecii şi alte rozătoare (pentru *E. multilocularis*) – ingeră ouăle, în organismul acestor gazde se dezvoltă chisturi. Atunci când un câine ingeră carne de vită sau de oaie conţinând chisturi, ciclul biologic e finalizat (6).

Manifestările clinice ale bolii depind de mărimea şi localizarea chisturilor. Majoritatea pacienţilor rămân asimptomatici până la vârste înaintate cum este şi cazul pacientei noastre. Chisturile produc disfuncţia organelor afectate inclusiv obstrucţie biliară, ciroză, obstrucţie bronşică, insuficienţă renală, hipertensiune intracraniană şi hidrocefalie. Pacienţii simptomatici cu hidatidoză hepatică prezintă de cele mai multe ori dureri abdominale sau o formaţiune palpabilă în cadranul superior drept. Compresia tractului biliar sau scurgerea lichidului din chist în arborele biliar poate mima litiaza biliară recurentă, obstrucţia biliară putând avea ca rezultat apariţia icterului. Ruptura sau scurgerea episodică dintr-un chist hidatic poate produce febră, prurit, urticarie, eozinofilie sau un episod anafilactic fatal.

Echinococcoza peritoneală poate apare în 2 circumstanţe (6): primitivă – prin migrarea unui chist hidatic hepatic în cavitatea peritoneală, localizarea fiind de obicei declivă (fosele iliace), uniloculară şi pe suprafaţa ficatului se pot detecta alte chisturi sau cicatrici; secundară – postoperator sau posttraumatic de la nivelul unui chist hidatic hepatic, localizarea fiind multiplă în toată cavitatea peritoneală.

Diagnosticul imagistic este important pentru detectarea şi evaluarea chisturilor hidatice. Radiografiile simple detectează chisturile pulmonare precum şi chisturile cu perete calcificat de exemplu de la nivel hepatic. Examenul ecografic şi computer tomografic pun în evidenţă leziunile primare

precum şi veziculele fiice (7, 17, 18). Examinarea prin rezonanță magnetică nucleară este superioară tomografiei computerizate pentru evaluarea recurențelor, leziunilor reziduale postchirurgicale precum şi pentru evidențierea unor determinări extrahepatice (19). Diagnosticul specific trebuie realizat prin examinarea lichidului aspirat în vederea evidențierii cârligelor scolecşilor, dar aspirația în scop diagnostic nu este recomandată, în mod normal, datorită riscului scurgerii lichidului hidatic şi producerii unor reacții anafilactice sau diseminării infecției. Analizele serologice pot fi utile (8), cu toate că un rezultat negativ nu exclude diagnosticul echinococcozei. Comparativ cu chisturile pulmonare, chisturile hepatice induc mult mai probabil răspunsuri imune umorale , dar pâna la 50% dintre indivizii afectați pot avea analize serologice negative (6).

Tratamentul constă în ablația chirurgicală (9, 10, 11, 12, 13, 21). De asemenea, administrarea pre- şi post - operator timp de o lună de Albendazol şi 2 săptămâni de Praziquantel ar trebui efectuată pentru sterilizarea chistului, scăderea tensiunii intrachistice, scăderea riscului de şoc anafilactic şi reducerea recăderilor postoperatorii (22). Studii recente afirmă necesitatea continuării tratamentului timp de 2 ani, fără efecte secundare semnificative (19, 20).

La cazul prezentat se remarcă descoperirea întâmplătoare a chistului hidatic hepatic la vârsta de 79 ani cu ocazia investigațiilor patologiei cardiovasculare. La această pacientă a coexistat un sindrom anemic care ar fi putut preta la probleme de diagnostic diferențial a formațiunii chistice intrahepatice precum şi alterarea organică funcțională complexă care a creat particularități de exprimare ale maladiei dar şi evoluția spre depreciere rapidă. În experiența clinică mondială sunt rar semnalate complicații asociate de tip suprainfectare a formațiunii chistice la nivelul unui organ coexistând cu diseminări la nivelul altor organe, ceea ce presupune evoluția fie primară a unei hidatidoze multiple de organe, fie fisurarea leziunii primare cu luni/ani anterior şi exprimarea clinică a complicațiilor infecțioase recent produse (6).

În aceste circumstanțe considerăm ca fiind o particularitate semnificativă absența reacțiilor alergice/anafilactice în antecedentele pacientei care ar fi putut exprima diseminarea unui chist hidatic hepatic fisurat. În literatură se semnalează că asemenea incidente sunt marcate de suprainfecția chistului şi/sau obstrucția tractului biliar sau arborelui bron şic având drept consecințe pneumonia, revărsatul pleural, pneumotoraxul, echinococcoza secundară în cavitatea pleurala, peritoneală sau pericardică (23).

Deşi pacienta şi familia sa au refuzat tratamentul chirurgical propus într-o internare precedentă, la descoperirea formațiunii chistice hepatice, evoluția ulterioară a cazului şi rezultatul anatomo-patologic necroptic susțin imposibilitatea rezolvării acestei patologii complexe în ciuda tratamentului recomandat în literatură (tratamentul chirurgical asociat cu chimioterapie specifica). Aceste cazuri, deşi rare, reprezintă un semnal de alarmă asupra subevaluării diagnostice mai ales în circumstanțele unui mod de viață precar igieno-sanitar care poate determina risc crescut de infestare. Simptomele clinice de tip imunoalergic, explorările ecografice abdominale efectuate cu atenție ar putea fi metode de diagnostic şi tratament în timp util. Abordarea diagnostică optimă este la nivel de medicină de famile deoarece pot fi apreciate cel mai corect condițiile de mediu ale pacienților şi verigile de legatură din etapele lanțului infestării.

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ASPECTE ALE PROFILULUI DE REZISTENȚĂ ANTIMICROBIANĂ ÎN INFECȚII CU ESCHERICHIA COLI ȘI KLEBSIELLA PNEUMONIAE LA PACIENȚII DIABETICI

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ASPECTS OF THE ANTIMICROBIAL RESISTENCE PROFILE IN INFECTIONS WITH ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE IN DIABETIC PATIENTS (Abstract): Infections in diabetic patient remains an important cause of morbidity and mortality, triggering and maintaining a prolonged metabolic imbalance. Emergence of extented spectrum beta-lactmase (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae* is a major concern, because of the atypical manner infection acts in this group of imunodepressed patients and also for the limited therapeutic solutions. For this reason we have evaluated the profile of antimicrobial resistance of these pathogens in both diabetic and non diabetic patients**. Material and method:** The aim of this study was to evaluate, in a retrospective case control study, the antibiotic susceptibility pattern in isolates of *E. coli* and *Klebsiella spp*. from different biological products in 49 diabetics and 150 non-diabetics admitted in The Clinical Hospital of Infectious Diseases Iași over a period of two years. **Results:** Most of strains of *E. coli* and *Klebsiella spp*. ESBL positive were found in uroculture. Significant differences in *E. coli* resistance rate between diabetics and non-diabetics were noted for amoxicillin-clavulanic acid and ciprofloxacin (31,4% vs.13,98%, p=0,04, respectively 52,9% vs. 24,46%, p=0,004). More isolates of ESBL positive *K. pneumoniae* were found in diabetic patients (50% vs. 24%). Ciprofloxacin resistance of *K. pneumoniae* was significantly higher in diabetics (75% vs 39%; p=0,05). There was no resistance in *E. coli* and *K. pneumoniae* isolates to imipenem in the diabetic group. **Conclusions:** The high resistance rate to quinolones and 3rd generation cefalosporins limits their use for the treatment of *Escherichia coli* and *K. pneumoniae* infections. Other alternatives for empiric therapy in community and nosocomial-acquired infections in diabetic patient remains carbapenems, aminoglycosides and colimycin. **Key words:** GRAM NEGATIVE INFECTIONS, DIABETIC INFECTION, ESBL, *KLEBSIELLA SPP., ESCHERICHIA COLI.*

Infecțiile cu bacili Gram negativ (BGN) la bolnavul diabetic constituie o cauză de morbiditate și mortalitate ridicată, fiind responsabile de declanșarea și menținerea

unui dezechilibru metabolic prelungit. Pacienţii cu diabet par a avea un risc particular crescut pentru infecţii cum ar fi otita externă invazivă, mucormicoza rinocerebrală, infecţii emfizematoase ale veziculei biliare, rinichilor şi vezicii urinare, cu o evoluţie şi un prognostic adesea nefavorabil.

Infecţiile formatoare de gaz de la nivel parenchimului renal, ureterului sau vezicii urinare survin în peste 90% din cazuri la pacienţii diabetici, patogeneza acestei boli fiind probabil legată de abilitatea unor microorganisme cum ar fi *E. coli* sau *K. pneumoniae* de a utiliza glucoza cu formare ulterioară de dioxid de carbon şi hidrogen. Mortalitatea globală în aceste situaţii ajunge la 18% (7).

K. pneumoniae este printre cele mai frecvente microorganisme întâlnite la nivel mondial, fiind implicată atât în infecţii nosocomiale (pneumonie, infecţii ale tractului urinar, infecţii intraabdominale), cât şi în infecţii comunitare. Pe lângă tabloul clinic dramatic al pneumoniei comunitare cu *Klebsiella* descris în studii din anii 1920-1960, cu o incidenţă în descreştere în ultima decadă (mai puţin de 1% de cazuri spitalizate în America de Nord), noi entităţi clinice ale infecţiei comunitare cu *K. pneumoniae* au fost obiectivate de diferiţi autori, în special în Asia (1, 2). Peste 900 de pacienţi cu abces hepatic cu *Klebsiella* au fost raportaţi în Taiwan în ultimii 10 ani, 70% dintre aceştia având diabet zaharat. În contrast, doar 23 de pacienţi au fost raportaţi cu aceeaşi condiţie în regiuni nonasiatice (3). Infecţiile cu tulpini de *E.coli* şi *K. pneumoniae* producătoare de beta-lactamaze cu spectru extins (BLSE) au fost descrise ca infecţii nosocomiale sau asociate serviciilor de îngrijiri medicale. Totuşi, date recente sugerează implicarea acestor microorganisme producătoare de BLSE şi în infecţii comunitare (6).

Deşi diabetul este recunoscut ca factor de risc pentru numeroase infecţii, un număr mare de factori amplifică eforturile de a stabili riscul infecţiei şi al complicaţiilor acesteia, studiile specifice comparative cu pacienţii non-diabetici fiind rare**.** Datele referitoare la rezistența *E. coli* și *Klebsiella spp*. la acest grup de pacienți imunodeprimați sunt în permanentă schimbare. Pentru acest motiv am evaluat profilul rezistenței la antibiotice al acestor microorganisme comparativ la pacienți diabetici și pacienți fără diabet.

MATERIAL ŞI METODĂ

S-a realizat un studiu caz-martor retrospectiv, în perioada ianuarie 2009-ianuarie 2011, în care am inclus 199 pacienți internați în Spitalul Clinic de Boli Infecțioase "Sf. Parascheva" Iași de la care s-au izolat tulpini de *E. coli* și *Klebsiella spp*. din diferite produse patologice. Pentru pacienții cu infecții recurente au fost incluse doar izolate de la primul episod infecțios. Cazurile au fost reprezentate de pacienții diagnosticați cu diabet zaharat (DZ) anterior internării și pacienți cu DZ de novo, decelați odată cu internarea în clinică. Din totalul populației studiate 49 pacienți cu diabet și 150 fără diabet. Criteriul utilizat pentru excluderea pacienților diabetici posibili din grupul de pacienți fără diabet a fost absența antecedentelor de DZ și glicemie bazală mai mică de 126 mg/dl. Au fost înregistrate date demografice (vârstă, sex), spitalizare și antibioterapie anterior internării în clinică, prezența sau absența sondei urinare. Infecțiile au fost clasificate în funcție de date clinice și de laborator. Riscul de infecții cu tulpini rezistente a fost apreciat prin evaluarea scorului Carmeli.

Testarea rezistenței la antibiotice a izolatelor bacteriene a fost realizată prin metoda standard difuzimetrică (CLSI) și E-test. A fost realizată o bază de date în Excel care a fost prelucrată ulterior cu ajutorul programelor *SPSS 16.0* și *Epi Info 3.5.1*. Variabilele studiate au fost analizate descriptiv și analitic și s-au considerat semnificative statistic valorile p< 0,05.

REZULTATE ŞI DISCUŢII

În timpul perioadei studiate din 199 pacienți, 39 erau diagnosticați cu diabet zaharat în momentul internării, iar la 10 pacienți infecţia a fost evenimentul declanşator al instalării dezechilibrului metabolic glucidic. Astfel au fost incluși în studiu 49 de cazuri (79,5% au fost femei), cu o vârstă medie de 66,2 ani. Pacienții non diabetici (43 bărbați, 107 femei) au avut vârste cuprinse între 1-83 ani (media 46,29 ani).

Au fost izolate 133 tulpini de *E. coli* (37 la diabetici și 96 la non-diabetici). Majoritatea tulpinilor au fost izolate din urocultură (UC) (93,9%). Doar la 2 cazuri de diabet a izolată *E. coli* din hemocultură (HC) și UC (o tulpină sensibilă la toate antibioticele testate, o tulpină rezistentă la fluoroquinolone (FQ) și amoxicilină-acid clavulanic). La pacienți non-diabetici au fost izolate tulpini și din HC (un caz), spută (2 cazuri), puroi (2 cazuri), secreții lohii (un caz). Tulpinile de *E. coli* producătoare de BLSE au fost izolate din UC la majoritatea cazurilor, doar la două cazuri la non-diabetici fiind izolate din puroi și spută.

TABELUL I

Rata de izolare a *E. coli* si *K. pneumoniae* la pacienți diabetici si non-diabetici

Microorganism izolat	Pacienți diabetici Nr/%	Pacienți non diabetici Nr/%
E. coli	37/27,8	96/72,1
E. coli ESBL+	5/13,5	16/16,6
Klebsiella spp.	12/18,1	54/81,8
K. pneumoniae ESBL+	6/50	13/24
Total *E.coli* + *K. pneumoniae* ESBL+	11/22,4	29/19,33

TABELUL II

Factori de risc asociați cu infecția cu *E. coli* si *K. pneumoniae* BLSE pozitive

Variabilă	DZ	NON DZ	OR*	IC	P	Semnificație statistică
Vârsta peste 60 ani	6	13	1,48	0,30 – 7,41	0,84	NS
Sex F	10	15	9,33	0,97 – 220,84	0,05	La limita SS
Antibioterapie anterioară	3	9	0,83	0,13 -4,80	0,87	NS
CG3	1	3	0,87	0,00 -11,66	0,63	NS
FQ	1	4	0,63	0,02 – 7,53	0,89	NS
Alte AB	2	6	0,85	0,10 – 6,27	0,75	NS
Sondă urinară	1	4	0,63	0,02 – 7,53	0,89	NS
Spitalizare anterioară	2	10	0,42	0,05 – 2,81	0,53	NS
Scor Carmeli 1	0	10	-	-	-	-
Scor Carmeli 2	0	11	-	-	-	-
Scor Carmeli 3	11	8	28,88	2,89 – 703,84	$0,06 \times 10^{-2}$	SS

*pentru valori < 5, s-a aplicat corecţia Yates; SS = semnificativ statistic;NS = nesemnificativ statistic; AB=antibiotice

Din 66 tulpini de *Klebsiella spp*., *K. pneumoniae* a fost izolată din UC la toți 12 pacienți diabetici, tulpini BLSE pozitive fiind depistate în jumătate din cazuri. (tab. I) În lotul de non-diabetici, din UC au fost izolate 59 tulpini (o tulpină de *K. oxytoca*, BLSE negativă), 13 fiind tulpini BLSE pozitive. Câte 1 tulpină de *K. pneumoniae* fost izolată respectiv din HC, lichid cefalorahidian, 3 din aspirat traheo-bronșic și 2 din puroi (în asociere cu *Staphylococcus aureus*, respectiv *Enterobacter spp*.).

Dintre cele 11 cazuri de diabet la care au fost identificate tulpini de *E.coli* și *K. pneumoniae* producătoare de BLSE, 6 cazuri aveau vârsta peste 60 ani, doar 3 cazuri au primit antibioterapie în ultimele 3 luni anterior spitalizării. Toate cazurile de diabet au avut scor Carmeli 3 (tab. II). În studiul nostru ratele rezistenței *E. coli* la antibiotice la diabetic comparative cu lotul de non-diabetici au fost: amoxicilină-acid clavulanic 31,4% vs. 13,98%, p=0,04; ceftazidimă 16,7 vs. 12%, p=0,70; ciprofloxacină 52,9% vs. 24,46%, p=0,004; gentamicină 13,3% vs. 21,5%, p=0,52. Rata rezistenței la cotrimoxazol a fost similară în cele 2 loturi (p=0,7). Nu au fost tulpini rezistente la imipenem la nici unul din grupuri. Este de remarcat o rezistență scăzută la cefoxitin atât la diabetici cât și la non-diabetici (11,76% vs. 4,7%; p=0,57). Doar una din 23 respectiv 16 tulpini testate au fost rezistente la nitrofurantoin, respectiv colimicină în grupul cu diabet (tab. III).

TABELUL III

Sensibilitatea la antibiotice a tulpinilor de *E. Coli*

Antibiotic	Pacienți diabetici	Pacienți non-diabetici	p
	Tulpini rez. /Tulpini testate N/%	Tulpini rez. /Tulpini testate N/%	
Amoxi-ac. clav.[#]	11/35 (31,4)	13/93(13,98)	0,04
Cefotaxim	6/34 (17,6)	9/45 (20)	0,97
Ceftazidimă	6/36 (16,7)	9/75 (12)	0,70
Cefoxitin	2/17(11,76)	4/85 (4,7)	0,57
Imipenem	0/8	0/89	-
Ciprofloxacin	18/34 (52,9)	23/94 (24,46)	0,004
Gentamicină	4/30 (13,3)	16/76 (21,05)	0,52
Amikacină	0	5/58 (8,62)	-
Cotrimoxazol	11/20 (55)	5/9 (55,5)	0,70
Nitrofurantoin	1/23 (4,34)	0	-
Colimicină	1/16 (6,25)	0	-
Tulpini multirezistente	6 (16,2)	8 (8,3)	0,31

* Tulpini multirezistente la 3 clase de ATB*: FQ, CG3 (ceftazidimă, cefotaxim), aminoglicozide; [#]amoxicilină-acid clavulanic; rez=rezistente

La pacienții diabetici s-a înregistrat un procent mai mare de tulpini de *K. pneumoniae* rezistentă la amoxicilină-acid clavulanic (54,55%), comparativ cu cei non-diabetici (39,6%), diferența fiind însă nesemnificativă din punct de vedere statistic (p>0,05). Rezistența la CG3 este de asemenea mai mare la diabetici.

TABELUL IV

Sensibilitatea la antibiotice a tulpinilor de *Klebsiella spp.*

Antibiotic	Pacienți diabetici	Pacienți non-diabetici	P
	Tulpini rez. /Tulpini testate N/%	Tulpini rez. /Tulpini testate N/%	
Amoxi-ac. clav.[#]	6/11 (54,55)	21/53 (39,6)	0,56
Cefotaxim	6/8 (75)	20/44 (45,5)	0,24
Ceftazidimă	7 /10(70)	20/45 (44,4)	0,26
Cefoxitin	1/9 (11)	11/39 (28,2)	0,52
Imipenem	0	2/37 (5,4)	-
Ciprofloxacin	9 /12(75)	21/54 (39)	0,05
Gentamicină	6 /10(60)	17/46 (37)	0,32
Amikacină	1 /5(20)	1/13 (7,7)	0,92
Cotrimoxazol	3/3 (100)	11/15 (73,3)	-
Nitrofurantoin	2/2 (100)	-	-
Colimicină	0/3	-	-
Tulpini multirezistente [*]	6 (50)	18 (33,3)	0,45

* Tulpini multirezistente la 3 clase de ATB*: FQ, CG3 (ceftazidimă, cefotaxim), aminoglicozide; [#]amoxicilină-acid clavulanic; rez=rezistente

La lotul de diabetici doar una din 9 tulpini de *K. pneumoniae* a fost rezistente la cefoxitin, comparativ cu lotul de non-diabetici (28,2%, p=0,52). Nu a fost nici o tulpină rezistentă la imipenem în grupul de diabetici. Rezistența la ciprofloxacină a înregistrat valori semnificativ mai mari la pacienții diabetici (75% *vs* 39%; p=0,05). La diabetici au fost decelate rate de rezistență crescute și la gentamicină, cotrimoxazol, nitrofurantoin, dar numărul mic de cazuri nu conferă semnificație statistică. (tab. IV)

DISCUŢII

Datele raportate în 2007 de către Sistemul de Supraveghere Europeană al Rezistenței la Antibiotice (EARSS) referitoare la tulpinile de *E. coli* arată faptul că, cea mai crescută rată a rezistenței a fost înregistrată pentru amino-peniciline, între 26% și 77 %. Rapiditatea cu care FQ își pierd activitatea împotriva *E.coli* este de neegalat, în 25 din 28 de țări evidențiindu-se o rezistență în creștere la acest grup de antibiotice (5).

Într-un studiu multicentric efectuat în 2007 în România, pentru *E. coli* datele au relevat 24,21% rezistență la FQ și 15,23% rezistența la cefalosporine de generația a 3-a (CG3). De asemenea, este de notat o sensibilitate în creștere la piperacilină-tazobactam și nitrofurantoin (4). În studiul prezent au fost înregistrate diferențe doar pentru amoxicilină-acid clavulanic și ciprofloxacină, rezistența în lotul de cazuri fiind semnificativ mai mare (31,4% *vs.*13,98%, p=0,04, respectiv 52,9% *vs.* 24,46%, p= 0,004) (tab. III).

Rezistența combinată survine frecvent, co-rezistență la patru clase de antibiotice, inclusive CG3 este deja una dintre cele mai comune patru forme de rezistență întâlnită în infecția invazivă cu *E. coli*, tendință în creștere în Europa (5).

În cercetarea noastră, multi-rezistența tulpinilor de *E. coli* nu a prezentat diferențe

semnificative între cele 2 grupuri (16,2% *vs*. 8,3%, p=0,31). Infecţii cu tulpini de *E. coli* BLSE pozitive au fost înregistrate doar la femei diabetice, un procent ridicat prezentând şi femeile fără diabet (62,5%).

În nouă studii europene publicate din 1990, doar 14 (2.3%) din 621 pacienţi au fost spitalizaţi pentru pneumonie comunitară severă având ca agent etiologic probabil *K. pneumoniae*. În Africa şi Asia, în schimb, pneumonia comunitară continuă să fie asociată cu *K. pneumoniae*. Într-un studiu prospectiv al lui Wen-Chien Ko şi colab. pneumonia comunitară însoţită de bacteriemie a fost raportată la 12 din 21 dintre pacienţii diabetici. Manifestări clinice distincte cu *K. pneumoniae* (abces hepatic, meningită, endoftalmită) comparate cu alte infecţii bacteriemice comunitare au fost semnificativ asociate cu DZ (60% vs 28%, p=0,0015) şi rezidenţa în Taiwan (3).

În studiul nostru jumătate din cazurile cu *K. pneumoniae* au fost diagnosticate cu sepsis, iar restul cu infecţii ale tractului urinar. La pacienţii diabetici au fost izolate mai multe tulpini producătoare de BLSE comparativ cu lotul de non-diabetici (50% vs. 24%) (tab. I). Majoritatea cazurilor cu tulpini BLSE pozitive au fost raportate la femei diabetice (83,3% vs. 38,5%) (tab. II). Referitor la factorii de risc asociaţi infecţiilor cu tulpini de *K. pneumoniae* BLSE pozitiv, doar 2 din 12 pacienţi diabetici au avut spitalizare anterioară, ceea ce ar sugera o tendinţă la emergenţa acestor tulpini şi în infecţii comunitare.

EARSS a evidenţiat, în studiul efectuat în 2007, o prevalenţă crescută în Europa de Est şi de Sud-Est a tulpinilor de *K. pneumoniae* rezistente la CG3, FQ şi aminoglicozide, cel mai frecvent fenotip fiind multi-rezistent la toate cele trei clase de antibiotice. În studiul nostru am remarcat o rată mai mare a multi-rezistenţei la pacienţii diabetici, comparativ cu cei non-diabetici (50% *vs*. 33,3%).

CONCLUZII

Rezistenţa crescută la fluoroquinolone şi la cefalosporinelede a 3-a generaţie observată şi în studiul nostru ar trebui să limiteze utilizarea acestora în terapia empirică, carbapenemele, aminoglicozidele şi colimicina reprezentând opţiuni ale terapiei de primo-intenţie. Recunoaşterea promptă şi abordarea multidisciplinară a infecţiilor comunitare sau nosocomiale, cu microorganisme multi-rezistente la bolnavul diabetic, cu monitorizarea strictă a dezechilibrelor metabolice şi intervenţia chirurgicală la timp sunt esenţiale pentru scăderea morbidităţii şi mortalităţii acestui grup.

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NOUTĂȚI

ASOCIEREA DISFUNCȚIEI OLFACTIVE CU MANIFESTĂRILE NEUROPSIHIATRICE ÎN BOALA PARKINSON

Manifestările non-motorii ale bolii Parkinson includ, în mod uzual, hiposmia, deficitul cognitiv şi afectarea psihiatrică, deşi legătura dintre ele este încă incomplet înţeleasă. Relaţia dintre disfuncţia olfactivă şi afectarea neuropsihiatrică a fost investigată prin intermediul unui studiu pe 248 de pacienţi înrolaţi din două clinici specializate în afectări ale mişcării. Analiza psihiatrică a inclus următoarele teste: GDS-15 (Geriatric Depression Scale-15), Inventarul Simptomelor Depresive, testul pentru Investigarea Statusului Anxios, Scala de Apatie şi Scala de Psihoză a bolii Parkinson. Analiza statusului cognitiv a inclus MMSE-ul (Mini Mental State Examination), Testul de Învăţare Verbală Hopkins, Testul Culoare-Cuvânt Stroop. Funcţia olfactivă a fost evaluată prin Testul de Identificare a Mirosurilor elaborat de Universitatea din Pennsylvania. Nici o asociere statistic semnificativă între olfacţie şi nivelul dispoziţiei pacienţilor nu a fost evidenţiată. Cu toate acestea, simptomele psihotice au fost mai frecvent înregistrate mai frecvent la acei pacienţi cu scoruri ale simţului olfactiv sub valoarea mediană (30% vs. 12%; $P < 0.001$). O capacitate olfactivă mai slabă a fost asociată cu o afectare mai severă a memoriei, iar scorul obţinut la Testul de Identificare a Mirosurilor a fost un factor predictiv important pentru rezultatul obţinut la testele cognitive. Relaţia dintre hiposmie, psihoză şi deficitele cognitive specifice ar putea reflecta distribuţia anatomică a corpilor Lewy; astfel, disfuncţia olfactivă ar putea reprezenta un marker adiţional pentru boala extranigrală. (Morley JF, Weintraub D, Mamikonyan E, Moberg PJ, Siderowf AD, Duda JE. Olfactory dysfunction is associated with neuropsychiatric manifestations in Parkinson's disease. Movement Disorder. 2011; 5:10-15).

Teodora Alexa

REGENERAREA NERVULUI PERIFERIC. STUDIU EXPERIMENTAL - REZULTATE DEFINITIVE

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REGENERATION CAPACITY OF THE PERIPHERAL NERVE: EXPERIMENTAL STUDY -FINAL RESULTS (Abstract): Any injury affecting peripheral nerve continuity is followed by a degenerative reaction from nerve itself and its muscle. Nerve regeneration is possible when the two ends are put in direct contact (direct suture), proximity (autologous or artificial grafts), or through muscle neurotization. The aim of this experimental study was to evaluate sciatic nerve degeneration and regeneration in rats after no suture (group 1), direct suture (group 2), nerve grafting (group 3) and muscle neurotization (group 4). Nerve samples were analyzed by optical microscopy at 8 and 16 weeks after surgery, and nerve degeneration (group 1), axonal growth (group 2) and neuromuscular regeneration (group 4) were assessed. Optical microscopy and different staining techniques confirmed nerve degeneration, regeneration, and axonal growth in different moments. Cholinesterase test after direct muscle neurotization showed enzymatic activity at the level of muscle implantation of nerve fibers, indirectly confirming the presence of neuromuscular synapse. **Key words**: PERIPHERAL NERVE INJURY, NERVE DEGENERATION, NERVE REGENERATION, MUSCLE NEUROTIZATION

Alterarea continuităţii nervului periferic, indiferent de cauză (striviri, compresiuni sau secţiuni) determină un proces de degenerare urmat firesc de tenativa de reparare, atât morfologică cât şi funcţională, proces reprezentat de reorganizarea structurală şi reacţiile metabolice (reacţii axonale) (1-4). Acest proces este posibil numai atunci când capetele nervului secţionat sunt în apropiere unul de celălalt. Prin regenerare se stabilesc conexiunile dintre centrii nervoşi şi organele terminale. Există o multitudine de tehnici chirurgicale prin care se obţine apropierea sau contactul direct dintre capetele secţionate ale unui nerv periferic, condiţie necesară debutului regenerării nervoase (1, 3-5).

Tehnicile chirurgicale prin care se restabileşte continuitatea fibrelor nervoase, în cazul secţiunii nervului periferic, pot include sutura directă, grefa de nerv (atunci când mărimea defectului nu permite sutura directă). În cazul defectelor de nerv pot fi utilizate şi alte ţesuturi, cum ar fi vena, muşchi în venă sau materiale sintetice de tipul tuburilor de silicon, pentru a putea crea un culoar de direcţionare a creşterii axonale (6-9). În cazul în care dimensiunea defectului nu permite utilizarea nici uneia

dintre aceste tehnici (acesta fiind mult prea mare sau ca urmare a smulgerii capătului distal), se va utiliza implantarea fibrelor nervoase direct în mușchi, tehnică numită neurotizare neuromusculară. Tehnica include disecția și separarea fibrelor nervoase și implantarea acestora în mușchi prin mici incizii practicate în acesta din urma, incizii realizate la profunzimi diferite în grosimea mușchiului. Se vor obține, prin acestă tehnică chirurgicală, formarea de noi sinapse neuromotorii la nivelul de implantare a fibrei nervoase în mușchi (10-12).

Scopul acestui studiu este de a pune în evidență, prin metode histologice, capacitatea de regenerarea axonală la nivelul unui nerv suturat direct, al unuia reconstruit prin interpunerea unei grefe de nerv, dar și punerea în evidență, prin utilizarea unor reacții enzimatice a formării sinapsei neuromotorii la locul de implantare a fibrei nervoase în mușchi atunci când este utilizată tehnica neurotizării neuromusculare.

MATERIAL ŞI METODĂ

Studiul actual urmăreşte a completa rezultatele preliminare prezentate într-un număr anterior al revistei (2). Studiul a fost realizat pe un lot de 30 de şobolani Wistar, femele, cu vârsta de 8 săptămâni şi greutăți cuprinse între 190 şi 220 de grame. Animalele au fost găzduite individual într-un mediu controlat 24 ore din 24 şi acces liber la apă şi alimente. Aclimatizarea animalelor s-a făcut cu 10 zile anterior intervențiilor chirurgicale. Cele 30 de animale nu au primit alimente cu o noapte înainte de intevenția chirurgicală. Sub anestezie generală, s-a descoperit nervul sciatic la nivelul membrului posterior drept, urmată de secționarea acestuia. Animalele au fost împărțite în 4 loturi în funcție de intervenția chirurgicală de care au beneficiat. Primul lot a cuprins un număr de 6 animale, la care nervul sciatic nu a fost suturat. În cazul celui de al II-lea lot, format dintr-un număr de 8 animale, s-a practicat sutura directă a nervului sciatic. Pentru lotul al III-lea, format dintr-un număr de 8 animale, reconstrucția nervului sciatic s-a efectuat prin interpoziția unei grefe de nerv liberă nevascularizată. Al IV-lea lot a inclus 8 animale, iar în acest caz s-a utilizat tehnica neurotizării neuromusculare, în care după disecția pe fascicule a nervului sciatic, s-a practicat implantarea acestora în mușchiul pe care îl inervează (fig. 1-5).

Implantarea fibrelor nervoase în mușchi s-a realizat pe diferite suprafețe și la diferite adâncimi, urmărindu-se punerea în evidență a sinapselor neuromotorii nou formate la acest nivel, și implicit utilitatea acestei tehnici chirurgicale.

Fig.1. Secțiune nerv sciatic

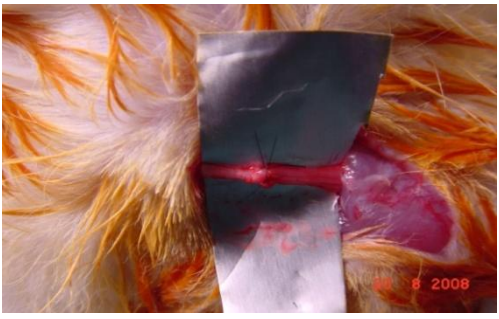

Fig. 2. Neurorafie directă nerv sciatic (lot I)

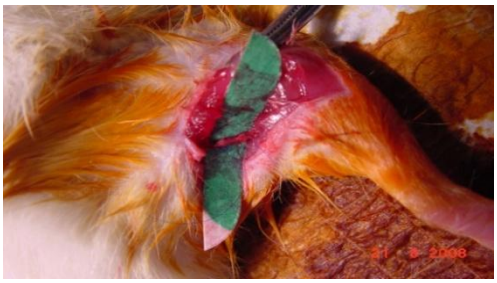

Fig. 3. Grefă de nerv (lot III)

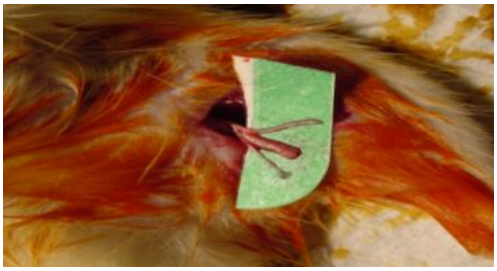

Fig. 4. Disecţia nervului sciatic în fascicule

Fig.5. Neurotizare neuromusculară directă (lot IV)

Un număr de 7 şobolani din lotul al III-lea au decedat în perioada postoperatorie imediată.

Lotul de 30 de animale a fost împărţit în două subloturi (A şi B) în funcţie de momentul în care s-a realizat recoltarea pieselor de analizat. Lotul A, la care recoltarea s-a efectuat la 8 săptămâni postoperator, a cuprins 11 animale (3 din lotul I, 4 din lotul II, 4 din lotul 4). Lotul B, la care recoltarea a fost realizată la 16 săptămâni de la operaţie a cuprins un număr de 12 animale (3 din lotul I, 4 din lotul II, 1 din lotul III si 4 din lotul IV).

Pentru studiul histologic, în toate cazurile, a fost folosită microscopia optică prin tehnica includerii la parafină. În prima etapă de colorare a secţiunilor s-au utilizat atât metode uzuale: coloraţia hematoxilină–eozină (HE) şi coloraţia tricromică (van Gieson), cât şi metode speciale: coloraţia tricromică C. Szekely şi coloraţia Gordon-Sweet. Pentru a pune în evidenţă sinapsele nou formate, în cazul implantării fibrelor nervoase direct în muşchi (neurotizare neuromusculară), s-a utilizat o reacţie enzimatică – tehnica colinesterazei. Tehnica colinesterazei sau metoda tiocolinei, propusă în 1959 de către Gerebtzoff, are la bază hidroliza substratului de acetiltiocolină de către activitatea enzimatică pentru producerea tiocolinei. Tiocolina se combină cu ionii de cupru prezenţi în mediul de incubare pentru a forma cupru-tiocolina. Precipitatul astfel format nu este vizibil în lumina microscopului. Fiind tratat cu o soluţie de sulfat de amoniu, va forma cupru-sulfatul, recunoscut astfel ca un precipitat de culoare maro, punându-se astfel în evidenţă activitatea enzimatică şi indirect a sinapselor nou formate.

REZULTATE

Rezultatele definitive le-au completat pe cele preliminare (prezentate într-un articol anterior) făcând comparaţia, din punct de vedere histologic, a zonelor de regenerare, în cazul diferitelor tipuri de tehnici de reconstrucţie nervoasă utilizate. Compararea s-a efectuat între cele două loturi, apreciindu-se astfel progresia regenerării. De această dată (faţă de studiul preliminar) evaluarea modificărilor degenerative cât şi a regenerării s-a făcut şi cu metodele speciale de colorare.

Pe animalele din primul lot, la care recoltarea s-a făcut la 8 săptămâni postoperator, s-au constatat inițial modificări degenerative incipiente cu fragmentarea fibrelor nervoase. La animalele din cel de-al II-lea lot, la care recoltarea s-a efectuat la 16 săptămâni, fenomenele de degenerare progresează, în structura nervului fiind remarcate fragmentări axonale mai importante. Modificări de acelaşi tip apar şi la nivelul mușchiului, degenerarea la nivelul fibrelor musculare fiind reprezentată de omogenizarea fibrelor şi dispariția nucleilor (fig. 6,7).

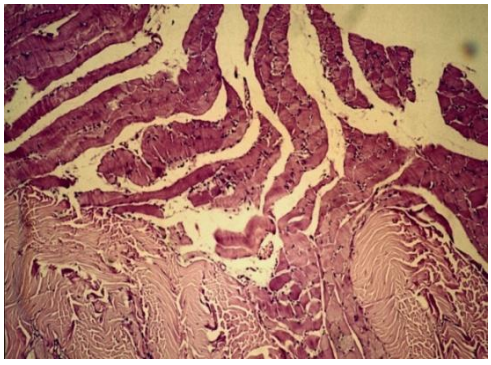

Fig. 6. Mușchi scheletal. Arii cu modificări degenerative ale fibrelor musculare, dispariția nucleilor, omogenizarea fibrelor (HE 20x)

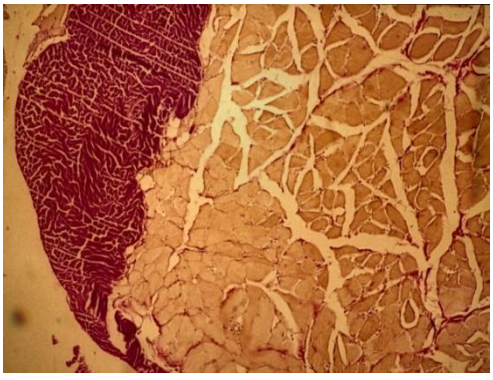

Fig. 7. Mușchi scheletal. Zonă cu modificări degenerative (van Gieson 20x)

Degenerarea a fost remarcată atât cu ajutorul tehnicii de colorare cu hematoxilină–eozină, cât şi în coloraţia van Gieson (fig. 8). Tehnica pentru reticulină evidențiază în nervul degenerat, zone restrânse de discontinuitate, în majoritate tecile Schwann cu membrana lor bazală păstrându-şi continuitatea (fig. 9). În aceeaşi coloraţie pentru reticulină, în zona musculară alterată, se remarcă o densificare a fibrelor reticulare cu apariția de fibre fine suplimentare, care gâtuie în cea mai mare parte fibrele musculare.

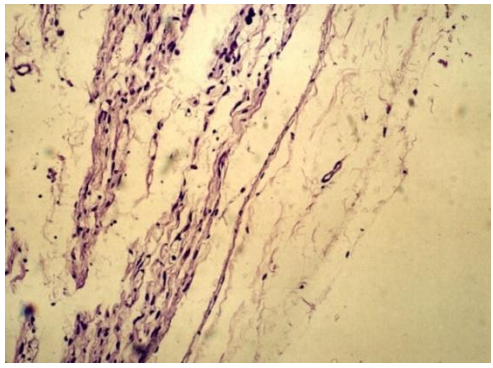

Fig. 8. Nerv periferic secționat, fragmentări axonale pronunțate (HE10x)

Fragmentul de nerv sciatic, recoltat la 8 săptămâni (lotul I), din segmentul distal neurorafiei, prezintă în coloraţia HE o reorganizare a histoarhitectonicii. Nucleii celulelor Schwann formează din nou şiraguri orientate în lungul nervului, iar în jurul lor există o cantitate mai mare de citoplasmă, cu o nuanță mai bazofilă. Printre fasciculele nervoase, din loc în loc, se observă mici lumene vasculare (fig. 9). După impregnare argentică, fibrele reticulare apar relativ paralele, în mare parte cu continuitate păstrată şi numai în unele zone se remarcă mici discontinuități (fig. 10). Țesutul muscular prelevat din zona distală neurorafiei, în cadrul aceluiaşi lot I, prezintă în

coloraţie HE, mănunchiuri de fibre eozinofile, cu nuclei periferici, organizate în fascicule, separate prin perimisium (fig. 11).

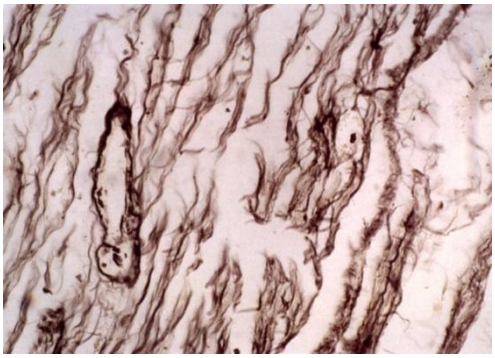

Fig. 9. Nerv periferic secţionat, fragment distal, modificări incipiente. (reticulină, impregnaţie argentică 40x)

Histoarhitectonia ţesutului muscular este păstrată. Impregnaţia argentică evidenţiază prezenţa fibrelor reticulare în endomisium, cu o perfectă organizare. În ceea ce priveşte lotul la care s-a utilizat neurotizarea neuromusculară, în cazul recoltării la 8 săptămâni postoperator, fragmentele de ţesut muscular prezintă aspecte diferite. În unele zone, la nivelul epimisiumului se remarcă lumene vasculare, ţesut adipos şi mici fascicule de fibre nervoase în vecinătatea unor fibre musculare striate, izolate între ele de ţesut conjunctiv bogat în adipocite (fig. 12). În alte zone, se remarcă la nivelul perimisiumului, fibre nervoase într-o atmosferă de ţesut conjunctiv lax. În preparatele tratate cu impregnaţie argentică, mănunchiurile de fibre nervoase apar evidente în perimisium, iar reţeua de fibre reticulare din ţesutul muscular învecinat, prezintă arhitectonie nemodificată. Prin tehnica de colorare tricromă, se remarcă prezenţa de mici fascicule de fibre nervoase în perimisiumul dintre fasciculele musculare striate (fig. 13). În zona de neurotizare, organizarea histoarhitectonică şi structura microscopică a fibrelor musculare striate, a fost integră, aspect evidenţiat atât în secţiuni longitudinale cât şi transversale.

Fig. 10. Nerv periferic cu neurorafie, segment distal.
(Reticulină impregnaţie argentică 40x)

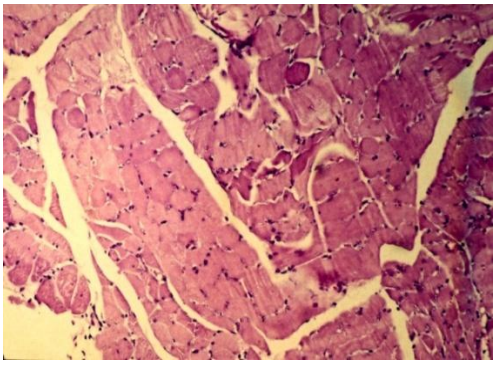

Fig. 11. Muşchi scheletal din zona de neurorafie. Structură conservată - HE (lot I)

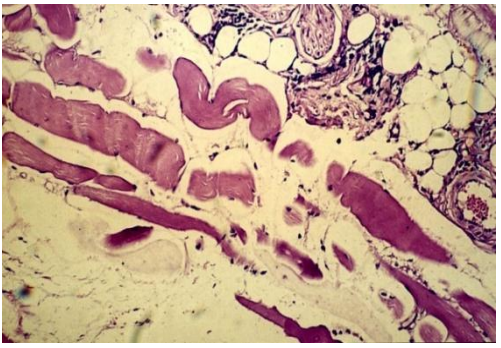

Fig. 12. Fibre nervoase în vecinătatea unor fibre musculare striate - HE 20x (lot I)

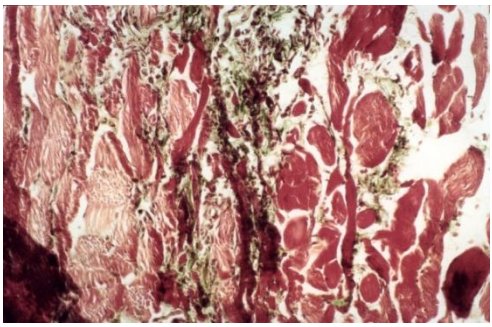

Fig. 13. Zonă de neurotizare. Fibre nervoase în vecinătatea fibrelor musculare - Tricrom Szekely 20x (lot I)

Fig. 14. Nerv periferic după neurorafie. Reticulină, impregnație argentică 20x (lot II)

În cazul lotului II, la care recoltarea s-a efectuat la 16 săptămâni postoperator, în colorație HE, segmentul distal al neurorafiei directe arată în secțiune longitudinală o structură conservată. Fibrele nervoase sunt organizate ordonat în ax longitudinal cu discrete sinuozități, cu celule Schwann aranjate în şiraguri, cu o densitate mai mică comparativ cu primul lot. Acelaşi fragment tratat pentru evidențierea fibrelor reticulare, arată o organizare a acestora în mănunchiuri orientate în axul lung al nervului, dispuse paralel şi cu un traiect uşor sinuos (fig. 14). În cazul neurotizării, unele fascicule musculare prezintă morfologie care sugerează regenerarea musculară avansată. Impregnarea argentică evidențiază un filet nervos într-o relație intimă cu fasciculele musculare (fig. 15). În numeroase arii examinate s-au pus în evidență pachete vasulo-nervoase ataşate suprafețelor musculaturii striate. Fragmentele musculare din zona de neurotizare au scos în evidență existența unor nervi periferici de dimensiuni diferite în perimisiumul țesutului muscular striat. Colorația tricromică scoate în evidență, deasemenea, prezența de fine fascicule nervoase în epimisium, în imediata vecinătate a fasciculelor musculare (fig. 16).

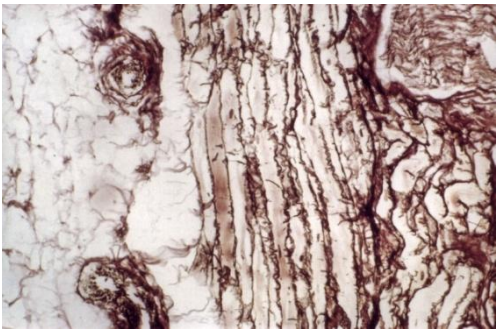

Fig. 15. Zonă de neurotizare mușchi striat cu structură conservată, în vecinătatea fibrelor nervoase. Reticulină, impregnație argentică

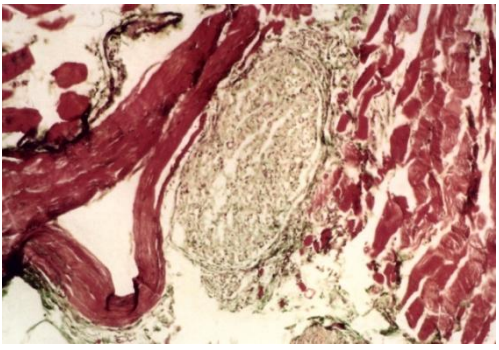

Fig. 16. Zonă de neurotizare. Mic nerv periferic şi lumen vascular printre fasciculele musculare. Tricrom Szekely 20x (lot II)

Depistarea sinapselor neuromusculare efectuată prin reacția histoenzimologică pentru colinesterază, a evidențiat prezența unei reacții pozitive de intensitate cores-

punzatoare, la nivelul fibrelor musculare striate, din zonele de neurotizare. Densitatea ariilor cu reacţii pozitive a fost variabilă, rămânând constant prezentă (fig. 17, 18).

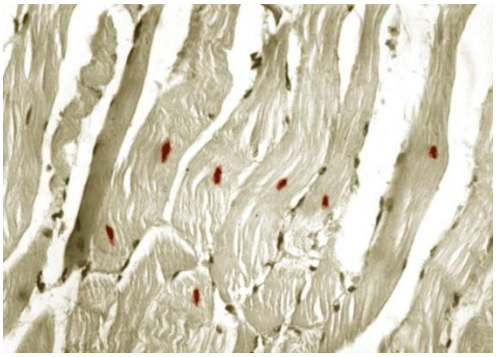

Fig. 17. Zonă de neurotizare cu rare sinapse. Colinesterază 20x (lot II)

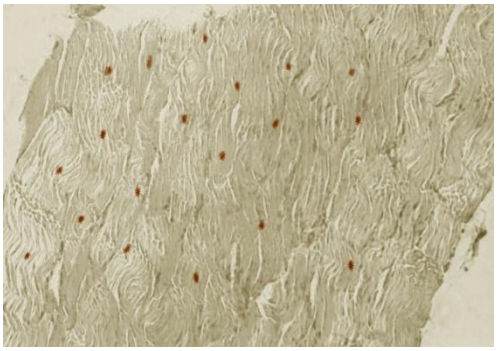

Fig. 18. Zonă de neurotizare: fibre musculare striate, prezenţa unor sinapse (secţiune longitudinală). Colinesterază 40x (lot II)

DISCUŢII

Capacitatea mare de regenerare a sistemului nervos periferic este dată de organizarea sa celulară. Orice traumatism la nivelul nervului periferic – strivire, secţiune sau smulgere – determină un proces de degenerare, care este urmat de tentativa de reparare morfologică şi funcţională. După denervare muşchiul îşi pierde contractilitatea voluntară şi devine flasc, mergând până la atrofie severă la un an de la leziune şi maximă la doi ani de la traumatism (13-15).

Traumatismele de la nivelul nervilor periferici sunt leziuni grave în urma cărora pacientul este scos din viaţa socială şi profesională pentru o perioadă lungă de timp. Repararea nervului periferic se face prin mai multe tehnici chirurgicale (sutura directă, grefa de nerv, neurotizare, etc), dar important este ca momentul la care se face reconstrucţia să fie cât mai aproape de cel al traumatismului (8, 9, 14,16).

Implantarea directă în muşchi a fibrelor nervoase dă posibilitatea formării de noi plăci neuromotorii gigante în zona aneurală a muşchiului, făcând astfel posibilă reinervarea muşchiului. Toate studiile făcute de-a lungul timpului au confirmat teoria neurotropismului şi sugerau că ţesutul nervos periferic este capabil de discriminarea unei ţinte specifice (10, 17, 18).

Studiul descris confirmă prezenţa modificărilor care au loc la nivelul capetelor unui nerv traumatizat (secţionat), precum şi modificările existente în muşchiul denervat.

La nivelul nervului periferic s-au observat alterările caracteristice degenerării walleriene, manifestate prin fragmentări ale fibrelor nervoase, constatate precoce, însoţite de modificări la nivelul tecii Schwann şi ale endonervului (19-21).

Pe preparatele examinate s-au putut constata zone de creştere ale celularităţii de-a lungul fibrelor nervoase din segmentul distal al nervului secţionat, datorită migrării macrofagelor şi stimulării celulelor Schwann, observaţie în concordanţă cu datele din literatură (6). Atrofia neurogenă s-a manifestat prin reducerea pronunţată în dimensiuni a fibrelor musculare cu angularea acestora şi apariţia modificărilor tinc-

torialității cu afinitate înspre fuxina acidă.

În prezent, se admite că regenerarea fibrelor nervoase pe seama segmentului proximal, începe imediat după secționarea nervului şi că eliberarea factorilor de creştere neurali (NGF) de către fibroblaste în matricea extracelulară a segmentului distal şi că expresia receptorilor NGF de către celulele Schwann creează un micromediu favorabil regenerării axonale (22, 23).

În experimentul efectuat, efectele favorabile ale neurorafiei s-au putut observa la ambele loturi, dar mai evident la cel de-al II-lea. În tehnica pentru reticulină, fibrele reticulare şi-au păstrat în mare măsura organizarea la nivelul bontului distal după neurorafie, ceea ce susține ideea că membrana bazală (care conține colagen IV şi colagen III) se reorganizează foarte precoce pe seama celulelor Schwann.

În ambele loturi, după practicarea neurorafiei s-au putut constata arii de fibre musculare striate fără modificări, sau arii restrânse cu aspect microscopic de regenerare. Acest aspect sugerează că regenerarea se realizează în mod progresiv în etape, în raport cu diferitele unități neuromotorii (4, 23).

În ceea ce privește neurotizarea neuromusculară s-au observat la ambele loturi, dar mai ales la cel de-al II-lea, numeroase structuri de tip nerv periferic, unele cu dimensiuni foarte înguste, aparent formate din 8-15 fibre nervoase cu tecile aferente prezente printre fibrele musculare striate, într-o atmosferă de țesut conjunctiv lax.

Observațiile studiului sugerează păstrarea în viața adultă a capacității fibrei nervoase de a răspunde la factorii locali de creştere, sintetizați în principal la nivelul țesutului conjunctiv, unde un rol important îl joacă laminina. Există de asemenea, factori care atrag conurile de creştere neuronală spre anumite ținte. În cazul neuronilor motori, conurile de creştere sunt atrase de către fibrele musculare de care aderă (10, 17, 18). Se consideră că sub efectul neurotransmițătorilor se vor organiza sinapse, realizându-se o inervare polineurală, care este tranzitorie şi care în final va deveni mononeurală prin eliminarea sinapselor mai puțin active. În experimentul prezent am putut constata variate relații de apropiere între fibrele nervoase şi fibrele musculare (2, 12, 25).

Cu ajutorul reacției histoenzimologice pentru evidențierea colinesterazei din sinapsă, am putut demonstra prezența sinapselor colinergice la nivelul fibrelor musculare din zona de neurotizare, cu o densitate mai mică la lotul I şi mai mare la lotul II. Brunelli şi colab. au demonstrat că neurotizarea neuromusculară directă este mai eficientă atunci când implantarea fibrelor nervoase la nivelul mușchiului se face mai superficial, obținându-se astfel o capacitate contractilă mai înaltă. Toate acestea se datorează, se pare, unei activități mai mari a enzimelor glicolitice din fibrele musculare periferice, fiind astfel scăzută insuficiența aparatului de ghidare şi formarea micronevroamelor intramusculare la joncțiunea neuromusculară (2, 17-19).

În toate cazurile un rol important îl au factorii de creştere nervoasă care sunt sintetizați în țesuturile inervate de nervi simpatici şi senzitivi şi sunt transportați de axonii terminali la corpurile celulare unde îşi exercită funcția de supraviețuire şi rolul trofic.

În cazul tuturor traumatismelor de la nivelul nervului periferic, recomandarea este ca reconstrucția nervului să fie una imediată (în urgență), pentru a crea condiții bune debutului creşterii axonale (2, 12, 14, 16).

CONCLUZII

Se poate spune că leziunile de la nivelul nervilor periferici sunt extrem de importante atât datorită deficitului motor şi senzitiv de la nivelul segmentului afectat, cât şi perioadei de timp mari de la momentul reconstrucției la momentul reintegrării socio-profesionale a pacientului. Pentru a obține rezultate mai rapide şi de o bună calitate repararea nervului traumatizat trebuie facută la un moment cât mai apropiat de cel al traumatismului. Pentru reconstrucția de nerv este de dorit a se evita tensiunea pe linia de sutură, se preferă sutura directă în favoarea grefei de nerv atunci când este posibil. Neurorafia directă duce la reorganizarea histoarhitectonicii. Nucleii celulelor Schwann formează din nou şiraguri orientate în lungul axului nervului, iar în jurul lor se poate remarca o cantitate mai mare de citoplasmă, cu o nuanță mai bazofilă, dând aspect general de hipertrofie a celulelor Schwann. Din loc în loc, printre fasciculele nervoase se pot observa mici lumene vasculare. Fibrele reticulare apar relativ paralele, după impregnarea argentică, în mare parte cu continuitatea păstrată şi numai în unele zone apar mici discontinuități.

Formarea sinapselor în zonele de implantare a fibrelor nervoase în muşchi arată eficiența tehnicii de neurotizare pentru reinervarea muşchiului denervat. Reacția histoenzimologică pentru colinesterază a evidențiat prezența unei reacții pozitive de intensitate corespunzătoare la nivelul fibrelor musculare striate din zonele de neurotizare, ceea ce reprezintă depistarea sinapselor neuromusculare.

Regenerarea nervoasă depinde de o multitudine de factori, cei mai importanți fiind vârsta, nivelul traumatismului leziunile asociate, nervul afectat, momentul reparării nervoase, tehnica utilizată, tratamentul adjuvant.

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SUTURELESS FUNCTIONALLY ADAPTIVE FIXATION METHOD FOR INGUINAL HERNIOPLASTY

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SUTURELESS FUNCTIONALLY ADAPTIVE FIXATION METHOD FOR INGUINAL HERNIOPLASTY (Abstract): Mesh fixation using glue instead of sutures for inguinal hernia (IH) repair has become popular during last decade. Existing literature database including multiple clinical trials is confusing, giving no exact answer whether mesh fixation with glue can reduce chronic pain without increasing the recurrence rate and/or causing other glue related side effects. **Aim:** To determine whether the use of glue to fix the mesh reduce pain, postoperative complications and 18 months reoccurrence rates after IH repair compared to Lichtenstein technique. Secondary objectives were to compare quality of life changes after sutured and glued mesh fixation. **Material and methods:** The study involved 94 primaries uncomplicated IH patients divided into 2 groups: control group of 71 (75.53%) patients, who underwent classic sutured Lichtenstein repair and study group of 23 (24.47%) patients with glued mesh fixation according to developed functionally adaptive fixation (FAF) technique combining albumin glue mesh fixation and mesh tailoring in accordance to anthropometric peculiarities. **Results:** Duration and expression of post-operative pain requiring painkillers, length of working capacity and physical activity restoration was shorter in study group compared to control. Quality of life according to SF-36 was better following FAF surgery, too. **Conclusions:** FAF demonstrated better or comparable results with less acute and chronic postoperative pain and better quality of life than sutures for Lichtenstein hernia repair. No FAF specific complications were demonstrated in this study. Further prospective studies are at need with wider cohort selection, better randomization, and standardization combined with different surgical glues assessment. **Keywords:** HERNIA REPAIR, INGUINAL HERNIA, GLUE, MESH FIXATION, PAIN, QUALITY OF LIFE, SUTURE.

Inguinal hernia (IH) is the most common abdominal wall hernia. IH account for about 75% of all abdominal wall hernias, with a lifetime risk of 27% in men and 3% in women; up to 50% of patients with IH are unaware of this condition and IH repair is one of the most commonly performed surgical procedures worldwide, with rates ranging from 10 per 100 000 of the population in the UK to 28 per 100 000 in the US.

IH are at risk of irreducibility or incarceration, which may result in strangulation and obstruction (1).

Risk factors for IH are well known, including gender, physical activity, and older age. However, similarly to other diseases, genetic predisposition to IH and connective tissue abnormalities in general (2) is discussed backed by both gender-associated nature of disease, modern morphological findings (3) and pathogenesis analogies with other conditions.

Conservative management and manual hernia reduction may be only applied in uncomplicated asymptomatic or minimally symptomatic IH (without or only minimal complaints). Surgery is the treatment of choice varying from currently outdated natural (own) tissue hernioplasty to Lichtenstein mesh and laparoscopic (TEP preferred to TAPP) repair. Non-mesh hernioplasty, according to EHS Guideline is only recommended for strangulated hernia repair in case of suspected infection. Endoscopic hernia repair requires both equipment end expertise, is comparatively expensive and more effective in bilateral IH. Compared with open repair, laparoscopic surgery for IH is associated with longer operation times, higher recurrence rates, but less severe postoperative pain, fewer complications, and more rapid return to normal activities. Tension-free Lichtenstein hernioplasty is comparatively simple, affordable and effective technique for IH repair; it has relatively low recurrence and complications rates, but several side effects (4). Most important of them, chronic groin pain syndrome may be a long-term problem related to mesh sutured fixation and operative trauma after Lichtenstein hernioplasty. Collection of blood or fluid, local inflammation and neuropathy are considered to be related to mesh and its fixation with sutures (5, 6).

As a result, mesh fixation using glue instead of sutures has become popular during last decade (7, 8). Existing literature database including multiple clinical trials is confusing, giving no exact answer whether mesh fixation with glue can reduce chronic pain without increasing the recurrence rate and/or causing other glue related side effects.

Therefore, *the aim of this study* was to determine whether the use of glue to fix the mesh instead of sutures reduce acute postoperative pain, postoperative complications, chronic pain and 18 months reoccurrence rates after IH repair compared to the own tissues and Lichtenstein technique. Secondary objectives were to compare quality of life changes after sutured and glued mesh fixation.

MATERIAL AND METHODS

The study involved 94 (mean age 34.17±6.49 yrs.) primary uncomplicated IH patients divided into two groups: *the control group* of 71 (75.53%) patients, who underwent classic sutured Lichtenstein repair and *study group* of 23 (24.47%) patients with glued mesh fixation according to developed functionally-adaptive fixation (FAF) technique. This study *conforms to international bioethical standards* (European Convention on Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on ethical principles of scientific medical research involving human subjects, GCP, EUC directive #609, etc.) and approved by Commission for Bioethics in Research of the Bukovinian State Medical University, Ukraine. All patients signed written permissions and obtained full information about the study prior to participation. Local anesthesia was combined with sedation and systemic painkillers (6).

Lichtenstein hernia repair (control group) performed with non-absorbable

polypropylene sutures for fixation of lightweight polypropylene mesh. FAF method (study group) included: -evaluation of patient's morphological type, determination of anthropometric indicators (abdominal indices, *distantia spinarum*, height of hypogastrium, and distances from the pubic symphysis to *spina iliaca* anterior superior and from the pubic tuberculum to the inguinal ring etc.), and assessment of anatomical peculiarities e.g. trauma or connective tissue conditions; use of albumin-based glue containing 45% albumin and 10% glutaraldehyde solutions in 4:1 proportions for mesh fixation. The *concept of FAF* method is based on use of albumin glue, less influencing local tissues compared to more common fibrin or cyanoacrylate glues (9, 10), but which may comparatively rapidly degrade due to local and systemic fibrinolysis (anti-fibrinolytic agents were added) stimulated by both surgical trauma, bleeding and grafting similarly to other surgeries (11); and tailoring the mesh according to anthropometric peculiarities of the patient, somehow similarly to E. Trabuco's technique (12). The last assists in keeping the mesh fixed on-site, providing additional *elastic fixation* by own tissues.

Set of clinical data including postoperative complications, pain syndrome, impact on physical capacity, and recurrence were evaluated by an independent blinded observer. SF-36 questionnaire was used for quality of life after hernioplasty assessment. *Statistical processing* was carried out using the *MS Excel* software.

RESULTS

Duration of post-operative pain requiring painkillers was slightly shorter in study group compared to control (tab. I). Pain, including chronic inguinal pain syndrome itself, paresthesia, and feeling of foreign body were the most common complication of hernioplasty in all groups. Higher than in control group percentage of fluid collection in study group (4.3% vs. 2.1% and 1.4%) is caused by smaller number of observations. Total number of fluid collections and wound suppurations was higher in suture group (control) compared to FAF (study group). Hernia reoccurrence rate for 18 months was minimal in both groups with minor differences between them.

TABLE I

Comparative evaluation of different inguinal hernioplasties (clinical data)

Indices	Control group (n=71)	Study group (n=23)
Duration of immediate post-operative pain (hrs.)	35.62±3.21	25.39±2.37 $p_1<0.05$
Restoration of basic physical activity (days)	1.80±0.22	1.71±0.35 p >0.05
Restoration of full working capacity (days)	31.12±2.27	30.17±3.62 p >0.05
Postoperative complications:		
- seromas	1 (1.4%)	1 (4.3%)
- hematomas	1 (1.4%)	–
- wound suppuration	1 (1.4%)	–
- paresthesia	6 (8.5%)	1 (4.3%)
- feeling of foreign body	11 (15.5%)	2 (8.6%)
- chronic inguinal pain syndrome	5 (7.0%)	–
- mesh deformation/dislocation	3 (4.2%)	1 (4.3%)
Hernia reoccurrence (18 months of observation)	1 (1.4%)	–

Notes: p – reliability of differences compared to control group data.

Use of surgical glue reliably improves physical activity and bodily pain indices (tab. II) during postoperative period. Other differences between both groups in other parts of SF-36 questionnaire were less significant including general health perceptions, vitality, and physical role functioning.

TABLE II

Quality of life after hernioplasty according to SF-36 questionnaire in different groups

Indices (points)	Control group (n=39)	II group (n=21)
Physical functioning	85.44±4.09	91.75±5.27 $p_1<0.05$
Bodily pain	84.73±3.91	90.22±5.27 $p_1<0.05$
Vitality	67.85±3.89	68.43±4.85 $p_1>0.05$
Social role functioning	86.41±5.41	86.64±4.18 $p_1>0.05$
Physical role functioning	82.28±4.31	81.46±3.84 $p_1>0.05$
Emotional role functioning	74.43±3.55	76.82±3.59 $p_1>0.05$
General health perceptions	87.46±4.77	87.33±4.34 $p_1>0.05$
Mental health	81.0±3.42	81.91±3.97 $p_1>0.05$

Notes: p – reliability of differences compared to control group data.

DISCUSSION

Tension-free IH repair is a method of choice since last decade. Use of mesh significantly simplified surgery itself, made it faster and safer, leading to very low recurrence rate. However, use of mesh for hernioplasty creates several conditions that may aggravate the outcome of surgery (8, 13). Among them chronic pain syndrome including pain itself, feeling of foreign body, and paresthesia (14) are the most common. Other mesh-associated postoperative complications not typical for surgery using own tissues are seromas, mesh dislocations and deformations.

Possible answer towards these issues is use of glue or anatomical structures for mesh fixation, which may potentially abolish conditions for mentioned above complications (5, 15).

However, suture-free E. Trabuco's technique requires specific graft and does not fully prevent chronic pain syndrome (4, 12). Most of existing comparative studies of use of glue for mesh fixation in groin hernioplasty demonstrate similar or better results compared to classic Lichtenstein technique proving its perspective, though both research bias, and differences of techniques may alleviate the value of obtained results (7).

In this study, we tried to combine both E. Trabuco's anatomical approach and use of other, previously unused glue for mesh fixation. While albumin glue is extensively used in cardiovascular surgery both as adhesive and sealant (9), due to its vulnerability against local fibrinolysis-proteolysis stimulated by both bleeding and surgical trauma including allotransplantation during hernioplasty, there was expectation of weaker mesh fixation compared to other techniques, leading to mesh dislocations, deformities, and higher recurrence rate. This study confirms similar or better results of FAF compared to I. Lichtenstein. Among FAF benefits facilitated chronic pain syndrome is the most important, lead-

ing to faster postoperative recovery and quality of life improvement.

CONCLUSIONS

FAF demonstrated significantly better results compared or comparable results with less acute and chronic postoperative pain and better quality of life than sutures for I. Lichtenstein hernia repair.

No FAF specific complications were demonstrated in this study. Further prospective studies are at need with wider cohort selection, better randomization, and standardization combined with different surgical glues assessment.

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CONTRIBUŢII LA CUNOAŞTEREA IMPLICĂRII UNOR AFECŢIUNI CARDIOVASCULARE ÎN APARIŢIA GLAUCOMULUI, OBSERVATE PE 996 PACIENŢI ASISTAŢI ÎN CLINICA DE OFTALMOLOGIE, A SPITALULUI DE URGENŢĂ "PROF. DR. N. OBLU" IAŞI

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CONTRIBUTIONS TO KNOW THE INVOLVEMENT OF CARDIO-VASCULAR DISEASES IN GLAUCOMA ETIOLOGY, STUDIED ON A SAMPLE OF 996 PATIENTS ASSISTED ÎN CLINICO OF OPHTALMOLOGY, "PROF. N. OBLU" EMERGENCY HOSPITAL, IASI (Abstract)**: Material and method:** The authors present the results of clinical and epidemiological research on a sample of 8118 patients assisted in the Ophthalmology Clinic of the Hospital "Prof. Dr. N. Oblu", including 996 (12.1%) with glaucoma, of which 54.1% primary open angle glaucoma (POAG), 22.6%, normal tension glaucoma (NTG), 5.7% primary angle closure-glaucoma (PACG) and 17.6% secondary glaucoma, during 2006-2010. **Results:** For patients with glaucoma authors assessed the prevalence of arterial hypertension (POAG: 33.1%; NTG: 32%; PACG: 8.7%, secondary: 8%), diabetes mellitus (POAG: 19.9%, TNG: 12.9%; PACG: 5.3%, secondary: 5.2%), atherosclerosis (POAG: 10.2%, TNG: 12%; PACG: 5.2%, secondary: 2.9%), vasospasm (POAG: 45 2%, TNG: 47.3%; PACG: 39.3%, secondary: 44.3%). **Key words**: GLAUCOMA, RISK FACTORS, CARDIOVASCULAR, DIABETES MELLITUS, OCULAR VASOSPASM

Deşi au fost identificaţi multipli factori de risc cu potenţial de a induce glaucomul primitiv cu unghi deschis (GPUD), numai unii din aceştia sunt unanim acceptaţi. Dovezile pentru factorii de risc implicaţi în etiopatogenia GPUD au fost obţinute prin studii caz-control, completate cu rezultatele investigaţiilor clinico-epidemiologice. Mulţi dintre factorii de risc pentru GPUD sunt implicaţi în evoluţia bolii şi se referă la cei cu acţiune generală, sistemică şi pentru structurile oculare (1,2).

Cel mai adesea, între factorii de risc sistemici, implicaţi în etiopatogenia glaucomului, se includ: diabetul zaharat, tensiunea arterială (hiper sau hipo), migrena, demenţa senilă Alzheimer, bolile endocrine, apneea de somn, infecţiile şi bolile autoimune (3,4,5,6).

MATERIAL ŞI METODE

Studiul retroprospectiv a inclus 8118 de pacienţi, asistaţi în Clinica a II-a Oftalmologie, Spitalul Clinic "Prof. dr. N. Oblu" Iaşi, în perioada 2006-2010. În baza examenului clinic general şi oftalmologic, pacienţii au fost împărţiţi în două loturi: lotul de cazuri, reprezentat de pacienţii diagnosticaţi cu glaucom (orice formă clinică) şi lotul martor format din cei internaţi pentru diverse patologii oculare. Au fost excluse internările multiple în cazul lotului cu patologie generală (lot

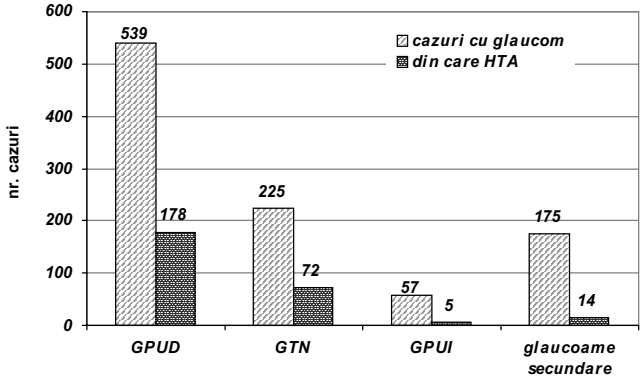

Fig. 1. Distribuţia valorilor de prevalenţă a cazurilor cu HTA în diverse subgrupuri ale pacienţilor cu glaucom, luaţi în studiu

martor), iar pentru lotul de cazuri (996 pacienţi cu glaucom) s-a urmărit evoluţia diferitelor patologii asociate, precum şi identificarea factorilor de risc generali şi oculari asociaţi. Datele evaluate şi incluse într-o fişă clinico-epidemiologică au fost prelucrate şi interpretate statistico-matematic.

REZULTATE ŞI DISCUŢII

1. Tensiunea arterială.

În perioada 2006-2010, din cei 8118 pacienţi cu internări multiple, 996 (12,1%) cazuri s-au prezentat cu glaucom şi au fost evaluaţi, la intervale regulate, atât clinic general, pentru a evidenţia afecţiunile asociate, cât şi oftalmologic. S-a cuantificat implicarea unor afecţiuni cardiovasculare ca factor de risc atât la prima internare cât şi ulterior. Grupul martor a fost format din restul pacienţilor internaţi în Clinica a II-a Oftalmologie, în perioada menţionată, pentru orice patologie oculară, alta decât glaucomul. Au fost excluşi din studiu pacienţii asistaţi în ambulatoriu, în principal datorită imposibilităţii urmăririi evoluţiei afecţiunilor cardiovasculare şi a factorilor de risc implicaţi. Date acumulate au fost incluse într-o fişă de observaţie oftalmologică, pe baza căreia s-a realizat cuantificarea retrospectivă a afecţiunilor cardiovasculare asociate, precum şi a factorilor de risc implicaţi atât în etiopatogenia comorbidităţilor, cât şi în apariţia şi progresia glaucomului (tensiune arterială, colesterol, fumat, diabet zaharat, vasospasm).

În grupul pacienţilor cu altă patologie decât cea glaucomatoasă, hipertensiunea arterială (HTA) a fost înregistrată la 571 (8%) cazuri, care au beneficiat de asistenţă în serviciile de medicină internă.

În relaţie cu forma clinică, pacienţii cu glaucom au fost împărţiţi în subgrupuri, incluzând : GPUD, glaucom cu tensiune normală (GTN), glaucom primitiv cu unghi închis (GPUI) şi forme secundare. Dintre pacienţii cu glaucom, 539 (54,1%) au prezentat GPUD, 225 (22,6%) GTN, 57 (5,7%) GPUI şi 175 (17,6%) cazuri de glaucom secundar.

În grupul cu glaucom, incidenţa HTA a fost de 269 (27,0%), adică de 3,6 ori mai mare decât cea din grupul martor (8,0%). În subgrupuri, s-a remarcat o pondere mai mare a pacienţilor cu HTA : 78 (33,0%) în cazul pacienţilor cu GPUD şi cu GTN. În subgrupurile cu GPUI şi în cel al glaucoamelor secundare, diferenţa incidenţei cazurilor cu HTA a fost nesemnificativă ($p > 0,05$), faţă de martori (GPUI - 8,7% şi glaucom secundar – 8,0%). A rezultat o preponderenţă a cazurilor cu HTA la grupurile cu GPUD şi GTN, fiind cunoscut faptul că factorul vascular poate fi implicat în patogenia creşterii presiunii intraoculare (PIO) (fig. 1).

Este luată în discuţie implicarea modificărilor microvasculare la nivelul capului

TABELUL I
Asocierea hipertensiunii arteriale cu diverse aspecte ale papilei optice în glaucomul primar cu unghi deschis

Tipul modificărilor papilei optice	Nr. total cazuri	Cazuri cu HTA	
		Nr.	%
Focal ischemic	113	33	29,2
Glaucomatos miopic	116	32	27,6
Senil sclerotic	120	57	47,5
Lărgire concentrică	105	41	39
Mixt	85	15	17,6

TABELUL II
Asocierea hipertensiunii arteriale la pacienţii din subgrupurile cu glaucom

Tipul glaucomului	Nr. total cazuri	Număr	%*
GPUD	539	56	10,4
GTN	225	34	15,1
GPUI	57	3	5,3
G secundar	175	6	3,4

* pentru valorile mai mici de 10, proporţiile sunt orientative

nervului optic în apariţia manifestărilor glaucomatoase de la acest nivel. Se ştie că sursele pentru circulaţia prelaminară şi laminară derivă din arterele coroidiene peripapilare şi din arterele ciliare posterioare. Fluxul sangvin poate fi compromis în glaucom prin următoarele mecanisme: afectarea selectivă a vascularizaţiei de la acest nivel, cu o pierdere egală a celulelor ganglionare şi a reţelei capilare, iar bolile microvasculare de tipul diabetului zaharat (DZ), favorizează, în mod semnificativ, instalarea GPUD (7, 8). În toate aceste circumstanţei, autoreglarea fluxului sangvin la nivelului capului nervului optic este perturbată în GPUD.

Studiul Rotterdam (5) a evidenţiat o asociere între o presiune sistolică ridicată şi GPUD, dar nu şi pentru GTN. În lotul studiat de noi, s-a observat o asociere aproximativ egală, atât pentru GPUD, cât şi pentru GTN, iar în relaţie cu aspectul papilei nervului optic în lotul studiat, în tipul senil sclerotic s-a remarcat o asociere semnificativ mai mare a cazurilor de HTA cu apariţia manifestărilor caracteristice pentru glaucom.

În lotul studiat, s-a evidenţiat o preponderenţă a HTA la pacienţii cu tipul senil sclerotic al papilei, pentru ca celelalte grupuri să înregistreze o incidenţă mai scăzută a comorbidităţii cardiovasculare (tab. I).

Unele cercetări (3) au arătat importanţa scăderilor episodice ale TA în glaucom, în special în GTN. Hipotensiunea arterială (hTA) nocturnă este implicată, de asemenea, în patogenia neuropatiei optice ischemice anterioare, legată de prezenţa GPUD şi a GTN, precum şi a progresiei acestora. Pe lotul studiat, incidenţa cazurilor cu hTA a evidenţiat o creştere moderată în grupul cu GTN, asociată posibil şi cu o hTA nocturnă, dificil de evaluat în absenţa unei supravegheri Holter, pentru 24 de ore.

Dintre pacienţii internaţi în Clinica a II-a Oftalmologie, 285 (4%) din grupul celor cu altă patologie decât glaucomul, prezentau hTA, iar la pacienţii cu glaucom, hTA sistemică s-a înregistrat la 9,9% din cazuri.

În relaţie cu forma clinică a glaucomului, se poate vedea o asociere mai frecventă a hTA la pacienţii cu GPUD şi GTN (tab. II).

Hipotensiunea arterială poate determina neuropatie optică glaucomatoasă la unii pacienţi, iar cei cu GTN care riscă această

TABELUL III
Incidenţa pacienţilor cu diabet zaharat în diverse ubgrupuri de glaucom

Tipul glaucomului	Nr. total cazuri	Număr	%*
GPUD	539	107	19,9
GTN	225	29	12,9
GPUI	57	3	5,3
G secundar	175	9	5,2

* pentru valorile mai mici de 10, proporţiile sunt orientative

TABELUL IV
Asocierea aterosclerozei şi a bolilor coronariene la pacienţii din subgrupurile cu glaucom

Tipul glaucomului	Nr. total cazuri	Ateroscleroză		Boli coronariene	
		Nr.	%*	Nr.	%*
GPUD	539	55	10,2	30	5,6
GTN	225	27	12,0	15	6,7
GPUI	57	3	5,2	1	1,8
G secundar	175	5	2,9	1	0,6
Total cumulativ	996	90	9,0	47	4,7

* pentru valorile mai mici de 10, proporţiile sunt orientative

complicaţie, tind să aibă o scădere mai mare a TA. Până la elucidarea implicării modificărilor TA la toţi pacienţii cu glaucom se consideră necesară măsurarea TA la una din consultaţiile oftalmologice, fără urmărirea Holter pe 24 ore, urmată de un consult cardiologic, care să asigure o terapie corectă.

2. Diabetul zaharat (DZ).

Valorile de prevalenţă ale GPUD sunt mai ridicate la bolnavii cu DZ, în unele studii (6, 7) dovedind prezenţa PIO uşor crescută, cu o prevalenţă mai mare a hipertensiunii oculare şi a glaucomului.

În studiul nostru, în grupul cu altă patologie decât glaucomul, au fost înregistrate 357 (5%) cazuri cu DZ. Din cei 996 de pacienţi cu glaucom, 148 (14,9%) aveau ca patologie asociată DZ. Această asociere ar putea fi explicată şi prin vârsta mai înaintată în grupul cu glaucom, prezenţa HTA, a aterosclerozei şi a altor patologii care determină afectare microvasculară.

Dintre cei 148 pacienţi cu glaucom asociat cu DZ, 82 (55,4%) prezentau şi, iar prevalenţa crescută a DZ în grupul pacienţilor glaucomatoşi arată că acesta este un factor de risc, deşi nu are o prezenţă constantă la pacienţii cu glaucom. S-a constatat o asociere mai frecventă a DZ la subgrupul cu GPUD şi cel cu GTN (tab. III).

Bolnavii cu GPUD par a fi mai receptivi la DZ, fără semnificaţie statistică ($p > 0,05$), deoarece sunt implicaţi ca factori de risc vârsta înaintată şi prezenţa unor comorbidităţi.

Diabetul, prin modificările microvasculare induse, poate determina afectarea autoreglării fluxului sangvin ocular, cu perturbări la nivelul porţiunii anterioare şi a capului nervului optic, care duc la apariţia neuropatiei optice glaucomatoase. De asemenea, DZ compromite funcţiile celulelor gliale şi ale neuronilor, precum şi a metabolismului retinian, care pot duce la o receptivitate crescută a celulelor ganglionare retiniene la variaţiile presionale (7). O afectare prelungită a retinei şi nervului optic prin factori vasculari, gliali şi neuronali poate fi asociată cu un risc mai mare de GPUD. Studiile lui Leske et al. (8) arată un rol potenţial al DZ în patogenia GPUD, pe când alte studii populaţionale (3) nu au evidenţiat o asociere semnificativă.

3. Ateroscleroza (ATS)

În clinica a II-a Oftalmologie, au fost internaţi 216 pacienţi cu ATS, incluşi în grupul celor care au înregistrat o altă patologie

TABELUL V

Asocierea unor aspecte ale vasospasmului subgrupurile cu glaucom, incluzând 7122 pacienţi

Tipul glaucomului	Nr. total cazuri	Extremităţi reci		Migrenă		Vasospasm	
		Nr.	%*	Nr.	%*	Nr.	%*
GPUD	511	165	32,3	115	22,5	231	45,2
GTN	298	95	31,8	62	20,8	141	47,3
GPUI	28	12	42,8	5	17,8	11	39,3
G secundar	79	32	40,5	12	15,2	35	44,3
Total cumulativ	916	304	31,2	194	21,2	418	45,6

* pentru valorile mai mici de 10, proporţiile sunt orientative

decât cea glaucomatoasă. În lotul de 996 pacienţi cu glaucom, prevalenţa celor cu ATS a fost moderat crescută (9%), pentru ca pacienţii cu GPUD şi cei cu GTN să înregistreze o prezenţă mai mare a ATS (10%, respectiv 27%) (tab. IV).

Ischemia cardiacă, angina paectorală şi infarctul miocardic au fost asociate, în variate proporţii, diferitelor forme de glaucom, deşi studiul prospectiv Rotterdam (5) şi alte cercetări nu au identificat o legătură semnificativă între ATS şi glaucom ($p > 0,05$). Hayreh (9) menţionează că serotonina, biomarker specific eliberat în timpul agregării plachetare la nivelul plăcii ateromatoase, la maimuţele cu ATS, produce vasospasmul arterei centrale a retinei şi pe arterele ciliare scurte posterioare, dar nici un studiu nu a permis o vizualizare directă a circulaţiei sangvine oculare la om, pentru a identifica modificările vasculare ale pacienţilor cu GPUD (1,2,7).

Leske et al. (8) se referă la istoria bolilor cardiovasculare ca predictor pentru progresia glaucomului, cu referiri la o incidenţă crescută a ischemiei miocardice silenţioase la pacienţii glaucomatoşi sau la relaţia dintre ischemia cerebrală, infarctul miocardic şi prezenţa glaucomului.

Deşi au fost raportate unele relaţii între factorii de risc aterosclerotici şi GPUD, influenţa directă a aterosclerozei în progresia glaucomului nu este pe deplin determinată.

4. Vasospasmul este o contracţie a vaselor sangvine persistentă, exagerată, rezultând o scădere a lumenului vascular şi a fluxului sangvin. Datele din fişele de observaţie medicală, pentru lotul în studiu, au evidenţiat prezenţa extremităţilor reci şi a migrenei, iar vasospasmul a fost relevat prin ecografia arterei carotide.

Prezenţa migrenei s-a înregistrat cu o prevalenţă de 22,5% la pacienţii cu GPUD şi 20,8% la cei cu GTN. Boala vasospastică fiind mai frecventă la femei la care este recunoscută asocierea, îndeosebi, cu GTN, iar terapia cu blocanţi de calciu poate ameliora hemodinamica retrobulbară (tab. V).

CONCLUZII

Glaucomul este o boală cu manifestări variate şi cu o varianţă mare în evoluţie. Sunt mulţi pacienţi a căror evoluţie este staţionară sau foarte lentă printr-o scădere a PIO. Sunt alţi pacienţi însă cu o reducere identică a PIO care au o progresie a bolii. Sunt factori de risc acceptaţi cum ar fi vârsta înaintată, miopia şi istoricul familial de glaucom. Studiile asupra factorilor vasculari sunt contradictorii, probabil datorită faptului că la doar o proporţie din pacienţii cu glaucom aceştia joacă un rol semnificativ. Este important de a identifica subgrupuri pentru a permite clinicianului o predicţie mai bună a incidenţei şi evoluţiei glaucomului. Aspectele papilei nervului optic este un concept bine definit în practică. Aceste aspecte, alături de forma clinică se pot corela, mecanismele patogenice putând fi similare.

Hipertensiunea arterială este un factor de risc probabil, numeroasele studii nu o-

feră însă o evidenţă clară a implicării HTA. Tensiunea arterială sistolică şi diastolică sunt cercetate independent, cea mai mare valoare etiopatogenică având în momentul de faţă tensiunea arterială diastolică. În lucrarea de faţă am observat o prevalenţă mai mare a hipertensiunii la pacienţii glaucomatoşi, mai evidentă la pacienţii cu glaucom cu unghi deschis şi la cei cu tensiune normală.

În funcţie de asocierile patologice, am observat că la anumite subtipuri ale papilei, recunoscute pentru asocierile cu alte boli cardiovasculare, proporţia pacienţilor cu hipertensiune arterială sistemică este mai mare. Aceasta s-a întâmplat în cazul pacienţilor cu aspect senil sclerotic şi la cei cu lărgire concentrică. Evidenţierea acestor tipuri poate duce la o acţiune mai bună atât asupra evoluţiei glaucomului cât şi a patologiei cardiovasculare asociate.

Modificările microvasculare au fost şi ele implicate în etiopatogenia glaucomului, diabetul zaharat fiind o patologie importantă asociată. Studiul nostru arată o prevalenţă mai mare a diabetului între pacienţii cu glaucom, literatura însă are numeroase studii contradictorii.

În studiul de faţă se vede o preponderenţă a vasospasmului la GTN, dar şi pacienţii cu GPUD au o incidenţă mai mare a manifestărilor periferice ale vasospasmului. Toate manifestările periferice (extremităţi reci), migrena conduc la ideea de scădere a fluxului sanguin în anumite teritorii prin acest mecanism. O evaluare mai bună a patologiei asociate poate conduce la o terapie adecvată, cunoscându-se unele studii care au testat diverse medicaţii (blocanţi ai canalelor de calciu).

În cazul subgrupurilor de glaucom cu unghi închis şi a celor secundare se observă o asemănare cu grupul martor, ştiindu-se mecanismele patogenice diferite. În unele cazuri se observă o incidenţă chiar mai scăzută a unor afecţiuni, probabil datorită cazurilor mai puţine, dar şi datorită vârstei care pentru glaucoamele secundare este mai mică, bolile cardiovasculare apărând la o vârstă mai înaintată.

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MANAGEMENT OF MALIGNANT THYROGLOSSAL DUCT CYSTS, A RARE CLINICAL ENTITY IN CERVICAL TUMOR PATHOLOGY

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MANAGEMENT OF MALIGNANT THYROGLOSSAL DUCT CYSTS, A RARE CLINICAL ENTITY IN CERVICAL TUMOR PATHOLOGY (Abstract): Thyroglossal duct cyst is a congenital malformation that occurs at cervical level following an embryonic developmental defect**.** The authors undertook a retrospective study on 143 patients diagnosed with malignant thyroglossal duct cyst in the ENT Clinic of "Sf. Spiridon" County Clinical Emergency Hospital Iasi, between 1992 and 2016 and only 2 cases came out as malignant thyroid gland cyst. This confirms the rarity of this type of lesion, consistent with the data presented in the literature. We present the case of a 51-year-old male case, hospitalized in the ENT Clinic of "Sf. Spiridon" County Clinical Emergency Hospital Iasi, treated by surgical excision of the thyroid gland cyst, performed by using the Sistrunk method. The post-operative histopathological examination of the thyroid gland cyst detected the malignancy. **Conclusions:** The oncology committee of the hospital decided to complete the therapeutic protocol by total thyroidectomy, bilateral neck dissection, radioiodine administration, followed by thyroid hormone replacement. Regular follow-up showed a favorable outcome without complications and no tumoral recurrence at 5 years. **Keywords**: THYROGLOSSAL MALIGNANT DUCT CYST, HISTOPATHOLOGY, RADIOLOGY, SURGERY, ONCOLOGICAL TREATMENT.

INTRODUCTION

Thyroglossal duct cyst is a congenital malformation that occurs at cervical level following an embryonic developmental defect. The thyroid gland developed at the foramen cecum level migrates to the lower third cervical level. The existing tract normally closes after birth. The persistence of this tract can generate the thyroid gland cyst, located between the base of the tongue and the thyroid, with an internal structure of respiratory epithelium and possible thyroid follicles at the external wall of the cyst. The formation is located in the antero-medial cervical section, below the hyoid bone and more rarely the left. It is initially asymptomatic and becomes manifest after an inflammatory episode (1, 2, 3).

In evolution, a fistula can develop, most frequently externally and rarely internally in the pharynx, due to infection. Rarely, the thyroid follicles can undergo malignant transformation. This malignant degeneration most commonly occurs as a thyroid papillary carcinoma developed from the cyst wall.

Differential diagnosis should exclude ectopic thyroid, adenopathy, dermoid cyst, Schwannoma and cystic lymphangiomas (4, 5). In case of thyroid gland cyst malignan-

cy, the accepted therapeutic protocol, that is also cited in the literature, is the complete excision of the thyroid gland and cervical lymph nodes, with post-surgical radioiodine administration followed by thyroid hormone replacement (6, 7).

Between 1992 and 2016, in the ENT Department of "Sf. Spiridon" Hospital from Iasi, Romania, 143 patients were diagnosed with thyroglossal duct cysts, 15 developed thyroglossal duct fistulas, out of which 2 cases were histopathological diagnosed as thyroglossal malignant duct cyst.

Thyroglossal duct cysts and fistulae predominates in the third and fourth decade of life (93 patients), respective 45 patients were female, and 98 patients were male.

The positive diagnosis was based on physical examination combined with cervical echography, scintigraphy, histopathological exam and CT scan for some cases. Patients with thyroglossal duct cysts had a prior asymptomatic mobile neck mass, which gradually expanded, located in the midline or left lateral part of the neck, usually under the hyoid bone. Some thyroglossal duct cysts develop infectious processes and a cutaneous fistula might appear.

All surgeries were performed under general anesthesia. Because the embryological development of the hyoid bone is closely linked to thyroglossal duct cysts, the middle portion of the hyoid bone was excised with the cyst (Sistrunk procedure). In case of malignant cysts, the protocol of the oncologic committee was applied. In five patients who presented fistulae, methylene blue was injected to ease the dissection of the thyroglossal duct cysts.

CASE REPORT

We present the case of a 51-year-old male case hospitalized in the ENT Clinic of "Sf. Spiridon" County Clinical Emergency Hospital Iasi for a cervical mass located below the hypoid bone, which occurred and was observed 2 months ago before being hospitalized, after one episode of upper respiratory tract viral infection. The ENT clinical examination revealed an elastic mass under the hyoid bone, mobile with swallowing.

Cervical echography associated with an endocrinological examination detected a well-defined cystic mass under the hyoid bone stretching to the anterior border of the thyroid cartilage with a normal appearance and topography of the thyroid gland (fig.1).

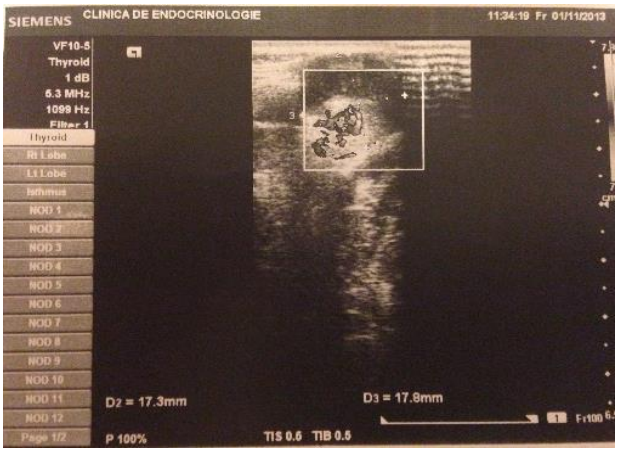

Fig. 1. Echographic image of the cervical mass

The dosage of thyroid hormones was within normal range.

The elected treatment was surgery under general anesthesia by external cervical approach. We performed the dissection of the mass which was adherent to the external perichondrium of the thyroid cartilage, the cranial part being adherent to the hyoid bone, having a tract to the base of the tongue. The whole cyst together with the body of the hyoid bone was excised, ligating the duct above the level of the hyoid bone, using the Sistrunk method (fig. 2-5). The histopathological result showed a cyst wall made up of fibrous tissue with moderate chronic inflammatory microhemorrhage infiltration and a cholesterol deposit, somewhere tapped by a squamous epithelium. Aspects of thyroid papillary carcinoma developed on the thyroid follicles where identified in the wall of the cyst. The examined sections identify adipose tissue and striated muscles with no tumor infiltration and bone fragments without tumor infiltration. The histopathological aspect corresponds to a thyroid papillary carcinoma, pT2NxG2 (fig. 6).

Immunohistochemical (IHC): CK19 - positive in malignant tumor cells, HBME-1 - positive in malignant tumor cells.

The histopathological aspects identified and the IHC profile corresponds to a thyroid papillary carcinoma originating in a thyroid gland cyst.

Fig. 2. Incision revealing the cyst

Fig. 3. Dissection of the cyst

Fig. 4. Post excision appearance

Fig. 5. Excised specimen (cyst + hyoid bone)

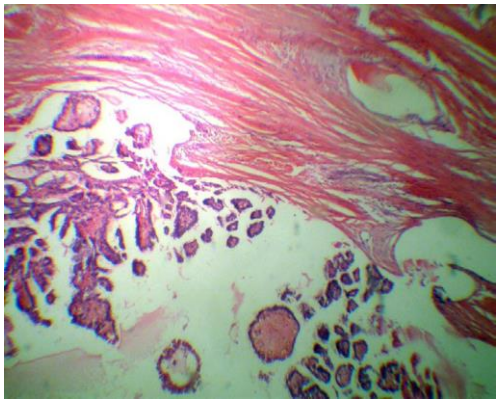

Fig. 6. Histopathological image – thyroid papillary carcinoma

Due to the malignant transformation of the thyroid gland cyst, it required the completion of the therapeutic protocol by performing second surgery for excision of the thyroid gland (total thyroidectomy), followed by application of radiotherapy according to a protocol conducted by the oncological committee.

The postoperative outcome was favorable, with follow-up performed at 1 month, 3 months, 6 months, one year and then annually up to 5 years, without tumor recurrence but requiring thyroid hormone replacement.

DISCUSSION

Thyroid gland cyst, frequent in young adults, is most commonly manifested by a respiratory infection that causes cyst superinfection with increased volume and possible appearance of a fistula.

The need for differential diagnosis to exclude other cervical swelling and thyroid gland pathology is imperative. For this purpose, radiological paraclinical examinations are performed and the patient is endocrinologically examined preoperatively (2).

Endocrinological evaluation, as well as the dynamic outcomes of hormone replacement by administration of Eutirox showed an optimal hormonal substitution level with clinical development in normal parameters that allowed normal social reinsertion of the patient.

The particularity of this case was the malignant transformation of the thyroid gland cyst, the second case encountered in our ENT department over the last 25 years, which corresponds to other statistics stated in the medical literature (7, 8).

A multidisciplinary team consisting of ENT surgeons, radiologists, endocrinologists, histopathologists and oncologists was needed in order to offer the best treatment plan for the patient (9, 10, 11).

The rest of the non-malignant thyroglossal duct cyst cases were resolved by using the Sistrunk surgical procedure, without postoperative complications and no relapse of the tumor (12).

CONCLUSIONS

Malignant thyroglossal duct cyst represents a rare complication of a congenital malformation in ENT pathology, diagnosed postoperatively following the histopathological examination.

The oncological therapeutic protocol requires excision of the thyroid gland as a complementary step, if considering the phylogenetic development of thyroid gland and thyroid gland cyst, followed by radioiodine administration and hormone replacement.

The surgical approach by block excision of the cyst and the hyoid bone body, with the ligature at the base of the tongue (Sistrunk procedure), prevents local relapse and prevents loco-regional complications.

The social reinsertion, the prognosis and the evolution of the patient under the conditions of applying a correct oncological protocol was favorable.

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NEWS

THE ROLE OF MATERNAL OMEGA-3 FATTY ACIDS IN METABOLIC DYSFUNCTIONS IN OFFSPRING

A study by Robertson *et al*. analyses the role of maternal diet during gestation and lactation on the development of the offspring's intestinal microbiota. Metabolic dysfunctions in adult life are influenced by the development of the early-life intestinal flora, which, in turn, is dependent on maternal fatty acid status. To prove this, Robertson *et al* used a transgenic model and showed that maternal n-3 polyunsaturated fatty acid production during gestation or lactation has different effects on male offspring compared to female offspring. Thus, maternal production of polyunsaturated fatty acid significantly reduced weight gain in males fed a high-fat diet but had no significant effect in female offspring. This effect on males persisted into adulthood life and was mediated by restructuring of gut microbiota composition. The microbiota in offspring is influenced more by the maternal fatty acid profile during lactation than in utero. The study shows that reduced exposure to maternal n-3 polyunsaturated fatty acid leads to significantly depleted *Bacteroides*, *Akkermansia* and *Epsilonproteobacteria* and higher relative abundance of *Clostridia* (Robertson RC, Kaliannan K, Strain CR, Ross RP, Stanton C, Kang JX. Maternal omega-3 fatty acids regulate offspring obesity through persistent modulation of gut microbiota. *Microbiome* 2018; 6(1): 95. Published 2018 May 24. doi:10.1186/s40168-018-0476-6).

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SINDROMUL OVARELOR POLICHISTICE – CONSIDERAŢII GENETICE

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POLYCYSTIC OVARY SYNDROME – GENETIC CONSIDERATIONS (Abstract): The Polycystic Ovarian Syndrome is one of the most frequent of the endocrinopathies, affecting in average 8% of the woman's that are in to reproductive age. It was first described by Chereau and Rokitanski, further associated with the amenorrhea encountered in the Stein and Levanthal syndrome, exposed by the Irving F, Stein and Michael Leventhal, in 1935. In many aspects this is a systemic disease also interfering with the process of reproduction. The studies of the genetic factor implication, by relating with the specialty literature, converging with a series of hypotesys launch over the years, show a poligenic affliction or a combination of the environment with the genetics factors. Due to the fact that this syndrome is the most frequently encountered cause of infertility among women's, with a very complex etiopathogeny, the comprehension of it, is most important, because the Polycystic Ovarian Syndrome is a metabolic long term disorder. The study of the genetic act implications is a name part in prophylaxis of the appearance of this syndrome or its complications. **Key words**: POLYCYSTIC OVARY SYNDROME, GENETICS REVIEW, APOPTOSIS, FOLLICULOGENESIS, Bcl2, ETHYOPATHOGENY

Sindromul ovarelor polichistice (SOPC) este cunoscut ca fiind cea mai frecventă afecţiune a femeilor aflate la vârsta reproductivă.

Acest sindrom a fost descris prima data de Irving F. Stein şi Michel L. Leventhal, ambii ginecologi din Chicago, în anul 1935, asociat frecvent cu anovulaţie, ce include: amenoree, hirsutism şi ovare marite în volum (micropolichistice), obezitate (nemenţionată, deşi apare în 3 din cele 7 cazuri studiate) (1).

Până în 1935 s-au publicat puţine lucrări care tratează aceleaşi vechi subiecte.

În perioada 1945 – 1955 preocupările sunt orientate spre fundamentarea clinică, anatomică şi morfopatologică, iar din 1955 – 1965, lucrările pun în evidenţa aspectul hormonal, datorită posibilităţii dozărilor în urină şi apoi în sânge. Dupa 1965 cercetările hormonale, devin mai precise graţie dozărilor RIA, a prelevării din venele ovariene şi suprarenale, a sintezelor „in vitro" făcute pe fragmente de ovare polichistice cultivate în prezenţa unor precursori hormonali marcaţi.

SOPC fiind în multe aspecte o boală sistemică, interferă nu numai cu reproducerea, diagnosticul şi tratamentul fiind esenţiale pentru pacientă, nu doar pentru restabilirea fertilităţii ci şi pentru prevenirea complicaţiilor sistemice. Studiul etiopatogeniei SOPC arată probabil o afectare poligenică sau o combinaţie a factorilor genetici cu cei de mediu. Chiar dacă implicarea unor sisteme adiacente în dereglările funcţionale ovariene a fost suspectată încă de la începutul „istoriei" acestei afecţiuni, ultimii ani au fost extrem de prolifici în suportul

conceptului patogenic multifactorial.

Lucrarea de faţă prezintă o relatare şi o urmărire a diverselor implicaţii ale factorului genetic în etiopatogenia SOPC.

În literatura de specialitate studiile demonstrează incidenţa crescută a acestui sindrom la rudele de sex feminin ale femeilor afectate. În istoricul familial al pacientelor regăsim diabet zaharat tip II, obezitate, hipertensiune arterială, hipertrigliceridemie (componente ale sindromului X metabolic).

Mulţi autori susţin ipoteza că androgenii crescuţi pot avea ca rezultat expresia unor gene legate de apoptoză şi consecutiv expresia factorilor de creştere şi/sau a receptorilor factorilor de creştere.

SINDROMUL OVARELOR POLICHISTIC – IMPLICAŢII GENETICE ÎN ETIOPATOGENIE

Există mai multe ipoteze majore care caută să explice apariţia sindromului ovarelor polichistice, deşi adesea se pot împleti în realitate.

Studiile relatate în literatura de specialitate arată o răspândire familială, anormalităţile gonadale (atât la femei cât şi la bărbaţi) fiind corelate cu o expresie crescută a EGF şi TGF alfa până la 80% dintr-o familie (2). Tendinţa la alopecie precoce (ce apare în familiile femeilor cu SOPC) a fost descrisă pe linie paterna sau materna, cu o transmitere autosomal dominanta (3).

Sunt o mulţime de ipoteze care încearcă sa puna în evidenţă genele responsabile de apariţia sindromului (4).

Una dintre ipoteze arata ca sinteza crescută de androgeni, care apare în acest sindrom, s-ar datora unui complex citocrom P450 C17 cu activitate crescută a enzimelor steroidogenetice (dehidrogenaze sau enzime din grupul de oxidaze). Citocromul P450 este numele generic atribuit unei clase de enzime oxidative. Termenul de 450 este atribuit datorită absorţiei unui pigment (450) în cursul reducerii. In cursul steroidogenezei sunt implicate următoarele enzime P450 : P450scc, P450c11, P450c17, P450arom. Steroidogeneza pe termen lung necesită transcripţie genetică şi sinteze proteice, în timp ce steroidogeneza pe termen scurt se produce independent de o nouă sinteză de ARN (5) .

Celulele tecale ovariene sunt caracterizate prin activitate steroidogenetică ca răspuns la hormonul luteinizant (LH), cu producere de precursori androgeni, prin activarea genelor responsabile de transcripţia P450scc, P450c17 şi a 3beta- hidroxisteroidogenazei (6). Aromatizarea androgenilor în estrogeni este o acţiune specifică celulelor granuloase ovariene, indusă de hormonul foliculostimulant (FSH) prin activarea genei P450arom.

Ar mai fi o altă ipoteză care consideră că există o deficienţă structurală de fond a ovarelor în aromatază, 17-hidroxilază şi 3--beta-hidroxisteroiddehidrogenază cu o sinteză ovariană scăzută de estrogeni şi o stimulare insuficientă a sintezei de gonadotrofine.

În cazul SOPC, absenţa ovulaţiei este asociată cu oprirea în creştere a foliculilor, la cca 10 mm diametru şi cu o producţie excesivă de androgeni de către celulele tecale ale foliculilor blocaţi. În termenii celor două celule (granuloasă şi tecală), degenerează compartimentul granulos, teaca rămânând să continue secreţia hormonală. Cauza primară (hipersecreţia de LH, de insulină sau o mutaţie a genei codante pentru enzima P450 care clivează lanţul de cholesterol), nu este cunoscută.

Foliculogeneza imperfectă poate fi datorată şi expresiei deficiente a factorului de diferenţiere a creşterii ovocitar (growth differentiation factor-9 - factor de creştere din familia TGFbeta. Inactivarea genei responsabile antrenează blocajul precoce al dezvoltării foliculare (7).

O ipoteza considera atrezia foliculară ca un proces apoptotic posibil în celulele granuloase ale foliculilor atretici, din cauza similarităţii morfologice între acestea şi celulele care suferă apoptoza (8).

Conceptul de moarte programată a celulelor, ca eveniment fiziologic a fost mai întâi analizat în ovar, în reglarea apoptozei având un rol important sistemul Fas (LH interferă cu acesta având efect antiapoptotic) (9).

Apoptoza trece prin etape distincte morfologic. Contrar necrozei sau morţii degenerative a celulelor, apoptoza este un mod controlat netoxic al morţii celulelor.

Ovarul, în timpul vieţii reproductive a femeii, suportă o remodelare extensivă şi există dovezi care sprijină rolul apoptozei ca mecanism de bază pentru menţinerea homeostaziei în ciclul ovarian.

Sunt cel puţin patru căi diferite prin care apoptoza poate afecta ovarul în SOPC :

1. rata crescută a apoptozei foliculilor poate avea ca rezultat atrezia foliculară crescută ;
2. apoptoza crescută a oocitelor poate avea ca rezultat o rată scăzută a ovulaţiei ;
3. apoptoza crescută a celulelor granuloasei foliculare poate exagera hiperandrogenismul, întrucât androgenii nu sunt convertiţi în estrogeni ;
4. atrezia foliculară totuşi, poate fi anormală în SOPC. Chisturile foliculare pot rezulta din atrezia atenuată în care programul normal al morţii celulare foliculare este atenuat (10).

Prelungirea intervalului de viaţă a foliculilor atretici poate duce la formarea de chisturi.

Genele reglează apoptoza, fie să promoveze, fie să prevină instalarea morţii programate a celulelor. Aceste gene pot fi activate de stimuli externi sau pot fi independente de factorii de mediu.

Foliculogeneza ovariană este caracterizată prin intense procese de proliferare, de diferenţiere celulară a celulelor granuloase în creştere (steroidogeneza) şi de asemenea şi de fenomene de moarte celulară programată în foliculii atretici. Inactivarea unor gene implicate în oricare din aceste procese duce la alterarea funcţiei ovariene. Astfel, inactivarea genei cyclinei D2 (Ccnd2) responsabilă în parte de proliferarea granuloasei, antrenează scăderea importantă a greutăţii ovarelor (11). Este deosebit de importantă observaţia că, în numeroase tumori ovariene, la femeie, au fost identificate niveluri crescute de mARN Ccnd2, care codează cyclina D2 (12).

O situaţie asemănătoare se produce şi prin inhibarea genei antiapoptotice Bcl2 (13). Ovarele cu această genă inhibată prezintă o diminuare cu 30% a numărului de foliculi primordiali (14).

Studiile în care s-a examinat apoptoza mediată de Fas 670 A/G în celulele granuloase şi cardiace umane au demonstrat că această genă joacă un rol important şi în patogeneza bolilor cardiace, asociate frecvent cu sindromul ovarelor polichistice (15).

Apoptoza alterată ar putea să contribuie şi la patogeneza tiroiditei, fie prin lezarea celulară iniţială care duce la răspuns imun, fie prin apoptoza mediată imun a celulelor foliculare tiroidiene care duce la hipotiroidism, un mare număr de femei cu SOPC prezentând o hipofuncţie tiroidiană (16). Hormonii tiroidieni, metabolismul lipidic şi nivelurile de insulină regleaza nivelurile serice ale leptinei – proteina obezităţii – care se găseşte în celulele granuloase prelevate din foliculii preovulatori şi acţionează pe căi autocrine şi paracrine, (implicată în reglarea greutăţii corporale, fiind cel mai bun marker pentru grăsimea totală din organism (17,18).

Mecanismul prin care hiperinsulinemia se asociază cu hiperandrogenismul este explicat printr-un defect în fosforilarea serinei lanţului beta al insulinei şi al enzimei P450c17 ovariană şi suprarenaliană. Fosforilarea serinei creşte, iar defosforilarea serinei descreşte activitatea enzimei 17, 20 liaza, implicată în producţia androgenilor (19,20). Astfel, fosforilarea serinei este un “buton de închidere (off)” pentru mecanismul de

transport al glucozei şi "buton de deschidere (on)" pentru producţia de androgeni.

Astfel, aceeaşi manifestare clinică poate apare în mai multe circumstanţe, printr-un defect al receptorilor de insulină sau afectare post receptor a funcţiei acestuia, având drept rezultat comun, fosforilarea crescută a insulinei (21).

Un procent de 40-50% dintre femeile cu SOPC şi obezitate vor dezvolta diabet zaharat sau toleranţă scăzută la glucoză până la vârsta de 30 ani. Hipertrigliceridemia, hipercolesterolemia, nivele scăzute de HDL sunt factori majori de risc pentru boala coronariană, risc ce se amplifică pe măsură ce pacientele înaintează în vârstă. Sunt studii care arată concentraţii crescute în ser a IMA (ischaemia-modified albumin) la paciente cu SOPC, un marker ischemic nou în boala coronariană (22). Rezistenţa la insulină determină consecinţe pe termen lung cu apariţia diabetului zaharat, a tulburărilor cardiovasculare, cât şi a celor de fibrinoliză, a hiperuricemiei a relaţiei cu inflamaţia sistemică care sunt dezbătute în continuare. SOPC prin asocierea cu obezitate şi insulinorezistenţă este un factor de risc pentru apariţia steatozei hepatice nonalcoolice (NASH) care duce la ciroză, hipertensiune portală, insuficienţă hepatică şi carcinom hepatocelular (complicaţii pe termen lung). În serul pacientelor cu acest sindrom s-au detectat nivele crescute ale capsazei 3, marker apoptotic pentru NASH (23).

SOPC şi diabetul zaharat tip II sunt forme clinice ale aceleiaşi rezistenţă la insulină cu aspecte fenotipice diferite funcţie de localizarea ovariană sau pancreatică a unui defect genetic. Un defect ar putea fi alterarea proprietăţilor stoichiometrice ale complexului proteinkinază A (PKA) – lipază hormono-senzitivă (HSL), ceea ce duce la o lipoliză crescută indusă în celulele adipoase viscerale (defect primar de lipoliză).

Având în vedere asocierea SOPC cu un risc crescut de diabet zaharat tip II, s-a propus studierea unor defecte genetice comune cu acesta (24). Enzima Calpain-10 este o cistein protează care participă la secreţia şi acţiunea insulinei (25). Variaţii ale genei care codifică Calpain-10 se asociază cu diabetul zaharat tip II (26).

În literatura de specialitate sunt studii care arată transmiterea autozomal dominantă, incidenţa hirsutismului, a calviţiei premature la barbaţi şi a oligomenoreei fiind de două ori mai frecvente decât în populaţia generală, dar cu manifestare fenotipică variabilă (27,28). De asemenea legăturile directe între hiperandrogenie şi hiperinsulinism sugerează că efectul stimulator al insulinei asupra producţiei ovariene de androgeni este influenţată de o predispoziţie sau susceptibilitate genetică. In familiile femeilor cu anovulaţie, hiperandrogenism şi ovare polichistice se constată o incidenţă crescută a hiperinsulinismului la femei şi a calviţiei premature la bărbaţi. Studiile genetice, aflate încă la început, implică în această afectare locusurile genetice pentru insulină şi pentru P450scc, nu şi pentru P450c17alfa. Este necesară investigarea familială în acest sens şi avertizarea familiilor cu SOPC privind predispoziţia pentru aceste afecţiuni.

Se consideră că interacţiunea unei anormalităţi a insulinei cu o tulburare a sintezei ovariene de androgeni, poate explica modificările din sindrom. S-a descris o asociere a acestui sindrom şi un genotip anume VNTR (variable number of tandem repeats) de la nivelul cromozomului 11p15,5 implicat în expresia insulinei, astfel, o alterare moştenită în secreţia insulinei ar putea fi implicată în etiopatogenia SOPC. Pornind de la citP450c17alfa, implicat în mai multe lanţuri patogenice, s-a încercat identificarea unui posibil defect asociat, însă studiile au exclus existenţa unui defect la nivelul CYP 17. Se pare însă că un defect la nivelul CYP11A care codifică P450scc, ar constitui,

alături de polimorfismul VNTR, bazele genetice ale SOPC (29,30). De asemenea, în cazul obezităţii, a cărei prevalenţă în SOPC a fost estimată la 50%, se estimează un defect genetic de 25%, chiar de 30-35% în cazul obezităţii troncoabdominale (31). Puternica asociere familială sugerează existenţa unui factor genetic în determinismul sindromului sau măcar al unor componente ale sale, dar heterogenitatea manifestărilor clinice şi biochimice pledează pentru o asociere multigenică.

Prima semnalare a asocierii între intoleranţa la glucoză şi hiperandrogenie a fost făcută de Archand şi Thiers în 1921, referitor la femeile diabetice cu "barbă" (32). Astăzi, relaţia între rezistenţa la insulină şi sindromul ovarului polichistic este unanim recunoscută.

Determinismul genetic al hiperinsulinemiei şi hiperandrogenismul este rar (33). Sunt descrise trei cauze principale ale rezistenţei periferice la insulină. Asocierea rezistenţă periferică la insulină hiperandrogenism şi achantozis nigricans, cunoscută sub numele de "sindrom tip A", poate fi datorată unei mutaţii a genei receptorului pentru insulină, care antrenează scăderea numărului receptorilor în organele ţintă. Leproşonismul (S Donohue) este un sindrom foarte rar ce debutează intrauterin sau imediat postpartum, manifestat prin talie scăzută (defect al receptorilor de creştere), rezistenţa la insulină (mutaţia genei receptorilor insulinici), ovare polichistice, hiperandrogenism, achantozis nigricans şi dismorfisme faciale (lepreschaun=cuvânt din irlandeza veche care semnifică o zână miraculoasă care este întruchiparea unui om bătrân) (34). Sindromul "tip B" constă în prezenţa autoanticorpilor receptorilor pentru insulină. Un alt grup, cel mai larg îl constituie pacientele la care nu se constată, nici anomalii ale receptorilor pentru insulină, nici autoanticorpi antireceptori, dar care prezintă hiperandrogenism şi hiperinsulinemie. Mecanismul posibil constă într-o afectare funcţională a acţiunii receptorului după cuplarea cu insulina.

CONCLUZII

Genele care reglează apoptoza în celulele granuloase sunt activate de factorii externi sau pot fi independente de factorii de mediu, factorii apoptotici şi cei de supravieţuire aflându-se în echilibru instabil. Controlul acestora de către diverse gene (Bcl2 antiapoptotică, gena codantă a ciclinei D2, a enzimelor steroidogenetice, a fosforilării serinei lanţului beta a insulinei, a enzimei Calpain 10, a factorilor de creştere intraovarieni) este încă neelucidat. Cert este că mediul intracelular determină responsivitatea celulelor granuloase la FSH, influenţând apoptoza acestora.

Etiopatogenia sindromului ovarului polichistic este complexă şi obscură, deoarece sindromul nu reprezintă o singură boală. Se admite că anomaliile genetice sau de reglare a unor factori de creştere intraovarieni, pot interveni în producerea sindromului determinând un exces de androgeni produs de suprarenală sau ovar, o secreţie anormală de LH, anomalii enzimatice sau intraovariene în sinteza steroizilor ovarieni şi hiperinsulinism.

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NOUTĂŢI

Escherichia coli O157 : H7 un pericol pentru cord şi rinichi

În primăvara anului 2000 reţeaua de alimentare cu apă din Walkerton, Ontario (Canada) a fost contaminată cu *E. coli* O157 : H7 şi *Campylobacter spp.*, rezultând 2300 de gastro-enterite, peste 750 de consultaţii în urgenţă, 65 de spitalizări, 27 de sindroame hemolitice şi uremice şi 7 decese. În 2002 a fost iniţiat un studiu de cohortă prospectiv (Walkerton Health Study), pentru a determina efectele pe termen lung ale acestei contaminări. Din cei 2000 de subiecţi recrutaţi pentru studiu, 1067 (54%) au fost infectaţi cu *E. coli O*157 : H7 şi *Campylobacter spp.*, 403 subiecţi (38%) prezentând o HTA pe parcursul supravegherii, faţă de 294 subiecţi (32%) necontaminaţi (Hazard Ratio HR = 1,33, interval de încredere 95% II 95% : 1,14-1,54). Riscul de patologie cardiovasculară (infarct, AVC sau insuficienţă cardiacă congestivă) a fost şi el crescut pentru subiecţii contaminaţi (HR = 2,13, II 95% : 1,03-4,43). Afectarea renală a fost confirmată prin dozarea microalbuminemiei (albumină /creatină > 2,0 mg/mmol pentru bărbaţi şi > 2,8 mg/mmol la femei) şi evaluarea filtrării glomerulare (> 60 ml/mim/1,73 m^2). Deşi doar 30 de subiecţi au prezentat modificarea simultană a ambilor parametri, aceştia au provenit în majoritate (22/8) din grupul subiecţilor infectaţi (HR3,41, II 95% : 1,51-7,71). Datele prezentului studiu impun o supraveghere atentă a pacienţilor care au prezentat în antecedente o gastroenterită acută simptomatică cu *E. coli O*157 : H7, recomandându-se controlul anual al tensiunii arteriale şi o supraveghere atentă a funcţiei lor renale. Epidemiile cu *E. coli O*157 : H7 sunt semnalate din ce în ce mai des în toată lumea : în SUA sunt semnalate anual 50000-120000 de gastroenterite acute şi 60 de decese. (Clark F et al. Long term risk for hypertension, renal impairement, and cardiovascular disease after gastroenteritis from drinking water contaminated with *E. coli O*157 : H7 : a prospective cohort study. *BMJ* 2010, 341 : c6020).

Al.D. Ciochină

INTRAPANCREATIC ACCESSORY SPLEEN: DIAGNOSTIC CHALLENGE AND REVIEW OF DATA FROM THE SPECIALTY LITERATURE

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INTRAPANCREATIC ACCESSORY SPLEEN: DIAGNOSTIC CHALLENGE AND REVIEW OF DATA FROM THE SPECIALTY LITERATURE (Abstract): Intrapancreatic accessory spleen is a diagnostic challenge requiring a differential diagnosis with retroperitoneal tumor, neuroendocrine pancreatic tumor, pancreas adenocarcinoma. We present a rare case of intrapancreatic accessory spleen, which was radiologically diagnosed as a pancreatic tumor. This is a 37-year-old female patient who showed fluctuating values of CA19-9 tumor marker. Initially, the endoscopic examination of the digestive tract (upper digestive endoscopy, colonoscopy) was performed, excluding the presence of a proliferative process at this level. In order to investigate the small intestine and other abdominal-pelvic organs we performed the abdomen-pelvic CT examination and the MRI examination. Hepatosplenic scintigram was performed to complete the investigations. The intrapancreatic accessory spleen should be considered as a differential diagnosis for pancreatic tumors. Complex paraclinical investigations (EDS, Colonoscopy, CT, MRI, Scintigraphy) can help diagnose and prevent surgical procedures that are not absolutely necessary. **Keywords**: INTRAPANCREATIC ACCESSORY SPLEEN, PANCREATIC TUMOURS.

The pancreas comprises a head, neck, body and tail. It lies retroperitoneally, separated from the stomach in front by the lesser sac. The spleen is the largest organ of the lymphatic system positioned between the fundus of the stomach and the diaphragm in the left hypochondriac region of the abdominal cavity, relatively below the left costal margin between the ninth and 11th ribs (1). Mesenchymal cells are the source from which the spleen is derived from, which are located between the tiers of the dorsal mesogastrium as early as the fifth and sixth weeks of fetal development (1). The rotation of the stomach during embryonic development causes the left mesogastrium surface fusion with the peritoneum above the left kidney and the resultant dorsal attachment of the lienorenal ligament. The yolk sac wall and near dorsal aorta are the sources of the cells needed for the hemopoietic function of the spleen. By

the second trimester, the spleen is capable of both RBC and WBC generation (1).

CASE PRESENTION

We present a rare case of intrapancreatic accessory spleen, which was radiologically diagnosed as a pancreatic tumor. This is a 37-year-old female patient who showed fluctuating values of CA19-9 tumor marker. Initially, the endoscopic examination of the digestive tract (upper digestive endoscopy, colonoscopy) was performed, excluding the presence of a proliferative process at this level. In order to investigate the small intestine and other abdominal-pelvic organs we performed the abdomen-pelvic CT examination and the MRI examination. Hepatosplenic scintigram was performed to complete the investigations.

The abdomen-pelvic CT examination

The abdomen-pelvic CT examination reveals a normal pancreas that contains two well-defined nodular formations located in the caudal pancreas: a distal one in contact with a descending colon loop situated postero-inferior to the splenic hilum, and dimensions of 26/21.5/11.5 mm (AP/T/CC), one located anterior, in the vicinity of the splenic hilum and having dimensions of 13/9.2/10 mm (AP/T/CC) (Fig.1,2). The aspect of the lesions of the caudal pancreas may be suggestive of pancreatic ectopic tissue / neuroendocrine tumor.

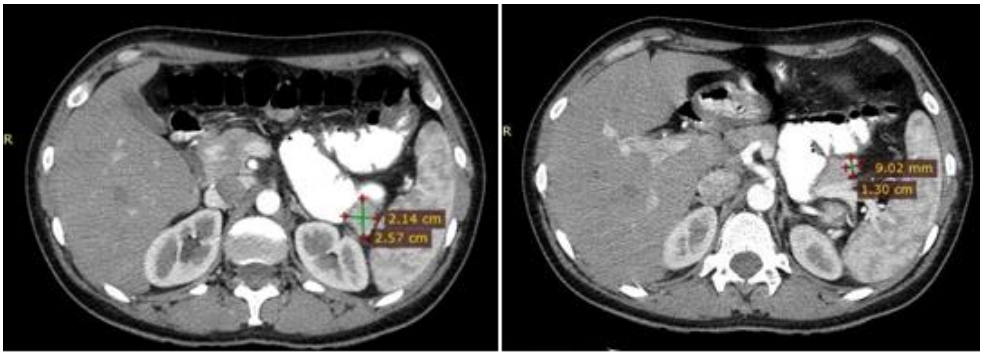

Fig. 1. The abdomen-pelvic CT examination-transverse section reveals a normal pancreas that contains two well-defined nodular formations located in the caudal pancreas

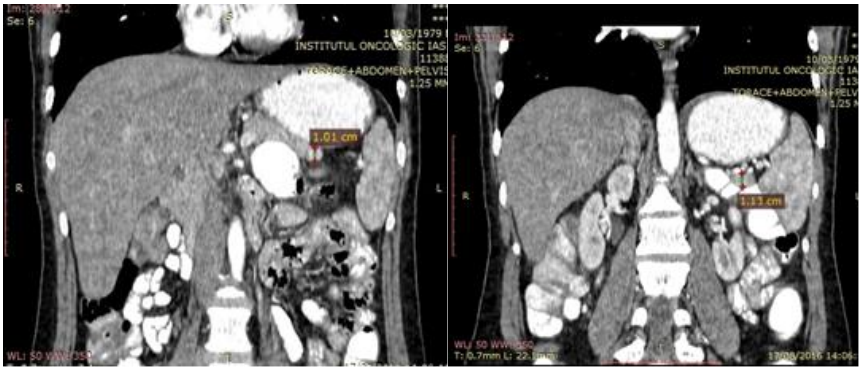

Fig. 2. The abdomen-pelvic CT examination-longitudinal section reveals a normal pancreas that contains two well-defined nodular formations located in the caudal pancreas

The MRI examination

Native MRI examination and MRI examination with contrast substance: pancreas with normal size, lesion in T1, intermediate T2 signal located on the topography of the pancreatic tail with diameter of 12 mm (fig. 3).

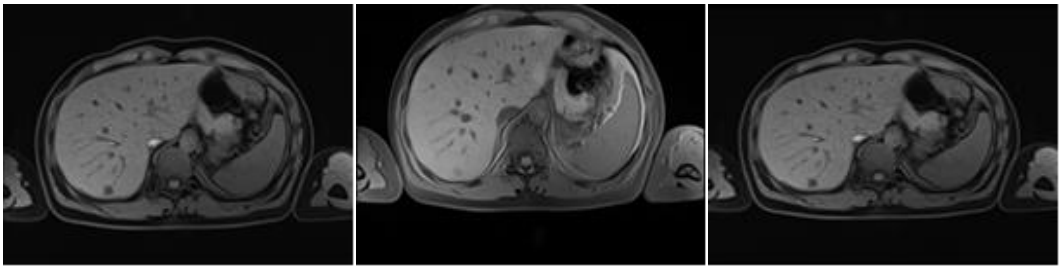

Fig. 3. MRI examination: solid lesion, net contoured, probably benign, caudal pancreas.

Hepato-splenic scintigraphy with 99mtc + NANOCOLL

Scintigraphy performed with 99mTc-NANOCOLL, 5 mCi, administration intravenous. The acquired images were: entire body; images in the abdomen, acquisition in dynamics in the first 5 minutes, SPECT and static (before and after the SPECT acquisition). The images showed the following (fig. 4, 5, 6):

- the heterogeneous hepatic fixation of the radiopharmaceutical (which may be related to the images suggestive of hepatic hemangiomas described in CT);
- fixation in the projection area of the spleen, which is homogeneous;
- on the anterior images and SPECT, medial of the splenic image, in an area that projects in the pancreatic caudal region, one can see a discreet fixation of the radiopharmaceutical, which is individualized and has a rounded contour and whose fixation decreases discreetly over time.

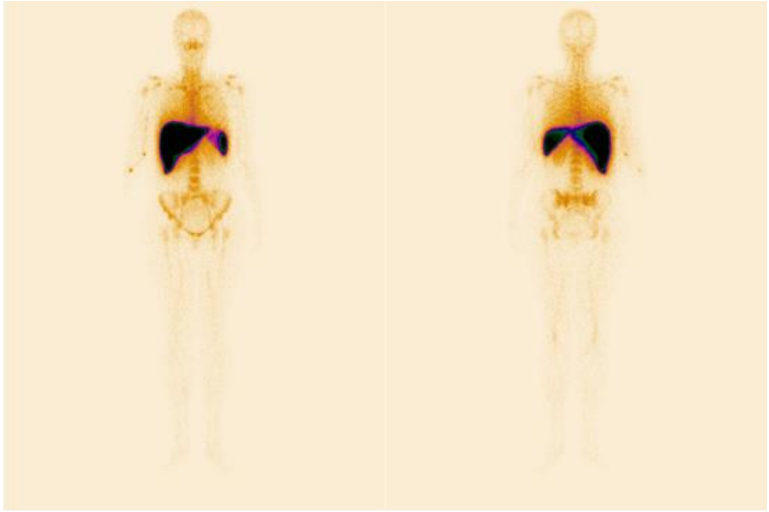

Fig. 4. Scintigraphy performed with 99mTc-NANOCOLL – whole body

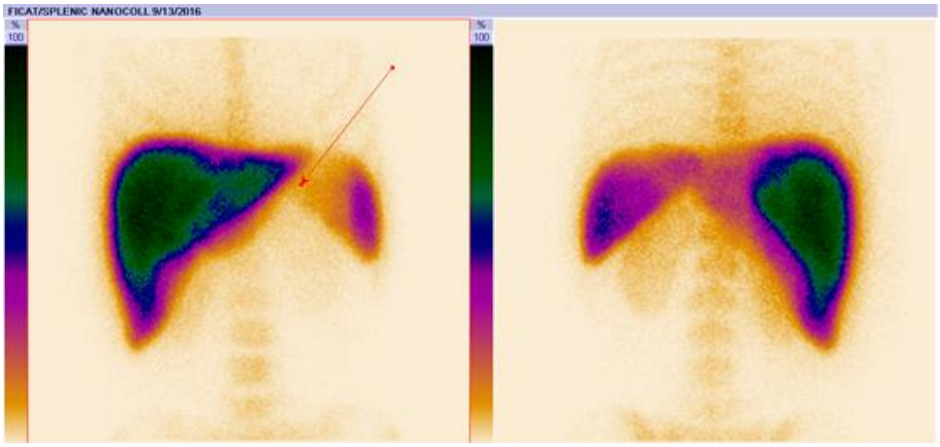

Fig. 5. Scintigraphy performed with 99mTc-NANOCOLL

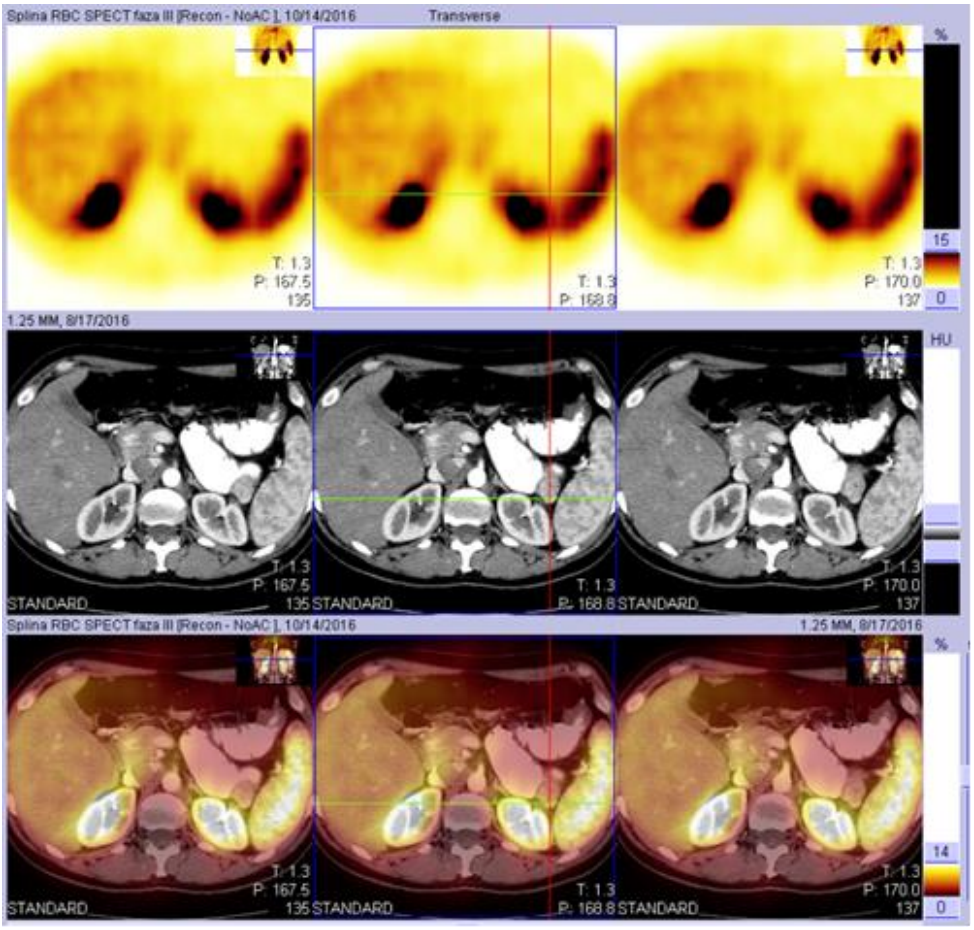

Fig. 6. Scintigraphy performed with 99mTc-NANOCOLL and CT images – Overlay

Conclusions of the hepato-splenic scintigraphy examination with 99mTc + NANOCOLL:

- Given the localization mechanism of the radiopharmaceutical, this image may be suggestive of an accessory spleen, as it would correspond to the formation identified on the CT images.
- We recommend confronting the images with splenic scintigraphic images with marked red blood cells (99mTc – PYP) and possibly with PET-CT images, to exclude with certainty a tumor of pancreatic nature.

Red blood cells scintigraphy marked with 99M TC-PYP

Hepato-splenic scintigraphy with red blood cells marked with 99mTc PYP (in vivo method), static and late static images, SPECT and the fused images SPECT-CT and SPECT with 99mTc PYP and SPECT with 99mTc Nanocoll, showed: very discrete fixation images of radiopharmaceutical in the projection area of the two formations identified on CT and images with 99mTc NANOCOLL.

Although the fixation is very small corresponding to the fixation in the spleen area, it is larger than the background noise (according to the quantification on the fused image).This low degree of fixation could be explained by the small size of the formations and, therefore, the small amount of tissue that would have to seize the marked red blood cells, in the situation of a spleen accessory tissue (fig. 7, 8)

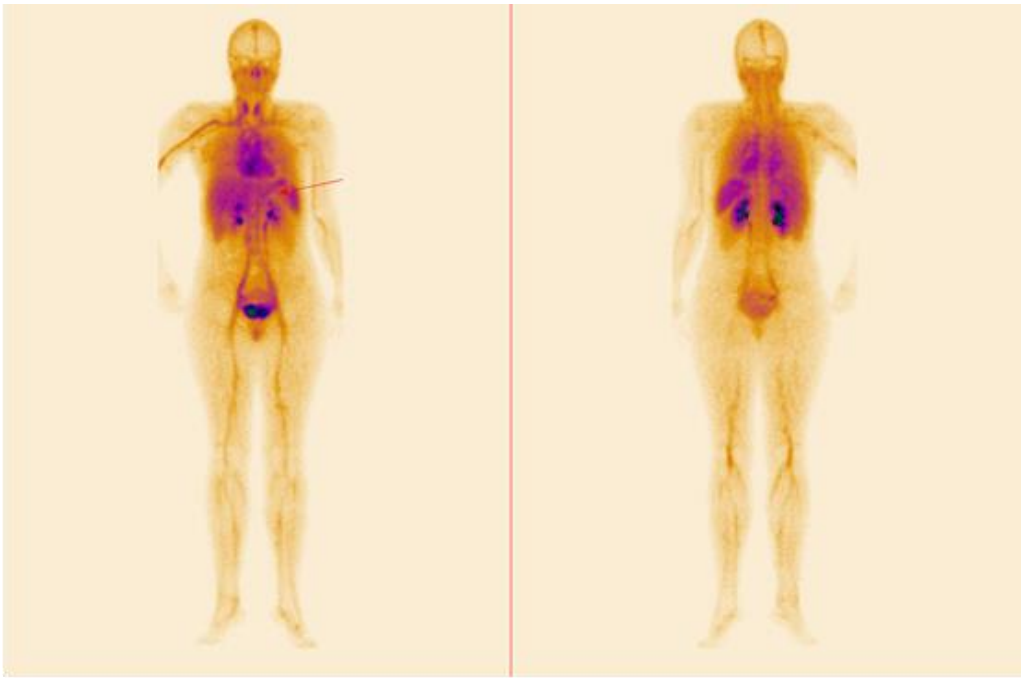

Fig. 7. Hepato-splenic scintigraphy with red blood cells marked with 99mTc PYP-whole body.

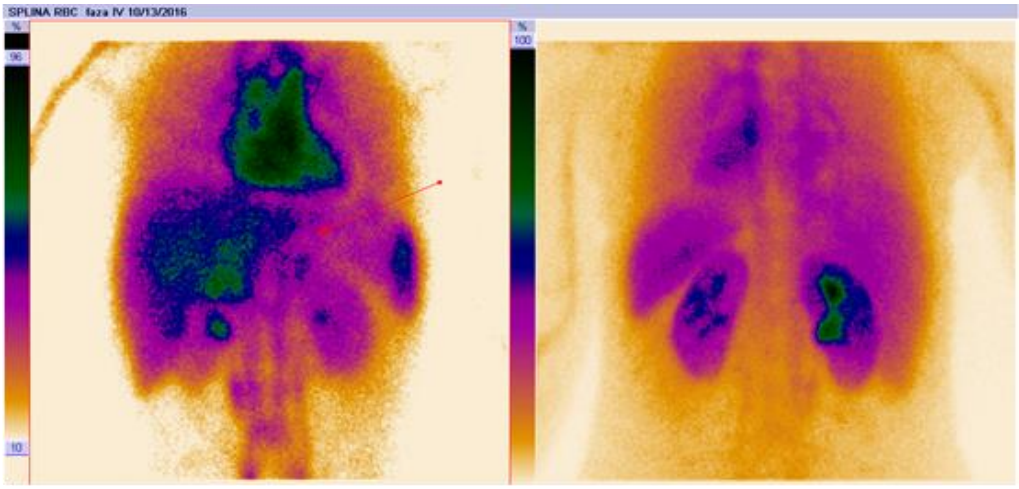

Fig. 8. Hepato-splenic scintigraphy with red blood cells marked with 99mTc PYP

DISCUSSION

The accessory spleen can be found relatively frequently in the general population - 10-15% according to the necroptotic results and according to other authors up to 20%. Among these, only 1-2% are located intrapancreatic (pancreatic tail), the majority being located in the splenic hill (2-5)**.** The accessory intrapancreatic spleen is a congenital anomaly in which there is a failure between a portion a splenic tissue and the main body of the spleen during the embryological development. The spleen appears at approximately the sixth week of embryologic life as a localized thickening of the coelomic epithelium of the dorsal mesogastrium near its cranial end. The proliferating cells invade the underlying angiogenetic mesenchyme, which becomes condensed and vascularized. The process occurs simultaneously in several adjoining areas, which soon fuse to form a lobulated spleen. In the subsequent periods of embryologic life, the earlier lobulated character of the spleen disappears but is indicated by the presence of notches on its upper border in the adult (6, 7). The accessory spleens ca be named also splenunculus (8).

Intrapancreatic accessory spleen is a diagnostic challenge requiring a differential diagnosis with retroperitoneal tumor, neuroendocrine pancreatic tumor, pancreas adenocarcinoma. The accessory intrapancreatic spleens are commonly presented as a hyper vascular node in the tail of the pancreas on abdominal CT and MRI, mimicking a tumor of the pancreas or pancreatic neuroendocrine tumor (10). An intrapancreatic accessory spleen (IPAS) is usually less than 3 cm in size and is generally an unexpected condition (11).

Although majority of the studies showed no clinical symptoms in patients or no pathological blood tests, in our case we found an elevated level of CA 19-9. This made hard to do differential diagnosis with a pancreatic tumor. Conclusions for red blood cells scintigraphy exam marked with 99mTc-PYP: there is a possibility that the formations described on the CT, and which show discrete fixation of 99mTc NANO-COLL (which would suggest a tissue structure with the retico-endothelial component) and discreet seizures of red blood cells

marked with 99mTc PYP to represent accessory spleen tissue, especially since the data from the literature suggest that histopathological there may be a relative variation in the accessory spleen tissue (for example intralesional cysts-which may even be associated with a growth of the seric CA 19-9 (12). The evolutive follow-up of these lesions by structural imaging methods, in a clinical and paraclinical context is required.

CONCLUSIONS

The intrapancreatic accessory spleen should be considered as a differential diagnosis for pancreatic tumors. Complex paraclinical investigations (CT, MRI, Scintigraphy) can help diagnose and prevent surgical procedures that are not absolutely necessary.

Knowledge of anatomy of spleen is fundamental to the radiologists during diagnostic procedures to prevent interpretation errors in diagnostic imaging.

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THE INFLUENCE OF MEDICAL EDUCATION ON MEDICAL STAFF COMPLIANCE WITH HAND HYGIENE GUIDELINES

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THE INFLUENCE OF MEDICAL EDUCATION ON MEDICAL STAFF COMPLIANCE WITH HAND HYGIENE GUIDELINES (Abstract): The objective of the study was to estimate the influence that continued medical education has on the correct disinfection of the hands during the work of different categories of medical staff. **Material and method:** The research was based on direct observation of the correctness of the hand disinfection, according to the WHO recommendations and the experimental validation of the hand disinfection rate using the Semmelweis Scanner developed by HandInScan. The study group included 553 physicians, nurses and auxiliary staff, from 6 hospitals located in Brasov. Data processing was carried out with iNZight software, through descriptive and interference statistics, *Chi-square test* and graphical view. The variables taken into consideration were medical education on hand hygiene, occupation, length of service, age and hospital profile. **Results** are in line with previously data published in literature and the *Chi square test* value (6.4756) confirms the hypothesis that the correct practice of hand disinfection depends on occupation. **Conclusions**: Young subjects and staff from surgical units are more compliant to the correct grasp of the 6-step technique in disinfecting the hand, compared to other age categories or other medical specialties. We recommend the development of a daily alert system for the permanent awareness of the medical staff regarding the frequent practice of hand disinfection. **Keywords**: HAND DISINFECTION, HOSPITAL, PHYSICIANS, NURSES, AUXILIARY STAFF, COMPLIANCE.

The share of healthcare-associated infections in developing countries is high compared to developed countries, thus requiring improvement in surveillance and infection control practices (1, 2). Although hand hygiene as a good medical practice in the hospital that prevents nosocomial transmission of germs is recommended by the WHO as a gold standard, compliance is usually modest (3, 4). The results of some research suggest that compliance with the rules of hand disinfection is significantly influenced by the behavior of medical staff and less by hospital amenities, such as the increased number of washbasins in the hospital rooms or the number of dispensers (5). Hand hygiene (HH) rates remain low among healthcare workers, despite the emphasis placed by trainers on the importance of hand hygiene (6, 7). Even when the staff is aware of being watched if they comply with the rules of hand hygiene and the disinfection technique is correct, the studies note that compliance rates do not change, remaining low (8).

Although there are entire chapters in the education curriculum on the importance of disinfecting teguments and performing correct disinfection techniques, reality shows that without control, education does not yield sustainable. At the individual level, it is necessary to augment a positive attitude towards hand hygiene, respecting the 6 mandatory steps in achieving a proper disinfection technique and reinforcing the belief that each individual can influence the professional behavior of the group to which it is contributing by improving compliance (9).

MATERIAL AND METHODS

This study was conducted in 6 hospitals from Brasov, on a group of 553 subjects, medical and auxiliary staff. The hospitals that participated in the study are including 3 emergency hospitals. Regarding the profile, 3 are single specialty hospitals and 3 multidisciplinary hospitals (including medical and surgical activities). Of the 6 hospitals covered by the study, 5 are state hospitals and only one is a private hospital. Of the 5 state hospitals, 4 are clinical hospitals, where educational activities are also under way, like training of future physicians and nurses.

The research was carried out by the method of direct observation of the correctness of hand disinfection without prior training and validation of the successful technique through the Semmelweis Scanner device. This device indicates the correct disinfection technique, when the surface coverage of hands with disinfectant exceeds 95%, which is considered as success rate. All values below 95% were interpreted as failure rate.

The criteria for inclusion in the study were as follows: the person has to be employed in the hospital, occupation (medical or auxiliary staff) and their participation agreement.

Two hypotheses were issued:

- Hypothesis 1: To what extent successful hand disinfection is influenced by occupation, age, seniority at work, medical or surgical profile of the activities.
- Hypothesis 2: Medical education continues to be valuable in time and significantly influences the hand disinfection technique.

The database was created in CSV format and the processing was done with iNZight software, developed by Professor Christopher J. Wild from the Department of Statistics at the University of Auckland, New Zealand.

RESULTS

Physicians participating in the study, working in medical or surgical specialties, are generally aged between 20 and 30 years and between 60 and 70 years respectively. The nurses working in the medical field department belong to the age group of 31-40 years, 61-70 years, compared to the ones working in the surgical specialties sector, who belong to the age group of 31-40 years. Auxiliary personnel in surgical or medical sections belong to the 51-60 age group. The lot is balanced on the two specialties, surgical and medical.

To all three professional studied categories were analyzed the following variables: hand hygiene course, correct and incorrect disinfection technique. It has been noticed that the highest rate of success in hand disinfection is done by nurses with a hand hygiene course. For auxiliary staff, the rate of failure in performing the disinfection technique is higher regardless whether they have taken a hand hygiene course or not. In the case of physicians, the success rate is higher regardless of whether or not the hand hygiene course is completed.

The distribution of medical staff by age groups, occupation and attendance to course of Hand Hygiene (HH) is presented in first figure.

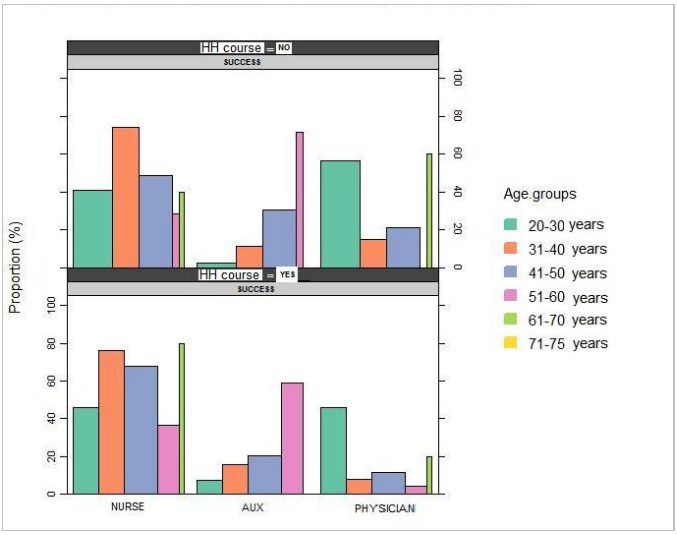

Fig. 1. Distribution of medical staff by age groups, occupation and attendance to Hand Hygiene course

Figure 2 shows the distribution of the comparative lot and the success rate. In 223 study participants, the hand disinfection cover area exceeded 95%, noted as the success rate, and in 330 subjects hand disinfection was incorrectly recorded as a failure rate. 112 people had performed the hand hygiene course and had successful disinfection rate of the hand. 111 people did not take the hand hygiene course but had a successful hand disinfection rate. 213 had not performed the hand hygiene course and had failed to disinfect hands. The highest rate of success is at nurses who attended the hand hygiene course.

The *Chi square test* (value 6.4756) confirms the hypothesis that the correct practice of hand disinfection depends on occupation. The p value was less than 0.05. In the same time, the *Chi square test* value demonstrates that the hand hygiene course influences differently the categories of people studied regarding the success or failure of the hand disinfection technique.

Chi-square test for equal distributions.

- Interference of the distribution by occupation (physician, nurse, auxiliary staff) (columns), by HH course (rows), for experiment success or unsuccess.
- Null Hypothesis: distribution of occupation (physician, nurse, auxiliary staff) does not depend on HH course.
- Alternative Hypothesis: distribution by occupation (physician, nurse, auxiliary staff) changes with HH course X^2=6.4756, df=2, p-value=0.039249.

The analyzed variables were seniority in work and correct hand disinfection technique. It has been noticed that the success rate is influenced by the seniority in work, namely all professional categories with a less seniority in work have a higher success rate. Low compliance has been registered with nurses with the highest seniority in work. In the case

of physicians, the rate of success has been among the young persons, with a lower seniority in work. Analyzing the successful hand disinfection rate, depending on the surgical or medical profile, there was an increase in the success rate for surgical specialties as opposed to medical ones.

Figure 3 summarizes the distribution of the study group with a success or failure rate by age groups and seniority in work. The highest compliance is highlighted in the group of 20-30 years. It is reconfirmed that professional categories with less seniority in work have a higher success rate.

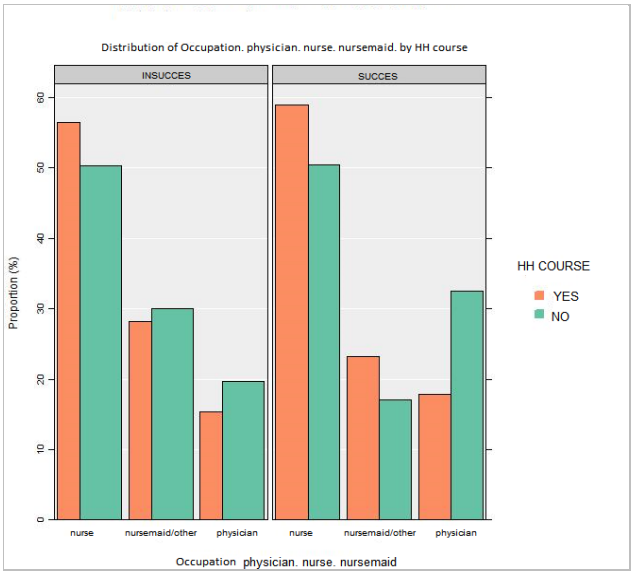

Fig. 2. Distribution of the study group by the rate of success or failure occupation and attendance HH course

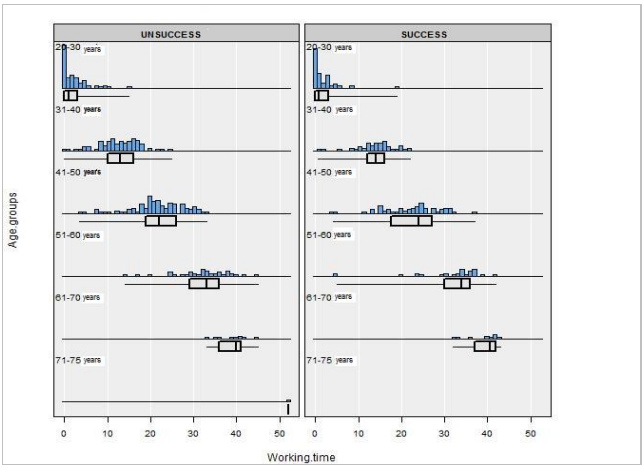

Fig. 3. Distribution of the study group with a success or failure rate by age groups and seniority in work

DISCUSSION

The effectiveness of medical education courses on hand hygiene is different for the subjects included in the study. Thus, in the case of physicians, the success rate is higher regardless whether or not hand hygiene course was completed, as explained by the knowledge gained during university studies. For nurses, the highest rate of success in disinfection is the one with the completed hand hygiene course, to which it is added the right technique that has been learned and practiced repeatedly during the schooling. The highest failure rate in hand disinfection is recorded by the auxiliary staff, whether they have a course or not, which raises the questions whether the hand hygiene course is adequately taught, and it is easy to understand for this professional category. The success rate in getting the right techniques for hand disinfection is higher for respondents with less seniority in work, for 10 years regardless of the profession exercised. This is supported by the willingness to have a higher compliance the more you are new in the profession, which is reconfirmed after the analysis of the success rate according to age groups and seniority in medical practice.

In the case of surgical specialties, all staff categories are more compliant with the hand hygiene concept than physicians, nurses and auxiliary staff who practice in medical specialties.

The specificity of their activities grants a greater importance to the following of aseptic and antisepsis techniques, hospital acquired infections being a possible and serious consequence.

CONCLUSIONS

Young subjects are more compliant with the correct grasp of the 6-step technique in disinfecting the hands, compared to the other age categories.

Staff from surgical specialties is more compliant with hand hygiene concept than the staff practicing in medical specialties.

We recommend the development of a daily alert system for the permanent awareness of the medical staff regarding the frequent practice of hand disinfection.

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NEWS

THERAPY FOR PULMONARY ARTERIAL HYPERTENSION IN ADULTS. UPDATE OF THE CHEST GUIDELINE AND EXPERT PANEL REPORT

Pulmonary arterial hypertension (PAH) carries a poor prognosis if not promptly diagnosed and appropriately treated. The development and approval of 14 medications over the last several decades have led to a rapidly evolving approach to therapy, and have necessitated periodic updating of evidence-based treatment guidelines. This guideline statement, which now includes a visual algorithm to enhance its clinical utility, represents the fourth iteration of the American College of Chest Physicians Guideline and Expert Panel Report on Pharmacotherapy for PAH. The guideline panel conducted an updated systematic review to identify studies published after those included in the 2014 guideline. A systematic literature search was conducted using MEDLINE via PubMed and the Cochrane Library. The quality of the body of evidence was assessed for each critical or important outcome of interest using the Grading of Recommendations Assessment, Development and Evaluation approach. Graded recommendations and ungraded consensus-based statements were developed and voted on using a modified Delphi technique to achieve consensus. Two new recommendations on combination therapy and two ungraded consensus-based statements on palliative care were developed. An evidence-based and consensus-driven treatment algorithm was created to guide the clinician through an organized approach to management, and to direct readers to the appropriate area of the document for more detailed information. Therapeutic options for the patient with PAH continue to expand through basic discovery, translational science, and clinical trials. Optimal use of new treatment options requires prompt evaluation at an expert center, utilization of current evidence-based guidelines, and collaborative care using sound clinical judgment (James R. Klinger, C. Gregory Elliott, Deborah J. Levine, Eduardo Bossone et al. *CHEST* 2019; 155(3): 565–586).

DEPRESSION, ANXIETY AND STRESS AMONG MEDICAL STUDENTS

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DEPRESSION, ANXIETY AND STRESS AMONG MEDICAL STUDENTS (Abstract): **The aim** of the study is to gather some data regarding the levels of depression, anxiety and stress among medical students. **Material and methods**: a number of 190 students provided answers to an online questionnaire. A socio-demographic data sheet and a psychological tool were administrated (Depression, Anxiety and Stress Scale - *DASS 21*). Data were analyzed using *SPSS program, version 21*. **Results**: The mean age for the sample is 21.38 (±2.18), with a majority of female participants (79.5%). *Independent samples t Tests* showed statistically significant differences between male and female students concerning depression ($t(88) = 4.40$, $p < .001$), anxiety ($t(88) = 4.25$, $p < .001$) and stress ($t(88) = 4.70$, $p < .001$). More specifically, women scored higher than men on all subscales of the *DASS-21*: depression (Mwomen = 18.58, Mmen = 10.92), anxiety (Mwomen = 14.95, Mmen = 7.94) and stress (Mwomen = 15.37, Mmen = 7.48. The results showed significant negative correlations between the three scales of the *DASS-21* and the hours students sleep per night: depression ($r = -.253$, $p < .001$), anxiety ($r = -.210$, $p = .004$), and stress ($r = -.249$, $p = .001$). Statistically significant differences in stress according to participants' body mass index were also identified. Students who rate themselves as consuming alcohol and carbonated drinks more frequently also score higher on depression**. Conclusions**: Stress, anxiety and depression are a real problem for medical students during their academic training. **Keywords**: MEDICAL STUDIES, STUDENTS, DEPRESSION, ANXIETY, STRESS, DASS-21, BMI.

Medical students are at risk for stress and burnout as medicine is a never-ending path and a career with high demands (1). It is widely accepted that medical studies entail a great amount of stress due to tremendous workload and great intellectual and emotional demands (2).

There are two categories of factors that can generate different levels of stress (3): endogenous (sex, personality traits, believes) and exogenous. Exogenous factors are divided into three categories: *academic stressors* (difficulties with understanding and learning new syllabus, difficulty with reading textbooks particularly if there is lack of a premedical university preparatory program (UPP), high workload, extensive medical curricula, long teaching hours, lack of leisure or recreation times; the poor quality of the educational process at the college; irregular schedules, frequency of tests, competition with peers and worries related to academic performance or achievement/ fear of failure), *psychosocial*

stressors (the high paternal expectations; home sickness; the new living accommodation; financial strain; fear of future failure in medical career), and *socio-demographic stressors* (gender; smoking; substance abuse, marital status; working during the study; the levels of parents' education; cultural background and the socioeconomic status of the family).

These stressors can trigger various mental health problems (3-6) and students cope by listening to music, talking to relatives or people close to them, resting or engaging in sports (7).

Of great concern is the fact that, of the students who screened positive for depression, only a low percentage sought psychiatric treatment: 15.7%, identified by Rotenstein *et al.* (8) and 12.9% by Puthran *et al.* (9).

Elliot and Tan (10) found that medical practitioners are also hesitant about seeking help when having mental health problems and the authors reported several barriers to help-seeking: concern about letting colleagues down; concern about stigma; concerns about losing medical staff privileges; concerns about losing medical license; negative impact on practice; confidentiality; letting patients down; letting colleagues down; lack of locum cover and career progression.

The rates of depression, anxiety and stress among medical students – results from different countries

GERMANY. Wege *et al.* (2) found, in a sample of German medical students, a 4.7% prevalence rate for major depression and 5.8% for other depressive symptoms. In their study, expected financial difficulties were associated with por mental health (major depression, other depressive symptoms, anxiety, panic disorders and psychosomatic symptoms). Also, in Burger and Scholz's (11) study, female medical students presented higher scores of depressiveness than male students.

EGYPT. Fawzy and Hamed (3) reported high frequencies of depression (65%), anxiety (73%) and stress (59.9%) among 700 Egyptian medical students. This high prevalence was found to be in relation to sex (being a female), living condition (away from families), the year of study (preclinical years) and academic achievement (poor academic achievement degrees).Wahed and Hassan (12) mentioned that 60.8% of the students in their sample had depression and higher depression scores were associated with increasing age, low socioeconomic standard and other governorates.

UNITED STATES OF AMERICA. In a study conducted by Wimsatt *et al.* (13), 14.7% of students reported a previous depression diagnosis. Moreover, most students declared that they would feel embarrassed if they were depressed and their colleagues knew. Moreover, Hardeman *et al.* (14) found that African American students had a 59 % greater risk of being classified as having depressive symptoms than White students and female medical students had a 39% greater risk of being classified as having depressive symptoms than male students.

Mousa *et al.* (15) found that 15.1% and 15.9% of postgraduates as well as 16.4% and 20.3% of medical students screened positive for major depressive disorder and generalized anxiety disorder. Also, both categories believe depression affects their academic performance (33.3% and 32% respectively). Additionally, according to Ludwig *et al.* (16), students in their third year of study are significantly at risk for

depression compared to their first year.

Wolf and Rosenstock (17) reported that 8% of students screened positive for depression at the beginning of the year and 9.3% in the middle of the year, with a positive association between burnout and depression. Also, the authors found positive depression screening, pathological sleepiness, and sleeping less than 7 hours by night to be independent predictors of burnout.

INDIA. Kumar *et al.* (18) reported an overall prevalence of depression of 21.50% in medical dentistry students. In their study, 3rd and 4th year students showed a significantly higher prevalence of depression than other years. The study of Iqbal *et al.* (19) revealed that 17.5% of medical undergraduate students had severe or extremely severe depression. Kumar and Narayan (5) found that 56% of the students in their sample presented depressive symptoms (there was no difference between male and female students) and they seemed to decrease as students progressed through their years of study. Jose *et al.* (20) also found a high prevalence of depression (78.2% of the students had a depression score higher than 49), with women scoring higher than men. The authors also found a positive association between depression and insomnia. An even higher prevalence of depression was reported by Elavarasan *et al.* (21). A rate of 85.3% of the students in their study were suffering from depression and they found it to be related to internet addiction. More specifically, 95% of the participants who were addicted to the internet were found to be suffering from depression and students with internet addiction had four times the risk of being depressed when compared with those without internet addiction.

BRAZIL. Pacheco *et al.* (22), in a meta-analysis including 25 studies on depression among medical students in Brazil, found the prevalence to be 30.6% and positive associations with several variables: female gender; desire to switch courses; later stages of the course; internship cycle; clinical cycle; dissatisfaction with the course; tobacco smoking; average (compared to good) academic performance; difficulties in relationships; emotional tension; evening-type preference; feeling pressured by parents; having concerns about the future; not having a parent who was a physician; not participating in social activities; parents were physicians; poor or reasonable physical health; thoughts of dropping out; religion other than Catholic; sedentary lifestyle; sporadic or rare involvement in leisure activities; uncertainty about professional future.

BANGLADESH. Hossain and Wahab (23) reported that 40.8% of the students of Armed Forces Medical College, Dhaka Cantonment, had depressive symptoms (with no gender differences) and they were very significantly higher in their 1st year than 2nd, 3rd and 4th year. Also, depression was significantly higher in Non-English Medium background than English Medium background participants. Another Bangladeshi study (24) reported an even higher level of depression (46.3%). Also, depression symptoms represented a strong predictor for Facebook addiction.

AUSTRALIA. Said *et al.* (25) reported that 8% of the Australian medical students reported having depression and they identified the following groups as vulnerable: women, 25-34-year-old, students on low income, and homosexual or bisexual students.

ROMANIA. Symptoms of depression,

ranging from mild to severe, were also found among first-year nursing students in a medical University from Romania (26). The prevalence of depression symptoms was 20% and the authors also found significant statistical correlations between depression and a series of variables: age, experience as a nurse, emotional exhaustion, introversion, emotional instability and job satisfaction. In another Romanian study, this time centered on dental and psychotherapy students, there was a moderate association between professional stress and symptoms of depression (27). More specifically, there was a positive association between the level of stress related to professional identity, faculty administration, workload and depression.

Taking into consideration the fact that poor mental health begins in medical school, it is important to gain insight into health-relevant factors early on in order to further career development of medical students. Given the fact that data on health status of medical students in Romania is scarce, we aimed at gathering some data regarding depression, anxiety and stress among them. More specifically, we examined the prevalence of the variables and their relationship with sex, sleeping hours per night, BMI, tobacco, alcohol, coffee, carbonated drinks, energy drinks, ethnobotanicals and drug use.

MATERIAL AND METHODS

Participants

A number of 190 medical students from all years of study provided answers to an online questionnaire. The participants were informed about the purpose of the study, about the confidentiality of data and the use of results. The questionnaire consisted of two parts: the first one gathered socio-demographic information, while the second one is a psychological instrument measuring the levels of *depression, anxiety and stress - DASS 21*.

Socio-demographic data and psychological tool

Students had to provide socio-demographic (sex, age, year of study, environment, faculty, living arrangements), anthropometrical (height, weight) and medical data (chronic diseases). They also had to provide information concerning dietary (tobacco, alcohol, coffee, carbonated drinks, energy drinks and drugs consumption) and sleeping habits. Academic results and family situation (siblings, level of education for both parents and having at least one parent working abroad) were also recorded.

The Depression, Anxiety and Stress Scale (*DASS 21)* (28) is a self-report instrument designed to measure the emotional states of depression, anxiety and stress. Each of its three scales consists of 7 items. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest / involvement, anhedonia and inertia. The anxiety scale assesses autonomic arousal, skeletal musculature effects, situational anxiety, and subjective experience of anxious affect. The stress scale assesses difficulty relaxing, nervous arousal, and being easily upset/agitated, irritable / over-reactive and impatient.

The present study employed the short version of the DASS (21 items) due to several advantages: fewer items, a cleaner factor structure, and smaller interfactor correlations (29).

Statistical analysis was performed using *SPSS Statistics version 21.0.* Means, standard deviations and percentages were used

for descriptive analysis. Comparative analysis was performed using Independent Samples *t-test* and *One-way ANOVA*. For correlational analyses we used *Pearson* and *Spearman* correlations.

RESULTS

Socio-demographic and health related data

The mean age for the sample is 21.38 (±2.18), with a majority of female participants (79.5%). Most of the students were enrolled in their third year of study (32.2%), followed by first (27%), second (23.8%), sixth year (7.4%), fourth (6.9%), and fifth year students (2.1%).

The majority of the participants were from urban areas (66.3%) and the vast majority was not married (95.8%). Most of the students (90.5%) declared no chronic disease.

Academic results and family situation

The baccalaureate mean for the sample is 9.04 (±.78) and the admission mean is 8.30 (±1.20). 42.1% of the students declared that one parent or both worked abroad. Almost half of them lived in dorm rooms or rental accommodation (48.4%). The rest of them lived with family (25.3%), life partner (13.7%) or alone (12.6%).

Anthropometric data, dietary and sleep habits

Mean weight for female students in our sample is 59.01 kg (±8.92), with a mean height of 165.02 cm (±14.89). Mean weight for male participants is 78.59 kg (±11.32), with a mean height of 181.15 cm (±6.76).

The body-mass index (BMI) for both genders was measured using self-declared data regarding weight and height. The values taken into consideration were WHO's standards for European population: a BMI < 18.5 kg/m^2 was categorized as underweight, 18.5-24.9 kg/m^2 as the normal range, 25.0-29.9 kg/m^2 as pre-obese, 30-34.9 kg/m^2 as obese class I, 35.0-39.9 kg/m^2 as obese class II and ≥ 40 kg/m^2 as obese Class III (30).

Most of the students have a normal BMI (73.4%), although we also identified underweight (11.2%), pre-obese (12.2%), and obese class I (3.2%) participants (tab. I).

TABLE I

BMI for men and women

Variables	Total results	Women	Men
Weight (kg)	63.03 ± 12.32	59.01 ± 8.92	78.59 ± 11.31
BMI	21.90 ± 3.21	21 ± 2.98	23.94 ± 3.31
Underweight	11.2%	13.4%	2.60%
Normal weight	73.4%	76.5%	61.5%
Pre-obese	12.2%	7.40%	30.8%
Obesity class I	3.20%	2.70%	5.10%

Table II presents students' smoking and drinking habits. More specifically, 16.3% of the subjects smoke on a daily basis, 11.6% of the students drink alcohol monthly or weekly, a high percentage (42.1%) declared they drank coffee every day, 51.1% occasionally drink carbonated drinks and 33.2% occasionally drink energy drinks. The majority of students claimed they never used ethnobotanicals (95.3%) or drugs (91.6%). A small percentage of participants used ethnobotanicals (4.7%) or drugs (7.9%), while the majority declared they never used them ((95.3%, 91.6% respectively).

TABLE II

The frequency of consumption (%)

Habit	never	occasionally	monthly	weekly	daily
Tobacco	60	18.9	.5	4.2	16.3
Alcohol	21.1	67.4	3.2	8.4	0
Coffee	14.7	30	3.7	9.5	42.1
Carbonated drinks	11.6	51.1	11.1	17.9	8.4
Energy drinks	58.4	33.2	2.6	2.1	3.7
Ethnobotanicals	95.3	4.7	0	0	0
Drugs	91.6	7.9	.5	0	0

Regarding sleeping hours, medical students reported a mean of 7.07 (±1.18) hours per night. Concerning the inquiry of whether they wake up during the night, the participants reported the following: *never* - 17.9%, *occasionally* - 68.4%, *monthly* - 1.1%, *weekly* - 4.2%, *every night* - 8.4%.

Depression, anxiety and stress

The results from the *DASS-21* show elevated levels of depression, anxiety, and stress among the students from our sample (tab. III). More specifically, more than a quarter of the participants reported moderate depression (28.9%), while 17.3% appear to have severe depression and 16.3% extremely severe depression. Concerning anxiety, 30.5% of the students present moderate symptoms, 9.5% severe symptoms, and almost a quarter (23.2%) of students suffer from extremely severe anxiety. In the case of stress, 26.3% of the participants in the sample reported levels ranging from moderate to extremely severe.

TABLE III

DASS 21 results

Level	Depression (%)	Anxiety (%)	Stress (%)
Normal	25.3	27.4	60.5
Mild	12.1	9.5	13.2
Moderate	28.9	30.5	11.6
Severe	17.4	9.5	10.0
Extremely severe	16.3	23.2	4.7

Correlation analysis

In order to identify the existing relationship between the *DASS-21* scales and the number of sleeping hours per night and BMI, Pearson correlation were performed. The results showed significant negative correlations between the three scales of the *DASS-21* and the hours students sleep per night: depression ($r = -.253$, $p < .001$), anxiety ($r = -.210$, $p = .004$), and stress ($r = -.249$, $p = .001$). More specifically, the more students sleep, the lower they score on depression, anxiety, and stress; conversely, the less they sleep, the higher they score on all scales of the DASS-21. In the case of BMI, there is no association between this variable and the scales of the *DASS-21*.

Spearman correlations were performed in order to establish the relationship between the three scales of the DASS-21 and tobacco, alcohol, coffee, carbonated drinks, energy drinks, ethno-botanicals and drug use. The results showed positive and significant

associations between depression and alcohol (r = .155, p = .03) and carbonated drinks consumption (r = .146, p = .04), meaning that students who rate themselves as consuming alcohol and carbonated drinks more frequently also score higher on depression.

Comparative analysis

Independent samples t tests showed statistically significant differences between male (N = 39) and female (N = 151) participants concerning depression (t(88) = 4.40, p < .001), anxiety (t(88) = 4.25, p < .001) and stress (t(88) = 4.70, p < .001). More specifically, women scored higher than men on all subscales of the DASS-21: depression (Mwomen = 18.58, Mmen = 10.92), anxiety (Mwomen = 14.95, Mmen = 7.94) and stress (Mwomen = 15.37, Mmen = 7.48).

One-Way ANOVA showed statistically significant differences in stress according to participants' BMI (F(184) = 3.81, p = .01). More specifically, participants with normal weight have lower scores (M = 13.11) than pre-obese (M=13.13) and obese class I participants (M=26.33). Concerning anxiety and depression, *One-Way ANOVA* analyses showed no statistically significant differences between participants according to their BMI.

DISCUSSION

Medical studies are characterized by the longest period invested in academic study, a continuously preparation for theoretical and practical achievement and require commitment, determination and responsibility. Students enrolled in medical faculties are under high stress due to high academic load, long working hours, new living accommodation, financial strain etc. The mental health of medical students worsens after they begin their studies and remains unsatisfactory all throughout training. More specifically, studies show high prevalence of depression among medical students from their first years of study to their last and even after completion (31). Also, studies report that medical students suffer from depression, anxiety, and stress. Yusoff *et al.* reported that healthy students develop depression and stress after commencing their medical education (32-34).

Our results reveled high rates of depression, anxiety and stress among female medical students and they tend to score higher than on these dimensions. These results are congruent with other research. For example, Brenneisen Mayer *et al.* (35) found a 41% prevalence of depression among medical students, with more depressive symptoms among female students and students from schools located in capital cities. Also, higher scores of depression, anxiety and stress were reported in students with the following characteristics: females, in lower semesters, younger and nonsmokers. Furthermore, students who were satisfied with their education had lower depression, anxiety and stress scores (19). Bore *et al.* (36) also found that female students in their sample were significantly more depressed compared to the female norms, while male depression did not differ from male norms. Another study conducted by Moutinho *et al.* (4) revealed a prevalence of 34.6% in medical students from all semesters of a Brazilian medical school. Assessing associated factors for depression, they found the following: female gender, intrinsic religiosity, anxiety, and stress. Also, Ibrahim and Abdelreheem (37) found a high prevalence of depression (57.9%), with a higher prevalence among female students. Also, medical students presented higher rates of depression compared to pharmaceutical students.

However, some other studies revealed no difference in depression considering gender as variable. Sharma *et al.* (31) reported that 31% of the students in their sample (private university) had depression (there were no gender differences) and it was higher in the first year compared to 3rd and 4th but increased after the completion of studies and the beginning of internship. They also found a significant association of depression with regularity of attendance the courses.

Also, some studies indicated the existence of a relationship between the DASS dimensions and the consumption of alcohol, tobacco and drugs (19). The present research showed that there are positive and significant associations between depression and alcohol ($r = .155$, $p = .03$) and carbonated drinks consumption ($r = .146$, $p = .04$), meaning that students who rate themselves as consuming alcohol and carbonated drinks more frequently also score higher on depression.

Sleep disorders are a real problem among medical students. One explanation could be that the university schedule is forcing them to mix theoretical and practical sessions with a hard investment of time for learning. Compared to general population scores, medical students sleep less and have more sleep-related problems. This is probably due to several reasons: the long duration of the academic achievement, demanding clinical study, a lot of practical duties that include overnight on-call activities, a hard work that can be emotionally challenging (increasing the level of burnout), a high level of consummation of energy drinks, coffee or dietary supplements, and, not to be neglected, their lifestyle choices (38). Also, sleep is closely related to good physical and psychological health and academic performance (39-41). The present study also found that sleeping hours have an impact on mental health. Specifically, the more students sleep, the lower they score on depression, anxiety, and stress; conversely, the less they sleep, the higher they score on all scales of the psychological instrument used for this research.

There is a great need for the promotion of mental health, given the fact that medical students present high rates of depression, with a focus on vulnerable groups: female students, students with financial difficulties, students with various addictions etc. Also, mental health professionals should focus on depression stigma (13) and should also address the fact that neither students nor healthcare professionals are inclined to seek help when they have mental health problems (8-10).

Our study showed that there is a statistically significant differences in stress according to participants' BMI (normal weight students having lower scores on stress comparing to over-weight students), but not when considering depression or anxiety.

CONCLUSIONS

High rates of depression, anxiety and stress are recorded among medical students. Increased rates of alcohol consumption, sleep-related problems, high body mass-index are found to be factors influencing students' levels of stress, anxiety and depression. Among them, females are more prone to experience these health problems. University teachers should include in their curricula topics aimed at preventing these psychological problems (in case they are related to lectures or clinical stages) or to arrange schedules in order to offer students more free time and proper sleep.

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TENDINŢE ALE EVOLUŢIEI EPIDEMIOLOGICE A HEPATITELOR ACUTE VIRALE B ŞI C LA COPIL, ÎN ROMÂNIA ŞI JUDEŢUL IAŞI, ÎN PERIOADA 1990-2009

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TRENDS IN EPIDEMIOLOGICAL EVOLUTION OF CHILD VIRAL HEPATITIS B AND C IN ROMANIA AND IAŞI COUNTY BETWEEN 1990 - 2009 (Abstract): Hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are the frequent causes of acute and chronic hepatitis worldwide and the leading causes for hepatic cirrhosis and liver cancer. There is a distinct geographical variation in VHB and VHC incidence in adult and child. **Aim**: To assess the evolution trend of VHB and VHC incidence in child, in Romania and Iasi County, during the last two decades. **Material and methods**: Data were obtained using information from the Reporting National System for acute viral hepatitis A, B, and C, from various geographical areas of Romania. Some systematical errors of information were identified, without significant influence of results and conclusions. **Results**: Results highlighted an incidence peak of VHB in Romania, in 1989, and in Iaşi county, in 1991 (43.1, respectively 41.2‰ inhabitants). The VHB incidence trend decreased after the implementation of vaccination programme, especially in children <4 years old. **Conclusions**: The study remarked the necessity of viral hepatitis surveillance programme continuity in Romania and the importance of prevention measures including, for VHB, an optimal vaccination. **Key words**: VIRAL HEPATITIS B, VIRAL HEPATITIS C, CHILD, VACCINATION PROGRAMME

Infecţia cu virusurile hepatitelor B şi C la copil se caracterizează prin unele aspecte epidemiologice şi clinice particulare. Riscul cronicizării este invers corelat cu vârsta producerii infecţiei, iar în cazul hepatitei virale B (HVB), poate să se situeze la 90% dintre copiii infectaţi în perioada de nounăscut, comparativ cu adulţii imunocompetenţi, care pot croniciza în 5% din cazuri. Dintre purtătorii cronici de virus, 15-20% pot dezvolta ciroză sau hepatocarcinom (1). Introducerea, în anul 1995, şi în România, a vaccinării anti-virus hepatitic B (VHB), în Programul Naţional de Imunizare, a schimbat, în mod semnificativ, evoluţia acestei infecţii în populaţia generală şi la grupurile cu risc, ceea ce se reflectă şi în tendinţa de reducere a incidenţei bolii, mai ales la copii (2).

Hepatita virală C (HVC) rămâne o provocare atât pentru patologia pediatrică, cât şi a adultului tânăr, iar consecinţele bolii, reflectate în nivelul de calitate a vieţii şi creşterea costurilor pentru asigurarea îngrijirilor sau a terapiei, face cu atât mai necesară aplicarea strategiilor de control şi de prevenţie generală a bolii, în absenţa vaccinării (3,4).

Studiul îşi propune să evalueze tendinţa în evoluţia incidenţei HVB şi HVC, la copil, în România şi particularizat pentru judeţul Iaşi, în perioada 1990-2009, perioadă ana-

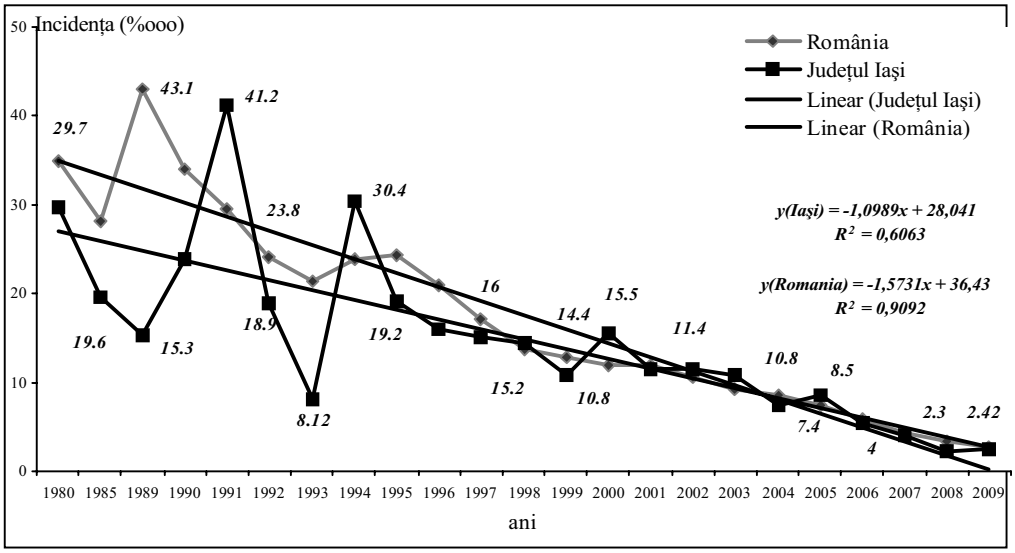

Fig. 1. Incidenţa hepatitei virale B, în România şi judeţul Iaşi, în perioada 1980-2009

lizată şi în relaţie cu momentul introducerii vaccinării anti-VHB, în Programul Naţional de Imunizare.

MATERIAL ŞI METODE

Datele incluse în studiu au fost obţinute prin Sistemul Naţional de Raportare a hepatitelor virale (HV), iniţiat încă din 1978, iar cele privind cazurile noi pe tipuri de hepatită, regiune, grupuri de vârstă şi rezidenţă au fost obţinute de la Centrul Naţional pentru Organizarea şi Asigurarea Sistemului Informaţional şi Informatic în Domeniul Sănătăţii din cadrul Ministerului Sănătăţii Publice (5). În desfăşurarea studiului, am identificat riscul producerii unor erori de informare prin utilizarea, în sistemul de raportare, a termenului de *hepatită nonA nonB* care reuneşte cazurile de boală hepatică acută virală infirmate, cu ajutorul examenelor de laborator, ca fiind cauzate de virusul A sau B şi încadrate în categoria HVC. În perioada 1990-2009, pentru toate cazurile de hepatită nonAnon B, diagnosticul de HVC a fost validat inclusiv prin dozarea anticorpilor anti-VHC.

Pentru analiza datelor înregistrate la copil, prin Sistemul Naţional de Raportare, în perioada de studiu la care ne referim, s-a remarcat o dificultate ca urmare a schimbării în raportare a structurării grupurilor de vârstă, ceea ce a determinat o analiză pe etape a fenomenului cercetat.

REZULTATE

*1. Evoluţia hepatitelor acute virale B şi C, în populaţia generală din România şi judeţul Iasi în perioada 1990-2009***.** Din datele de incidenţă înregistrate la nivel naţional, pentru toate grupurile de vârstă, se remarcă nivelul crescut al HVB în deceniile 8 şi 9 (43,1 şi 24,4‰ loc. în 1989, respectiv 1995), pentru ca, în ultimii 9 ani, numărul cazurilor să scadă semnificativ la 6,1 în anul 2000 şi 2,42‰ în 2009 (fig. 1).

Incidenţa HVC în România s-a încadrat între valorile cele mai scăzute de 0,8 şi 1,3‰ în 1980, respectiv 1989 şi cele mai crescute de 7,6 ‰ în 2001. Pentru judeţul Iaşi, incidenţa urmează tendinţa înregistrată în întreaga ţară, cu excepţia anului 1998 când au fost raportate valori

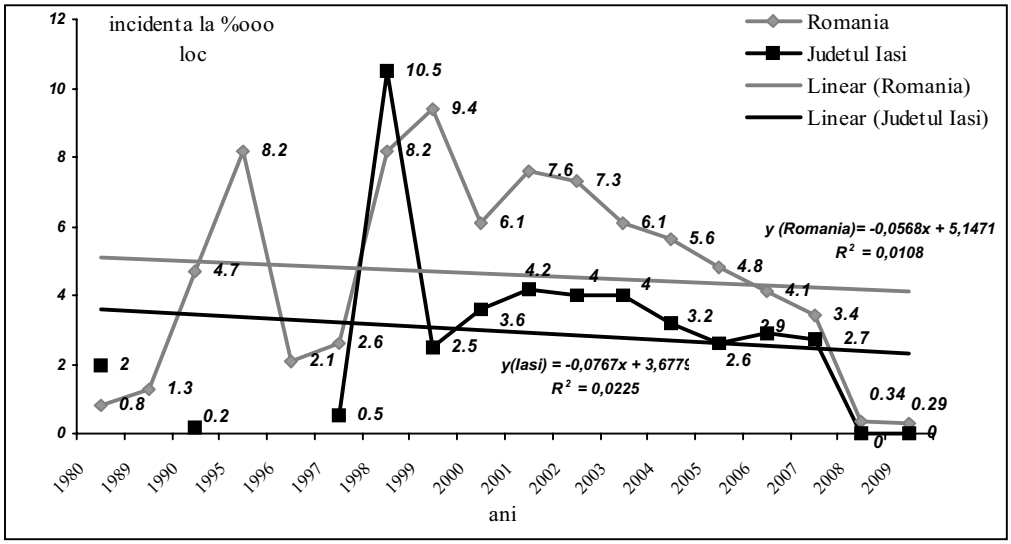

Fig. 2. Incidenţa hepatitei virale C, în România, în perioada 1980-2009

de 10,5%ooo, comparativ cu România, de 8,2%ooo.

2. Evoluţia hepatitei acute virale B şi C la copil, în România şi Judeţul Iaşi, în perioada 1990-2009. Scăderea numărului de cazuri de HVB la copil, în România, atât la grupul de vârstă <1 an, cât şi la 1-14 ani, a fost semnificativ influenţata de schimbarea politicii de vaccinare, începând cu 1995 (tab. I).

După un interval de 5 ani de la introducerea vaccinării anti-VHB, în Programul Naţional de Imunizare, cele mai multe cazuri au fost înregistrate la grupurile de vârstă 5-9, respectiv 10-14 ani, ceea ce demonstrează că imunizarea a avut un impact pozitiv în protecţia specifică a copiilor din România şi respectiv din Judeţul Iaşi (tab. II, fig. 3).

În judeţul Iaşi, situaţia înregistrată după anul 2000 (la 5 ani de la introducerea vaccinării), a fost similară cu cea înregistrată în întreaga ţară, fiind justificată de raportarea celor mai multe cazuri la copiii din grupurile de vârstă 5-9, respectiv 10-14 ani.

Pentru HVC, numărul de cazuri raportate la copii cu vârsta de 1-14 ani a fost redus atât la nivelul României, cât şi al judeţului Iaşi. Cazurile au fost înregistrate la diverse vârste din intervalul 1-14 ani (tab. III, fig. 4).

DISCUŢII

Incidenţa HVB şi HVC în ţările din Uniunea Europeană variază în funcţie de zona geografică, având o tendinţă comună de scădere pentru HVB şi de creştere pentru HVC. Prevalenţa purtătorilor de VHB s-a încadrat în intervalul 0,1-8,0% (6% valoarea raportată pentru România) şi 0,1-6,0% pentru VHC (6,7,8). În România, evoluţia HVB, în perioada anterioară introducerii vaccinării anti-VHB, a fost marcată de un nivel al incidenţei bolii ridicat, mai ales la copil.

Studiile efectuate în România (2,8,9,10, 11,12,13,14), în diverse zone geografice, relevă caracterul de endemicitate a HVB cu tendinţă de scădere a numărului cazurilor noi de boală, la copii, ceea ce constituie dovada eficienţei tuturor măsurilor preventionale, aplicate după anii '90: folosirea seringilor de unică utilizare în activităţile

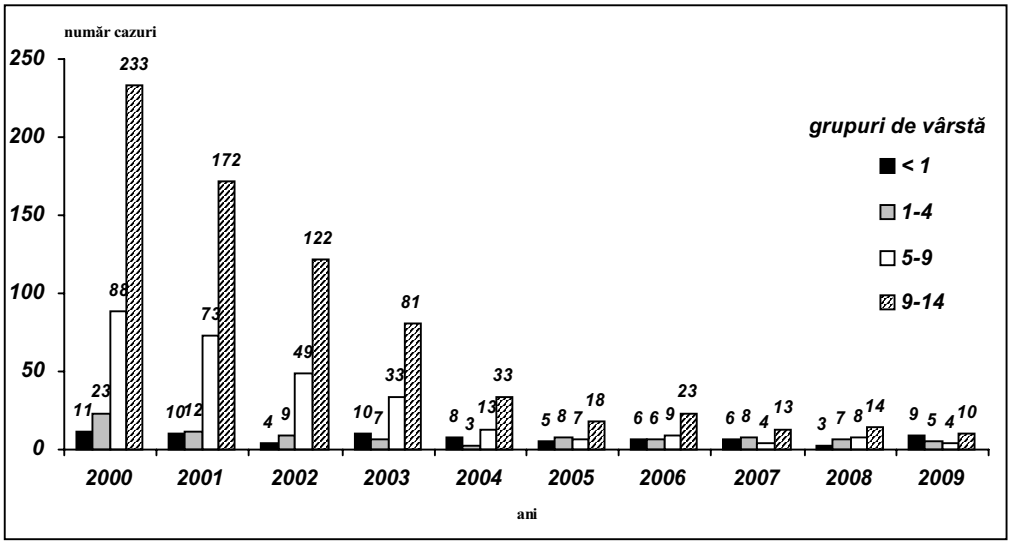

Fig. 3. Evoluţia cazurilor de hepatită virală B, în România, după 5 ani de la introducerea vaccinării anti –VHB, în Programul Naţional de Imunizare

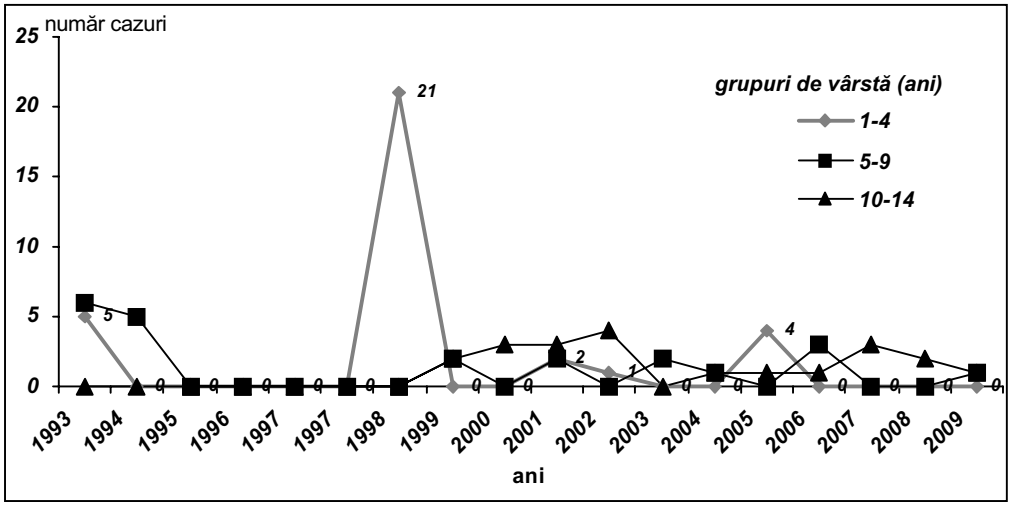

Fig. 4. Repartiţia cazurilor de hepatită virală C raportate în judeţul Iaşi, la copiii din grupurile de vârstă 1- 14 ani, în perioada 1993-2009

medicale (vaccinare, tratamente etc.) (1991) ; introducerea testelor imunoenzimatice pentru controlul sângelui donatorilor sau în stabilirea diagnosticului serologic de hepatită virală (1992) ; introducerea imunizării nou-născuţilor, a personalului cu risc din instituţiile medicale (1995) şi ulterior a copiilor cu vârsta de 9 ani, născuţi înainte de introducerea vaccinării în Programul Naţional de Imunizare, a elevilor şi studenţilor din structurile de învăţământ medical (1999) ; extinderea vaccinării la adulţii tineri din

TABELUL I
Evoluţia cazurilor de hepatită virală B la copiii din România din grupele de vârstă < 1 an si 1-14 ani in perioada 1990-1999

Anul / Grupuri de vârstă (ani)	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
<1	93	108	59	31	17	24	18	11	10	5
1-14	2602	2403	1646	1299	1299	1683	1988	1550	1044	576

TABELUL II
Numărul de cazuri noi de hepatită virală B diagnosticate în judeţul Iaşi, în perioada 1990–1999, respectiv 2000-2009

Anul / Grupuri de vârstă (ani)	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
< 1	4	5	2	0	0	0	0	2	0	0
1-4	29	27	12	0	10	4	6	3	3	0
5-9	19	47	13	3	15	18	16	9	5	1
10-14	27	61	13	5	16	21	31	12	7	2

TABELUL II (continuare)
Numărul de cazuri noi de hepatită virală B diagnosticate în judeţul Iaşi, în perioada 1990–1999, respectiv 2000-2009

Anul / Grupuri de vârstă (ani)	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
<1	0	0	0	**1**	0	**1**	0	0	0	0
1-4	0	0	0	0	0	0	0	0	0	0
5-9	**2**	0	**2**	0	0	0	0	0	0	0
10-14	**8**	**4**	**4**	0	0	0	**2**	**2**	0	0

ultima clasă de liceu (2004) (2).

Vaccinarea efectuată în mod sistematic, cu 3 doze după schema 0-2-6 luni de viaţă, la nou-născut sau la un interval de 0-1-6 luni la alte grupuri de vârstă (copiii de 9 ani neimunizaţi la naştere), cu întreruperi accidentale cauzate de lipsa preparatului vaccinal, a generat o acoperire vaccinală, prin titruri protective de anticorpi specifici anti-VHB, de peste 95% (2) şi respectiv la nou-născut de 99,9% în anii 1997 şi 1998, de 98,0% în 1999, în 2000 de 99,0 % şi 98,0% în 2001 (15). Cazurile raportate după 1995, au fost înregistrate preponderent la copiii născuţi înainte de 1995 (grupurile de vârstă de peste 1 an), sporadic la copiii rămaşi nevaccinaţi sau la cei vaccinaţi, la care răspunsul imun post-vaccinare a fost sub nivelul protector.

În judeţul Iasi, incidenţa HVB a fost sub valoarea înregistrată la nivel naţional, cu excepţia anilor 1991, 1994 şi 2000. La copii, imunizarea corect aplicată şi monitorizată a dus la scăderea importantă a incidenţei cazurilor. Strategia actuală, la nivelul judeţului Iasi, este de menţinerea acestei

TABELUL III
Numărul de cazuri noi de hepatită virală C raportate la copii, în România, în perioada 1999-2009

Anul / Grupuri de vârstă (ani)	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009
<1	**19**	0	**1**	0	0	0	**1**	0	0	0	0
1-4	0	0	0	**1**	0	**1**	**5**	0	0	0	**2**
5-9	0	**1**	**2**	0	**2**	0	0	0	**1**	0	0
10-14	0	0	3	1	0	0	0	2	0	**1**	0

situaţii favorabile privind incidenţa HVB şi, mai ales, evitarea golurilor de imunizare.

În cazul infecţiei cu VHC, chiar în situaţia subraportării posibile, datorită formelor clinice asimptomatice nedepistate sau a dificultăţilor de confirmare a diagnosticului prin examenul de laborator, în perioada 1995--2005, au fost raportate 250.000 de cazuri de HVC în 24 ţări ale UE. Prevalenţa infecţiei cu VHC a variat în intervalul de 0,1--6,0%, România fiind inclusă în categoria ţărilor cu nivelul cel mai înalt, de 6% (16).

Incidenţa HVC pediatrică în România, cu excepţia anului 1999, când au fost raportate 19 cazuri la copiii < 1 an la nivelul întregii ţări şi respectiv 21 cazuri în anul 1998, înregistrate la grupul de vârstă 1-4 ani, în judeţul Iaşi, a avut un nivel scăzut. Explicaţia pentru nivelul redus al incidenţei la copil poate consta în specificitatea transmiterii virusului, mai puţin frecvent de la mamă la copil, precum şi ca rezultat al măsurilor de prevenţie şi control, care au fost aplicate pentru toate infecţiile produse de virusurile cu transmitere prin sânge, derivatele sale sau alte produse biologice provenite de la bolnavi sau purtători.

Hepatita virală C la copil, în judeţul Iaşi, nu pare a pune probleme dacă am analiza fenomenul numai prin evaluarea incidenţei cazurilor. Privind aceste cazuri diagnosticate ca fiind un posibil vârf al „iceberg-ului" şi apreciind ca dificilă îngrijirea şi aplicarea terapiei adecvate la cazurile depistate, considerăm că această infecţie trebuie să rămână în atenţia medicilor pediatri şi a tuturor specialiştilor (infecţionişti, virusologi, epidemiologi etc.) care contribuie la ţinerea sub control a fenomenului.

CONCLUZII

România care face parte, de mai mulţi ani, din reţelele europene de supraveghere a hepatitelor virale,beneficiază de un sistem de supraveghere de tip pasiv, bazat, mai ales, pe raportarea cazurilor acute. Studiile de morbiditate şi de seroprevalenţă, efectuate în ultimele două decenii, au completat golurile informaţionale privind această patologie, la diversele grupuri de vârstă (sau de risc), inclusiv la copil. În România, se menţine caracterul de endemicitate pentru HVB şi HVC, fiind remarcată scăderea semnificativă a incidenţei HVB, semnificativ la copil, după aplicarea strategiei de imunizare, prin introducerea vaccinării anti-VHB, încă de la naştere sau, ulterior, la vârsta adolescenţei.

Cazurile apărute la copil, după 1995, pot avea mai multe cauze care pot fi stabilite pe baza evaluărilor analitice ale factorilor de risc, identificaţi prin anchetele epidemiologice. Demonstrarea existenţei unor goluri imunitare, în populaţia infantilă, ca urmare a unor factori individuali, de răspuns la preparatul vaccinal, de infecţie cu anumite genotipuri de VHB sau ca urmare a unor disfuncţionalităţi tehnice de administrare a vaccinului, poate constitui un subiect particular, care să fie analizat în vederea optimizării sistemului de prevenţie a infecţiilor cu VH în România.

Continuarea strategiei de supraveghere a infecţiei cu VHB, după standardele europene, precum şi menţinerea unei acoperiri vaccinale optime, ca urmare a aplicării programului de imunizare adecvat situaţiei epidemiologice din România, constituie o speranţă în ţinerea sub control a acestei infecţii şi, mai ales, de reducere a consecinţelor legate de povara bolii, la copil.

Hepatita virală C, încă subestimată, ca urmare a limitelor de depistare şi diagnostic, înregistrează niveluri în creştere, dar semnificativ mult reduse faţă de HVB. Pentru România, chiar dacă, în ultimii ani, numărul de cazuri noi de boală, la copil, a fost redus, iar posibilitatea transmiterii mult diminuată prin măsurile preventionale, o supraveghere continuă a infecţiei este necesară, mai ales în contextul în care perspectiva protecţiei specifice prin vaccinare este mult îndepărtată, iar posibilităţile actuale de monitorizare şi terapie a cazurilor, limitate.

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ADNEXAL MASSES IN PREGNANCY: A TWO- CENTER EXPERIENCE

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ADNEXAL MASSES IN PREGNANCY: A TWO-CENTER EXPERIENCE (Abstract): **Aim**: To evaluate the characteristics of adnexal masses in pregnancy diagnosed and managed in our centers. **Material and methods**: This study was conducted in the interval January 2008-December 2017 in two major centers. Included in the study were pregnant women diagnosed with adnexal masses detected by ultrasound or incidentally during cesarean delivery. Adnexal masses were evaluated ultrasonically and histopathologicaly. **Results:** The incidence of adnexal masses in pregnancy identified in our study was 0.31 %. Prenatal diagnosis of adnexal masses was made in 140 cases (66.6 %), 70 cases (33.3 %) being diagnosed during cesarean section. The mean size of all adnexal masses was 6.8 cm. Histopathologically, the vast majority of adnexal masses were benign tumors. The most common complication of adnexal masses during pregnancy was ovarian torsion. **Conclusions:** It is very important to know that adnexal masses can progress rapidly and can be life-threatening for the mother and fetus; thus it is absolutely necessary to develop and implement specific diagnostic and treatment protocols. **Keywords:** ANNEXAL MASSES, PREGNANCY, ULTRASONOGRAPHY.

Adnexal masses have become increasingly common in daily obstetric practice, representing a diagnostic and therapeutic challenge for the physician, especially in the absence of specific management protocols. The precise incidence of adnexal masses in pregnancy is unknown and varies widely, being variably reported as 0.15-5.7% (1-3). It is clear that due to repeated ultrasound examinations specific to pregnancy monitoring protocols, the diagnosis of adnexal masses in the early stages of gestation has increased, many of them being asymptomatic (4). In some situations, the presence of these masses is an unexpected intraoperative finding, given the increasing cesarean delivery rate. Adnexal masses can be found in pregnant women of any age. They have their origin in the ovaries, fallopian tubes, or surrounding tissue and are characterized by high clinical, laboratory and histological variability (5). The majority of adnexal masses in pregnancy are benign, and the incidence of malignancy is approximately 6% (2). It is very important to know that the progression

of adnexal masses can be fast and life-threatening for both the mother and fetus, being identified in any trimester of pregnancy, and the therapeutic approach must take into account the gestational age and maternofetal status. Also, during pregnancy, surgical management of adnexal masses and their complications is more difficult and more challenging (1). In this context, the aim of this retrospective study is to evaluate the characteristics of adnexal masses in pregnancy, diagnosed and managed in two centers.

MATERIAL AND METHOD

This study was conducted in the interval January 2008 - December 2017 in two centers: "Cuza-Vodă" University Hospital, Iași, Romania, a tertiary referral obstetrics and gynecology center and "Elena-Doamna" University Hospital Iași, Romania. The medical records of pregnant women were reviewed retrospectively using the specific codes of the World Health Organization (WHO) - International Classification of Diseases (ICD): C56, C57.0, C 57.1, C57.2, C57.3, C57.4, D27, D39.1, N83.0, N83.1, N83.2, N83.5, N83.9. The inclusion criterion was represented by pregnant women diagnosed with adnexal masses at ultrasound examination or detected incidentally during cesarean delivery. Eligible women were identified by the research coordinator and/or the medical staff involved in project. Data on patients' clinical course and their operative records were collected from the hospital computerized database. The following parameters were evaluated: maternal age and parity, clinical presentations, ultrasound features of the detected masses and gestational age at diagnosis, adnexal mass-associated complications, gestational age at the time of surgery, and histopathological diagnosis of adnexal masses. Prenatal screening in these cases was performed according to specific protocols by blood tests and ultrasound: dating / viability at 7-9 weeks, first trimester screening between 11-14 weeks, scan for fetal anomalies between 19-22 weeks and fetal wellbeing ultrasound in the third trimester. Adnexal masses were ultrasonically evaluated whenever necessary in our centers (transvaginal ultrasound) in terms of tumor size and location, morphological and vascular features. The differential diagnosis included malignant ovarian tumors (borderline tumors included), and in the cases requiring surgery the extemporaneous examination and the final histopathological report. These patients were treated by classical laparotomy, the main indications for surgery being symptomatic adnexal mass (adnexal torsion), enlarging mass, or suspicion of malignancy. Exclusion criteria were non-gynecologic pelvic masses, pregnancies of unknown or ectopic location, and ovarian stimulation treatment. *Statistical analysis* was performed using SPSS version 17.0 software. Data were expressed as mean ± SD. Mean, standard error of the mean, and standard deviation were determined for quantitative variables. Categorical variables were expressed as number and percentage (%) of patients. The study was approved by the local Ethics Committee (No:790/25.01.2017) and conducted in accordance with the Helsinki Declaration.

RESULTS

Between January 2008 and December 2017 at the Iasi "Cuza-Vodă" University Hospital and "Elena-Doamna" University Hospital, 70,779 births were recorded, of which 226 (0.31 %) were diagnosed with

adnexal masses in pregnancy. Of all these patient, only for 210 cases we were able to collect all data required for a complete statistical processing. The mean age of the patients at the time of adnexal mass detection was 28 years +/- 5.18 years (mean +/- SD) and the mean biometric age was 33 weeks of amenorrhea +/- 10.19 weeks (mean +/- SD). A hundred and thirty-six patients were primipara (64.7 %), and 74 patients were multipara (35.2%). In the study group, 13 patients (6.1 %) had spontaneous abortions (before 20 weeks of gestation, none after surgery for complication of adnexal masses), 77 patients (36.6%) had cesarean (C-section). Prenatal diagnosis of adnexal masses was made in 140 cases (66.6 %), 70 cases (33.3 %) being diagnosed during C- section. Only three patients (1.4 %) underwent surgery during the antepartum period: two in the first trimester (at 7 and 9 weeks of gestation, respectively) and one in second trimester at 14 weeks of gestation. In 66 cases (31.4 %) adnexal mass surgery was performed at the same time as the C-section. The masses were detected on the right side in 84 cases (40 %), on the left side in 124 cases (59 %), and bilaterally in 2 cases (fig. 1). The mean size of all adnexal masses was 6.8 cm.

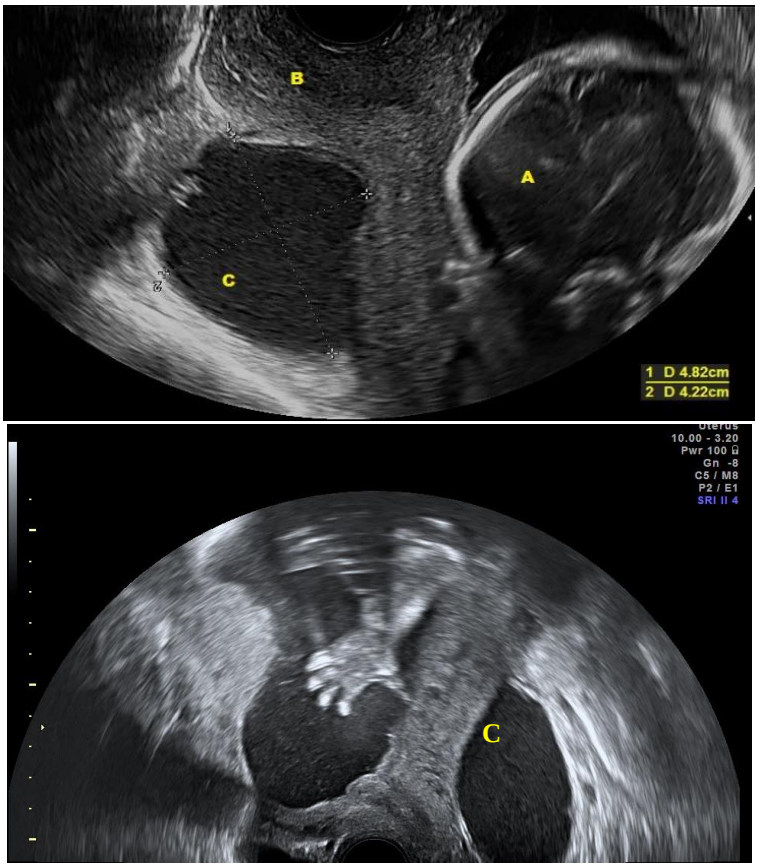

Fig. 1. 26-year-old pregnant woman- a transvaginal ultrasound obtained at 18 weeks' of gestation: A. fetal head; B. cervix; C. adnexal mass-ovarian cyst

The clinical manifestations of adnexal masses were dominated by nonspecific diffuse abdominal pain. The most common complication of adnexal masses during pregnancy was ovarian torsion- 6 cases (2.85 %, 4 in the first trimester and 2 in the second trimester- who needed emergency surgery), followed by hysterectomy in 3 cases of ovarian cancer confirmed by histopathological extemporaneous examination. Pathology results and surgical procedures used in the management of adnexal masses are presented in Table I. In 2 cases, the ovarian cysts were removed by transabdominal puncture and aspiration at 13 weeks and 32 weeks of gestation.

TABLE I

Pathology results and clinical characteristics of adnexal masses in surgically managed patients

Sr. No.	Pathology	No. (% of all cases)	Clinical manifestation (No. cases)	Mean size (cm)	Surgical procedure (No. cases)
1	Serous cystadenoma	23 (10.9%)	asymptomatic (13) symptomatic (10)	10.5	salpingo-oophorectomy (13) cystectomy (8) release of torsion (2)
2	Functional ovarian cyst	12 (5.7%)	asymptomatic (10) symptomatic (2)	6.5	salpingo-oophorectomy (6) cystectomy (6)
3	Para tubal cyst	10 4.7%)	asymptomatic (9) symptomatic (1)	6	cystectomy (8) salpingectomy (2)
4	Endometrioma	10 (4.7%)	asymptomatic (7) symptomatic (3)	6.5	cystectomy (10)
5	Dermoid Cyst	8 (3.8%)	asymptomatic (7) symptomatic (1)	5.8	salpingo-oophorectomy (2) cystectomy (6)
6	Cystadenocarcinoma (including borderline tumor)	3 (1.4%)	asymptomatic (3)	6.7	Hysterectomy with bilateral salpingo-oophorectomy (3)
7	Hemorrhagic cyst	3 (1.4%)	symptomatic (3)	6.2	salpingo-oophorectomy (2) cystectomy (1)

DISCUSSION

The incidence of adnexal masses in pregnancy identified in our study was 0.31 %, which is in agreement with the incidence reported by most authors. Although the vast majority of adnexal masses are diagnosed by ultrasound in the first trimester (21.4–75.7% of cases) (6), in our case the advanced gestational age at the time of diagnosis (33 weeks) can be explained by the absence of specific monitoring of pregnancy. Many of these patients are of low socioeconomic status without immediate access to specialized prenatal care. Also, although we have identified a spontaneous abortion rate of 6.1 %, it is difficult to say that this is entirely due to the presence of adnexal masses, which may however be associated with a poor obstetric outcome, depending on their size and location (6). Only 16% of

cases were diagnosed in the first trimester of pregnancy. Analyzing the databases, it is evident that in some studies the specialists opt for a conservative attitude of observing the progression of adnexal masses, whereas in others, elective surgeries in the first and second trimester are preferred (7). In our study, in the vast majority of cases we opted for a conservative approach of adnexal masses. This option was based on the finding that the vast majority of ovarian cystic masses identified in the first trimester of pregnancy, disappear later (8,9). A very important role in the monitoring of adnexal cystic masses and the identification of suspected malignancies is played by ultrasound examination, analyzing such specific aspects as: size, septations, internal cyst walls, acoustic shadows, bilaterally of masses, irregular unilocular/multilocular tumor, ascites, papillary structures and increased vascularity as determined by Doppler ultrasound- International Ovarian Tumor Analysis (IOTA) rules to diagnose ovarian cancer (10). In this context it is very important to remember that IOTA simple rules can be used to classify 75% of all adnexal masses as benign or malignant. However, it should be noted that evaluating the ultrasound characteristics of adnexal masses in the third trimester of pregnancy can be difficult.

Ultrasonography is the primary imaging modality used to assess adnexal masses, but in some cases the findings may be nonspecific and it is MRI (Magnetic Resonance Imaging) that can provide new data regarding the diagnosis (7). Although in some of our cases MRI was performed, we were unable to obtain adequate data for a more detailed analysis. The measurement of serum tumor marker CA-125 was not included in the differential diagnosis of adnexal masses due in particular to its lack of specificity and high levels in pregnancy, which can lead to false positive results for the risk of malignancy (4). Levels of other markers, such as alpha-fetoprotein (AFP), human chorionic gonadotropin and lactate dehydrogenase can be elevated during pregnancy normally or as a result of abnormal placentation or fetal anomaly (12). The malignancy rate of adnexal masses was 1.4 %, which is comparable with the reported rates of 2% to 6% (1). It should be noted that Doppler evaluation of potentially malignant adnexal masses in pregnancy is difficult due to the fact that the vessels and blood flow surrounding the gravid uterus mainly have high velocity and low resistance characteristics (13). From the histopathological point of view, the vast majority of adnexal masses were benign tumors, respectively cystadenomas /functional ovarian cyst, which is in agreement with the data provided by other authors (14). Unlike functional cysts that are hormonally influenced and may disappear in pregnancy, cystadenomas are not hormonally sensitive and do not resolve after 16 weeks of pregnancy (15). Although most of the adnexal masses are asymptomatic, we have identified 6 cases of adnexal torsion (patients presenting severe pelvic pain, nausea, vomiting), which corresponds to the frequency reported by most authors. Most cases of torsion were diagnosed in the first trimester of pregnancy, especially on the right side, which is in accordance with other reports in showing that this complication mainly occurs during the first trimester of pregnancy when adnexal mass is larger than 5 cm (16). In general, starting with the second trimester of pregnancy, the incidence of the adnexal torsion is lower due to the increase in size of the gravid uterus. In 2 cases, surgery was performed for the suspicion of appendicitis, subsequently adnexal torsion being identified. Moreover, due to the nonspecific pain symptomatolo-

gy in pregnancy, adnexal torsion is difficult to differentiate from appendicitis and other diseases such as pyelonephritis and renal lithiasis (17). Regarding the therapeutic management of adnexal masses in pregnancy, the main problem is the lack of a specific protocol and a general consensus. In this context, although the surgical technique used differs, the vast majority of practicing specialists prefer laparoscopy. In our study we used laparotomic surgery in the management of adnexal masses, mainly due to the fact that the vast majority of adnexal masses were operated during cesarean section. Also, the inexperienced surgeons as well as the technical difficulties associated with laparoscopic interventions in pregnancy can be taken into consideration. The laparoscopic removal of adnexal masses in the first trimester of pregnancy is generally avoided, due in particular to the risk of miscarriage. However, there were no significant differences in the risk of miscarriage between women who underwent laparoscopy and those who underwent laparotomy. In the second trimester of pregnancy laparoscopic surgery may represent a technical challenge due to the complications associated with the use of the Veress needle or trocar (injury of the gravid uterus) and lack of visibility because of the limited space between the laparoscope and the adnexal mass (2). Transabdominal puncture and aspiration of ovarian cysts (performed in 2 study cases) is only indicated if they cause pain or obstruct labor (18).

CONCLUSIONS

The presence of adnexal masses in pregnancy can be a cause of anxiety for both the patient and the specialist. Although the vast majority of them disappear spontaneously in pregnancy, some of them may develop complications that may affect the maternal-fetal prognosis. In this context, it is absolutely necessary to develop and implement specific diagnostic and treatment protocols.

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NEWS

VEOLIZUMAB – A CHALLENGE IN THE TREATMENT OF CROHN'S DISEASE

Vedolizumab is a humanized monoclonal antibody against α4β7 integrin, which prevents mucosal addressin cell adhesion molecule-1-mediated lymphocyte homing to the gut. It is effective as induction and maintenance therapy for patients with moderate to severe Crohn's disease (CD) and ulcerative colitis (UC).This was a prospective observational study of consecutive inflammatory bowel disease (IBD) patients who started vedolizumab treatment at a single tertiary referral center between May 2016 and June 2017. The main new finding of the study is the predictive value of induction and early maintenance vedolizumab trough levels for robust combined endoscopic and clinical remission by the end of the first year of vedolizumab treatment. An important finding of the study was the 96% negative predictive value of the vedolizumab trough level threshold of 17 mg/mL at week 6 for combined endoscopic and clinical remission. Trough levels below this threshold were observed in a high proportion of the patients. Early therapeutic drug monitoring could help identify patients at risk of unfavorable outcomes and provide an early opportunity for therapeutic intervention. This prospective real-life observational study with centrally evaluated endoscopy identified vedolizumab trough levels at week 6 to be a predictor of combined endoscopic and clinical remission, as defined by the Selecting Therapeutic Targets in Inflammatory Bowel Disease (STRIDE) programme. Taken together with the findings of others, trough levels at week 6 appear to be a promising tool to assist clinical decisions. The hypothesis-generating study warrants further research to explore whether patients with a low likelihood of combined remission (i.e. those with week 6 trough levels<17 mg/mL) could benefit from early dosing interval shortening, possibly including other relevant patient-reported outcomes (Hanzel J, Sever N, Ferkolj I, *et al*. Early vedolizumab trough levels predict combined endoscopic and clinical remission in inflammatory bowel disease. *United European Gastroenterol J.* 2019; 7(6): 741-749).

THE SIGNIFICANCE OF NEUTROPHIL TO LYMPHOCYTE RATIO, PLATELET TO LYMPHOCYTE RATIO AND LYMPHOCYTE TO MONOCYTE RATIO IN PERIPHERAL BLOOD OF PATIENTS WITH BENIGN AND MALIGNANT ORAL LESIONS

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THE SIGNIFICANCE OF NEUTROPHIL TO LYMPHOCYTE RATIO, PLATELET TO LYMPHOCYTE RATIO AND LYMPHOCYTE TO MONOCYTE RATIO IN PERIPHERAL BLOOD OF PATIENTS WITH BENIGN AND MALIGNANT ORAL LESIONS (Abstract): Local inflammation appears to play a very important role in the apparition and development of local tumors and metastases. The aim of this study was to determine the importance of neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) in patients with benign and malign tumors in the oral maxillofacial (OMF) region. **Material and methods:** Medical records of 387 patients diagnosed with oral maxillofacial malignant or benign tumor between January 2017 and June 2019 were retrospectively analyzed. Pretreatment neutrophil, lymphocyte, platelet and monocyte counts and their ratio neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR) and platelet-to-lymphocyte ratio (PLR) were investigated. **Results**: From preoperative complete blood count (CBC) of 387 patients, 151 female and 236 male, 141 benign and 246 malign we found that NLR, PLR and LMR in patients with benign oral lesions were 2.26 (2.03-2.48; SD: 1.35). 107.97 (100.4473-115.5079; SD: 45.2274) and 4.88 (4.5423 to 5.2244; SD: 2.0484) while patients with malign oral lesions had 3.13 (2.85-3.41; SD: 2.95). 129.83 (122.0907-137.5865; SD: 61.6954) and 3.9 (3.6258 to 4.2452; SD: 2.4663). **Conclusions:** Determining NLR, PLR and LMR can be done with a simple investigation and can help determine the prognosis of patients with oral cavity cancer. Mean NLR and PLR in malign tumors was found to be higher than in benign lesions, while LMR was found to be lower in malign tumors than in benign ones. Furthermore, when combining them there is a greater chance of distinguishing a benign tumor from a malignant one. **Keywords**: LYMPHOCYTE-TO-MONOCYTE RATIO, NEUTROPHIL-TO-LYMPHOCYTE RATIO, AND PLATELET-TO-LYMPHOCYTE RATIO, ORAL CAVITY CANCER, BENIGN MALIGN LESION.

Oral cancer is one of the most common cancers worldwide. In 2002, 274,000 cases of oral cavity cancer were reported, of which nearly two thirds in men, thus occupying the 12th place in the world (1). In 2008 oral cancer ranks 15th in the world with 263,000 cases and 127,000 deaths (2) while in 2012 approximatively 300,000 new cases of oral cancer were reported, meaning 2.1% of the world total, with two-

thirds occurring in men and only one-third in women with almost 145,000 deaths (1.8% of the world total), of which 77% were in the less developed regions. The most common pathological type being squamous cell carcinoma (SCC) (3).

Local inflammation appears to play a very important role in the apparition and development of local tumors and metastases. Inflammation has a key role in cancer physiology by promoting carcinogenesis, primary tumor growth, stimulating the proliferation of tumor cells, inhibiting cellular apoptosis and increasing mitotic rates (4).

Inflammation is a complex biological response to cellular damage in which the immune system tries to eliminate or neutralize the infection and start the healing and regenerative processes.

It is possible that the tumor promoting inflammation, whether it precedes or follows the tumor apparition, is a normal process in response to local injury and infection that it is used by cancer cells to develop itself (5).

One such example of a connection between acute inflammation and cancer is shown in a recent study which emphasizes a higher risk of pancreatic cancer in patients who had acute pancreatitis (6).

Chronic infections are known to contribute to cancer initiation and progression by promoting angiogenesis. Also, chronic infections are considered etiological factors for many human cancers, Hepatitis B, Hepatitis C, hepatomas, *H. pylori* and gastric cancer, oral and cervical cancers (7).

Rapid differentiation of a benign oral lesion from a malignant one can be a real challenge in some cases. A correct clinical examination of the patient by inspection and palpation of the lesion is essential for a diagnosis. In addition to histopathological examination of certainty, there are also new non-invasive methods of detecting tumor cells in the oral cavity such as fluorescence imaging and salivary protoporphyrin examination (8).

NLR is a known marker for systemic inflammation that is easily obtained and measured with a simple blood tests and it has been shown to be effective in predicting the prognosis of cancer treatments.

Mean NLR values in healthy adult people was shown to be 1.65 (±1.96 SD: 0.78-3.53) (95%CI (0.75-0.81) in a study on 413 patients (9) while a study made on 12,160 patients without any cancer or other medical history showed a mean NLR across all ages of 1.65, with a mean NLR of 1.63 (0.76) for men and a slightly higher mean NLR for women of 1.66 (0.82) (10).

An elevated NLR indicates an aggressive or advanced tumor and an inflammatory microenvironment that enables a tumor to spread. A high NLR can mean a low lymphocyte who have tumor suppressing effects and/or a high neutrophil count which can cause angiogenesis enabling the tumor to spread (11).

Neutrophils are the most abundant cells found in early stage of acute inflammation while lymphocytes are mostly involved in chronic infections. Neutrophils and lymphocytes have reverse effects in cancer development. Neutrophils increase angiogenesis through vascular endothelial growth factor while T-lymphocytes can inhibit the spread of tumor cells.

Therefore, an elevated NLR, caused by neutrophilia and lymphopenia, is commonly observed in malign tumors (12).

The exact mechanism that can explain the association of high monocytes and a poor cancer prognosis is still unclear. Monocytes create proinflammatory cyto-

kines like IL-1, IL-6, IL-10 and TNF that are associated with poor prognosis and shorter overall survival in cancer. Furthermore, when stimulated they can produce monocyte chemo-attractant protein-1 that can mediate migration and infiltration of tumor associated macrophage which could promote angiogenesis and distant metastases in malign tumors (13).

MATERIAL AND METHODS

Information was collected from patients with histopathological confirmed oral and maxillofacial benign or malignant tumors admitted to the Oral Maxillofacial Clinic of the Craiova County Clinical Emergency Hospital between January 2017 and June 2019 resulting a number of 550 cases. Patients having any acute or chronic systemic inflammatory condition or no recorded neutrophil, lymphocyte, platelets and monocyte counts (n=163) were excluded resulting in a total of 387 patients available for analyses.

A positive diagnosis of oral maxillofacial benign or malign lesion has been established based on histopathological examination of an incisional biopsy, by taking part of an abnormal area of cells, or an excisional biopsy by removing all the area with abnormal cells within oncological safety margins.

All study subjects were divided into two groups based on histopathologic results. All malignant cases were placed in group 1 while the remaining nonmalignant cases, benign or premalignant tumors were included in group 2.

The data collected included: year of admission, sex, age, histopathological results, type of tumor (benign or malign), monocytes, neutrophils, platelets, leukocytes, AST, ALT, hemoglobin, creatinine, urea, glucose, secondary diagnosis, complications, surgery type and post-hospitalization evolution.

The neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR) were calculated and statistical analysis was performed using *MedCalc* 18.11.3 and *Microsoft Excel* 2016 v 1809. Using independent samples t-test and Receiver Operating Characteristic (ROC) we considered a value of $p < 0.05$ statistically significant.

The statistical analysis will be discussed from the perspective of: NLR, PLR, LMR, sex and age ratio, male and female, benign and malign.

RESULTS

There were totally 387 patients with an oral cavity lesion available with preoperative complete blood count (CBC) record and histopathological confirmation. Of these 387 subjects. 246 (63.56%) of them were diagnosed with malignant tumor and only 141 (36.43%) with nonmalignant lesion. 151 females (39%) and 236 males (61%) with an average age of 62.8675 ± 2.74 years and 61.8008 ± 1.62 years respectively. The malignant group was found to be older (65.5 ± 1.48 years) compared with the benign group (56.47 ± 2.82 years) (tab. I).

NLR mean for patients with benign oral lesions was 2.26 (2.03-2.48) with a SD 1.35. while patients with malign oral lesions had an NLR mean of 3.13 (2.85-3.41) and SD 2.25. This means a difference of 0.8725 NLR mean between benign and malign lesions, moreover, emphasized by the p result of 0.001 showing a clear difference in the inflammatory response between malign and benign lesions, with a higher inflammatory response in malign batch (tab. II, fig. 1).

TABLE I
Mean age

Age	Benign	Malign
Sample size	141	246
Arithmetic mean	56.4752	65.5081
95% CI for the mean	53.6478 to 59.3025	64.0265 to 66.9897
Variance	288.3655	139.1897
Standard deviation	16.9813	11.7979
Standard error of the mean	1.4301	0.7522
F-test for equal variances	P < 0.001	

TABLE II
NLR benign *vs.* malign

LR	Benign	Malign
Sample size	141	246
Arithmetic mean	2.2614	3.1339
95% CI for the mean	2.0359 to 2.4868	2.8507 to 3.4171
Variance	1.8337	5.0849
Standard deviation	1.3541	2.2550
Standard error of the mean	0.1140	0.1438
F-test for equal variances	p < 0.001	

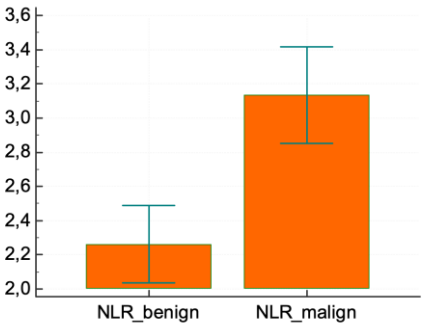

Fig. 1. NLR benign *vs.* NLR malign

Out of the 387 patients with oral lesions 141 were benign, 72 female and 69 males. NLR mean female was 2.26 (1.88-2.63) while NLR mean male was 2.25 (2-2.51). Thus, there is no significant difference between the male and female inflammatory response to benign lesions (tab. III).

From 387 patients with oral lesions, 246 were malign, 79 (32.1%) female and 167 (67.8%) males. NLR mean female was 2.62 (2.27-2.97) while NLR mean male was

3.37 (2.99-3.75). Also seen from the p value of 0.001 when assessing the inflammatory response in malign lesions there is a significant difference between men and female, with an increased response in male subjects (tab. IV).

The mean PLR in 141 oral benign lesions was 107.9 while PLR mean in 246 malign oral lesions was 129.8. The difference was caused because of lymphopenia in patients with malign lesions.

With a p value of 0.001 there is a significant difference in response between benign and malign groups (tab. V). Mean LMR was 4.88 (4.54-5.22) in patients with benign oral lesions and 3.9 (3.62-4.24) in malign oral lesions. With a p value of 0.016 there is a high association between the increased level of monocytes and the presence of malign or benign lesions (tab. VI).

TABLE III

NLR in benign group, women *vs.* men

NLR Benign	Women	Men
Sample size	72	69
Arithmetic mean	2.2631	2.2596
95% CI for the mean	1.8897 to 2.6364	2.0031 to 2.5161
Variance	2.5238	1.1402
Standard deviation	1.5886	1.0678
Standard error of the mean	0.1872	0.1285
F-test for equal variances	p= 0.001	

TABLE IV

NLR in malign, women and men

NLR Malign	Women	Men
Sample size	79	167
Arithmetic mean	2.6283	3.3730
95% CI for the mean	2.2791 to 2.9775	2.9931 to 3.7530
Variance	2.4301	6.1838
Standard deviation	1.5589	2.4867
Standard error of the mean	0.1754	0.1924
F-test for equal variances	p < 0.001	

TABLE V

PLR benign *vs.* malign

PLR	Benign	Malign
Sample size	141	246
Arithmetic mean	107.9776	129.8386
95% CI for the mean	100.4473 to 115.5079	122.0907 to 137.5865
Variance	2045.5175	3806.3280
Standard deviation	45.2274	61.6954
Standard error of the mean	3.8088	3.9336
F-test for equal variances	p < 0.001	

TABLE VI

LMR benign *vs.* malign

LMR	Benign	Malign
Sample size	141	246
Arithmetic mean	4.8834	3.9355
95% CI for the mean	4.5423 to 5.2244	3.6258 to 4.2452
Variance	4.1960	6.0826
Standard deviation	2.0484	2.4663
Standard error of the mean	0.1725	0.1572
F-test for equal variances	p = 0.016	

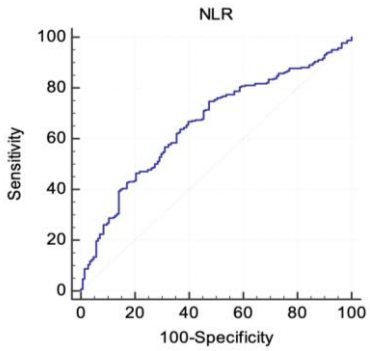

Fig. 2. Receiver-operating characteristics curve (ROC) analysis for NLR.

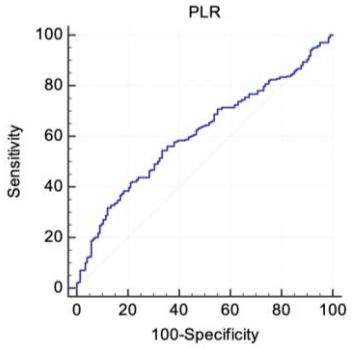

Fig. 3. Receiver-operating characteristics curve (ROC) analysis for PLR.

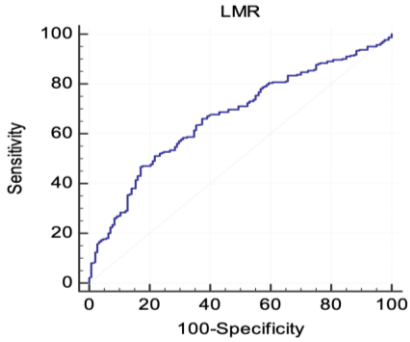

Fig. 4. Receiver-operating characteristics curve (ROC) analysis for LMR.

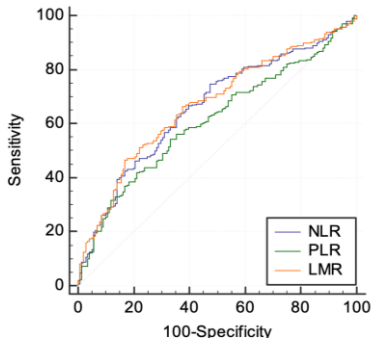

Fig. 5. Receiver-operating characteristics curve (ROC) analysis for NLR, PLR and LMR.

The ROC analysis suggested cutoff value of 1.9609 for NLR in predicting malignancy (AUC: 0.660, sensitivity 74.8%, specificity 52.5 %) (fig. 2).

The ROC analysis suggested cutoff value of 113.9565 for PLR in predicting malignancy (AUC: 0.610, sensitivity 54.5%, specificity 66.7%) (fig. 3).

The ROC analysis suggested cutoff value of 3.2 for LMR in predicting malignancy (AUC: 0.669, sensitivity 46.7%, specificity 83%) (fig. 4).

The ROC analysis suggested cutoff value of 1.9609 for NLR, 113.9565 for PLR and 3.2 for LMR in predicting malignancy (fig. 5).

TABLE VII

Statistics on all 387 patients

	Men	Women	Mean age	benign	malign	left	right	bilateral	Total
lower lip	28	14	65,4	8	34	6	10	26	42
upper lip	1	4	72,6	1	4	0	0	5	5
commissure	12	1	59,3	0	13	7	6	0	13
buccal region	11	14	66,9	12	13	14	11	0	25
jugal	3	3	66,3	5	1	5	1	0	6
submandibular	17	10	53,7	17	10	16	9	2	27
laterocervical	20	8	54,3	13	15	11	13	4	28
tongue	14	14	58,2	3	25	9	12	7	28
mouth floor	27	1	59,6	3	25	4	10	14	28
mouth floor + tongue	10	0	57,6	0	10	4	4	2	10
mandible	17	11	63,3	13	15	10	12	6	28
upper jaw	16	8	65,2	9	15	12	7	5	24
orbital region	4	6	71	2	8	6	4	0	10
hard palate	4	3	53,5	2	5	0	2	5	7
parotid	41	42	63	49	34	35	48	0	83
nasal region	6	8	72,9	1	13	4	5	5	14
others	5	4	65,4	3	6	3	5	1	9
Total	236	151	62,83529	141	246	146	159	82	387

DISCUSSION

Many meta-analysis on patients with oral maxillofacial cancer have shown that a high value of the NLR ratio is an unfavorable prognosis (14, 15).

Several studies on patients with oral

tongue cancer showed that for a pretreatment mean NLR above 2.93-2.95 there is an exponentially increasing chance of having a large invasive tumor with perineural invasion and neck metastases even in clinically negative neck (16, 17). In our study on 387 patients with malign oral tumor a mean NLR of 3.68 (2.96-4.4) was found in 58 (15%) patients (47 men and 11 women) that had neck metastases while mean NLR in subjects with no metastases was only 2.96 (2.66-3.26).

A recent study made on patients with oral cancer showed that LMR can be associated with T and N classification and pathologic stage while NLR is associated with T classification and pathologic stage and PLR with N classification and pathologic stage (18).

In oral squamous cell carcinoma (OSCC) higher neutrophil count correlate with lower overall survival (OS) while higher lymphocytes positively correlate with OS (7) resulting an NLR that can predict the OS in oral cavity cancer. Elevated NLR associated with C-reactive protein is significantly associated with malignant tumor, metastases, overall survival and disease-free survival (19).

In a study on 202 patients with oral cancer it was shown that in oral cavity cancer, the peripheral total white blood cell count, neutrophil and monocyte counts as well as NLR increased with the advancement of clinical stage while lymphocyte count was decreasing. Further, patients with lymph node metastases had monocyte count increased (20).

There is a growing interest in researching the correlation between inflammation, infection and cancer as it has been shown in several studies the existence of a direct connection between NLR, PLR, LMR and cancer prognostic.

The mean NLR values in healthy adult people was shown to be 1.65 (±1.96 SD: 0.78-3.53) (9, 10) while in our analysis on 387 patients, 151 female and 236 male, 141 benign and 146 malign we found NLR mean for patients with benign oral lesions was 2.26 (2.03-2.48) with a SD 1.35. while patients with malign oral lesions had an NLR mean of 3.13 (2.85-3.41) and SD 2.25. The ROC analysis suggested cutoff value of 1.9609 for NLR in predicting malignancy (fig. 2).

Elevated platelet count and platelet-to-lymphocyte ratio (PLR) was shown to be associated with poor prognosis in a meta-analysis made by (21). Mean PLR in healthy adult people is 132.4 (10) while we found a 107.97 (100.4473 - 115.5079) mean PLR in benign lesions and 129.83 (122,0907-137.5865) PLR mean in malign oral lesions.

In our study on patients with oral lesions, mean LMR was 4.88 (4.5423 to 5.2244) in patients with benign oral lesions and 3.9 (3.6258 to 4.2452) in malign oral lesions while mean LMR in healthy people is 5.31 (10). The ROC analysis suggested cutoff value of 3.2 for LMR in predicting malignancy (fig. 4).

Even though there is a small difference in the age-related variance between the two groups the data analyzed is relevant.

In our study, of the 387 patients, only 83 (21.44%) had all three inflammatory markers, namely NLR, PLR and LMR, in the malignant area suggested by the ROC curve analysis. Of these, only 12 (14.45%) had benign tumors and 71 (85.54%) malignant ones.

We further found 67 cases (17.31%)

who had NLR and PLR suggesting malignant tumor while LMR indicating benign of which 47 (70.15%) were histopathological confirmed to be malignant lesions and 20 (29.85%) benignant (tab. VII).

CONCLUSIONS

Patients with oral maxillofacial benign lesions had higher count of neutrophils and lower lymphocytes, meaning a higher NLR than healthy people while patients with oral malignant lesions had a mean NLR even higher. These results were in consonance with other studies that suggest the diagnostic role of NLR in differentiating nonmalignant oral lesions from oral cancers.

Stratifying the analysis, we have found no significant association between gender and inflammatory response in the benign batch of samples as opposed to the malignant counterpart where the association was significant with a higher relevance in the count of NLR in men.

The PLR ratio was also significant between malign and non-malign groups, while when referring to the LMR ration we have discovered a high association between malign and benign with a heavier load in the benign group, confirming the literature.

Furthermore, when combining the NLR, PLR and LMR in patients lacking any acute or chronic systemic inflammatory condition and that are not under any anti-inflammatory treatment, there is a greater chance of distinguishing a benign tumor from a malignant one.

There are also some limitations to our study which we have to emphasize, respectively the relatively small number of enrolled subjects which can be corrected in other studies by continuing the enrollment.

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C. Munteanu and A. Camen contributed equally to this work as the first author.

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EPIDEMIOLOGICAL ASPECTS OF SYPHILIS

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EPIDEMIOLOGICAL ASPECTS OF SYPHILIS (Abstract): Syphilis is the main venereal disease globally and one of the main problems of modern medicine, although its incidence is continuously declining. Aim: to gather data regarding the incidence of syphilis over the last 5-6 years in North-eastern Romania. **Material and methods**: Retrospective study on the main forms of syphilis (primary syphilis, secondary syphilis, latent syphilis, congenital syphilis, neurosyphilis) which included a total of 304 patients admitted and treated to the Clinic of Dermatovenerology of the Iasi County "Sf. Spiridon" Emergency Hospital between 2013-2018**. Results:** The incidence of the different clinical forms of syphilis was as follows: primary syphilis 1%, secondary syphilis 16%, recent latent syphilis 37%, late latent syphilis 22%, early congenital syphilis 3%, late congenital syphilis 11%, results on agreement with literature data. **Conclusions**: Our study demonstrates a linear decrease in the incidence of syphilis over the last 5-6 years in Northeastern Romania, but it still ranks first among EU countries. Further research is needed to understand the social determinants that lead to the spread of sexually transmitted diseases in Romania. **Keywords**: SYPHILIS, EPIDEMIOLOGY, SEXUALLY TRANSMITTED DISEASE.

Syphilis, a sexually transmitted infectious disease in most cases, has been the main venereal disease globally and is still one of the most important problems of modern medicine (1). In 1905, Schandinn and Hoffman (2) identified the causal agent of syphilis, *Treponema pallidum*.

Syphilis is an infectious disease with chronic, undulating course caused by the spirochete *Treponema pallidum*, which is transmitted through the skin and mucous membranes damaging all organs over time. Since the 16th century, syphilis is the most feared sexually transmitted disease (STD) worldwide (3-5). Syphilis is classified as acquired and congenital. Congenital syphilis is caused by the transplacental transmission of spirochetes and is associated with an increased rate of congenital malformations. In its turn, acquired syphilis is divided into early and late syphilis. The early form is classified as primary, secondary and early latent, and late syphilis includes late latent and tertiary syphilis (3,6).

Untreated syphilis can be fatal in over 60% of cases, mainly due to complications during the tertiary stage, and also facilitates the transmission of HIV infection. Most patients with syphilis are asymptomatic and do not realize that anything is wrong, the disease being detected following routine investigations (7, 8).

Annually, approximately six million new cases of syphilis are recorded globally in people aged 15 to 49 years. Over 300,000 fetal and neonatal deaths are attributed to syphilis. Syphilis rates in Eastern Europe increased dramatically after the collapse of the Soviet Union, contributing to changes in health infrastructure, sexual behavior and the emergence of the HIV/AIDS pandemic (8).

In Romania, during 1980-2009, the average annual incidence of syphilis was 25.2 cases per 100,000 population. The incidence had an upward trend during 1980-2002, subsequently declining dramatically during 2002-2009. From 2009 until now, the incidence of syphilis has been steadily declining, but is still one of the highest in the EU countries (9, 10).

Modes of direct transmission of acquired syphilis are sexual contact (90% of cases), extragenital route (wet kiss, accidental inoculation through stings, bites, continuity solutions, rarely encountered in practice), transmission through infected blood, saliva, semen, urine; indirect transmission through contaminated objects is exceptional (11).

The epidemiology of syphilis is influenced by a social, economic and civilization "macro context", as well as by a "micro context" related to individual factors: behavior, level of education and culture (12).

Globally, WHO estimates that most cases of syphilis are recorded in Asia and Africa and that in Europe 30,000-40,000 new cases occur annually. Epidemiological data regarding the incidence of syphilis in Eastern Europe are rare and inconsistent. The incidence of this disease is underestimated because some countries do not report all cases or report them with significant delays. On the other hand, some patients do not see a doctor when lesions occur, or the diagnosis is overlooked due to the similarity to many other conditions (7,13-14).

In Romania, in the last 50 years, the highest morbidity was recorded in 1972 (27 cases per 100,000 population) and the lowest in 1982 (5 cases per 100,000 population) (1). In 2007, Romania reported the highest incidence of syphilis in the European Union (2).

MATERIAL AND METHODS

Our study was a retrospective and observational one that included a series of 304 patients admitted to the Dermatovenerology Clinic "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, with the diagnosis of syphilis during 2013-2018. For each study patient the following data were collected: sex, age, area of residence, and disease status. 61% were male and 39% female, male/female ratio 1.53.

Statistical analysis. The data were centralized in a *SPSS version 18.0* database and processed with the statistical functions to which they are expected, at the significance level of 95%.

RESULTS

Of the total number of admission to the Dermatovenerology Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital from Iasi, during 2013-2018, 3.2% were syphilis cases.

As shown below, 59% of the study patients resided in urban areas. The annual distribution of the study cases revealed a linear decline in the number of new syphilis cases. This constant decrease has a positive population-level impact, considering the socioeconomic issues this disease implies (fig. 1).

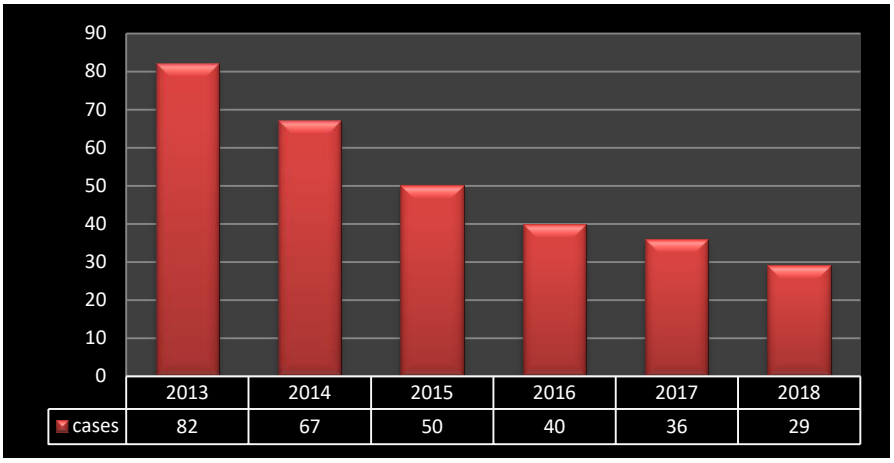

Fig. 1. Incidence of new syphilis cases during 2013-2018

Age group incidence rates were the highest in patients aged 20-49 years, which is the sexually active period (fig. 2). The youngest study patient was 15 years (female, with the diagnosis of secondary syphilis) and the oldest 82 years (male, with early latent syphilis). Mean age at the onset of syphilis was 41 years.

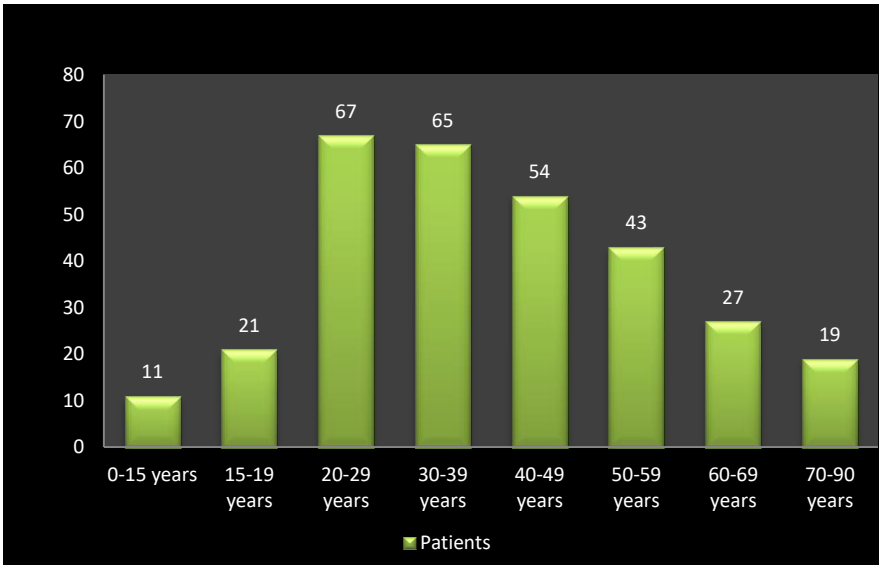

Fig. 2. Age-group distribution of the study patients

Of the 304 patients included in the study, 37% were diagnosed with early latent syphilis followed by 22% late latent syphilis, 16% secondary syphilis and 11% primary syphilis. A diagnosis of congenital syphilis was made in 40 patients, of which 11% had late congenital syphilis and 3% early congenital syphilis. In the interval 2013-2018, only one case of neurosyphilis was recorded (fig. 3).

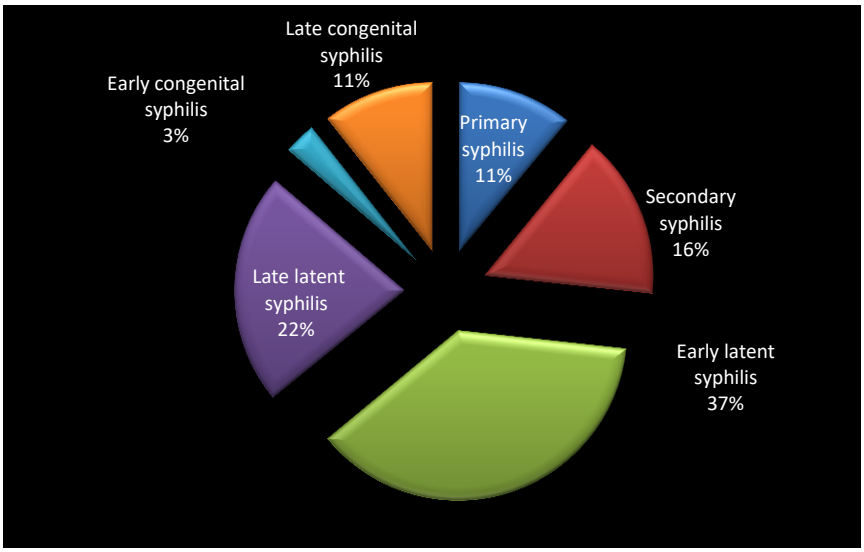

Fig. 3. Early latent syphilis, late latent syphilis, early congenital syphilis, late congenital syphilis, neurosyphilis, primary syphilis, secondary syphilis

DISCUSSION

Bjekic *et al*. (15) demonstrated in a 2014 study analyzing the epidemiological situation of syphilis in the population of Belgrade that the incidence of syphilis was higher in men. Also, in our study, the ratio of men to women was 1.53 and a possible explanation would be the first intercourse that occurs earlier in men that in women (16).

Most of the study patients resided in urban area, this agreeing with the data in the literature. In a study conducted at the Colentina Hospital in Bucharest, Sălăvăstru *et al*. (7) showed that the prevalence of syphilis is higher in the urban compared with rural population. The higher prevalence of syphilis cases in urban areas may be due to both better medical supervision and easier access of the population to medical services, which result in higher rates of diagnosis and appropriate case reporting. Similarly, urban residence has been associated with a high syphilis rates in studies conducted in America and Estonia.

Similar to our study in which young adults (20-39 years) were predominantly affected, Neghina *et al*. (9) showed a higher incidence of syphilis in the same age category in Romania between 1980-2009, Chesson *et al*. (17) found the highest incidence in persons aged 25 years in the United States (2007) and Rodriquez de Mingo *et al*. (18) showed that most cases (46.3%) registered at a health center in Madrid, Spain were under 35 years. Socioeconomic

and educational factors, pimping, as well as sexual assault of juveniles led to increased syphilis morbidity at ever younger ages (5).

These paper demonstrated that the incidence of syphilis in the Northeastern Romania is constantly decreasing (2013 - 82 cases, 2014 - 67 cases, 2015 - 50 cases, 2016 - 40 cases, 2017 - 36 cases, 2018 - 29 cases). Similar to these results, many studies in the literature indicate a decrease in syphilis cases. This reduction in the annual incidence of syphilis demonstrates improved screening and treatment programs in recent years, as well as a higher level of population health education. However, the incidence data obtained in Romania in recent years are significantly higher compared to those reported in other Western European countries (9).

Regarding the disease stage at the time of diagnosis, the majority of patients were in the early latent or late latent syphilis stages, which highlights that the disease was detected following routine serological tests. These data are in accordance to those obtained by Salăvăstru *et al.* in the study carried out at the "Colentina" Hospital in Bucharest between 2002 and 2006 (7). There are no visible symptoms at this stage, but serological evidence of ongoing disease can be found. Screening, and particularly of some population groups, such as pregnant women or medical students, is very important for detecting asymptomatic cases (19). Particular attention should be paid to congenital syphilis rate, as it may be underestimated. The diagnosis of congenital syphilis is challenging, more than half of all children being asymptomatic, and the signs in symptomatic infants can be subtle and nonspecific (9). In the last 70 years, with the decrease in syphilis rate due to the introduction of penicillin treatment, neurosyphilis rate has also decreased. This accounts for the fact that in the interval 2013-2018 only one case of neurosyphilis was reported in our clinic. Often when a patient presents with such neuropsychiatric manifestations as psychosis, mania, cognitive disorders, behavior changes, the diagnosis of syphilis is not the first suspected. Often, the patients with such symptoms were initially treated with psychiatric medication, although the appropriate treatment was penicillin. Thus, performing serologic tests for Treponema pallidum in patients with psychiatric manifestations is justified (19).

CONCLUSIONS

Syphilis is the third most common sexually transmitted disease in Europe. Raising awareness of the need for testing, knowledge of the main symptoms and signs of this disease along with improving the case reporting systems should be the most appropriate future measures to control the spread of disease. In 2016, the World Health Organization (WHO) launched a new strategy on sexually transmitted infections for 2016- 2021. The priority of this strategy is the elimination of congenital syphilis by implementing complex screening methods for pregnant women as well as for some target populations in order to reduce the number of cases by 90% .

It has been suggested that generally the spread of sexually transmitted diseases is related to international travel and migration. In Romania, this hypothesis is supported by high incidence rates in large cities, such as Bucharest and Constanța, the largest Black Sea port.

Our study demonstrates a linear decrease in the incidence of syphilis over the last 5-6 years in Northeastern Romania, but it still

ranks first among EU countries. Further research is needed to understand the social determinants that lead to the spread of sexually transmitted diseases in Romania.

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CONSIDERAŢII ASUPRA TRATAMENTULUI CHIRURGICAL ÎN CANCERUL DE COL UTERIN AVANSAT

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ADVANCED CERVICAL CANCER SURGICAL TREATMENT CONSIDERATIONS (Abstract) : The aim of this study was to highlight the importance of surgical treatment in advanced cervical cancer (IIB-IIIB). **Material and method**: Data from 179 patients with cervical cancer, admitted in the Clinic of Emergency Surgery, "Sf. Ioan" Hospital, Iaşi, between January, 1st, 2003 and December, 31st, 2009, were collected. **Results**: A number of 11 cases (6.1%) cases were without any clinical response, so that they benefit by radical radiotherapy ; a radical surgical intervention was performed in the other 168 cases (93.7%), in 4-6 weeks after chemotherapy. No intraoperative complications were evidenced, but after surgical intervention we recorded : urinary troubles - 10 (6.5%) ; lymphedema – 3 (1.9%) ; posttoperative intestinal occlusions - 2 (1.3%) ; extended dynamic ileus - 2 (1.3%) ; phlebitis – 2 (1.3%) cases. **Key words**: CERVICAL CANCER, SURGERY, SURVEILLANCE

DUMITRU BAGDASAR - MEDICAL MALPRACTICE?

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DUMITRU BAGDASAR- MEDICAL MALPRACTICE? (Abstract): The Law 95/2006 defines malpractice as "the professional error committed in exercising the medical or medical-pharmaceutical act, generating prejudices on the patient, involving civil liability of the medical personnel and the supplier of medical, sanitary and pharmaceutical products and services". Although the law mentioned above does not have a known history, through 1930s, acts of medical malpractice have existed since the begging of the first doctors. The well-known Romanian physician Dumitru Bagdasar, the founder of the Romanian Neurosurgery School, in 1935 was accused of having chosen to go on holiday in the province instead of checking on his patient who suffered from a brain tumor. While he was gone, the patient died following a puncture, but his survival chances would have been inexistent even if the renowned neurosurgeon had spent every minute by his bedside. Whereas generally the first reaction is to accuse the treating physician, this cannot always be the case. The physician's liability cannot be invoked in this case, given that there was no professional blame. Dumitru Bagdasar bequeathed to future generations a multitude of valuable information that can be put into practice even nowadays. Most of the time, given that is difficult to accept the idea that a loved one is no longer with us, the blame lies with the doctor who, although he makes every effort to help an individual, fails to save his life. **Keywords:** DUMITRU BAGDASAR, MALPRACTICE, PHYSICIAN'S LIABILITY.

"It is hard being a physician and mostly being a surgeon ! The nervousness surrounding the surgery itself; the anxiety of potential death, inherent for this profession; so many emotional states shattering your health and maybe altering the primitive feeling of human mercy". This is what the well-known Romanian physician Dumitru Bagdasar, the son of a peasant from the village of Roșiesti (1) – the founder of the Romanian Neurosurgery School – wrote in his dairy on the 9th of November 1935.

Nonetheless, his passion for neurosurgery never made him become overwhelmed with the emotions of the operating room. Indeed, his professional performance has been praised to this day (2).

Medicine and law are among the oldest human activities (3). The Law 95/2006 defines malpractice as *"the professional error committed in exercising the medical or medical-pharmaceutical act, generating prejudices on the patient, involving civil liability of the medical personnel and the supplier of medical, sanitary and pharma-*

ceutical products and services". Hence, medical errors may be committed regardless of a physician's specialty; the consequence is not only civil liability, but also criminal liability, which brings prejudice to both parties (3).

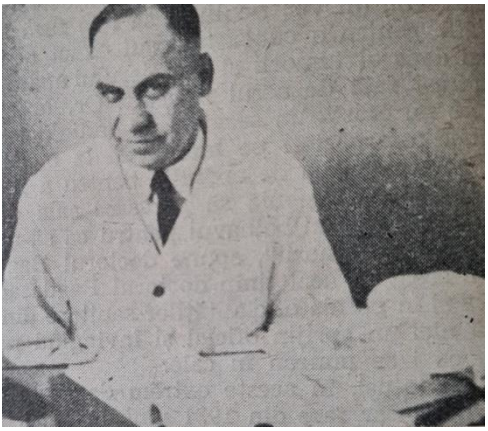

Fig. 1. Doctor Dumitru Bagdasar. (Photo source: Kreindler A, *Viața și opera profesorului Dr. Dumitru Bagdasar*) (2)

A physician's obligation is one of means; exceptionally, there are situations when they face the obligation of results. The prejudiced situation would prove the fact that they were the victim of a professional error. This solution may be found not only in our legislation, but also in Canada, Italy, Spain, or Belgium (4).

Many things have been written thus far concerning such cases; the authors were both legal and medical experts who – upon noticing certain things going wrong – have tried to issue a warning sign.

Across time, surgeons have developed a competitive spirit providing them with the opportunity to innovate, to change paths in many areas of medicine, but it has also brought them in an area of penumbra: I refer here to professional envy, accuses, or denunciations. The history of universal medicine and mostly the history of surgery abound in examples of the last category. The Romanian space is no exception. Important names of scalpel artists have been involved in malignant conflicts, such as the one between Thoma Ionescu and Victor Gomoiu. The Surgery Society – acknowledged from 1923 as a moral and legal entity – has included many meetings over time, where they discussed difficult patient cases, papers, surgical equipment presentations, and standpoints concerning the military situation and the war surgery. Dan Setlacec states – in a well-known writing – that "in our society, except for a few years with a low-key activity, the atmosphere was vibrant", "discussions were sometimes animated", and "scientific and non-scientific" controverses were expressed (5).

Deontological debates have also found a place among the discussions carried on by the members of the society, though they would make many of them feel rather embarrassed. Amza Jianu and Dimitrie Gerota brought up in 1928 a rather difficult topic for many years in the amphitheater: the abuse of surgical interventions, having nothing to do with a patient's affliction. Other sensitive topics included: self-granting scientific titles; details of course syllabuses; the selection criteria for physicians within various clinics; or the abuses and inconsistencies in the Criminal Code. The Surgery Society brought to the attention of its members the infamous Bagdasar-Vasiliu conflict (5).

On the 8th of September 1935, the journal named "Curentul" featured a piece titled *"For the redetermination of certain facts"* (6). Actually, one day prior, the same publication included an articled called *"The physician takes a walk"* (7). According to the authors, the latest article

had brought "a serious and unjust moral offence" against the famous physician Bagdasar; the authors wished to requalify the facts as they had occurred, for everybody to understand.

Therefore, the paper titled *"The physician takes a walk"* states that a physician's liability is a conscience-related matter, given that the patients put their trust in the physician. Persuaded that the specialists hold the knowledge necessary to heal them and that their moral values are noble, patients put their lives in the hands of a person with great surgical skills. However, the lines published in the journal *"Curentul"* describe the situation of a patient who died because of the negligence of a medical practitioner. Nicolae Popovici – the son of a well-known former school inspector – suffered from a brain tumor, reason for which he was admitted to the central hospital (7).

The well-known physician Dumitru Bagdasar was accused of having neglected to check on his patient, by *"remembering that summer was almost over and that he simply needed another relaxing escape to the province, to take advantage of the fine weather"* (6, 7) - the hospital ward remained under the supervision of an inexperienced young doctor. While he was gone, the patient died following a puncture, which stirred the anger of the person who wrote the article.

Therefore, in early 1934, N. Ionescu, MD and Ion T. Niculescu, MD – the authors of the article *"For the redetermination of certain facts"* – received the mission of consulting (along with other colleagues) Nicolae Popovici, admitted to the ophthalmology ward of the "Colțea" Hospital. The young man's condition worsened; symptoms indicated a brain tumor, and his life was in danger. However, the surgical intervention that saved him was performed by Dumitru Bagdasar; this intervention was recommended by all the neurosurgeons having consulted the individual.

In August 1935, Nicolae Popovici's health status declined. He was brought to Bucharest again, where he was admitted to the "French" Sanatorium. Along with the renowned physician D. Bagdasar, N. Ionescu, M.D. and Ion T. Niculescu, M.D. were summoned for a new consult. Given that the patient was not stable, the three decided to tell the family that it was impossible to carry out a surgery, because it was highly likely for the young man to die on the table; they recommended waiting for the patient to become stable and the family was informed expressly in this respect.

Meanwhile, the patient was transferred to the "Central" Hospital, in Bagdasar's ward. However, due to his very serious medical condition, Nicolae Popovici died but his survival chances would have been inexistent even if the renowned neurosurgeon had spent every minute by his bedside.

Whereas generally the first reaction is to accuse the treating physician, this cannot always be the case. The letter sent by N. Ionescu, M.D. and Ion T. Niculescu, M.D. had the role of clarifying that doctor Dumitru Bagdasar was not to blame; that he was passionate about his work and devoted to the service; all of his efforts –however great– had been in vain, unfortunately.

Most of the times, surgeons make superhuman efforts to save the lives of patients. It is extremely important to practice the profession diligently; to show compassion and patience in order to attain optimal results. The physician's liability cannot be invoked in the aforementioned case, given that there was no professional blame.

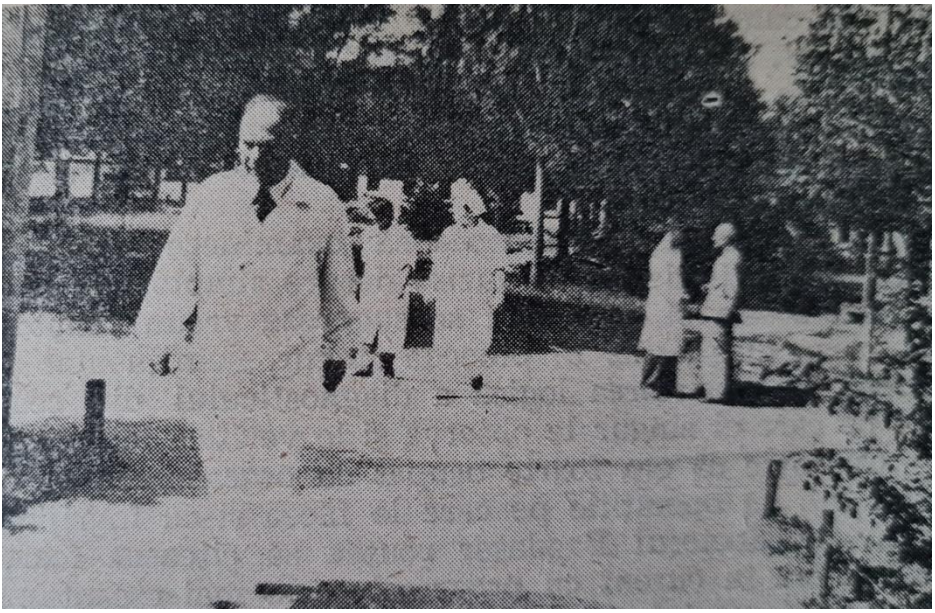

Fig. 2. Dr. Bagdasar at the Central Hospital.
(Photo source: Kreindler A, *Viața și opera profesorului Dr. Dumitru Bagdasar*) (2)

Dumitru Bagdasar represents a model for many neurosurgeons nowadays. His research in the fields of spinal cordotomies, of cerebral tuberculomas, and of spinal cord tumor pathology has been crucial for the current medical progress.

Despite all the struggles in his personal life, his medical passion was limitless: he helped those in need regardless of their social status.

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7. *** Curentul. *Medicul la plimbare*, year VIII, no. 2727, 7th of September 1935, 1.

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TO WHAT EXTENT IS ROMANIA PREPARED TO JOIN HEALTH TECHNOLOGY ASSESSMENT COOPERATION AT EUROPEAN LEVEL

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TO WHAT EXTENT IS ROMANIA PREPARED TO JOIN HEALTH TECHNOLOGY ASSESSMENT COOPERATION AT EUROPEAN LEVEL (Abstract): An increasing number of EU States are using the Health Technology Assessment (HTA) approach, and as health technologies, pharmaceuticals are one of the main fields of application in HTA. This is a review aiming to identify the current status of the HTA collaboration efforts in EU and in Romania, taking into consideration the growing role of HTA in decisions about the level of price and reimbursement in some EU countries or, in other countries, in decision on whether to reimburse a new product or to reject its funding totally. Romanian HTA system needs further development, both in terms of financial resources and, probably more important, human resources; the efforts in the long run will bring great returns to the entire health system, allowing better, safer, and more efficient use of pharmaceuticals and other health technologies. The scientific evidence provided may support the decision-making process. **Keywords**: HEALTH TECHNOLOGY ASSESSMENT, EVIDENCE BASED DECISION-MAKING HTA CORE MODEL.

The evidenced based decision-making process relays increasingly at EU level on assessing technologies that includes pharmaceuticals, devices, procedures, programmes, and even organizational aspects of health care systems. A widely accepted definition of Health Technology Assessment (HTA) was provided by European Network for Health Technology Assessment (EUnetHTA): "a multidisciplinary process that summarises information about the medical, social, economic and ethical issues related to the use of a health technology in a systematic, transparent, unbiased, and robust manner" (1).

HTA concept has been introduced in Europe stepwise since 1970s, and the formal collaboration among the national HTA agencies started in 1990s with financial support from the European Commission (EC) (2).

EUnetHTA Collaboration (3) was launched in November 2008, following an EC funded project aiming to connect national HTA agencies, research institutions and health ministries in order to enable an effective exchange of information on health technologies effectiveness thus assisting the EU Member States and candidate countries to plan, deliver and monitor health

services effectively (3). The first EUnetHTA Joint Action (2010-2012) built on the previous project results for developing an effective and sustainable HTA collaboration in Europe (3). EUnetHTA Joint Action 2 (2012-2015) improved the EUnetHTA tools and methodologies based on results of cross-border collaborative practical application of these (3). EUnetHTA Joint Action 3 (2016-2020) is strengthening the collaboration among EU Member States by an increased number of commonly evaluated technologies, revision of methodologies, development of quality standards and mechanisms to ensure uptake at national level, in order to establish a permanent HTA working structure for Europe (3).

Despite all attempts starting in early 1990s, Romania has set an HTA agency in 2012 only as a consequence of Directive 2011/24 EU. Initially established as a unit within the Ministry of Health, since 2014 it passed under the National Agency for Medicines and Medical Devices (NAMMD) (4). A 2017 World Bank financed project on Technical Assistance for institution building of HTA structure, including training for the NAMMD had the objective of making recommendations on further development of HTA system in Romania.

MATERIAL AND METHODS

The current study is a review of the main EU regulations, of national HTA legislation, of literature and working documents regarding the HTA Romanian system and EU cooperation in HTA. For the main consulted documents see first table.

TABLE I

Main consulted documents

- Directive 2011/24/EU of the European Parliament and of the Council of 9 March 2011 on the application of patients' rights in cross-border healthcare;
- Commission Implementing Decision of 26 June 2013 providing the rules for the establishment, management and transparent functioning of the Network of national authorities or bodies responsible for health technology assessment;
- Rules of Procedure of the Health Technology Assessment Network adopted by the HTA Network on 10 november2016;
- Proposal for a Regulation of The European Parliament and of The Council on Health Technology Assessment and amending Directive 2011/24/EU;
- Commission Staff Working Document on Impact Assessment Strengthening of the EU Cooperation on Health Technology Assessment (HTA) Accompanying the Proposal for a Regulation of the European Parliament and of the Council on Health Technology Assessment and amending Directive 2011/24/EU;
- Law 95/2006 on the Healthcare Reform;
- Ministry of Health Order 861/2014 for the approval of HTA criteria, documentation to be submitted by applicants, methodological instruments used in the assessment process concerning inclusion, extension of indications, non-inclusion or exclusion of medicines in/from the List of INNs of medicines from which the insured persons benefit, as well as INNs of medicines granted in national health programmes, and the means of challenge;
- Studies on mapping of HTA organizations and methodologies commissioned by European Commission;
- Technical assistance reports of the World Bank project conducted in Romania.

The list of the main consulted documents

A comparative analysis was performed following the developments of HTA at the European Union level and the development of HTA system in Romania.

RESULTS AND DISCUSSION

EU cooperation on HTA.

It is unanimously accepted all over EU that HTA has an important input on evidence-based decision-making.

The EC has supported the cooperation between HTA bodies, through substantial financing of three Joint Actions (JAs): 2010-2012 JA1 (6 million Euros), 2012-2015 JA2 (9.5 million Euros), 2016-2020 JA3 (20 million Euros) (fig. 1).

EU has facilitated the cooperation on HTA in Europe by creating the legal basis of an HTA Network. The article 15 of Directive 2011/24/EU on the application of patients' rights in cross-border healthcare introduces the setting up of a voluntary network to support cooperation between national authorities or bodies responsible for HTA (5). All EU countries have applied for membership and participate. Romania applied for membership and participated in all the Network's meetings through the MoH, mandated by the Law 95/2006, article 922 (6).

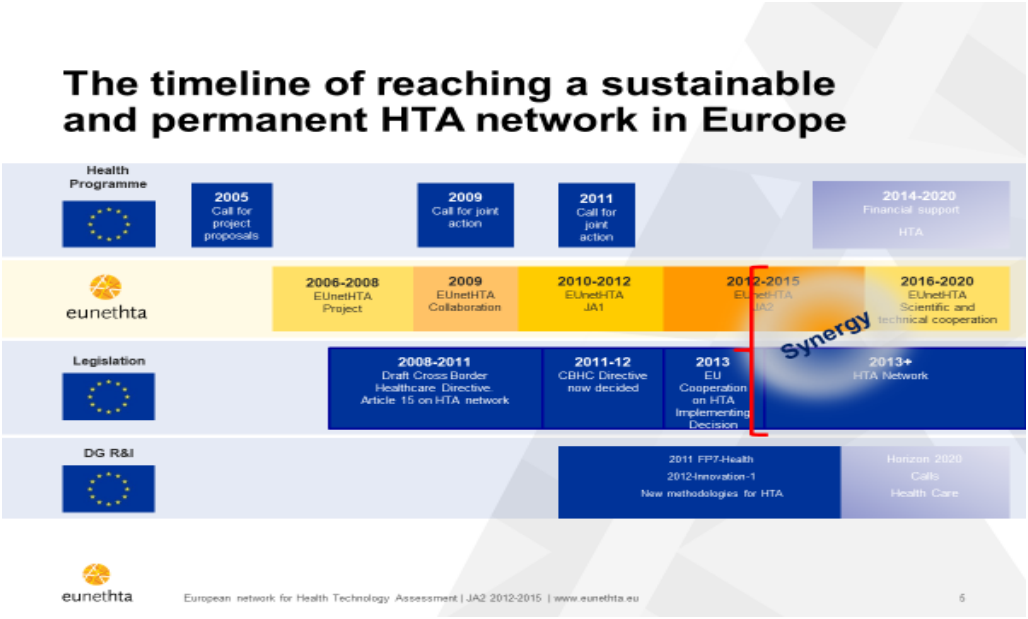

Fig. 1. The timeline of reaching a sustainable and permanent HTA network in Europe
Source: General Presentation on EUnetHTA
(https://www.eunethta.eu/general-presentation-on-eunethta)

The HTA Network's activity is regulated by the Commission Implementing Decision of 26 June 2013 providing rules for the establishment, management, and transparent functioning of the Network of national authorities or bodies responsible for HTA, that created a synergy between HTA Network and EUnetHTA Joint Actions (7),

stating "HTA Network shall be supported by a scientific and technical cooperation mechanism" (8). The HTA Network has also adopted owns Rules of procedures, a 2016-2020 Strategy for EU cooperation on HTA and developed Multiannual Working Programmes.

Thus, while the HTA Network is focusing on strategic issues and takes decisions relevant to EU cooperation on HTA, the currently EUnetHTA JA3 ensures scientific and technical cooperation for the HTA Network.

EUnetHTA collaboration. The scientific work in EUnetHTA Joint Actions, over the last 10 years, resulted in a specific set of tools and methodologies, handbooks and toolkits for users, teaching materials, internal strategies and methodological standards, guidelines and procedures manuals, databases, recommendations on the implementation of sustainable European network for HTA, position papers and reports. The final outcome of these JA consisted in the Joint or Collaborative Assessments of different health technologies produced by partners from different European countries.

The Network has been growing fast. If the first EUnetHTA JA counted 33 partners and 26 collaborators from 25 countries, the 3rd JA includes 81 organizations from 29 countries. The National School of Public Health, Management and Professional Development Bucharest joined EUnetHTA in 2011, and two more Romanian institutions in 2016: The National Institute of Public Health and the "Babes-Bolyai" University from Cluj-Napoca (3).

The main methodological framework for production and sharing of HTA information is *HTA Core Model* that collects information on nine domains: Health problem and current use of technology, Description of health technology, Safety, Clinical effectiveness, Cost and economic effectiveness, Ethical analysis, Organisational aspects, Social aspects, Legal aspects. The model reflects the broad scope and multidisciplinary nature of HTA (9).

Designed in EUnetHTA 2006-2008 project, the model has been refined along the 3 JAs, especially following its piloting, being currently at its 3rd version, and is still under revision. The HTA Core Model for the production of core HTAs (version 3.0) consists of three main components: (1) Standardised set of HTA questions (the ontology) that helps to define specific research questions; (2) Methodological guidance to assist in answering the research questions; (3) Common reporting structure. In its development, the Core model has been piloted on seven non-pharmaceutical technologies. The HTA Core Model is accessible free of charge but subject to the HTA Core Model Licence (10).

Given the complexity of the HTA Core Model, it was also developed an application for rapid assessments – *the HTA Core Model for Rapid Relative Effectiveness Assessments (REA)* that covers only four domains: Health problem and current use of technology, Description of health technology, Safety and Clinical effectiveness (fig. 2). REA was initially developed for pharmaceuticals in order to allow the assessment in the limited timeframe of 90-180 days imposed by the European Transparency Directive 89/105/EEC. Equally the Full Core Model, the Core Model for REA was continuously improved based on several pilots and its application was expanded to non-pharmaceutical technologies such as devices, diagnostics, surgical interventions, and screening (10).

The aim of EUnetHTA JA 3 is to define and implement a sustainable model for cooperation on HTA in Europe after 2020 by supporting the collaborative production of HTA and encourages the use of the HTA reports at national level (11).

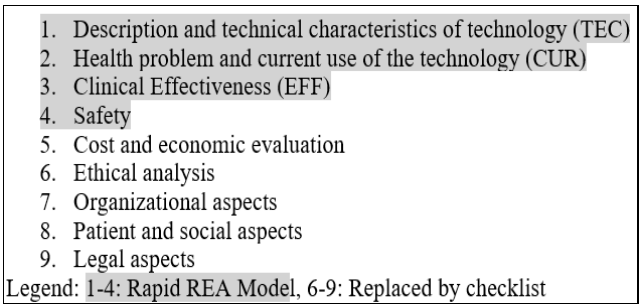

Fig. 2. The domains of the HTA Core Model and relative effectiveness assessment. Source: EUnetHTA WP5. HTA Core Model for rapid relative effectiveness, 2015

The updated list of assessments performed during EUnetHTA JA3 or planned to be performed is available on EUnetHTA website (12). The National School of Public Health, Management and Professional Development Bucharest (NSPHMPDB) has been involved as co-author in five REAs, and as dedicated reviewer in one REA, all for non-pharmaceutical technologies, adding on the experience acquired in JA2 from participation of the piloting of three Full Core HTA assessments. A list of technologies assessed during the EUnetHTA JA 3 with NSPHMPDB' contribution is present ed in second table.

TABLE II

List of technologies assessed during the EUnetHTA JA 3, with contribution of NSPHMPDB.

Pharmaceutical technologies assessed	Publication of Final Assessment
Hypoglossal nerve stimulation systems for treatment of obstructive sleep apnoea	21.05.2020
Biodegradable rectum spacers to reduce toxicity for prostate cancer	24.04.2020
Point-of-care Tests (POCT): D-Dimer and Troponin	14.08.2019
Screening for osteoporosis in the general population	19.12.2018
Bioresorbable Stents in cardiovascular indications (coronary artery disease)	20.08.2018
Antibacterial-coated sutures versus non-antibacterial-coated sutures for the prevention of abdominal, superficial, and deep, surgical site infection (SSI)	06.10.2016
Use of Intravenous immunoglobulins for Alzheimer's disease including Mild Cognitive Impairment.	30.11.2015
Structured telephone support (STS) for adult patients with chronic heart failure.	30.11.2015
Fecal Immunochemical Test (FIT) versus guaiac-based fecal occult blood test (FOBT for colorectal cancer screening.	31.07.2014

Source: Assessments REA (2016 – 2021) (https://eunethta.eu/rapid-reas/)

EU collaboration after EUnetHTA. During JA3, there were performed several evaluations to identify challenges and shortcomings of EU collaboration on HTA

after 2020, either by EUnetHTA, having in mind the aim of preparing for a sustainable network post 2020, or by the European Commission, in the view of preparing further regulations.

According to a study commissioned by EC in 2017, there is a large variety of organizational structures of national HTA models in EU and EEA countries, from large single HTA bodies, specially assigned working groups in the Ministry of Health, to systems with two or more organizations performing various functions in the national HTA processes or constituted in regional networks (13).

As well, an impact assessment performed in the view of issuing the Proposal for a Regulation of the European Parliament and of the Council on HTA and amending Directive 2011/24/EU, reveals that national HTA systems in Member States differ not only in the procedural frameworks and methodologies, but also there are significant differences in the available resources. Having identified all shortcomings, the study also presents an in-depth analysis of four policy options as further steps: (1) No Joint Actions after 2020; (2) Project-based cooperation on HTA activities; (3) Permanent cooperation on common tools, procedures, and early dialogues; (4) Permanent cooperation on common tools, procedures, early dialogues, and joint REA (14).

Based on the previously mentioned study, the EC adopted in 2018 a legislative proposal by which to reinforce cooperation amongst Member States, building on the policy option 4. Thus, the Member States will work together on joint clinical assessments of new medicines and certain new medical devices, on joint scientific consultations for developers, on identification of emerging health technologies and on voluntary cooperation in other areas. Individual EU countries are responsible for assessing non-clinical (e.g., economic, social, ethical) aspects of health technology and making decisions on pricing and reimbursement (15). The proposal is still under discussions, even if according to initial planning it should have been adopted by the Parliament and the Council in 2019. The last published progress report dates from June 2019, when the Council was under Romanian Presidency (16).

Stage of development of HTA system in Romania. The national competent authority for HTA in Romania is set as a unit within the National Agency for Medicines and Medical Devices, and the evaluation methodology is provided by the Ministry of Health Order no. 861/2014. The methodology is not relaying on assessment of evidence but is based on a scorecard system that relays mainly on decision-making in other countries (4,17,18).

As well, the HTA unit is not supported by adequate human resource capacities. A World Bank project was undertaken in Romania in order to provide recommendations for the further development of the national HTA system. In June 2017, the Technical Assistance Report presented three alternative models for the institutionalisation of HTA in Romania, taking into consideration the spread limited capacities in the country (19). At the time of writing no decision was taken in this regard.

Until developing the capacity of doing own assessments, Romania cannot actively participate to the joint clinical assessments as envisaged by the European Commission for the further EU collaboration on HTA. For being able to participate to this process, further steps should be taken in developing

the legislation, training human resources, and ensuring collaboration between existing experts in the field at national level.

CONCLUSIONS

The EU collaboration on HTA still faces a lot of challenges and barriers, from language use and reporting structure to differences in national processes and methodologies and the use of HTA report in decision-making. The EUnetHTA collaboration is aiming to develop an effective and sustainable HTA collaboration, through 1) reducing repetitions and improve the efficiency in the use of resources; 2) facilitate growing impact of HTA in EU Member States; 3) consolidation the relation between HTA and health care decision-making; and 4) supporting countries with less experience in HTAs. The benefits for HTA agencies that contribute to and use EUnetHTA assessments are resource- and time-savings. But, to achieve this, the national agencies should align to EUnetHTA practices in terms of topic selection, scope, and quality of the assessment (11). These initiatives intend to harmonize or standardize the HTA processes so to improve their level of transparency, quality and comprehensiveness, and facilitate the extraction of relevant information that can then be transferred cross-border.

In many countries, even high-income countries, the staffing levels of public authorities tasked with HTA, pharmaceutical pricing, procurement and reimbursement are low, and this is also true for the Romanian health system (20).

As well, EU collaboration on HTA require not only HTA agencies commitment, but also clear involvement and engagement from all stakeholders: industry, patients, healthcare providers and payers (18,19,21). From this perspective it can be argued the role of HTA in Romania is still undervalued, even if calls for increased efficiency and better methods for reimbursement and pricing for drugs are ten years old (20,22). In order to be able to use HTA and to participate to EU collaboration, Romanian HTA system needs further development, both in terms of financial resources and, probably more important, human resources; the efforts in the long run will bring great returns to the entire health system, allowing better, safer, and more efficient use of pharmaceuticals and other health technologies.

CONFLICT OF INTEREST

The authors reveal no conflict of interest concerning the manuscript.

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BRÂNCUȘI-GEROTA "ÉCORCHÉ" – 120TH ANNIVERSARY SINCE ITS COMPLETION

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BRÂNCUȘI-GEROTA "ÉCORCHÉ" 120TH ANNIVERSARY SINCE ITS COMPLETION (Abstract): The Écorché or "Ecorșeul", is the first of the many works of art done by Constantin Brâncuși, the world-renowned Romanian artist. It was achieved in collaboration with the famous anatomist Prof. Dr. Dimitrie Gerota and represents a national premiere in being the first anatomical work conceived in such detail in 20th century Romania. The debutant artist assimilated knowledge about the human anatomy from the dissection of human bodies, this being a process which harmoniously binds art with the study of anatomy. Inspired by Antinous's statue, Brâncuși used, in his work, its stature and flamboyant pose. Awe-inspiring are the copies of Brâncusi's Écorché, which, worked in plaster and attentively supervised by Brâncuși. They were donated later to Medical Schools and Art Schools around Romania for academic study. The Écorché, which was realized with intense efforts, brings national and international renown to the young sculptor, at the very start of his carrier, which became, with the passing of time, a work linked closely to his name, despite the modernist and abstract current which embodied his whole carrier. **Keywords:** CONSTANTIN BRÂNCUȘI, DIMITRIE GEROTA, ÉCORCHÉ, HUMAN ANATOMY, HISTORY OF MEDICINE.

EARLY LIFE

Constantin Brâncuși, the absolute artist of Romania, a faithful militant of the infinity he created with his own hands, is considered the parent of modern international sculpture.

The genius with magic hands, has learned his trade at the beginning of the 20th century, when he finished his studies at the National Arts School in just four years, where he cultivated his perseverance and workmanship.

The pinnacle of his career, however, was to be found in Paris, where after great sacrifices, he managed to be admitted to the *"Ecole Nationale Superior des Beaux Arts".*

It is here, in Paris, where he spent a large part of his later years, after having completed some of his greatest works and after having received a substantial number of distinctions awarded by multiple French art association.

THE ÉCORCHÉ, MORE THAN A WORK OF ART

The "Écorché", or "Ecorșeul", in Romanian, represented the first large-scale

work of young Brâncuși and it is an art piece which proves his artistic genius to this day. It was realized during his studies in Bucharest and represents one of Brâncuși's most different and impressing work of art.

The Romanian word "Ecorșeu" comes from French and has the same root word as in English, namely "Écorché". It means a representation by drawing, painting, or sculpture of the human body devoid of skin and its three layers.

As stated in the abstract, the work was done under the careful guidance of Prof. Dr. Dimitrie Gerota, who helped young Brâncuși acquire the foundations of anatomical knowledge, so essential for the correct realization of the "Écorché".

In this regard, Prof. Dr. Dimitrie Gerota, procured the dissection materials "according to nature, directly human, the bodies being recovered from the Bucharest Faculty of Medicine, from professional works and from the city's mortuary" relates the "Brâncușian exegesis expert" V. G. Paleolog.

The work of dissection was carried out by both Brâncuși and Prof. D. Gerota, the goal of which was the establishment of the perfect proportions for the anatomical casts, which were later used to create the baseline through which the "Écorché" was made.

Brâncuși received his ideas from "The Artistic Anatomy" of Paul Richet and from the dissection courses which he follows at the Bucharest Faculty of Medicine, with the goal of bettering his knowledge (1).

The genius sculptor does all these works in a spirit of sacrifice, "for art's sake", which after morbid experiences, he begins to detest "the carnal", and even calls the human flesh "biftec" (Romanian word for beefsteak) (1).

The repulsion, which the artist develops, will be reflected in his future sculptures in which human traits are replaced by perfection, shaping the human ideal according to his aesthetic eye.

The "Skinned" (as Brâncuși nicknames the Écorché) was a subject that, indeed, treated the human body from both the perspective of art and the perspective of anatomy. This conviction is reinforced by the very model that Brâncuși will be inspired by, namely a copy of the sculpture 'Hermes Capitoline', located at the Capitoline Museum in Rome. This replica is present at the School of Fine Arts in Bucharest (fig. 1), and it was known as "Antinous" at the time. It was made available to the young sculptor who, will use the stature and pose of the statue, to achieve the "Écorché" (2).

From the artist's account to V. G. Paleolog, we learn that the muscles on some bodies were dissected individually and compared directly to the statue of Antinous. Therefore, we can say that the "Écorché" is an "ecorched" "Hermes Capitoline", or as Brâncuși would say, skinned (2).

In the summer of 1901, with the anatomical study soon to be completed, Brâncuși is rewarded at the Anatomy Competition (judged by Prof. Gerota) with the Bronze Medal.

The distinction seeming to have, indeed, been a thorough encouragement for the young artist. There were also some scholarships that he subsequently obtained for the carrying out and completing of the work.

It bears reminding that with the completion of the Écorché, he receives national notoriety, from which point on he is rewarded a number of distinctions at Artistic Youth exhibitions in Romania (3). This is

the moment when he becomes consecrated, in the minds of, if not the international public, yet, certainly the national one, as a master sculptor in his own rights.

The completion of the work takes place sometime in 1902, the exact date being unknown until nowadays.

The first exhibit of the Écorché, towards the greater public happens at the Romanian Athenaeum, in Bucharest, in 1903, it enjoyed nation-wide recognition ever after.

We shall now mention the friendship that Prof. Gerota and Constantin Brâncuși have had, because of their collaboration (4).

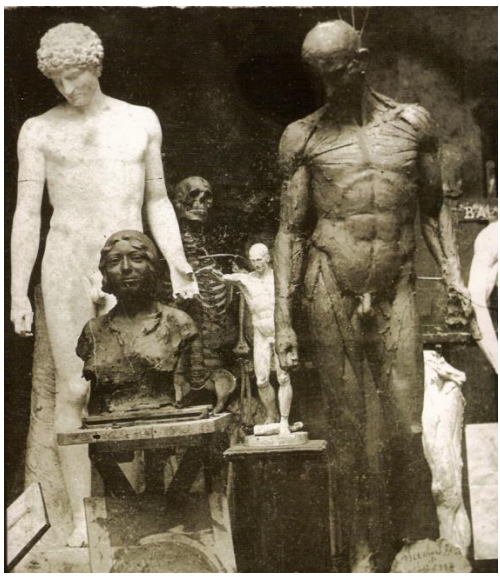

Fig. 1. Original photo held by Brâncuși, depicting the écorché, in progress, next to the statue of Antinous (taken from: Dumitrescu E, "Ecorșeul Brâncuși-Gerota istoria unei lucrări realizate la Școala de Belle Arte din București") (1)

Despite the age difference of nine years, the two continued their relationship after the artist left for Paris, with the remaining correspondence proving this. The connection between them may well be due to their common origin, the two being from the same region of Romania, Oltenia.

DIMITRIE GEROTA'S CONTRIBUTION

Prof. Dimitrie Gerota (fig. 2) was considered the first radiologist in Romania, offering a huge contribution to the foundation of this discipline in our country. At the same time, he was a passionate anatomist, being the founder of a museum of anatomical-surgical casts for students. Prof. Gerota, a true teacher, supported Brâncuși's work with the desire to offer a model of anatomical study at the level of the entire Romanian Universitary Community.

And that he did, ordering faithful copies of the Écorché, recreated in plaster, which were donated/sold to medical schools all over the country.

One of the most well-known and well-preserved specimens is still in Iassy, where it was sent by Prof. Gerota through the Ministry of Cultural Affairs in June 1903.

This colorful mold was used both at the Faculty of Medicine in Iassy and, at the School of Fine Arts for the studies of plastic anatomy.

The renowned anatomist from Iassy, Dr. Ștefan Possa had bought the Écorché for the sum of 10,000 lei, a considerable amount at that time (4).

Since two World Wars followed the purchase, it was assumed that the location of the Iassy "Écorché'' has been changed many times, without appearing on the inventory recorded by the educational institutions.

It appeared in several archives from the interwar period, and then only in 1966 is it recalled in a letter accompanied by a photograph.

Fig. 2. Professor Doctor Dimitrie Gerota (taken from: Dumitrescu E, "Ecorșeul Brâncuși-Gerota istoria unei lucrări realizate la Școala de Belle Arte din București")

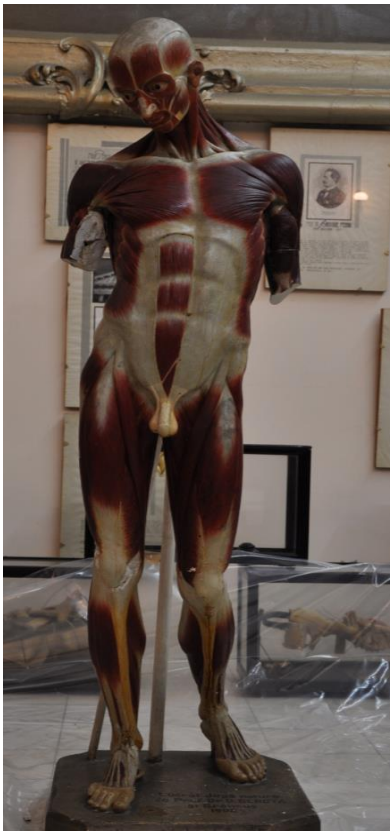

Fig. 3. The original from Iassy, during the restoration of the piece, sometime at the end of 2012 / original photography by Felix Aftene

Over time, small reconditioning took place on this specimen, but the most important was carried out in 2012 under the guidance of Felix Aftene. Both arms/hands of the mold were restored, these having been the most altered components. Today, the faithful copy of the Écorché (fig. 3) is located at the Anatomy Museum of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, along with other "ecorched" pieces and anatomical preparations, which constitute an impressive heritage of this prestigious institution.

Significant for the Iassy copy, is the mold socket, where the two authors were signed: "Prof. Dr. Gerota and Brâncuși" in 1902.

Today, there are known to be four authentic copies, which are located throughout Romania, in Iassy, Cluj-Napoca, Craiova and in Bucharest. The Original (fig. 4) can be seen till today, at the entrance of the National Art University in Bucharest, its dimensions being 177 x 52 x 32 cm (5).

As a mention, at the exhibition organized at the Brummer Gallery in New York, in the year 1933, Brâncuși especially wanted that on one of the walls to be placed a photo of the "Écorché'', a work that all biographers catalogued as a "schoolwork" (5). This was done to show the critics of his art style, that the evolution of his artworks had a natural course and that all the synthesis capacities he had attained through his masterpieces were derived from a deep and thorough knowledge of the human body.

CONCLUSIONS

We can firmly affirm that the joint work of Constantin Brâncuși and Professor Doctor Dimitrie Gerota revolutionized the study of myology in Romania, taking on a vital importance for Anatomy schools and

Fine Arts schools throughout the country. We cannot imagine the learning difficulties that the Romanian students of the 20th century would have gone through in the absence of materials such as the "Écorché" and its special copies. Today, young people have at hand the technology that, through various 3D applications, presents the anatomy of the human body at the click of a button, but then, in the absence of teaching materials, it was almost impossible to provide an overview of the "skinned" body, of human myology, and as a result, Brâncuși's creation was like "a breath of fresh air" for the anatomical study in Romania at the time.

Undoubtedly, the "Écorché" has become an anatomical and artistic symbol that will endure over the centuries.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NEWS

THE IMPACT OF VIRTUAL REALITY IN EATING BEHAVIOR

Obesity prevalence has increased in the last decade all over the world. Environment is one of the many factors that have an impact on eating behaviors and the prevalence of obesity. In reality, eating behavior such as of portion size perception, energy density, and taste, has changed over the years, as a result of the decrease of home-cooked meals consumption. Virtual reality can be used as a tool to study the impact of environments on eating behaviors, offering sensory information, as well as environment change. In this study participants masticatory cycles, heart rate, as well as skin temperature were tested in various scenarios: a restaurant and an empty room with a table. In both scenarios participants consumed pizza, masticatory rhythm and heart rate modification, as well as skin temperature variation, occurring only amongst those exposed in the restaurant context. Thus, virtual reality can conduct to a better understanding of environments impact on eating behaviors (Oliver JH, Hollis JH. Virtual Reality as a Tool to Study the Influence of the Eating Environment on eating Behavior: A feasibility Study. *Foods* 2021; 10(1): 89 / doi: 10.3390/foods10010089.).

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LEONARDO DA VINCI (1452-1519): OVERVIEW OF NEUROANATOMY CONTRIBUTION

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EARLY LYFE

Leonardo da Vinci (born April 15, 1452, in Florence, died May 2, 1519 in France) (fig. 1) was an Italian painter, engineer, encyclopedic personality and remarkable neuroanatomist. He was born from Leonardo di ser Piero da Vinci, a wealthy notary and landlord from Florence, and Caterina de Meo Lippi, a young peasant woman. Due to his wealth status, da Vinci couldn't afford a superior education, so he received an elementary education from his paternal grandfather Antonio, which consisted in reading, writing and learning arithmetic skills.

After his father took him to Florence, when he was 15 years old, he had the opportunity to develop his drawing interest from artist Andrea del Verrocchio (1435-1488), which introduced the young da Vinci in painting, sculpture, technical-mechanical arts, geometry, arithmetic, chemistry, metallurgy and woodworking. During his stay at Verrocchio's workshops, he met famous painters such as Botticelli (1445-1510), Ghirlandaio (1448-1494) and also worked in the workshop of artist Antonio del Pollaiuolo (1429-1498). Working simultaneously in two famous artists workshop was a defining experience for young Leonardo, and in 1472, at only 20 years old was accepted into the Painters' Guild of Florence, a secular organization that controlled the arts in Florence during Renaissance Era (1). He continued to work with Verrocchio for five more years before started his independent career, when his main point was to find a higher sense to human existence (2).

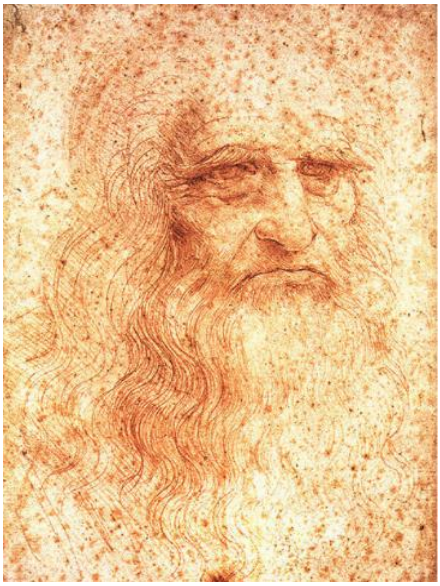

Fig. 1. "The portrait of a man in red chalk", self portrait of Leonardo da Vinci, ca. 1510 (public domain)

BEGINNINGS IN HUMAN ANATOMY

While working for Ludovico Sforza, Duke of Milan (1452-1508), his interest for understanding human anatomy and physiology began with anatomical drawings of animals. The practice of animal dissection was commonly used during da Vinci time for a better understanding of past scholars such as Hippocrates (460-370 BC) and Galen (129-216 AD) who studied usually on animals, to compare with human anatomy. Because human dissection was forbidden during Renaissance Era, da Vinci performed animal dissection until 1489. In a document dated from 1485-1487, da Vinci firstly described the pitching of a frog: "The frog retains life for some hours when deprived of its head and heart and all its bowels. And if you puncture the said nerve (spinal medulla), it immediately twitches and dies" concluding the fact that spinal cord was not only the source of semen, as beforementioned by Hippocrates and Plato, but actually the center of the body, naming it *virtu gienjitua* (generative power) (fig. 2) (3).

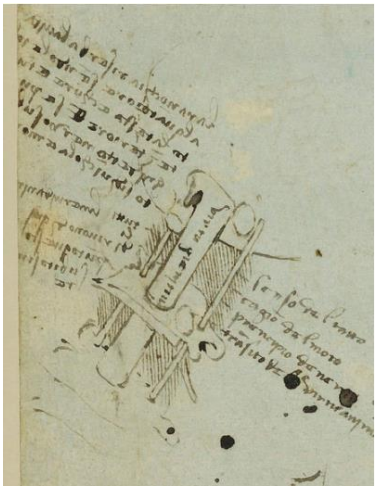

Fig. 2. Experiment with pithing the frog spinal cord, ca. 1485-1487 (public domain)

The study of anatomy was seen by da Vinci as a necessity for a better technique in his painting, his obsession with understanding nature processes and correlating anatomical parts with their function pushed him to perform human dissections, without any medical training, in the times when this practice was forbidden by the Catholic Church. Therefore, during 1489-1513 he actively learned through dissection on dead bodies and successfully described for the first time multiple anatomical structures (4).

REMARKABLE NEUROANATOMY AND NEUROSCIENCE DISCOVERIES

Da Vinci drawings were comprehensive, containing elements from both the Central Nervous System (CNS) and Peripheral Nervous System (PNS). In regard to CNS, he studied the morphological aspect of the skull and multiple intracranial structures (figs. 3, 4), to better the correlation between the eyes, brain and sight (5). Moreover, he performed dissections on nerve plexuses and peripheral nerves, precisely describing the main trunks of the brachial plexus and the ulnar, radial and median nerves (fig. 5), additionally. He explained in detail how sensitive and motor deficits are caused according to the specific area damaged, being a pioneer in the study of physiology (6). For lumbosacral plexus, he noted "There are as many nerves as muscles in the thigh" (7), and in a precise drawing of his, femoral, obturator and sciatic nerves can be observed (fig. 6). In the case of lower limb physiology, he noted the action of agonist and antagonist muscles before it was described by Sherrington (1857-1952).

Between the years 1506 and 1508 he mastered his knowledge of using molds for bronze sculptures, thus inventing a novel technique for studying neuroanatomy by injecting wax into the brains of oxen to create casts (8). This technique consisted in

injecting the molten wax inside the fourth ventricle and with this experiment, he was the first to accurately describe the lateral third and fourth ventricle, and also an interventricular foramen (fig. 7), who was described 200 years later by the Scottish physician Alexander Monro (1733-1817), named after himself as foramen of Monro. Leonardo da Vinci described the ventricular system being formed by three structures: *imprensiva* (anterior), *senso comune* (middle) and *memoria* (posterior). In plus, in his drawings, we can observe the foramen of Monro and aqueduct of Sylvius.

Fig. 3.Skull studies: coronal hemi section, showing frontal and maxillary sinuses, ca. 1489 (public domain)

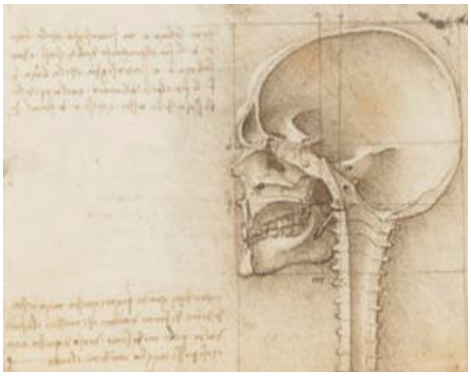

Fig. 4. Skull studies: Sagittal section, including cervical vertebrae, ca. 1489 (public domain)

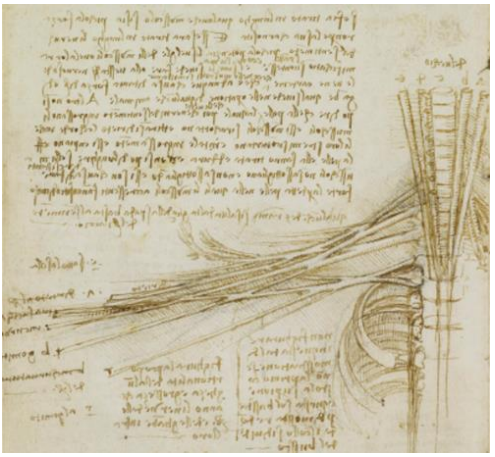

Fig. 5. Brachial plexus: superior, middle and inferior trunks are represented in detail, ca. 1508 (public domain)

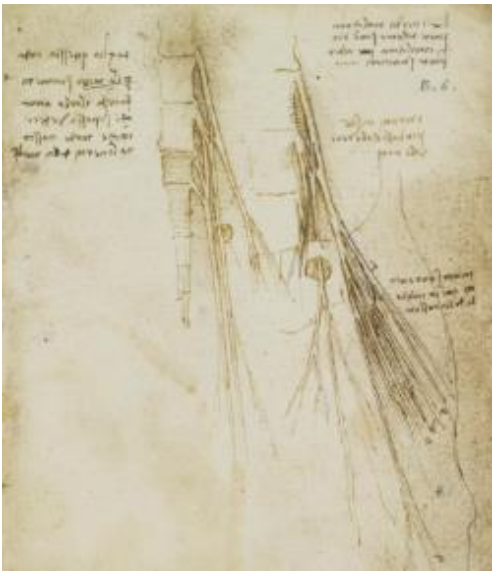

Fig. 6. Lumbosacral plexus: femoral nerve with his multiple branches, obturator nerve and a duplicated sciatic nerve (error), ca. 1508 (public domain)

Between the years 1506 and 1508 he mastered his knowledge of using molds for bronze sculptures, thus inventing a novel

technique for studying neuroanatomy by injecting wax into the brains of oxen to create casts (8). This technique consisted in injecting the molten wax inside the fourth ventricle and with this experiment, he was the first to accurately describe the lateral third and fourth ventricle, and also an interventricular foramen (fig. 7), who was described 200 years later by the Scottish physician Alexander Monro (1733-1817), named after himself as foramen of Monro. Leonardo da Vinci described the ventricular system being formed by three structures: *imprensiva* (anterior), *senso comune* (middle) and *memoria* (posterior). In plus, in his drawings, we can observe the foramen of Monro and aqueduct of Sylvius.

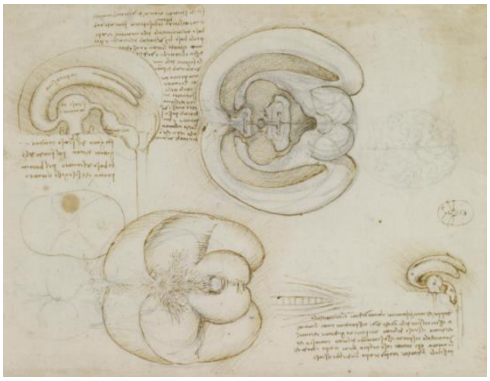

Fig. 7 – Ox brain injected with wax: ventricular system described as *imprensiva* (anterior), *senso comune* (middle) and *memoria* (posterior), ca. 1508 (public domain)

In a famous drawing of da Vinci, where he accurately describes anterior, middle and posterior fossae for the first time, and an impression of the anterior and middle meningeal arteries, the artist demonstrated the location of the *sensus comunis* (fig. 8), an intersection of three lines – an anterior-posterior axis, a lateral axis approximately along the ridge of the *dorsum sellae*, and a vertical axis through the upper point of the skull (9). All of those lines, intersect over the optic foramina, being on the anterior part of the third ventricle. He considered the soul as being located in the senso comune, maintaining the vision of others Galenic physicians (10).

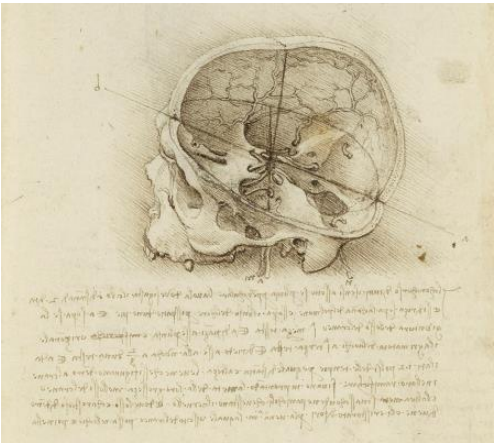

Fig. 8. Skull studies: senso comune represented by the optic chiasm, ca. 1489 (public domain)

One of the major objectives of Leonardo was to find a correlation between the nature and the soul, through the sense of sight (11). After many dissections on this matter, he concluded that we could see objects due to eyes receiving light. With this observation he contradicted classical thinkers like Plato, who considered the ability to see as a property of the eyes. Da Vinci noted “the eye, the window of the soul, is the chief means whereby the understanding can most fully and abundantly appreciate the infinite works of Nature” maintaining his point regarding a higher sense of the sight. Moreover, he was the first to describe the optic pathway and the first to consider the olfactory bulb as a cranial nerve (fig. 9) (12).

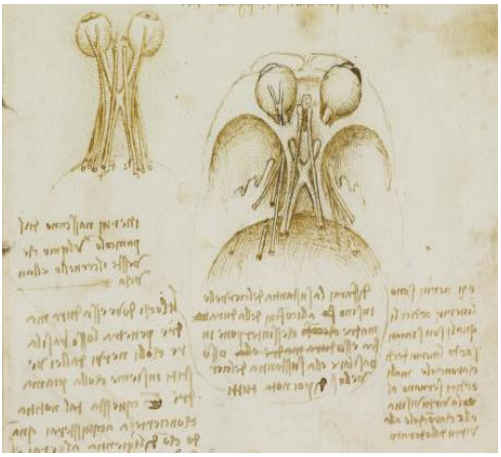

Fig. 9. Optic pathway: optic nerves, optic chiasm and olfactory bulb, in plus, the drawing includes the oculomotor, abducens and branches of the trigeminal nerve ca. 1508 (public domain)

The progresses of Leonardo da Vinci in understanding the neuroanatomy of human are comprehensively explained in the "Weimar anatomical sheet" (fig. 10) (13), a drawing from 1508 who contains two figures of the nervous system. This outstanding representation of the nervous system includes the main discoveries made by da Vinci in the field of neuroanatomy, from *sensus comunis* to cerebral ventricles, and an overall look of the layers of the head, to which he compared to the layers of an onion "If you will cut an onion through the middle, you will be able to see and enumerate all the coats or rinds, which circularly clothe the center of the onion", "Similarly, if you cut through the middle of the head of a man, you will first cut the hairs, then the scalp, then the muscular flesh and pericranium, then the cranium; and inside, the dura mater, the pia mater and the brain; then again the pia mater and dura mater and the rete mirabile and then the bone, their foundation" (14).

Fig. 10. The "Weimar anatomical sheet": ventricular system, brain and cranial nerves in pairs, ca. 1508 (public domain)

CONCLUSIONS

Leonardo da Vinci was a famous artist in his lifetime and his fame grew over time. In the field of science, through his discoveries, he is considered a pioneer in anatomy and physiology of the nervous system. He made astonishing observations in understanding neuroanatomy and neuroscience, unfortunately, those unique accomplishments did not receive a well-deserved appreciation during Renaissance Era. His papers in anatomy were not published before the period 1898-1916, so his influence on the history of anatomy was minor during the Renaissance Era. We realize the importance of his discoveries, knowing the nowadays proven truth of science, but if we look into the context in which da Vinci was, they are many reasons why he was not recognized as a neuroanatomist during his life. First of all, a court painter would not have access to academic circles that will give him the

opportunity to publish his discoveries, even for Andreas Vesalius (1514-1564) a university professor at Padua, highly respected by academic groups, it took Vesalius decades before his treatise *"De Humani Corporis Fabrica Libri Septem"* achieved recognition. Secondly, da Vinci did not study anatomy from a scientific point of view, he wanted to understand the human body more from an empirical perspective, to explain the unity of man and nature in an integrated system. His drawings were encrypted and disorganized, so he never synthesized his theories and observations into a coherent scientific paper, even nowadays, in these technologically advanced times, it took scientists many years of work to decrypt his manuscripts. We pay tribute to Leonardo work and what he represented for the humanity, a complex encyclopedic personality with a distinguish style and character who well represented the Renaissance Era.

CONFLICT OF INTEREST

The authors declare no conflict of interest and received no specific funding regarding this scientific research

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CBCT ASSESSMENT OF MANDIBULAR BONE QUALITY AND QUANTITY IN PATIENTS WITH OSTEOPOROSIS

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CBCT ASSESSMENT OF MANDIBULAR BONE QUALITY AND QUANTITY IN PATIENTS WITH OSTEOPOROSIS (Abstract): Osteoporosis is characterized by weakened bones that put people at higher risk of fracture, especially in patients who have an advanced degree of damage and who follow or not treatment for osteoporosis. The **aim** of the study was to compare mandibular indices and mandibular bone density with the skeletal status investigated by osteodensitometry in patients with osteoporosis, with or without treatment. **Material and methods**: 40 patients with osteoporosis, divided into two equal groups, were evaluated by CBCT for the assessment of some mandibular indices that indicate the bone density at the cortical and cancellous bone level. The statistical analysis was performed with SPSS20.0, p=0.05. **Results**: For most of the evaluated parameters, higher values were recorded in the group of participants with osteoporosis but without treatment compared to the group of participants with osteoporosis, with treatment. The results showed a significant association between the mandibular cortical index and the type of treatment, with G1 patients presenting in a proportion of 25% the mandibular cortical index type 2, while G2 patients presented the mandibular cortical index type 2 in 58.33% of cases. In the case of G2 patients the cortical bone density values are significantly higher compared to the values corresponding to G1 patients. There are no significant differences in regard to cancellous bone density values between groups. **Conclusions:** The density of the mandibular bone is closely correlated with changes in the density and implicitly of the state of the skeleton of patients with osteoporosis, usually evaluated by bone osteodensitometry. The CBCT technique provides sufficient radiographic information that helps dentists and oral and maxillofacial surgeons to play an important role in the early diagnosis of mandibular osteoporosis**.** **Keywords:** CBCT, OSTEOPOROSIS, OSTEODENSITOMETRY.

Osteoporosis is characterized by weakened bones that put people at higher risk of fracture (1). Bone strength is a reflection of the fusion of bone quality and density are the two main characteristics. Bone density, which can be measured as grams of bone

mineral per bone area, is thought to be responsible for 60-70% of bone strength. The rate of bone turnover, mineralization, material characteristics, microarchitecture (loss of horizontal struts, loss of connectivity), and geometry are all indicators of bone quality. Age-related bone loss creates deeper resorption cavities that concentrate mechanical stress that degrades bone quality and makes those areas more prone to fracture (2, 3).

Women are disproportionately affected by osteoporosis, which causes fractures that are related to it. The number of fractures and the proportion of women with osteoporosis will rise due to current demographic trends, despite decreases in hip fracture rates. Age, low BMD, and a history of fractures are just a few of the risk factors for fracture that have been identified. The identification of women at high risk should be made easier by targeting those with multiple risk factors (3).

The mandible is an active bone and is influenced by the force of the masticatory muscles. Therefore, reducing the number of teeth causes a gradual decrease in bone mass, (4) thus creating a thinner layer of cortical bone. The mandible can be affected by systemic diseases or various drugs in terms of bone quantity and quality. It is known that after a tooth extraction, alveolar bone resorption occurs, which can be accelerated by the presence of a systemic condition such as osteoporosis. The changes that can occur in the bone tissues of the skeleton and the mandible show numerous risk factors, such as: age, race, menopause, genetic factors, various medications and family history of a disease (5).

Several studies have been conducted in the last decade that demonstrate the presence of a link between the changes that can occur in the mandibular bone and the overall BMD status of the skeleton. Some writers (6) have clearly argued that there is a substantial relationship between mandibular cortical thickness or appearance and systemic osteoporosis. Other studies have discovered no such associations, and their confidence is low (7). The World Health Organization (WHO) classified osteoporosis only based on the T score, as a decrease in BMD of less than 2.5 standard deviations below the average of a young adult of the same sex (Osteopenia Score T -1 -2.5 SD, Osteoporosis Score T-2,5 SD).

The clinicians emphasized that radiomorphometric indices and the density of the jaw bones can be calculated on imaging studies, which can serve as a diagnostic criterion of skeletal BMD, allowing them to select patients with potential BMD changes (8, 9). These radiomorphometric indices, which included the upper and lower panoramic mandibular indexes, the chin index, and the cortical mandibular index, were assessed using orthopantomography to determine the quality of the mandibular bone. Few studies have employed these indices on CBCT images to assess mandibular bone (10).

The aim of the study was to compare mandibular indices and mandibular bone density investigated by osteodensitometry in patients with osteoporosis, with or without treatment.

MATERIAL AND METHODS

The study was carried out in the Medimagis Clinique of oral and Maxillo-Facial Radiology, Iasi. In this study, 40 patients with osteoporosis were included, who presented for CBCT investigations to plan the surgical treatment. Patients signed an informed consent prior to their inclusion

in the study. The study was approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (No. 281/5.03.2023).

The inclusion criteria of patients in this study were: only women before menopause, aged between 40 and 80 years, diagnosed with osteoporosis and different classes of mandibular edentulousness. Exclusion criteria were local conditions affecting the mandible, postmenopausal status and other secondary causes of osteoporosis. The participants were equally divided into two groups: group 1 - participants with treatment for osteoporosis (G1), group 2 - participant without treatment for osteoporosis (G2).

All these patients presented clinical observation sheets, from which additional information was extracted regarding the status of the disease and its complications (hemoleucogram, CTX). An anthropometric assessment was also performed which included data on height, weight and abdominal circumference.

CBCT examination of the mandible

This research is part of a large study related to the radiological determination of bone characteristics in patients with general pathologies. This research is part of a large study related to the radiological determination of bone characteristics in patients with general pathologies. The working technique is described in detail in the study carried out by Nemtoi *et al.* (2019)(11). The Planmeca Promax 3D Mid (Planmeca OY, Helsinki, Finland) CBCT equipment was utilized to do the scan that was carried out with a FOV of 100x60 mm with the following exposure parameters: 85-90 kV, 11-12 mA, 12,02 seconds, voxel size of 200 mm, and 578 DAP (mGyxcm2). Romexis 4.4.0 software (Planmeca, Helsinki, Finland) was used to produce the initial and final reconstructions. CBCT reconstructions with a thickness of 1 mm and a distance of 1 mm were used to create sagittal and panoramic sections.

Mandibular bone analysis, quantitative and qualitative

Radiomorphometric indices tailored FOR CBCT images were utilized for quantitative and qualitative examination of the mandible according to Ledgerton's categorization (Ledgerton *et al*, 1999) the following indices were measured on CBCT images:

1) CBCT upper mandibular index, which represents the ratio between the thickness of the lower cortex and the distance from the level of the upper edge of the chin foramen to the lower edge of the mandible.

2) CBCT lower mandibular index, which represents the ratio between the lower cortical thickness and the distance from the lower edge of the chin foramen to the lower edge of the mandible.

3) The CBCT chin index, which represents the thickness of the lower cortex of the mandible, near the chin foramen.

4) CBCT mandibular cortical index, which is classified as follows:

✓ Type 1: the endosteal margin of the basilar bone cortex appears smooth and regular.

✓ Type 2: the endosteal edge of the basilar bone cortex presents semilunar defects or 1-2 layers of cortical debris.

✓ Type 3: the endosteal margin shows numerous (> 3) cortical remnants and is obviously porous.

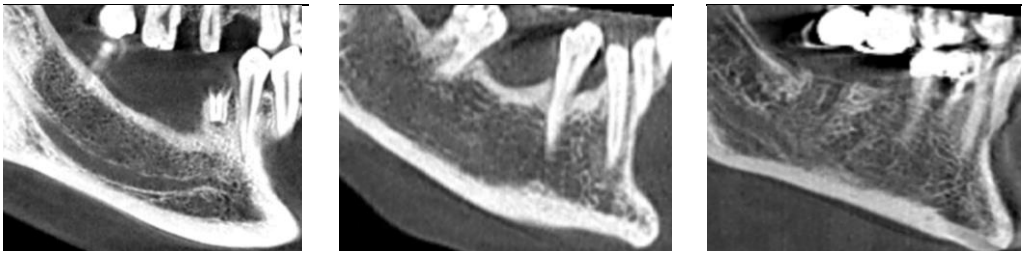
In addition, mean bone density was estimated for the regions of interest for qualitative analysis.

A B C

Fig. 1. CBCT aspect of measurements made on sagittal sections. A. The distance from the level of the upper border of the chin foramen (S) to the lower border of the mandible. B. The distance from the lower edge of the chin foramen (i) to the lower edge of the mandible. C. G represents the thickness of the lower cortex of the mandible, near the chin foramen (Medimagis collection)

These measures were taken from the sagittal (fig. 2) and panoramic (fig. 3) reconstructions produced by the CBCT examination.

A radiologist with long practice in oral and maxillofacial radiology used Romexis 4.4.0 software (Planmeca, Helsinki, Finland) to do all measurements.



A B C

Fig. 2. CBCT aspect of the mandibular cortex. A. Type 1: the endosteal margin of the basilar cortical bone appears smooth and regular. B. Type 2: the endosteal margin of the basilar bone cortex shows crescentic defects or 1 - 2 layers of cortical debris. C. Type 3: endosteal margin shows numerous (> 3) cortical debris and is obviously porous (Medimagis collection).

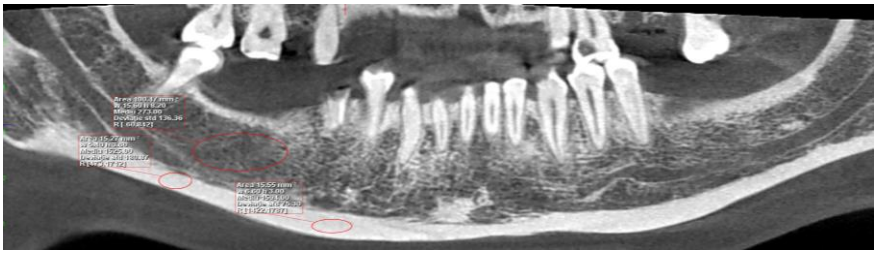


Fig. 3. CBCT evaluation of cortical and trabecular bone density in the regions of interest (Medimagis collection)

Statistical analysis

Data were analyzed with a statistical software package (*SPSS* version 20.0, SPSS Inc, Chicago, IL). Statistical tests were used to measure the differences between mandibular bone quality, bone mineral density at the level of the mandible: frequencies (%), average values, minimum and maximum values, ANOVA and Kruskal-Wallis test. Comparisons were made between groups. Statistical significance was established at p=0.05.

RESULTS

Both the right lower mandibular index (F=0.0018, p=0.892) and the left lower mandibular index (F=0.323, p=0.5729) show comparable values without significant differences in the case of G2 patients compared to G1 patients (tab. I).

TABLE I.

Statistical indicators for IMI $_{right}$ /$_{left}$ values. *vs.* groups

	Group	Av. IMI	Average		SD	SE	Min	Max	Q25	Median	Q75
			-95%	+95%							
IMI right	G1	0.45	0.29	0.62	0.31	0.08	0.11	0.98	0.14	0.40	0.78
	G2	0.44	0.34	0.54	0.24	0.05	0.18	0.90	0.23	0.43	0.61
IMI left	G1	0.44	0.29	0.59	0.29	0.07	0.12	0.89	0.15	0.38	0.74
	G2	0.48	0.39	0.58	0.22	0.04	0.20	0.91	0.27	0.46	0.64

The study of the values of the right upper mandibular index and the left upper mandibular index demonstrated the absence of significant differences depending on the group, the average values being in the case of G2 patients of IMS right → 0.50±0.32 SD and IMS left → 0.53±0.31 SD, and in the case of G1 patients, the average values were IMS right → 0.57±0.27 SD and IMS left → 0.59±0.25 SD (tab. II).

TABLE II.

Statistical indicators for IMS $_{right\ /\ left}$ values *vs.* groups

	Groups	Av. IMS	Average		SD	SE	Min	Max	Q25	Median	Q75
			-95%	+95%							
IMS right	G1	0.50	0.33	0.67	0.32	0.08	0.14	0.99	0.17	0.44	0.80
	G2	0.57	0.46	0.69	0.27	0.05	0.19	0.98	0.29	0.57	0.80
IMS left	G1	0.53	0.37	0.70	0.31	0.08	0.17	0.98	0.21	0.46	0.82
	G2	0.59	0.48	0.69	0.25	0.05	0.21	0.98	0.33	0.63	0.76

The analysis of the values of the right chin index according to the type of treatment, demonstrates significant values (F=6.67, higher for patients with G2 (2.47±0.8 SD) compared to patients with G1 (1.67±0.8 SD).

The values of the left chin index are significantly (F=6.95, p=0.0121, 95% CI) higher in patients with G2 (1.8±1.13 SD) compared to the values recorded in patients with G1 (2.58±0.75 SD) (tab. III).

The study of the degree of association between the mandibular cortical index and the type of treatment demonstrated the presence of a significant correlation, with G1 patients presenting in a proportion of 25% the mandibular cortical index type 2, while G2 patients presented the mandibular cortical index type 2 in 58.33% of cases (r=-0.298, χ^2=8.46, p=0.0145, 95% CI) (fig. 4, tab. IV).

TABLE III.

Statistical indicators for IMn $_{right/left}$ values *vs.* groups

	Groups	Av. IMn	Average		SD	SE	Min	Max	Q25	Median	Q75
			-95%	+95%							
IMn right	G1	1.67	1.05	2.29	1.17	0.29	0.20	4.20	0.65	1.60	2.55
	G2	2.47	2.13	2.81	0.80	0.16	1.20	3.50	1.80	2.70	3.25
IMn left	G1	1.80	1.20	2.40	1.13	0.28	0.30	3.80	0.80	1.90	2.70
	G2	2.58	2.27	2.90	0.75	0.15	1.40	3.80	1.90	2.80	3.25

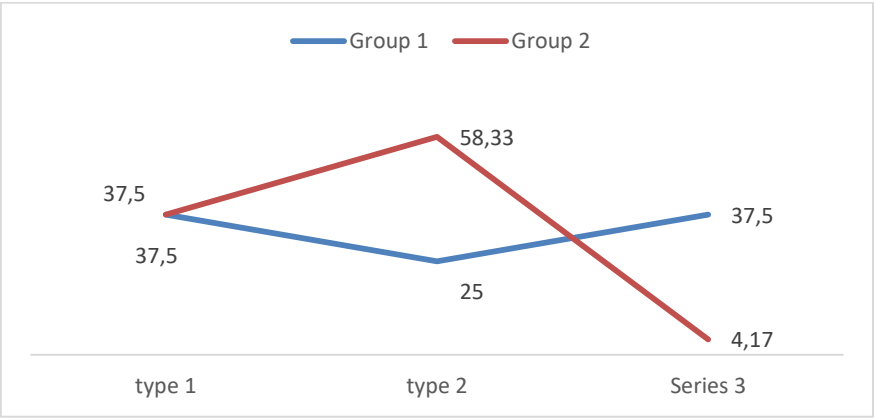


Fig. 4. Distribution of cases according to mandibular cortical index *vs.* groups

TABLE IV.

The estimated parameters in testing the association of the mandibular cortical index *vs.* groups

df=2	Chi-square χ^2	p 95% CI
Pearson Chi-square - χ^2	8.465609	0.01451
M-L Chi-square	8.839532	0.01204
Correlation coefficient (Spearman Rank R)	-0.298507	0.23613

The analysis of the edentulous region according to type of treatment reveals the fact that in the case of G1patients the edentulous region is frequently found anteriorly, while in the case of G2 patients the edentulous region is found in the posterior region (fig. 5). These results demonstrate the significant association between type of osteoporosis treatment and the edentulous region (r=0.793, χ^2=8.83, p=0.00221, 95%CI) (tab. V).

The analysis highlighted the fact that in the case of G2 patients the cortical bone density values (704.38±352.1 SD) are significantly higher (F=4.38, p=0.0430, 95% CI) compared to the values corresponding to G1 patients (896.86±230.8 SD).

In the case of G2 patients, the cancellous bone density values (738.96±259 SD) do not show significant differences (F=3.607, p=0.065, 95% CI) compared to the values corresponding to G1 patients (562±359 SD). Since the distribution of values is not normal in this case, the results of the analysis considered the Kruskal-Wallis test, which is specific to these types of data (tab. VI).

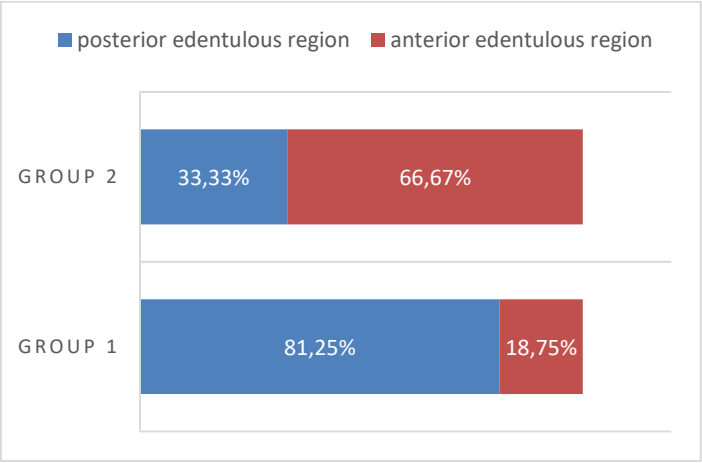


Fig. 5. Evaluation of the localization of mandibular edentation according to groups

TABLE V.

Estimated parameters in testing the association of mandibular bone density *vs.* groups

df=1	Chi-square χ^2	p 95% CI
Pearson Chi-square - χ^2	8.838763	0.00295
M-L Chi-square	9.356570	0.00222
Correlation coefficient (Spearman Rank R)	0.7931035	0.00221

TABLE VI.

The statistical indicators for the values of cortical bone density DOC (UH) and cancellous bone density (UH) *vs.* groups

	Groups	Value DOC/UH	Average		SD	SE	Min	Max	Q25	Median	Q75
			-95%	+95%							
DOC	G1	704.38	516.74	892.01	352.12	88.03	234.00	1298.00	345.50	775.50	983.50
	G2	896.87	799.41	994.34	230.81	47.11	569.00	1298.00	716.00	967.00	1016.50
UH	G1	562.0	386.7	737.3	329.0	82.2	209.0	1267.0	234.0	499.5	856.0
	G2	739.0	629.6	848.3	259.0	52.9	430.0	1256.0	444.0	856.0	890.0

DISSCUSION

This study investigated the mandibular bone by applying two different radiological techniques whose results were compared. His aim was to assess the quality and quantity of mandibular bone in a population of treated and untreated patients with osteoporosis, as the quality and quantity of mandibular bone is of particular importance in certain dental treatments.

Bones differ in anatomical structure, with a different distribution of trabecular and cortical bone. Trabecular bone has a larger surface area and responds more rapidly to metabolic changes than cortical bone (12). The changes that can occur in the thickness of the mandibular cortex and the occurrence of systemic osteoporosis at the level of the skeleton have been studied by numerous researchers (6).

Preoperative CBCT measurements will give the practitioner useful information on

bone quality and amount. Radiomorphometric indices developed for CBCT pictures were utilized for quantitative and qualitative examination of the mandible, and the findings obtained were significant for the study's goal.

In the case of G2 patients compared to G1 patients, the lower mandibular index reveals comparable values with no significant changes. The upper mandibular index showed no significant variations based on the group, with average values in all six groups.

The examination of the values of the right chin index based on treatment type reveals that patients with G2 had significantly greater values for both sides than patients with G1.

The findings revealed a substantial relationship between the mandibular cortex index and the kind of therapy, with G1 patients exhibiting the mandibular cortical index type 2 in 25% of instances and G2 patients exhibiting the mandibular cortical index type 2 in 58.33% of cases.

The examination of the edentulous region based on treatment type indicates that in the case of G1 patients, the edentulous region is usually located anteriorly, but in the case of G2 patients, the edentulous region is frequently found posteriorly. In our investigation, we discovered a strong relationship between the kind of osteoporosis therapy and the edentulous area.

Cortical bone density readings in G2 patients are substantially greater than values in G1 patients. There are no statistically significant differences in cancellous bone density values across groups.

The fact that the parameters obtained in the group of patients receiving osteoporosis therapy were lower than in the group of participants with osteoporosis but not receiving treatment may be explained through the lens of the kind of drug supplied and the course of osteoporosis. Several studies have been conducted to demonstrate the function of antiresorptive medicine in enhancing bone density

Thus, Bouxsein *et al.* (13) discovered increases in bone mineral density following raloxifene therapy, as well as a substantial reduction in fracture risk. According to Nils Heim *et al.*, (14) the impacts on bone density and microarchitecture are restricted to cortical bone and not cancellous bone. This is because bone remodeling is dependent on a balance of osteoclastic (bone-resorbing) and osteoblastic (bone-formation) activity. Antiresorptive drugs inhibit osteoclasts and reduce bone resorption by binding to active areas of bone remodeling. In the absence of therapy, bone density drops by 1.8% in the first 5 years after start, and the average N-telopeptide of cross-linked collagen type 1 is reduced by 70% below baseline, according to Bone *et al.*

The group sizes were one of the stud's limitations. More research with a bigger sample size, as well as an evaluation of the association between CBCT findings and BMD of other bone locations in osteoporosis patients, are required.

CONCLUSIONS

CBCT is a 3D type of imaging method used in dentistry. The CBCT approach gives adequate radiographic information to assist dentists and oral and maxillofacial surgeons in the early detection of mandibular osteoporosis. Furthermore, this study reveals that the density of mandibular bone is directly connected to changes in the density and, implicitly, the status of the skeleton in individuals with osteoporosis, as measured by bone osteodensitometry.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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INNOVATIVE SYNTHESIS OF ZINC AND SELENIUM COMPLEXES WITH GALLIC ACID: EXPLORING THEIR ANTIOXIDANT POTENTIAL

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INNOVATIVE SYNTHESIS OF ZINC AND SELENIUM COMPLEXES WITH GALLIC ACID: EXPLORING THEIR ANTIOXIDANT POTENTIAL (Abstract): Polyphenols are naturally occurring compounds found largely in fruits, vegetables, cereals and beverages. Fruits like grapes, apple, pear, cherries and berries contain up to 200–300 mg polyphenols per 100 grams fresh weight. Among these compounds, gallic acid, (GA), a trihydroxybenzoic acid, stands out for its selective cytotoxicity against various tumor cell lines. This study focuses on the synthesis and analysis of a new complex of Zn(II) with GA, but also Se(IV) with gallic acid, exploring its comparative antioxidant potential. **Materials and methods:** The complex, synthesized in a 1:2 molar ratio of GA to zinc acetate dihydrate, respectively sodium selenite pentahydrate, showed various promising therapeutic roles in vitro. GA and zinc both possess antioxidant properties, and their coordination in the complex increases these attributes depending on the concentration. **Results:** GA, known for its powerful antioxidant effects, outperforms popular antioxidants like ascorbic acid in various tests. The study evaluates the ability of GA to remove reactive species, showing its superior efficiency. In addition, GA and its ester derivatives demonstrate protective effects against lipid peroxidation, a important process in cellular damage. **Conclusions:** The synthesized Zn(II) complex, with its structure-activity relationship suggesting Zn(O4) coordination, opens avenues for understanding its antioxidant potential. Overall, this research reveals the multifaceted pharmacological potential of GA and its complexes, providing insights into their antioxidant effects. **Keywords:** METAL-ION-COMPLEX, GALLIC ACID-ZINC-COMPLEX, ANTIOXIDANT CAPACITIES.

Polyphenols, also known as phenolics, are ubiquitous in nature and represent a structurally diverse group of compounds, particularly prevalent in plant species (1, 2). Among them, condensed tannins, the most abundant polyphenols, are found across various plant families, including lower organisms such as mushrooms (3-6).

Certain polyphenols, such as phytoalexins produced by plants in response to pathogen intrusion, exhibit a hypersensitive reaction (7-10).

The presence of polyphenols can contribute to environmental contamination in proximity to processing facilities engaged in the production of olive oil, coffee, paper, and within the carpet industry. Conversely, numerous polyphenols are renowned for their antioxidative (11, 12), anti-inflammatory (13), and anticancer properties (14, 15), warranting attention in the realm of drug development, particularly for combating cancer (16, 17, 18, 19).

One prominent polyphenol of note is GA, (3,4,5-trihydroxybenzoic acid) (fig. 1), which holds significant relevance due to its selective cytotoxicity against various tumor cell lines.

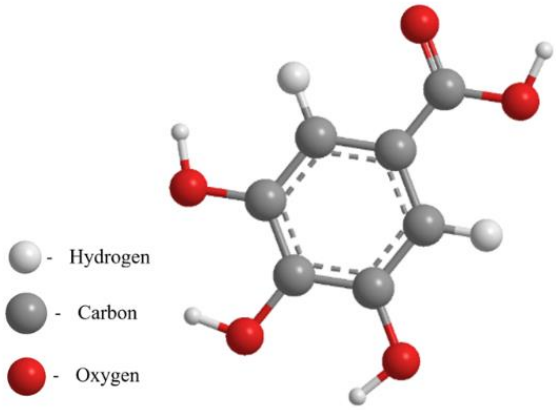


Fig. 1. 3D rendering of the GA molecule (made by using Chem3D)

GA is an organic compound belonging to the class of polyphenols. It is marketed as a crystalline powder, either white or pale yellow. It is a trihydroxybenzoic acid with a benzene ring in its structure, to which a carboxylic group providing acidity to the molecule (-COOH) is attached, along with three hydroxyl groups (-OH) located at positions 3, 4, and 5 of the ring (20, 21).

GA is an endogenous plant polyphenol abundant in tea, grapes, berries, and other fruits, as well as in certain hardwood species like oak (*Quercus robur*) and chestnut (*Castanea sativa* L.). It manifests as yellowish-white crystals, possesses a molecular mass of 170.12 g/mol, a melting point of 250°C, and exhibits water solubility of 1.1% at 20°C (22, 23).

Known for its profound impact on several pharmacological and biochemical pathways, GA demonstrates potent antioxidant, anti-inflammatory, antimutagenic, and anticancer properties (24, 25, 26). Moreover, GA exhibits pro-oxidant characteristics in a concentration-dependent manner in the presence of metal ions, a trait that has been identified as an inducer of apoptosis in cancer cell lines (27). Studies have also confirmed the protective role of GA against chemically induced carcinogenesis (28, 29).

MATERIALS AND METHODS

For the synthesis and analysis of the studied complex we have used the follow-

ing reagents and equipment:

- Polyphenol (GA) –analytical grade purity, purchased from Sigma-Aldrich;
- Metalic ion salts (Zinc acetate, Sodium selenite) Sigma-Aldrich;
- Solvents (Distilled water, Methanol, Absolute ethanol, 96% Ethanol) (Sigma-Aldrich);
- Magnetic stirring plate;
- Laboratory grade glassware (Erlenmeyer flasks, beakers, magnet for stirring, thermometer);
- Filter paper;
- Analytical balance Mettler (Mettler Toledo, Greifensee, Switzerland);
- Magnetic stirring FALC-30ST;
- spectrophotometer Specord® 210 PLUS

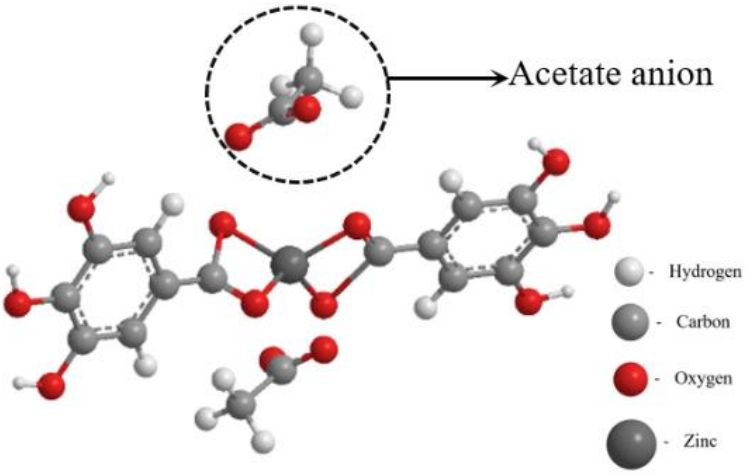


Fig. 2. 3D rendering of the synthesized Gallic acid-Zn(II) complex (made by using Chem3D)

INSTRUMENTATION

Instrumentation for the structural characterization of the formed complex involved Fourier-transform infrared spectroscopy (FTIR). The FTIR spectra were acquired using an FT-IR Bruker Vertex 70 Spectrophotometer in reflexional mode with the attenuated total reflectance (ATR) technique. Spectral analysis was conducted at a scan rate of 40/s within the range of 4000 cm-1 to 380 cm^{-1}, using approximately 1 mg of sample.

A ^{1}H NMR spectrum reveals the chemical shifts corresponding to various protons within the tested samples (30, 31).

The Nuclear Magnetic Resonance spectra ^{1}H-RMN, were registered using a Bruker Vance III apparatus, at 500 MHz in deuterated dimethyl sulfoxide (DMSO-d_6). The displaced chemical signals present in the registered spectra, were represented using δ values in parts per million (ppm), and the coupling constancies J in Hz.

Synthesis of the gallic acid and Zinc(II) complex

The complex was synthesized using zinc acetate dihydrate and GA in a 1 :2 molar ratio, our study has followed this method with slight alterations. The synthesis started by weighing the reagents and dissolving 376.3 mg of GA monohydrate

and 219.51 mg of zinc acetate dihydrate separately in 5 mL of methanol each. After this, the solutions were gradually stirred using a magnetic stirring plate. The complex, Fig. 2, formed as a white, almost milky gelatinous precipitate, which was separated by filtration.

The resulting precipitate was washed multiple times with a 50% methanol solution, dried and stored at standard room temperature in air-tight glass vials.

Synthesis of the gallic acid and Se(VI) complex

The complex was synthesized using sodium selenite and GA, our study has followed the method described in the literature with slight alterations (32). The synthesis started by weighing the reagents and dissolving 376 mg of GA monohydrate and 263 mg of sodium selenite pentahydrate separately in 5 mL of methanol each. After this, the solutions were gradually stirred using a magnetic stirring plate. The complex, formed as a dark grey precipitate which was separated by filtration.

The resulting precipitate was washed multiple times with a 50% methanol solution, dried and stored at standard room temperature in air-tight glass vials.

Zinc(II) exhibits a preferential tetrahedral coordination but can also adopt higher coordination geometries, such as octahedral. It tends to favor ligands that form relatively weak bonds, making zinc less prone to forming highly stable complexes with strongly chelating ligands compared to other transition metals. Zinc complexes are relatively stable and find applications in various pharmaceutical and industrial settings. Due to their moderate stability, zinc complexes are ideal for applications requiring controlled release of the ligand (33, 34).

In the complex with zinc(II) ions, bonds are formed with GA molecules. These bonds can be coordinative, where the oxygen atoms of the functional groups in GA bind to zinc ions. Both zinc and GA possess antioxidant properties. GA, among numerous plant-derived polyphenols, possesses both antioxidative and antidiabetic pharmacological potential (35, 36).

Antioxidant activity

The DPPH scavenging activity of the complex was measured using a modified version of a previously described method by Sanni O *et al.* (37). The assay mixture comprised 75 µl of various concentrations (ranging from 3.75 to 60 µM in the reaction mixture) of the complex, along with its precursor GA, zinc(II) acetate, sodium selenite (IV), or standards such as ascorbic acid, or their respective solvents (control). Additionally, 37.5 µl of a 0.3 mM DPPH solution was added to the mixture in a 96-well plate. Following a 30 minutes incubation period in the dark, absorbance readings were taken at 517 nm using a Specord® 210 PLUS spectrophotometer. Sample blank or sample solvent was utilized as the blank. The following formula was employed to calculate the percentage of DPPH radical scavenging activity:

$$\% \text{ inhibition} = 100 \times (A_{control} - A_{final}) / A_{control}$$

where:

$A_{control}$ is the absorbance of DPPH before adding the GA or ots complexes

A_{final} is the absorbance of the DPPH mixture after adding the GA or its complexes and incubating in a dark place.

The evaluation of the antiradical potential of antioxidants commonly employs the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, where DPPH, a stable free radical

with an absorbance band at 517 nm, undergoes reduction upon interaction with an antiradical compound (38, 39). The statistical evaluation was performed by determining the mean values of five readings for each sample and for each concentration used. The data included in the graphs represent the average ± the standard deviation.

RESULTS AND DISCUSSION

In our study, the most important assessed parameter was the nature of the solvent used for synthesis. It can be observed in Figure 3, that the superior yield was obtained when using methanol as the solvent for the complexation reactions. The polarity of the solvent and its capacity to form hydrogen bonds has an impact on the interactions between the metallic ions and the ligand used.

The confirmation of complex formation was achieved through ^{1}H-NMR spectroscopy in DMSO-d_6. The absence of the signal corresponding to the proton of the carboxylic group is observed at 13-12 ppm. The region between 10-9 ppm for both complexes undergoes slight modifications, with no change in the region of aromatic protons.

The structure of GA was confirmed through the ^{1}H-NMR proton spectrum in DMSO-d_6. The region at 13-12 ppm corresponds to the proton of the carboxylic group (-COOH), being the most shielded. The region of 10-9 ppm corresponds to the protons of the hydroxyl groups (-OH), and the region of 7-6.7 ppm is attributed to the aromatic protons in the benzene ring GA.

The characteristic bands of the phenolic hydroxyl group were observed in the FTIR absorption spectrum of GA and its complex in the range of 3200-3400 cm^{-1}, representing stretching vibrations due to intramolecular hydrogen bonds.

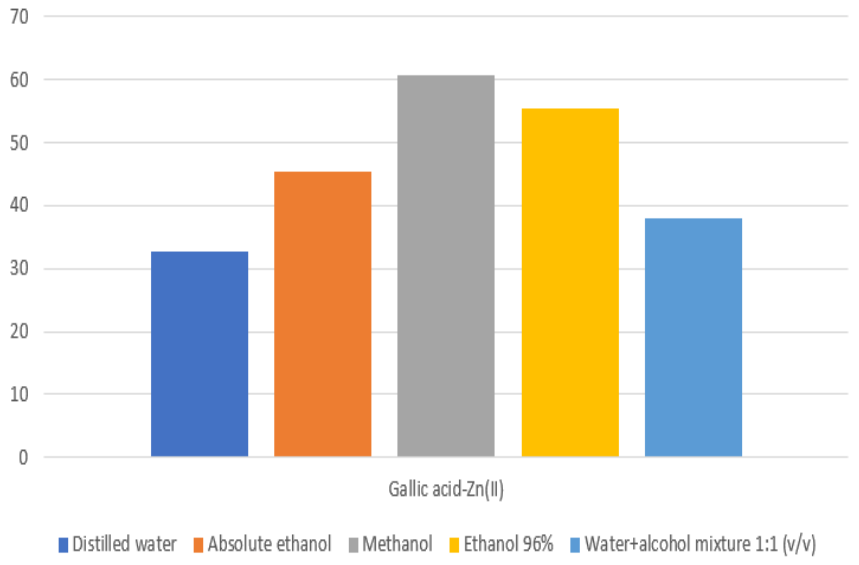


Fig. 3. The yield in relation to the solvent used

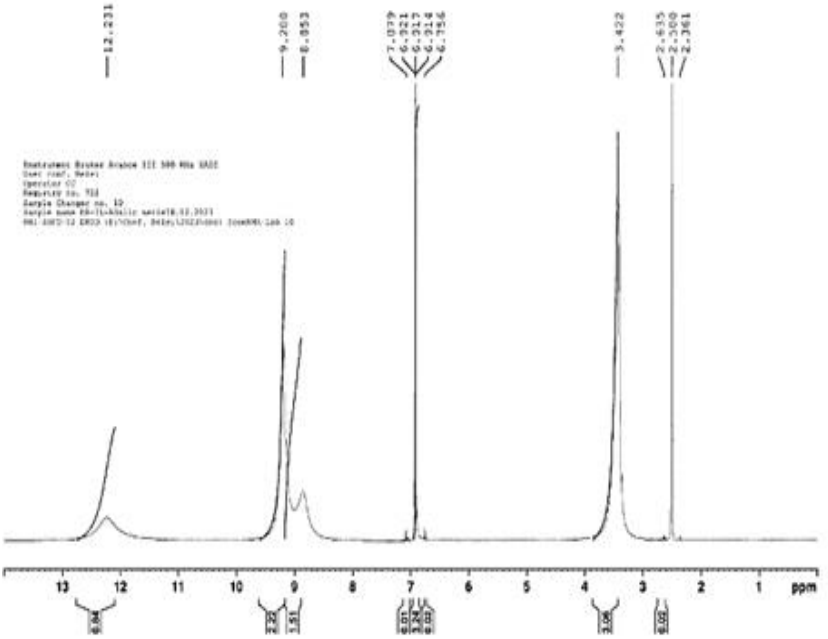


Fig. 4. ^{1}H-NMR spectrum of GA

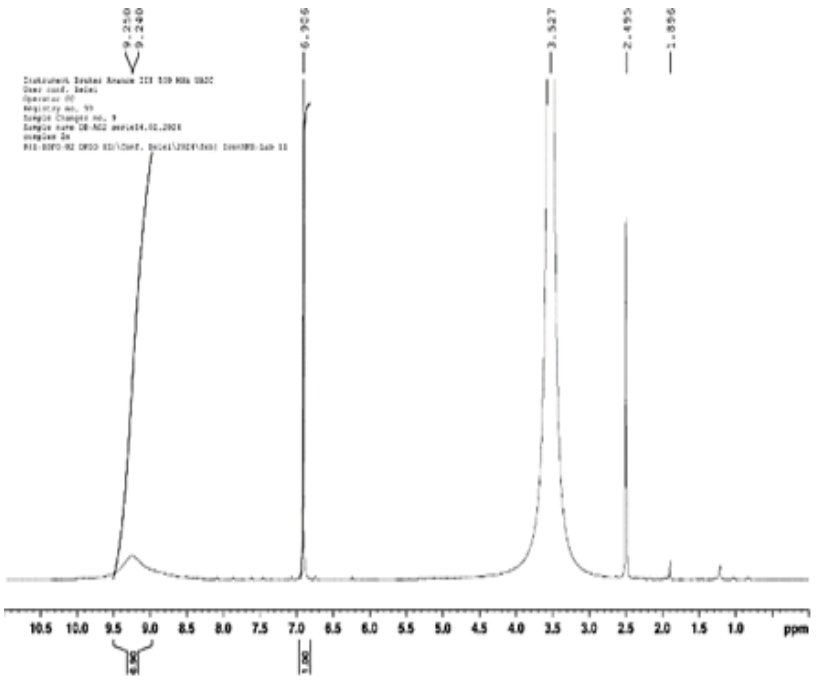


Fig. 5. ^{1}H-NMR spectrum of gallic acid-Zn(II)

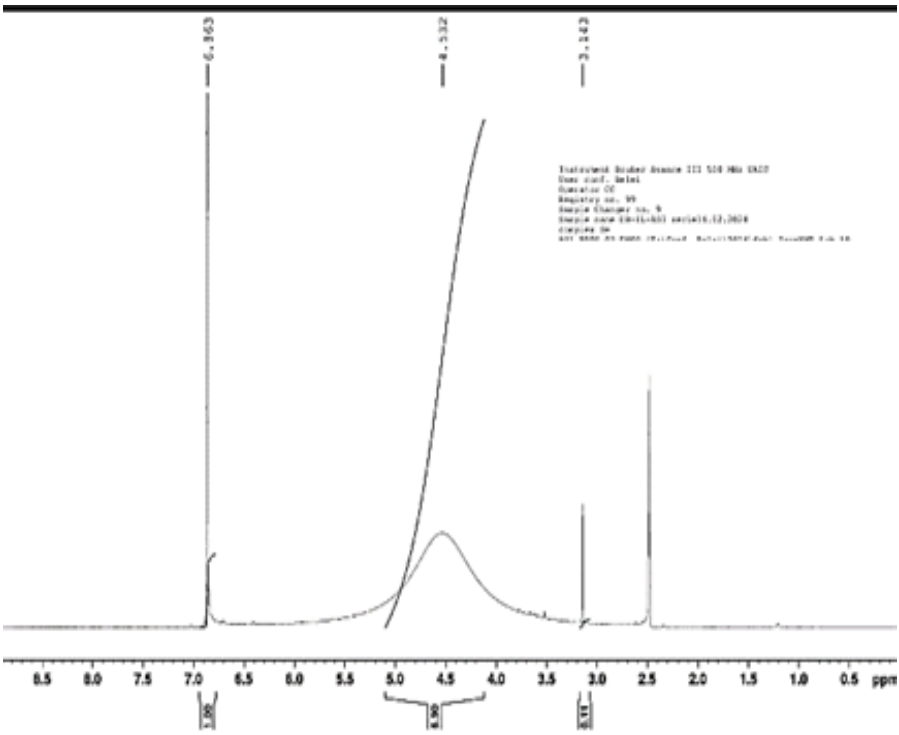


Fig. 6. ^{1}H-NMR spectrum of gallic acid-Se(IV)

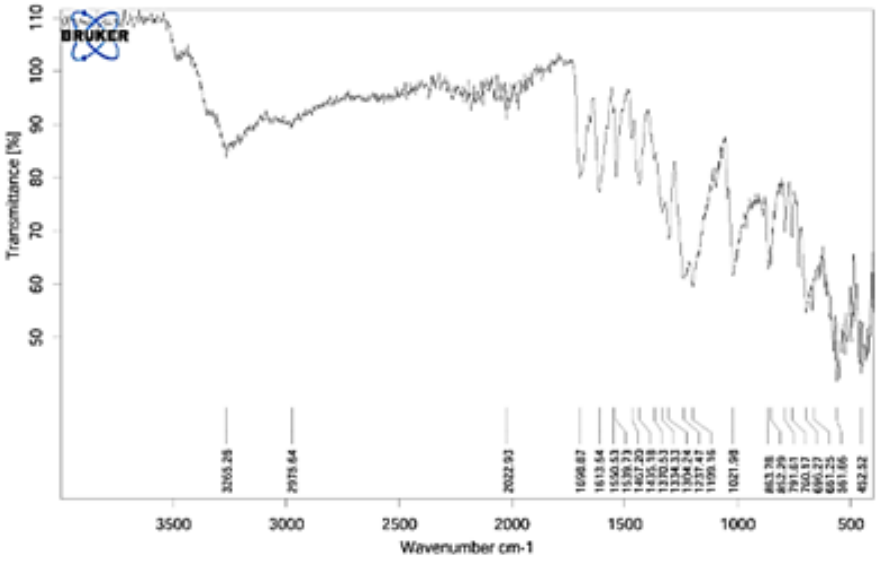


Fig. 7. FTIR absorption spectrum of GA

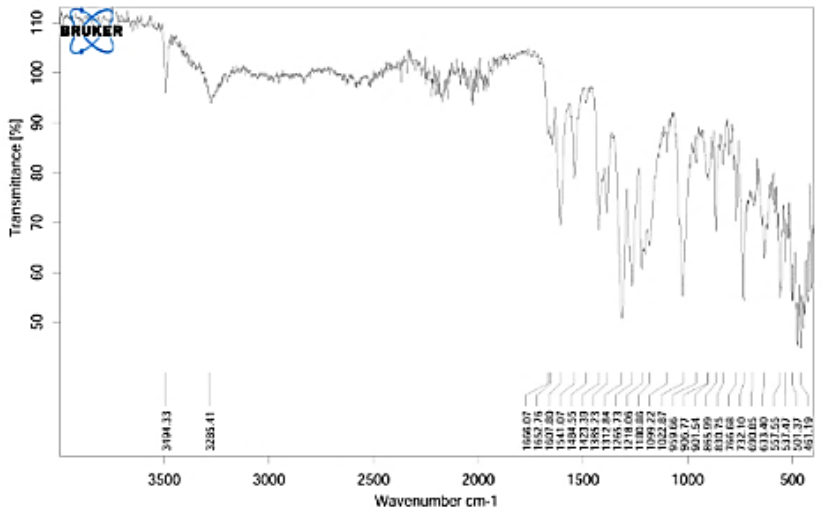


Fig. 8. FTIR absorption spectrum of gallic acid-Zn(II)

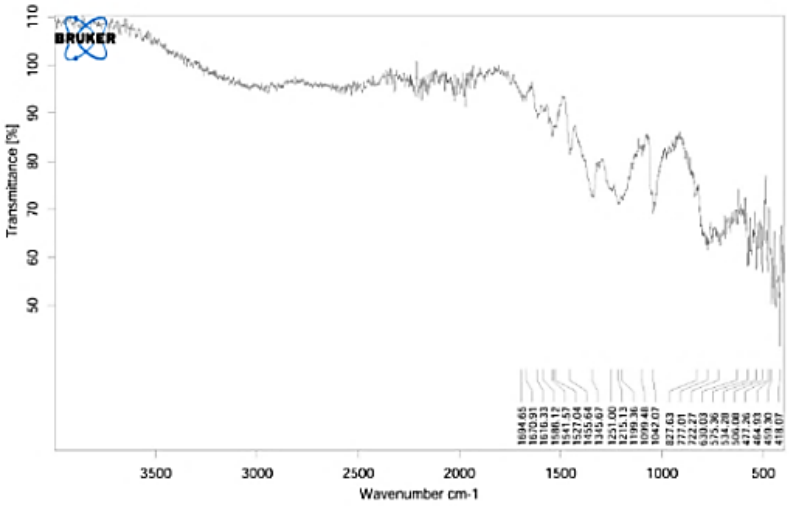


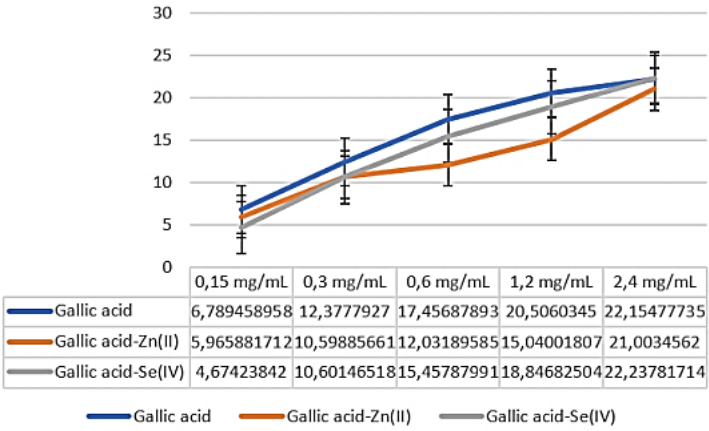
Fig. 9. FTIR absorption spectrum of gallic acid-Se(IV)

The presence and structure of the gallic acid-Zn(II) complex is confirmed by the presence of bands observed in GA at 1378 and 1306 cm^{-1} and in its complex with zinc acetate at 1374, 1336, and 1307 cm^{-1}, representing specific bending vibrations of aromatic nuclei. The band at 3085-3024 cm^{-1} is characteristic of the stretching vibration of the C-H bond in the aromatic ring. In the ranges of 2995-2863 cm^{-1} and 1648-1582 cm^{-1}, bands appear due to stretching vibrations of –CH3 groups and the >C=C< bond, respectively. Bending vibrations of the aromatic CH bond and the –CH3 group occur at

1458 cm^{-1} and 1357 cm^{-1}, respectively. Bands in the ranges of 1247-1000 cm^{-1} and 567-501 cm^{-1} are attributed to in-plane and out-of-plane deformation vibrations of the aromatic ring.

The presence and structure of the gallic acid-Se(IV) complex is confirmed by the presence of bands observed in GA at 1378 and 1306 cm^{-1} and in its complex with sodium selenite at 1345, 1251, and 1215 cm^{-1}, representing specific bending vibrations of aromatic nuclei. The band at 3085-3024 cm^{-1} is characteristic of the stretching vibration of the C-H bond in the aromatic ring, and in the range of 1648-1582 cm^{-1}, bands are present due to the >C=C< bond. Bending vibrations of the aromatic CH bond occur at 1458 cm^{-1} and 1357 cm^{-1}, respectively. Bands in the ranges of 1247-1000 cm^{-1} and 567-501 cm^{-1} are attributed to in-plane and out-of-plane deformation vibrations of the aromatic ring. The confirmation of complex formation can also be supported by the absence of vibration bands at 3263 cm^{-1} corresponding to the -OH groups.

The complex combinations of GA with zinc(II), selenium(IV), as well as free GA, were subjected to examination, and their antioxidant activity was tested through recorded absorbance.



	0,15 mg/mL	0,3 mg/mL	0,6 mg/mL	1,2 mg/mL	2,4 mg/mL
Gallic acid	6,789458958	12,3777927	17,45687893	20,5060345	22,15477735
Gallic acid-Zn(II)	5,965881712	10,59885661	12,03189585	15,04001807	21,0034562
Gallic acid-Se(IV)	4,67423842	10,60146518	15,45787991	18,84682504	22,23781714

Fig. 10. DPPH• % inhibition of gallic acid and its complexes with Zn(II) and Se(IV)

GA complexed with selenium ions exhibited the highest antioxidant activity at the highest concentration. However, it can be observed that at different concentrations, GA demonstrates stronger antioxidant action. The action also depends on the molar ratio used in synthesis, in this case, GA: zinc salt = 1:2. Referring to the structure of GA, the newly formed bonds resulted in a decreased antioxidant activity.

Comparing our results to the literature, the findings were confirmed by Samy M. and colleagues (40), who also obtained lower absorbance for free GA at maximum concentration and higher absorbance for the gallic acid-selenium(IV) complex. In another study conducted by Denice M. Motloung and colleagues (41), the gallic acid-zinc(II) complex recorded a higher inhibition percentage at concentrations of 15, 30, and 60 μM compared to free GA and ascorbic acid.

Under these conditions, chelating GA with selenium(IV) enhances the antioxidant activity of the polyphenolic acid, depending on the molar ratio, solvent, and the

concentration of the tested complex. The antioxidant properties of zinc acetate, used for synthesizing the complex with GA, were also tested, but the results obtained were very small, almost irrelevant for its use as an antioxidant.

An important aspect is that these metal ions can be used in combination with polyphenols, improving the antioxidant activity of the complex.

Studies by Abdelwahed *et al* (42) reveal that GA and 1,2,3,4,6-pentagalloylglucose (PGA) employ a hydrogen-donating mechanism to scavenge DPPH radicals, proving more effective than Vitamin E. The antioxidant activity order, ranks GA above caffeic acid, ascorbic acid, Trolox, sinapinic acid, and isoeugenol (43).

Beyond the DPPH assay, GA excels as a potent antioxidant in various assays such as Trolox equivalent antioxidant capacity, total radical-trapping antioxidant parameter (TRAP), photochemiluminescence (PCL), and ferric reducing ability of plasma (FRAP). It outperforms popular antioxidants like ascorbic acid, Trolox, and uric acid. Computational studies further confirm GA's superior antioxidant efficiency compared to various counterparts. At a concentration of 4.17 mM, GA exhibits significant scavenging effects on DPPH and H_2O_2 radicals. GA and n-alkyl gallates effectively scavenge other reactive species, HOCl, at low concentrations, protecting a1-antiproteinase and reducing ox brain phospholipid peroxidation (44, 45).

Moreover, GA and its ester derivatives showcase antioxidant capacity against various reactive oxygen and nitrogen species, including peroxyl, azide, and hydroperoxyl radicals (46, 47).

CONCLUSIONS

The complex produced by the combination of zinc acetate and GA resulted in good yield when methanol was used as solvent and NaOH as co-regulator. The same mechanism was in the case of the reaction between GA and sodium selenite.

Various modern techniques have confirmed the formation of complex combinations. Moreover, the antioxidant capacity did not show very large variations in the case of the tested compounds, the activity being dependent on the concentration of the sample to be analyzed.

It can be concluded that the antioxidant action is much better for the complex with zinc or selenium ions compared to pure salt.

Other antioxidant tests are needed to describe a more complex mechanism of the complex combination.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest and no funding regarding this scientific research.

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THE PREVALENCE OF IMPLANT SUCCESS *VERSUS* FAILURE IN A ROMANIAN SAMPLE POPULATION. A RETROSPECTIVE STUDY

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THE PREVALENCE OF IMPLANT SUCCESS VERSUS FAILURE IN A ROMANIAN SAMPLE POPULATION. A RETROSPECTIVE STUDY (Abstract**):** Research has revealed that patterns of dental implant failure often occur in groups within individuals and are linked to various factors such as dietary preferences, intake of cold/hot beverages. **Materials and methods**: this research was to investigate the potential correlation between changes in the frequency of dental implant loss due to consumption of hot/cold beverages and dietary habits. Information was gathered from the patient records of individuals seeking implant-prosthetic treatments at the "M. Kogălniceanu" Dental Education Base. **Results:** Out of the examined database, 47 cases were chosen based on the selection criteria. The average age of the study group was 41.6 ± 5.051 years (with a minimum age of 27 years and a maximum age of 49 years). Over half of the participants were male. It has been noted that the intake of solid foods is linked to dental implant loss, showing a positive but moderate level of association (r=0.573). In terms of the impact of consuming hot beverages on the lifespan of implants, the statistical analysis suggests a positive relationship between the two factors, albeit with a relatively weak correlation (r=0.382). **Conclusions:** It is recommended that additional research be conducted to identify the possible risk of implant failure associated with the regular intake of extremely hot beverages, which patients rarely report. **Keywords:** DENTAL IMPLANT, DIETARY HABITS, TEMPERATURE.

INTRODUCTION

In recent years, technological advancements and research in the field of biomechanics have brought about a series of innovations in the realm of implant-prosthetic restorations (1). From new materials and computer-aided design techniques to improvements in implant placement and integration, these new discoveries have revolutionized the way these restorations are planned, fabricated, and implemented (2, 3).

The introduction of advancements in the field of biomechanics applied to implant-prosthetic restorations represents an essential approach to understanding the complexity of interactions between implants, bone, soft tissues, and restoration as a whole (4, 5).

The biomechanics of implant-prosthetic restorations involves the study of forces and stresses acting on these structures during normal oral cavity function (6). It is crucial to understand how these forces are distributed and how they can be managed to prevent restoration failures and ensure optimal long-term functionality (7, 8). From the initial conception of a treatment

plan to the completion of the restoration, bio-mechanical aspects must be considered at every stage of the process (9-11).

Nevertheless, there are certain drawbacks to this treatment approach. Various studies have shown that dental implants have failure rates anywhere from 1% to 19% (12, 13). The abutment connection time determined whether the failure occurred before or after functional loading. In the former case, the failure occurred before occlusal loading or the first removal of the provisional restoration, while in the latter case, the failure occurred after (14).

A dental implant is considered to have failed early if osseointegration cannot be achieved, and a dental implant is considered to have failed late if either osseointegration or function cannot be maintained (15). In early failure, there are just biologic difficulties (16), but in late failure, there could be mechanical as well as biologic issues. Resorption of both soft and hard tissues is a common symptom of peri-implantitis, which can lead to biologic problems (17, 18). Inadequate implant loading design is a potential source of mechanical problems, which can cause the implant body, screw body, or implant suprastructure to fracture (19).

The aim of this study was to find the existence of a link, between the increase/decrease the frequency of losing dental implants because of the intake of hot/cold beverages and dietary preferences.

Thus, patients, doctors, and prosthodontists/restorative experts may all benefit from a better understanding of the causes of late dental implant failure in order to better anticipate treatment results and avoid disagreements.

MATERIALS AND METHODS

The retrospective study aimed at detecting correlations between dental implant loss and the presence of certain risk factors. Data were collected from the patient records of those who presented at the "M. Kogalniceanu" Dental Education Base for implant-prosthetic treatments.

The selection criteria for the clinical cases were as follows: patients who lost one or more implants due to peri-implantitis, without associated systemic diseases, without dental trauma, without periodontal disease, non-smokers, and patients aged between 25 and 50 years.

From the patient's record, data were extracted regarding the patient's age, gender, origin environment, number of implants lost, their location, the type of prosthesis made on these implants, the duration of their survival on the arch, the type of opposing arch, the type of diet (consistency), as well as the consumption of hot beverages, factors that can affect the integrity and persistence on the arch of the implants.

To achieve the purpose of this study, associations between the number of implants lost and the type of diet and beverages consumed were analyzed.

The statistical analysis was conducted using *SPSS 26.0* (IBM, NY, USA). The results of the statistical analysis were presented in the form of mean values and frequencies. Statistical differences between genders and origin environments were analyzed. Pearson's correlation coefficient was used to analyze the correlations between variables. The level of statistical significance was set at $p=0.05$.

RESULTS

From the analyzed database, 47 cases were selected that met the selection criteria. The study group had an average age of 41.6±5.051 (min. age - 27 years, max. age = 49 years). More than half of the participants were males (62.2%), with 82.2% of participants coming from an urban environment (tab. I).

TABLE I.
Demographic characteristics of the study group

	No	%
Age	41.6±5.051 (min. age - 27 years old, max. age = 49 years old)	
Sex		
Females	17	37.8
Males	28	62.2
Place of residence		
Urban Area	37	82.2
Rural	8	17.8

In Table 2, information is presented regarding the distribution of cases based on demographic characteristics and the status of the lost implants. 63.8% of cases lost 2 implants, with more than half of these having a survival period on the arch of 6-10 years (59.6%). 61.7% of the implants were overdenture with removable prostheses, the implants being placed predominantly in the lateral areas. The type of opposing arch was 40% natural or mixed (31.9%). The majority of lost implants were recorded in male subjects from urban areas, many of the implants having a survival period on the arch of 6-10 years.

TABLE II.
Distribution of cases

			Gender		Residence	
	No.	**%**	**Female**	**Male**	**Urban**	**Rural**
Number of implants						
1 implant	17	36.2	35.3%	64.7%	88.2%	11.8%
2 implants	30	63.8	36.7%	63.3%	80.0%	20.0%
p			0.591		0.385	
Number of years since implantation						
<5 years	11	23.4	45.5%	54.5%	90.9%	9.1%
6-10 years	28	59.6	28.6%	71.4%	85.7%	14.3%
>10 years	8	17.0	50.0%	50.0%	62.5%	37.5%
p			0.412		0.222	
Type of denture						
Dental bridges	18	38.3	90.9%	9.1%	88.9%	11.1%
Removable denture	29	61.7	85.7%	14.3%	79.3%	20.7%
p			0.107		0.334	
Type of opposing arch						
natural arch	19	40.4	26.3%	73.7%	84.2%	15.8%
mixed arch	15	31.9	46.7%	53.3%	86.7%	13.3%
artificial arch	13	27.7	38.5%	61.5%	76.9%	23.1%
p			0.462		0.778	
Implant location						
frontal area	17	36.2	35.3%	64.7%	82.4%	17.6%
lateral premolar area	15	31.9	53.3%	46.7%	80.0%	20.0%
Lateral molar area	15	31.9	20.0%	80.0%	86.7%	13.3%
p			0.164		0.885	

The statistical analysis results of the associations between the number of lost implants and the dietary preferences of participants are presented in table III. It is observed that the consumption of foods with a hard consistency is associated with the loss of dental implants, with a positive but moderate intensity of association (r=0.573); however, the differences in the number of implants lost are statistically insignificant (p=0.840). Regarding the effect of consuming hot beverages on the survival period of implants, the statistical analysis indicates a positive association between the two variables, though the correlation is relatively weak (r=0.382).

TABLE III.
Correlations between number of lost implants and dietary preferences

	No of lost implants		p	r
	1 implant	2 implants		
Dietary preferences				
Soft food	5.9%	3.3%	0.840	0.573
Hard food	82.4%	80.0%		
Both	11.8%	16.7%		
Beverage preferences				
Cold beverages	23.5%	13.3%	0.306	0.382
Hot beverages	76.5%	86.7%		
Frequency of hard food consumption				
daily	88.2%	90.0%	0.603	0.854
occasionally	11.8%	10.0%		
Frequency of hot/cold beverage consumption				
daily	76.5%	83.3%	0.417	0.875
occasionally	23.5%	16.7%		

Significant positive correlations were recorded between implant loss and the frequency of daily consumption of hard foods and hot beverages. Thus, participants who consumed hard foods or hot beverages daily lost more implants compared to those who consumed them occasionally (r=0.854 for hard foods and r=0.875 for hot beverages).

DISCUSSION

Systemic diseases including diabetes and osteoporosis are more common among older individuals seeking implant rehabilitation. The stability of osseointegration could be jeopardized if these illnesses negatively impact bone metabolism. Because we aimed on the dietary habits of the patients, we excluded from the study this type of patient, in order not to interfere with the results.

Dental implants can only last if the bone is healthy and the place is prepared correctly. Research has linked factors like cigarette use to clustering of dental implant failure patterns within participants. It is now well acknowledged that the bone should sustain minimum mechanical and thermal damage when the location is prepared for an implant. No research in Romanian literature has examined temperature variations in and around implants during normal oral functions, despite the fact that heat-induced bone injury may happen during uncontrolled drilling or acrylic polymerization near an implant abutment.

Exposure to heat, in terms of both tem-

perature and time, is the determinant of thermal damage. There has been reports of hyperemia at 40°C. At 42°C, proteins such procollagen and collagen I, which are essential in bone remodeling. Additionally, exposing cortical bone to 47°C for one-minute produces necrosis (20-23).

The oral cavity temperature can rise to 67°C16 and even 76°C to 77°C after drinking hot beverages, according to previous research (24, 25).Ex vivo simulations of implanted bovine mandibles showed that the highest temperatures recorded at the implant-bone interfaces were higher above the temperature threshold for transitory alterations in bone, which is 42°C (24). The importance of minimizing mechanical and thermal stress on the bone during the preparation of the implant site has recently been acknowledged.

A significant risk factor for late failure in the oral history is a history of periodontitis. A need for implant rehabilitation might be prompted by periodontitis, which is a leading cause of tooth loss. Not only that, but a prior review found that periodontitis is a risk factor for peri-implantitis, which in turn increases the likelihood of late failure (24). Inflammation around implants, known as peri-implantitis, is strongly linked to a patient's history of periodontitis, according to research by Jemt *et al.* (25). The transfer of periodontal bacteria from natural teeth to the implant may be the cause of this impact (26). Although peri-implantitis is an indication of late failure, only one study (11) looked at implant loss and not the correlation between a periodontitis diagnosis and late failure.

It is intriguing to note that according to two investigations, every individual who had a late loss had also suffered an early loss (25, 27). There was no statistical study done, however while treating patients who have experienced early implant loss, doctors should keep in mind that there is a substantial association between early and late implant loss.

While some studies have shown a link between posterior implant placement and late failure (17, 28, 29), others have failed to find such a correlation (30, 31). The reason behind the substantial association result could be because the occlusal forces on the back teeth are three times larger than on the front teeth (32). There is evidence that dental plaque accumulates more readily in posterior regions than in anterior ones, and that this buildup causes inflammation of the gums and the beginning of various oral disorders, all of which can contribute to the failure of dental implants (33).

The impact of maxilla implant site on late failure is debatable, despite the fact that it is known to increase the likelihood of early failure (34). Some research have suggested that implants put in the mandible (25, 35) or the maxilla (29) could increase the likelihood of late failure, while the majority of studies have shown that implant placement site does not significantly affect late failure (30, 31, 36).

Implant implantation in the maxilla may be associated with late failure because the cortical bone is thinner and the trabecular bone is less dense compared to the mandible (37, 38).

A decreased initial stability due to weakened bone structure increases the likelihood of dental implant failure in the long run (25). At the same time, it is not known why implants placed in the mandible are associated with late failure.

CONCLUSIONS

Osseointegration is the most important factor in the success of dental implant therapy. While dental implants are a reliable and popular option for individuals who are missing teeth, there is a risk that the high temperatures used to place the implants could harm the surrounding tissues and organs, leading to

unfavorable consequences. To sum up, we have tackled the intriguing issue of dental implants experiencing temperature variations and transitory heat transfer when hot liquids are consumed in this work.

According to our research, one possible cause of clustered implant failures is the regular drinking of very hot/cold drinks. It is possible to warn patients about the risk of harm from prolonged exposure to temperatures greater than 42°C or even 47°C at the interface between osseointegrated implants and bone, even if this risk has not been adequately studied.

Further clinical studies are required to determine whether the habitual consumption of hot food and beverages might be considered a risk factor in the success of implant-supported restorations.

It is recommended that additional research to be conducted in order to identify the possible risk of implant failure associated with the regular intake of extremely hot beverages, which patients rarely report.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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NUTRITIONAL HEALTH IN ADULTS WITH DOWN SYNDROME LIVING IN URBAN NORTH-EASTERN ROMANIA

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NUTRITIONAL HEALTH IN ADULTS WITH DOWN SYNDROME LIVING IN URBAN NORTH-EASTERN ROMANIA **(**Abstract**):** Down syndrome is a genetic disorder typically linked with developmental delays, mild to moderate intellectual disability, and distinctive physical features. With a high prevalence of overweight and obesity, weight management strategies designed for the general population may not be suitable due to physiological and cognitive disparities in adults with DS. Weight issues in individuals with DS often stems from excessive calorie intake and inadequate physical activity, underscoring the importance of addressing dietary patterns. **Materials and methods:** We conducted a cross sectional study in the Cardiovascular Rehabilitation Clinic at Iasi Clinical Rehabilitation Hospital between July 1, 2022, and February 1, 2024. The study included 28 patients diagnosed with DS. We used SGA to assess nutritional status. **Results:** Among the patients, 25% were overweight and 39.3% were obese. Most obese individuals were from single-parent families (72.2%), performed minimal physical activity (81.81%), and exhibited poor dietary habits. In contrast, the overweight group had a more balanced diet, and superior hand grip strength, even outperforming the normal weight group. The obese group was the only one where we found scores of SGA over 9 points indicating an immediate need for nutritional intervention. **Conclusions**: Healthcare providers must address the unique challenges faced by adults with DS, including health conditions, feeding difficulties, and barriers to physical activity, while recognizing the critical role and stress experienced by caregivers. Despite the heightened risk of obesity, effective weight management is achievable with proper screening and intervention. **Keywords**: DOWN SYNDROME, NUTRITIONAL HEALTH, OBESITY.

Historically, weight gain was seen as a sign of health and prosperity. However, with industrialization and better living standards, obesity has become a major global health concern, affecting both children and adults (1). Obesity is now recognized as a leading cause of death, surpassing traditional health issues like infectious diseases (2). Despite advancements in diet, exercise, and treatments, obesity rates continue to rise, posing significant public health challenges (3).

The concept of genetic susceptibility to obesity was introduced in the early 20th century. Advances in genetics have since clarified specific forms of obesity linked to genetic defects (4).

BMI is commonly used to classify obesity in adults, defined by a value of 30 kg/m² or higher. However, BMI does not differentiate between muscle and adipose tissue. Waist circumference is often used as a practical measure of abdominal fat and related health risks (5, 6).

The International Association for the Scientific Study of Intellectual Disabilities (IASSIID) highlights the impact of obesity on individuals with intellectual disabilities, including Down Syndrome (DS) (7). DS is a genetic disorder characterized by intellectual disability and distinct physical traits, which currently has a life expectancy of around 60 years. (8, 9).

Recent studies show that adults with DS are at a higher risk of obesity due to factors like slower metabolism and low muscle tone, combined with challenges in maintaining healthy habits. Nutritional deficiencies and thyroid dysfunction are also more common in this group, requiring careful dietary management (10, 11). While general nutritional strategies apply, assessing nutritional status in adults with DS remains challenging due to limited research (12).

To our knowledge, this study represents the first comprehensive evaluation of the nutritional status within this particular population, addressing a significant gap in the literature, as no prior research has been conducted on the nutritional health of adults with Down syndrome in Romania.

MATERIALS AND METHODS

Study design

We conducted a descriptive cross-sectional study at the Cardiovascular Rehabilitation Clinic of the Iasi Clinical Rehabilitation Hospital between July 1st, 2022, and February 1st, 2024. The study cohort comprised 28 patients diagnosed with DS through genetic testing, selected from a total of 505 patients admitted to the clinic. Inclusion criteria for the study were: age over 18 years, a confirmed diagnosis of DS through genetic testing, and provision of informed consent. There were excluded: patients with recent surgical procedures, neoplasia, and those who were unable to complete the proposed assessments.

All participants were evaluated using a standardized clinical protocol by a single investigator. Demographic and clinical data included age, gender, body mass index (BMI), abdominal and calf circumference and handgrip strength. Additionally, data on family support, and whether the patient was from a single-parent household were gathered. The anamnesis also covered dietary habits, meal planning, and water intake.

The Subjective Global Assessment (SGA) is a widely validated tool for assessing malnutrition, combining clinical judgment with a detailed nutrition history and physical exam. It evaluates nine parameters: weight changes, food intake, gastrointestinal symptoms, functional capacity, comorbidities, subcutaneous fat, muscle loss, BMI, and serum albumin. Scores of 0-1 require reassessment, 2-3 suggest patient education, 4-8 call for dietary intervention, and scores over 9 need immediate nutritional intervention (13, 14).

Muscle strength was evaluated using an EH101 electronic dynamometer. Handgrip strength was measured with one familiarization trial and one test trial for both hands,

assessing maximal contraction over 4 seconds with verbal encouragement. We applied cut-off values from the European Working Group on Sarcopenia in Older People (EWGSOP): <30 kg for men and <20 kg for women.

Informed consent was obtained from all participants prior to their inclusion in the study. The study protocol received approval from the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iasi (208/9.07.2022) as well as the Iasi Clinical Rehabilitation Hospital (13/22. 06. 2023).

Data analysis

Statistical analysis was performed using SPSS version 20.0 (Statistical Package for the Social Sciences, Chicago, IL, USA). Variables that followed a normal distribution were reported as mean values with standard deviations and compared using the Student's t-test. For variables that did not follow a normal distribution, data were presented as medians with interquartile ranges and analyzed using the Mann-Whitney U test. Statistical significance was determined at a threshold of $p \leq 0.05$ for all analyses.

RESULTS

Our study included 28 patients, with a mean age of 28.07±9.51. All lived in urban area, were non-smokers and had family support.

As indicated in first table i, 25% of the patients were classified as overweight, and 39.3% as obese, predominately men. Within the obese group, 72.2% were from single-parent households and exhibited low physical activity levels (81.81%).

TABLE I.
General characteristics of the study group

	Normal Weight (n=10)	**Overweight** (n=7)	**Obese** (n=11)	p
Gender				
- Women	4 (40%)	3 (42.85%)	4 (36.36%)	0.96
- Men	6 (60%)	4 (57.15%)	7 (63.63%)	
Age	27.80±11.82	25.57±5.62	29.90±9.56	0.65
Environment				
- Urban	10 (100%)	7 (100%)	11 (100%)	-
- Rural	0 (0%)	0 (0%)	0 (0%)	
Smoker	0 (0%)	0 (0%)	0 (0%)	-
Physical activity				
- Minimal	5 (50%)	6 (85.71%)	9 (81.81%)	0.06
- Moderate	5 (50%)	1 (14.28%)	2 (18.19%)	
Family support	10 (100%)	7 (100%)	11 (100%)	-
Single parent family	5 (50%)	4 (57.15%)	8 (72.72%)	0.58

Obese adults with DS included in the study demonstrated poor dietary habits, including frequent fast food consumption (81.81%), low water intake (90.90% con-

suming only 500-1000 ml/day), and inadequate protein intake (only 36.36% regularly eating meat and 9.10% consuming fruits/vegetables more than twice a week or dairy daily). Hence, SGA scores above 9 points were found only in the obese group.

The mean BMI for the overweight group was towards the lower end of the range. In addition, they had the lowest mean SGA value (3.71 points) (tab. II).

TABLE II.
Physical and nutritional evaluation of the study group

	Normal Weight (n=10)	**Overweight** (n=7)	**Obese** (n=11)	p
Calf circumference (cm)	32.2±1.87	36.21±1.21	42.68±4.33	<0.00001
Abdominal circumference (cm)	85±9.93	91.57±6.79	114.77±12.47	<0.00001
Body Mass Index (kg/m^2)	23.17±1.93	26.77±1.14	38.69±8.52	<0.0001
Handgrip strength (kgf)				
- Dominant hand	14.70±2.89	15.21±4.14	13.90±2.76	0.68
- Nondominant hand	14.41±3.62	15.28±5.34	13.64±3.33	
SGA	4.5±2.01	3.71±1.60	5.90±3.44	0.21
SGA				
- 0-1	0 (0%)	1(14.28%)	0 (0%)	-
- 2-3	4 (40%)	1 (14.28%)	3 (27.27%)	0.53
- 4-8	6 (60%)	5 (71.42%)	6 (54.54%)	0.79
- 9 and above	0 (0%)	0 (0%)	2 (18.19%)	-
Number of meals (per day)	2.4±0.51	2.71±0.48	2.63±0.50	0.40
Milk/Cheese (daily)	8 (80%)	6 (85.71%)	1 (9.10%)	0.0001
Eggs (at least 1 per week)	10 (100%)	7 (100%)	11 (100%)	-
Meat (daily)	7 (70%)	3 (42.85%)	4 (36.36%)	0.30
Fruits/vegetables (>2/week)	3 (30%)	3 (42.85%)	1 (9.10%)	0.26
Fast-food	0 (0%)	1 (14.28%)	9 (81.81%)	-
Water intake				
- < 500ml/day	0 (%)	0 (%)	0 (%)	0.009
- 500-1000ml/day	4 (40%)	2 (28.57%)	10 (90.90%)	
- > 1000ml/day	6 (60%)	5 (71.43%)	1 (9.10%)	

In terms of lipid profile, we observed statistically significant differences in mean LDL cholesterol values. Although not statistically significant, it is worth mentioning that the obese group had the highest average of triglycerides, TSH levels and accentuated inflammatory status (tab. III).

TABLE III.
Biological parameters of the study group

	Normal Weight (n=10)	Overweight (n=7)	Obese (n=11)	p
Hemoglobin (mg/ dl)	14.30±1.12	14.37±1.40	14.38±1.08	0.98
Cholesterol (mg/ dl)	181.70±37.96	215.85±44.63	204.72±30.02	0.16
HDLc (mg/ dl)	59.08±10.42	56.31±22.04	58.57±8.10	0.90
LDLc (mg/ dl)	103.74±25.64	129.68±42.32	134.40±31	0.09
Triglycerides (mg/ dl)	100.78±30.89	88.14±26.21	117.99±33.12	0.14
Total proteins g/ l	7.34±0.45	7.38±0.90	7.25±0.48	0.89
Glucose (mg/ dl)	98.69±9.89	98.85±12.36	96.26±8.65	0.81
TSH (µUI/ml)	3.90 (2.67;6.51)	4.34 (2.53;6.09)	8.24(3.31;9.09)	0.49
ESR (mm/ h)	8 (5;17.75)	10 (7;14)	12 (7;26)	0.37
eGRF (ml/ min/1,73 m^2 sc)	76.45±9.41	81.68±13.94	87.16±13.42	0.15

DISCUSSION

Calories intake versus number of meals

The results in our study show that obese individuals have fewer meals per day compared to those who are overweight. One possible explanation is that meals, even few in number, are much higher in calories.

Our findings are consistent with those in the literature. In a Spanish cross-sectional study of 23 adults with DS, researchers used a consumption frequency questionnaire and the Mediterranean Diet Prevention Questionnaire to qualitatively assess dietary patterns. The study showed low intakes of vegetables, fruits, and dairy products, while the consumption of meat and sweet snacks were notably high (more than twice a week) (15).

Dratwa *et al.* (16) described in a study from 2023, the dietary habits of children and young adults with DS, pointing the importance of parental dietary mistakes. The study found that 62.6% of children had no nutritional restrictions, but some parents opted for a gluten-free (26.2%), lactose-free (28.7%), or no sugar diet (13.3%). The study concluded that while most children with DS have a balanced diet, parents often make nutritional errors.

Glucose levels

The results of the present study showed a mean glucose levels within normal ranges across the entire study group. Nevertheless, the mean glycemic index was notably lower in obese patients. Accordingly, a study conducted by Braunschweig *et al.* (17) in the United States (U.S.) while assessing the nutritional status of 48 adults with Down syndrome, revealed that 89% of participants were either overweight or obese, 54% had large waist circumferences, and none met the recommended guidelines for fruit and vegetable intake. Despite these findings, average glucose levels remained within normal ranges, and the prevalence of hypertension, elevated lipids, and glucose levels was lower compared to the general U.S. population.

Lipid profile

When analyzing the lipid profile, an intriguing paradox emerges: triglyceride levels exhibit the lowest mean values in overweight patients, while total cholesterol levels are highest among this group compared to the other patient categories. Overall, the only parameter that consistently

increases with weight is LDL cholesterol.

In an attempt to understand what leads to possible changes in the lipid variables of people with DS, some hypotheses were raised.

Excess weight and body fat, especially in the abdominal region, common among people with DS, may possibly interfere with lipid levels. Ordonez-Munoz *et al.* (18) found significant correlations between anthropometric variables (BMI, AC) and the lipid profile of young people with DS. However, Asua *et al.* (19) found that adult participants with abdominal obesity, did not present total cholesterol, LDLc, HDLc or triglyceride values different from those who were not identified with excess abdominal fat.

The understanding of thyroid function and its link to obesity in adults with DS is limited due to the scarcity of long-term follow-up data. As to the alterations in the lipid profile of the patients included in our study, hypothyroidism may also be related to changes in cholesterol, especially increased LDL. Studies and scientific papers have demonstrated that reduced thyroid hormone levels lead to decreased synthesis and expression of LDL receptors resulting in high serum levels of LDL and, consequently, increased cholesterol levels. (20).

Subjective Global Assessment

Findings in our study reveal that overweight patients had the best mean SGA score (3.71 points). Notably, 71% of these patients had scores ranging from 4 to a maximum of 8, indicating a clear need for dietary intervention. Conversely, the obese group showed a more urgent requirement for symptom management or nutritional intervention, with 54% of patients scoring between 4 and 8 and 20% achieving scores exceeding 9 points.

This discrepancy may be attributed to the fact that overweight individuals maintained a more balanced diet, with regular dairy and meat intake (70% and 80%, respectively), and a higher rate of adequate water consumption (60% drinking 1000 ml/day). This group also exhibited superior hand grip strength (15.21±4.14 and 15.28±5.34), even compared to the normal weight group, although only 14.28% engaged in moderate physical activity.

Recent literature indicates that SGA is a highly effective tool for nutritional diagnosis, often outperforming traditional methods such as anthropometry and laboratory data. However, its application in individuals with DS has not been extensively explored. This is a notable gap, considering the limited research on nutrition for individuals with DS, where many studies depend on less precise nutritional questionnaires and caregiver assessments (13, 21, 22).

Water intake

Previous research has consistently highlighted a concerning trend of dehydration among individuals with DS (23, 24). This issue is often linked to anatomical changes or muscle hypotonia. Additionally, studies have shown a troubling preference for sweet beverages over plain water in this population (15, 16). This underscores the vital role that family members and caregivers play in steering adults with DS toward healthier dietary and hydration habits.

Our study's findings resonate strongly with these observations, revealing not only insufficient water intake across all patient groups but also a worrying tendency toward fast food consumption, particularly among those with obesity. Most alarmingly, our results indicate a predominant association with a mean renal function gradient (RFG) suggestive of renal distress. One

possible explanation for this issue may be closely tied to the high intake of animal and vegetable protein (70%), further emphasizing the need for targeted interventions to improve dietary habits and prevent serious health complications in this vulnerable population.

Weight management strategies for individuals with DS include portion control, increased fiber and vegetables, and regular physical activity tailored to their abilities. The "GLOBAL Medical Care Guidelines for Adults with DS" provide recommendations, but more research is needed on the combined effects of diet, exercise, and calorie management in this population (25).

Limitations of the study

The limitations of our study include the small sample size and the lack of a control group without Down syndrome DS. These factors may limit the ability to generalize the findings and might impact the strength of the conclusions. They also present challenges in relating variables to accurately represent the broader DS population. Furthermore, the relatively small number of studies on adults with DS, compared to those focused on pediatric populations, underscores the need for additional research targeting this specific group.

CONCLUSSIONS

This study highlights that while overweight status can be relatively manageable for a patient with DS, progression to obesity presents significant health risks and can lead to rapid deterioration in health outcomes. The data emphasize the urgent need for early intervention upon the onset of obesity. It is imperative that families receive targeted education regarding nutritional guidelines and management strategies specifically designed for individuals with DS, due to their distinct obesity-related challenges.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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EPIDEMIOLOGY AND DEMOGRAPHIC PATTERNS OF RADIAL HEAD FRACTURES: A TWELVE-YEAR ANALYSIS FROM A TERTIARY TRAUMA CENTER

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EPIDEMIOLOGY AND DEMOGRAPHIC PATTERNS OF RADIAL HEAD FRACTURES: A TWELVE-YEAR ANALYSIS FROM A TERTIARY TRAUMA CENTER (Abstract): Radial head fractures represent approximately 33% of all elbow fractures, displaying a bimodal incidence pattern predominantly affecting younger males through high-energy impacts and older females due to low-energy trauma. Classified by the Mason system, these fractures range from minor displacements to complex, comminuted fractures that often require surgical management. **Materials and methods:** This retrospective and prospective study included 311 patients with radial head fractures admitted between 2011 and 2023 to the Department of Orthopedics-Traumatology at "Sf. Spiridon" County Clinical Emergency Hospital Iasi We collected data encompassed demographic information, fracture classification, associated injuries and aimed to identify demographic patterns and risk factors associated with radial head fractures. Statistical analyses were conducted using *SPSS version 18.0*. **Results:** The study confirmed a bimodal distribution in fracture incidence, with Mason III and Mason IV types being most common. Gender analysis showed that males were four times more likely to have the terrible triad fracture pattern compared to females (OR = 4.00; 95% CI: 1.09-14.6; p = 0.025). Age-related analysis indicated that olecranon-associated fractures were 1.69 times more common in patients aged 45 and above (OR = 1.69; 95% CI: 1.36-2.10; p = 0.001), whereas younger patients were more prone to the terrible triad, though statistical significance was marginal (p = 0.085). **Conclusions:** Radial head fractures exhibit distinct demographic patterns, with age and gender influencing fracture type distribution. These insights underline the importance of targeted diagnostic and management strategies, particularly for high-risk fracture patterns, to improve patient outcomes in radial head fractures. **Keywords:** RADIAL HEAD FRACTURE, MASON CLASSIFICATION, EPIDEMIOLOGY, AGE AND GENDER DISTRIBUTION, RISK FACTORS.

INTRODUCTION

The radial head is essential for elbow joint biomechanics, particularly in stabilizing the elbow through the radio-capitellar joint. Is the primary structure providing longitudinal stability for the forearm. Roughly 60% of the mechanical load on the elbow is absorbed through the radial column

(1, 2).

Radial head fractures account for 17-19% of elbow trauma cases and constitute 33% of elbow fractures (1) and approximately 1.7% to 5.4% of all fractures (3), with an estimated annual incidence rate of 25 to 55.4/100,000 people. This rate has been increasing, likely due to an aging population and a widespread participation in high-risk activities (4, 5). Epidemiologically, radial head fractures display a bimodal distribution: younger males are more prone to high-energy impacts, such as sports injuries or falls from height, resulting in complex fractures, (6) while older females often sustain simple fractures from low-energy mechanism, potentially due to osteoporosis (7, 8). The peak incidence is around 37 years for men and 52 years for women (9).

The Mason classification, initially introduced in 1954 (10) and modified by Johnston in 1962 (11), is widely used for evaluating radial head fractures, guiding both preoperative planning and prognostic assessment. The system categorizes fractures from Type I (non-displaced) to Type IV (displaced with dislocation), with treatment ranging from non-operative approaches to open reduction and internal fixation (ORIF) or radial head arthroplasty (RHA) for more complex injuries. Recently, RHA has gained popularity for complex fractures due to its stability benefits, particularly in cases where excision or non-operative treatment may not provide adequate joint support (12). This shift underscores the importance of individualized treatment, considering factors like age, trauma mechanism, and fracture complexity to optimize outcomes.

MATERIALS AND METHODS

The study included 311 adult patients diagnosed with radial head fractures, admitted between 2011 and 2023 to the Department of Orthopedics-Traumatology at "Sf. Spiridon" County Clinical Emergency Hospital from Iasi. Data were gathered both prospectively and retrospectively from our computerized patient records database, containing variables such as age, gender, socio-economic status, injury mechanism, method of treatment, period of hospitalization and immobilization after surgery, comorbidities and associated injuries.

Radial head fractures were categorized using the Mason classification. Statistical analysis was conducted using *SPSS version 18.0,* employing both parametric and non-parametric tests. The choice of statistical test depended on the nature of the data: t-tests and F-tests (ANOVA) were applied for comparing quantitative variables, while chi-square tests were used for categorical data to evaluate frequency distributions. We maintained a 95% confidence interval (CI) for all tests to ensure reliable statistical significance, with chi-square tests applied to assess differences in fracture distribution by age and gender.

To examine fracture incidence trends over time, linear regression analyses were conducted on the annual hospital admissions. The coefficient of variation (CV%) was calculated to assess homogeneity within data sets, and the Skewness test was applied to validate the normality of the data for continuous variables.

RESULTS

Over the 12-year study period, annual hospital admissions showed a decreasing trend ($y = 2727 – 47x$), projecting approximately 2,116 admissions for 2024. However, the distribution of radial head fracture cases initially increased, peaking in 2018 at 9.4% of admissions, before declining in recent years ($y = 9.68 - 0.34x$). Projections

estimate a 5% frequency in 2024 (tab. I). The distribution of cases was bimodal, with 12.2% in 2015 and 12.9% in 2022, showing an upward trend (y = 4.92 + 0.40x), predicting an incidence of approximately 10.5% in 2024 (fig. 1). Throughout the months of the study, an increased frequency of cases was observed in May (11.6%), July (11.3%), and September (11.3%). The monthly percentage distribution during the analyzed period showed no significant differences (y = 7.99 + 0.06x; p = 0.094).

TABLE I.

Annual admissions and case distribution of radial head on year study

Year study	Admissions	% from total	Cases of radial head fracture	Cases / 1,000 admissions
2011	1771	5.7	7	3.95
2012	2762	8.8	14	5.07
2013	2750	8.8	20	7.27
2014	2769	8.9	24	8.67
2015	2813	9.0	38	13.51
2016	2362	7.6	35	14.82
2017	2874	9.2	14	4.87
2018	2934	9.4	21	7.16
2019	2816	9.0	31	11.01
2020	1400	4.5	14	10.00
2021	1630	5.2	22	13.50
2022	2022	6.5	40	19.78
2023	2313	7.4	31	13.40
Total	31216	100.0	311	9.96

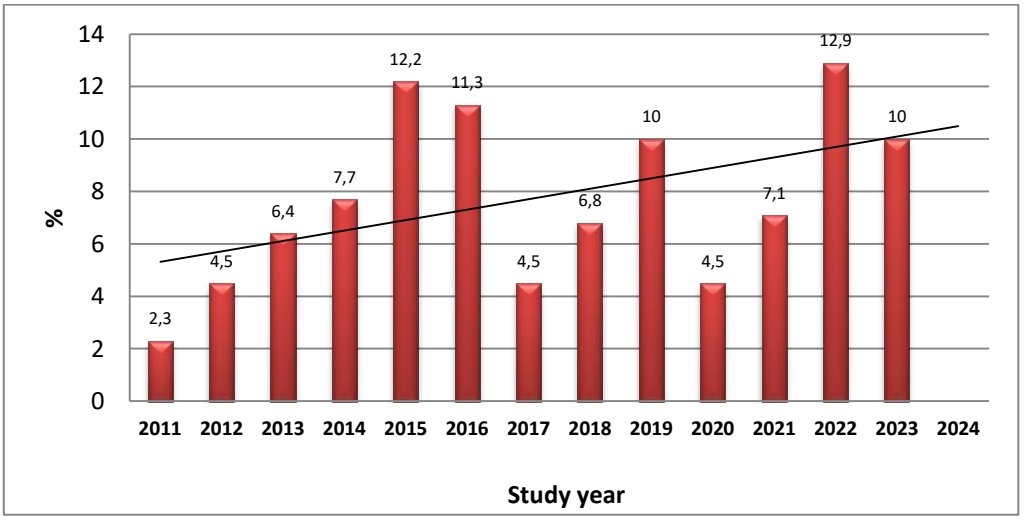

Fig. 1. Case distribution by year of study

The study population ranged from 18 to 94 years, with a mean age of 46.56 ± 15.86 years, close to the median age (45 years), with the Skewness test result (s = 0.266) suggesting homogeneity in the data series, allowing for the application of statistical significance tests. Gender distribution showed no significant differences, with 49.2% men and 50.8% women. The mean age was significantly higher in females (38.56 *vs.* 54.31 years; p = 0.001). In the age group under 45 years, male cases predominated (71.2%), while in the age group over 45 years, female cases were more prevalent (72.8%) (p = 0.001) (fig. 2).

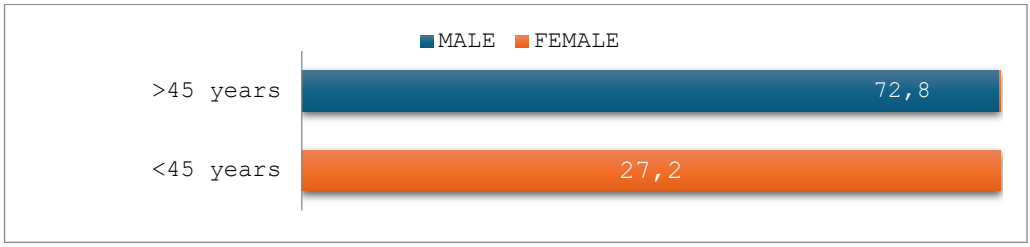

Fig. 2. Case distribution based on gender and age group

Among study participants, 23.2% of patients were unemployed, while over half were employed (50.2%). Educational status was low for 45% of the study group, though most patients had completed high school (43.7%) (fig. 3).

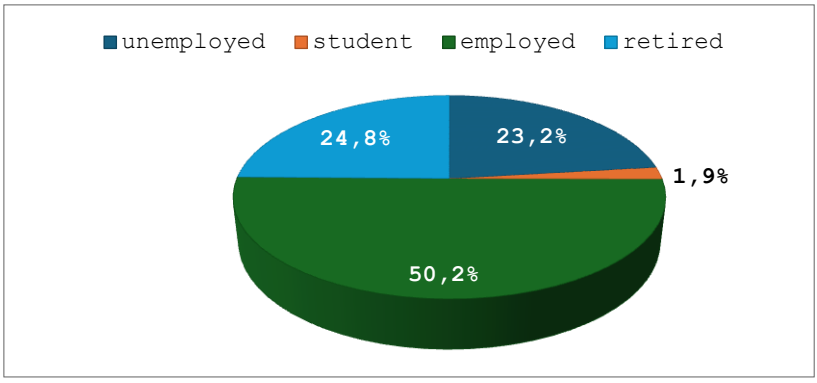

Fig. 3. Distribution of patients by educational status

The findings related to weight status in the study group show a total of 48.6% of cases were overweight, and 15.4% had grade I obesity. Obesity was observed in 14.5% of men and 29.8% of women (p = 0.008) (tab. II).

The left limb was more frequently affected (53.4%). Among patients with the left limb affected, 52.2% were male (p = 0.272), and 52.4% were over 45 years of age (p = 0.633). For patients with the right limb affected, 55.4% were female (p = 0.274), and 50.4% were over 45 years of age (p = 0.637). Among patients with both limbs affected, 86.7% were male (p = 0.272), and 66.7% were under 45 years of age (p = 0.633).

TABLE II.
Weight status by gender

Weight status	Male (n=153)		Female (n=158)	
	n	%	n	%
Normal weight	43	28.1	48	30.4
Overweight	88	57.5	63	39.9
Obesity Ist degree	16	10.5	32	20.3
Obesity IInd degree	5	3.3	12	7.6
Obesity IIIrd degree	1	0.7	3	1.9

The most common associated fractures were of the olecranon (8.7%), followed by the coronoid process (7.7%) and distal radius (4.8%) or Monteggia-like lesion (4.8%). The estimated risk (RR), by gender (tab. III), of an associated olecranon fracture was 1.52 times higher in females (RR = 1.52; 95% CI: 1.18-1.96; p = 0.01) and of an associated coronoid process fracture was 1.30 times higher in males (RR = 1.30; 95% CI: 0.93-1.81; p = 0.173). The risk of an associated Monteggia-type dislocation was 1.19 times higher in females (RR = 1.19; 95% CI: 0.78-1.83; p = 0.464).

TABLE III.
Estimated risk of associated fractures by gender

Associated fractures	Male (n=153)		Female (n=158)		p	RR	95% CI
	n	%	n	%			
Olecranon	7	4.6	20	12.7	**0.010**	1.52$_F$	1.18-1.96
Coronoid process	15	9.8	9	5.7	0.173	1.30$_M$	0.93-1.81
Distal radius	8	5.2	7	4.4	0.742	1.09$_M$	0.67-1.77
Monteggia-like lesion	6	3.9	9	5.7	0.464	1.19$_F$	0.78-1.83

By age group (tab. IV), the risk of an associated olecranon fracture was estimated to be 1.69 times higher in the age group over 45 years (RR = 1.69; 95% CI: 1.36-2.10; p = 0.001) and of an associated coronoid process fracture was estimated to be 1.31 times higher in the age group under 45 years (RR = 1.31; 95% CI: 0.94-1.83; p = 0.163). The risk of an associated Monteggia-like lesion was estimated to be 1.76 times higher in the age group over 45 years (RR = 1.76; 95% CI: 1.40-2.21; p = 0.003). In the gender-based distribution (tab. V). fractures were categorized by the Mason classification types I to IV. Essex-Lopresti fracture-dislocation (radial head fracture, rupture of the forearm interosseous membrane and distal radio-ulnar joint dislocation), and the terrible triad (radial head fracture, coronoid process fracture and elbow dislocation). Among males (n=153) and females (n=158), the most common fracture types were Mason III and Mason IV. The chi-square test showed no statistically significant gender

difference for most fracture types, except for the terrible triad, which was more common in males (7.2%) compared to females (1.9%) with a significant p-value of 0.025.

The odds ratio indicated that males had a fourfold higher likelihood of experiencing terrible triad fractures (OR = 4.00, 95% CI: 1.09-14.6).

TABLE. IV

Estimated risk of associated fractures by age

Associated fractures	≥ 45 years (n=159)		< 45 years (n=152)		p	RR	95% CI
	n	%	n	%			
Olecranon	22	13.8	5	3.3	**0.001**	1.69 $_{45+}$	1.36-2.10
Coronoid process	9	5.7	15	9.9	0.163	1.31 $_{<45}$	0.94-1.83
Distal radius	8	5.0	7	4.6	0.861	1.05 $_{45+}$	0.64-1.70
Monteggia-like lesion	13	8.2	2	1.3	**0.003**	1.76 $_{45+}$	1.40-2.21

TABLE V.

Distribution of cases by gender correlated with fracture type

Fracture type	Male (n=153)		Female (n=158)		Chi^2 test p	OR	95% CI
	n	%	n	%			
MASON I	23	15.0	31	19.6	0.286	1.38$_F$	0.76-2.49
MASON II	25	16.3	34	21.5	0.245	1.40$_F$	0.79-2.49
MASON III	63	41.2	58	36.7	0.420	1.21$_M$	0.76-1.91
MASON IV	25	16.3	28	17.7	0.746	1.10$_F$	0.61-1.99
ESSEX-LOPRESTI	2	1.3	2	1.3	0.974	1.03$_M$	0.14-7.42
TERRIBLE TRIAD	11	7.2	3	1.9	**0.025**	4.00$_M$	1.09-14.6
MASON I+II	0	0.0	1	0.6	0.325	-	-
MASON I+III	1	0.7	1	0.6	0.982	1.03$_M$	0.06-16.7
MASON II+III	3	2.0	0	0.0	0.077	-	-

For age-based distribution (tab. VI), patients were split into two groups: those aged ≥45 years (n=159) and those under 45 years (n=152). The analysis indicated that Mason III and Mason IV fractures were the most prevalent across both age groups, showing no significant differences by age. However, Essex-Lopresti fracture-dislocation were more frequent in patients under 45 years (2.0%) compared to older patients (0.6%), though this finding was not statistically significant (p=0.294). The terrible triad was more common in patients younger than 45 (6.6%) compared to those 45 and older (2.5%), with an OR of 2.73, though the p-value was 0.085, indicating a trend but not statistical significance.

TABLE VI.
Distribution of cases by group age correlated with fracture type

Fracture type	≥ 45 years (n=159)		< 45 years (n=152)		Chi² test p	OR	95% CI
	n	%	n	%			
MASON I	25	15.7	29	19.1	0.436	$1.26_{<45}$	0.70-2.28
MASON II	35	22.0	24	15.8	0.162	$1.51_{\geq 45}$	0.85-2.68
MASON III	62	39.0	59	38.8	0.974	$1.01_{\geq 45}$	0.64-1.59
MASON IV	30	18.9	23	15.1	0.382	$1.30_{\geq 45}$	0.72-2.37
ESSEX-LOPRESTI	1	0.6	3	2.0	0.294	$3.18_{\geq 45}$	0.33-30.9
TERRIBLE TRIAD	4	2.5	10	6.6	0.085	$2.73_{\geq 45}$	0.84-8.90
MASON I+II	0	0.0	1	0.7	0.317	-	-
MASON I+III	2	1.3	0	0.0	0.166	-	-
MASON II+III	0	0.0	3	2.0	0.076	-	-

DISCUSSION

This study provides valuable insights into the epidemiological and demographic patterns associated with radial head fractures. Consistent with prior research (13), we found a bimodal age and gender distribution, where younger males frequently sustained high-energy injuries resulting in complex fractures, while older females were more likely to experience low-energy fractures, possibly due to osteoporosis and other age-related factors.

The increased prevalence of Mason III and IV fractures aligns with previous studies, which also identified these types as common in radial head fractures requiring surgical intervention. Associated injuries with radial head fractures are increasingly recognized for their clinical significance (14, 15). Our findings regarding the terrible triad pattern are particularly noteworthy. Consistent with the literature, this study revealed that younger males had a significantly higher likelihood of experiencing terrible triad fractures (OR = 4.00, p = 0.025), a finding that underscores the impact of high-energy trauma in this demographic. The higher rate of olecranon fractures in older females similarly echoes findings from other epidemiological studies, suggesting a heightened vulnerability to specific fracture patterns in this group due to bone density loss.

Our analysis also highlighted the distinct risk factors associated with gender and age. The increased risk of olecranon and Monteggia-like fractures in patients over 45 years reinforces the need for targeted diagnostic approaches in elderly patients, especially females. These age- and gender-specific findings suggest that healthcare providers should consider demographic factors when assessing and planning treatment for radial head fractures.

The demographic insights gained in this study have implications for clinical practice, particularly in the development of tailored treatment protocols. For example, males under 45 with high-energy injuries may benefit from more intensive imaging

to identify the potential for complex fracture patterns like the terrible triad. For females over 45, early intervention strategies focusing on osteoporosis management could reduce the likelihood of low-energy fractures and improve patient outcomes.

While this study presents robust data from a large sample over 12 years, some limitations must be acknowledged. The single-center design may limit the generalizability of our findings to other regions or populations. Additionally, although the study includes comprehensive demographic and clinical data, factors such as lifestyle and physical activity levels, which may influence fracture risk, were not assessed. Future studies that incorporate these variables could provide a more holistic view of fracture risk factors.

Also, future research should focus on longitudinal studies correlating fracture types and surgical procedures with clinical outcomes. Evaluating the effectiveness of interventions like radial head arthroplasty across various fracture types and patient demographics would provide valuable data to refine treatment protocols.

Additionally, exploring the relationship between bone health interventions and fracture incidence in older patients, especially postmenopausal women, may help establish preventative strategies.

CONCLUSIONS

In conclusion, this 12-year study on radial head fractures highlights distinct demographic patterns, with younger males more prone to high-energy fractures and older females to low-energy fractures. The findings confirm that Mason III and IV fractures are the most prevalent and often require surgical intervention. Gender and age disparities, such as the higher incidence of the terrible triad in males and olecranon-associated fractures in females, underscore the importance of individualized diagnostic and treatment approaches tailored to patient demographics.

This study's large sample size provides valuable insights into the epidemiology and demographics of radial head fractures, emphasizing that targeted management strategies can enhance patient outcomes. Further research into the relationship between fracture types, clinical outcomes, and specific treatment approaches may help refine guidelines and improve patient care.

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CONFLICT OF INTEREST AND FUNDING

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NON-TRAUMATIC NON-COVID-19 RELATED FINDINGS IN EMERGENCY CHEST MULTIDETECTOR COMPUTED TOMOGRAPHY. A RETROSPECTIVE STUDY

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NON-TRAUMATIC NON-COVID-19 RELATED FINDINGS IN EMERGENCY CHEST MULTIDETECTOR COMPUTED TOMOGRAPHY: A RETROSPECTIVE STUDY (Abstract): This study **aims** to analyze the non-traumatic and non-COVID-19-related findings detected in patients presenting to the emergency department with acute chest pain who underwent chest multidetector computed tomography (MDCT) and the relationship with patient gender and treatment solutions. **Materials and methods:** We retrospectively selected and reviewed the radiological reports of a cohort of 378 patients who underwent chest MDCT in emergency at "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania, between January 1, 2021, and July 1, 2024. Exclusion criteria were recent trauma history, a positive PCR test for COVID-19, and acute myocardial infarction. MDCT examinations were performed using a 16-slice Siemens Somatom Emotion CT scanner and a 64-slice Philips Incisive CT scanner. **Results:** Among the 378 patients, 222 were males (58.7%) and 156 were females (41.3%), with a mean age of 73.8 years (SD 10.767; CI 72.55-75.06; range 35-94 years). We classified the lesions into four categories: pleuro-pulmonary (51.58%), cardiac (24.9%), vascular (16.55%), malignant (6.97%). Life-threatening findings were present in only 21.42% of cases, including pulmonary embolism (14.8%), acute pulmonary edema (2.3%), descending aorta dissection (0.7%), and massive pleural effusion (0.7%). The most common primary malignancy was lung cancer. **Conclusions**: This study underlines the importance of correctly identifying and managing the chest CT findings in the case of patients examined in emergencies. **Keywords:** CHEST EMERGENCY, COMPUTED TOMOGRAPHY, NON-TRAUMATIC, NON-COVID-19, ACUTE CHEST PAIN.

INTRODUCTION

Chest multidetector computed tomography (MDCT) became an essential tool in emergency departments due to its ability to provide fast, accurate, high-resolution diagnostic images and to evaluate the cause and extent of the disease (1, 2, 3, 4).

Chest pain and shortness of breath are determined only in a small percentage by acute coronary syndrome, even if ischemic heart disease is frequent and often clinically indistinguishable from other chest conditions (5-10); the differential diagnosis includes gastro-esophageal diseases, chest-wall syn-dromes,

and less frequent causes: pulmonary embolism, pleural and pericardial lesions, pneumonia, lung cancer, aortic dissection, and herpes zoster. Among them, there are several life-threatening causes: pulmonary embolism (PE), tension pneumothorax, pericardial tamponade, aortic dissection, and esophageal perforation, which can be confidently and rapidly diagnosed by chest CT (8, 9, 10). PE is the third most common cause of cardiovascular-related deaths, following myocardial ischemia and stroke (11-13), and for its diagnosis, the gold-standard investigation is computed tomography pulmonary angiography (CTPA) (13, 14), with a high sensitivity (96-100%) and specificity (97-98%) (14, 15).

Previous studies have established the efficacy of MDCT in evaluating thoracic emergencies (7, 8, 16, 17); however, regional data, particularly from Eastern Europe, remain limited (18, 19).

MATERIALS AND METHODS

We retrospectively searched and randomly selected from the hospital databases 378 patients who presented to the emergency department (ED) at "Sf. Spiridon" County Clinical Emergency Hospital, Iasi, Romania, between January 1, 2021, and August 31, 2024, with acute chest pain and undergone a chest MDCT. We reviewed all the reports and DICOM images of 191 non-enhanced chest CT (for evaluating lung lesions described on chest x rays) and 187 contrast-enhanced chest CT (to rule out mainly pulmonary embolism or a thoracic aortic dissection). The exclusion criteria from the study were: a recent traumatic history, a positive PCR test for COVID-19 or an acute myocardial infarction.

CT examinations were performed on a 16-slice Siemens Somatom Emotion CT scanner and a 64-slice Philips Incisive CT scanner using standard protocol settings. A non-contrast CT chest protocol was conducted on all patients. To rule out a life-threatening cause of acute chest pain, such as a pulmonary embolism or aortic dissection, the non-enhanced scan was followed by a contrast-enhanced CT with the administration of non-ionic iodinated contrast material (Iopromidum, Ultravist 370, 370 mg/mL, Bayer, Leverkusen, Germany).

Statistical analyses were conducted using *SPSS software for Mac version 29.0* (SPSS, Chicago, IL, USA). Continuous variables are provided as the mean ± standard deviation (SD), while nominal variables are shown as frequencies and percentages. There were six main categories for nominal variables: pleural and pulmonary findings, (non-coronary) cardiac findings, vascular findings, urgent findings, malignant findings, and other findings. To identify significant parameters in our population study, we used Pearson's chi-squared test (non-parametric analysis) for categorical variables. A p-value less than or equal to 0.05 was considered statistically significant.

RESULTS

Among the 378 patients, 222 were males (58.7%), and 156 females (41.3%), with a mean age of 73.8 years (SD 10.767, CI 72.55-75.06; range 35-94 years), and we discovered only 81 cases (21.42%) that needed immediate treatment (emergencies). We classified the non-emergency findings into four categories: pleuro-pulmonary lesions (51.48%) heart diseases (24.9%), vascular conditions (16.55%), malignant findings (6.97%). Most of the patients had several lesions present, and the most common findings were pleural effusion (n=156, 41.2%), valvular calcifications (n=84, 22.2%), cardiomegaly (n=73, 19.3%), pulmonary embolism (n=56, 14.8%).

Emergency findings. The most important discoveries were the emergency diagnostics, representing 21.42% (n=81),

the most frequent of all being pulmonary embolism, found on 14.8% of patients, followed by acute pulmonary edema (2.3%, n = 9), descending aorta dissection (0.7%, n = 3), and massive pleural effusion (0.7%, n = 3). These patients have been referred to specialists for immediate treatment.

In our study the localization of emboli was as following: in main pulmonary arteries (4.4%, n = 17), lobar (3.9%, n = 15), and segmental arteries (5%, n = 19), but the filling defects in subsegmental arteries are difficult to depict, probably that's why we detected them only in 5 cases (1.3%); nevertheless, subsegmental pulmonary embolism is not considered to be significant for the diagnosis (18,19). Pulmonary infarction was present in 2.1 cases (8%).

There was no significant gender association in either pulmonary embolism or pleural effusion, both having approximately equal distribution in both genders.

Pulmonary embolism was strongly correlated with pulmonary hypertension (dilated pulmonary trunk > 33 mm) ($p < 0.001$).

Pleural effusion often accompanies pulmonary embolism, due to the hemodynamic changes and increased pressure in the pulmonary circulation caused by emboli ($p<0.05$)

Patients presenting with acute pulmonary edema often had cardiomegaly, due to underlying heart conditions contributing to fluid accumulation in the lungs ($p<0.05$).

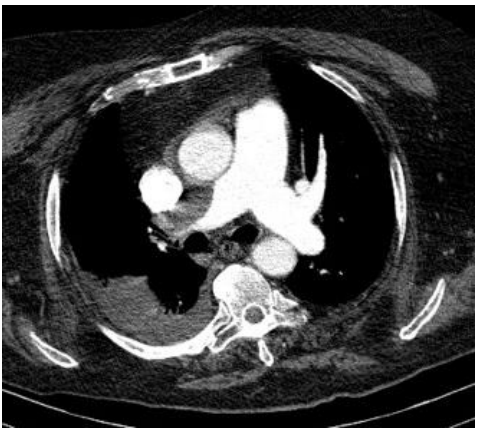

Fig. 1. CTPA-thrombus in the right pulmonary artery, enlarged main pulmonary artery, right pleural effusion, and adjacent lung collapse**.**

Descending aorta dissection was significantly correlated with hypertension ($p < 0.001$). There was a correlation between massive pleural effusion and underlying malignancies, such as bronchopulmonary cancer ($p= 0.04$).

Examining CT images, we depicted on the abdominal sections, beside chest emergencies, also some abdominal lesion worth mentioning (tab. I); for these diagnostics also an immediate treatment was done. These insights can be valuable for understanding the complexity of patient conditions and planning appropriate diagnostic and treatment strategies.

TABLE I.

Summary of emergency diagnostics

Emergencies	No. of patients=81	21.42%
Acute pulmonary embolism	56	14.81
Acute pulmonary edema	9	2.38
Descending aorta dissection	3	0.79
Mesenteric ischemia	3	0.79
Massive pleural effusion	3	0.79
Tension pneumothorax	3	0.79
Small bowel obstruction	2	0.52
Acute pancreatitis	2	0.52

Pleuro-pulmonary findings (tab. II) were found in 195 patients (51.58%) with multiple findings for each patient; the most common are the following: pleural effusion (41.2 %, n = 156), fibrotic bands (33 %, n = 125), non-calcified pulmonary nodules (18.2 %, n = 69), pulmonary emphysema (17.9 %, n = 68), and pulmonary tuberculosis sequelae (12.4 %, n = 47).

Pleural effusion (causing pleuritic chest pain, dyspnea, and a dry cough) (20) was the most common finding in our study (41.2%, n = 156), but only in three cases (0.7%) with massive pleural effusion an immediate treatment was necessary. The study found no significant correlation with gender (p=0.52), but there was a significant association between pleural effusion and pulmonary hypertension (pulmonary trunk > 33 mm), pericardial effusion, and cardiomegaly ($p < 0.001$).

Pulmonary emphysema is one of the most frequent lung conditions (21), depicted in our study in 68 cases (17.9%). Males are more likely to be affected ($p < 0.001$). Patients with pulmonary emphysema have also fibrotic bands ($p < 0.01$).

Non-calcified pulmonary nodules were found in 69 patients (18.2%). According to updated Fleischner Society Guidelines for managing incidental non-calcified pulmonary nodules; were divided by structure in two groups (solid and subsolid) and by dimension in three groups (< 6 mm, 6-8 mm and > 8 mm).

TABLE II.

Summary of pleuro-pulmonary findings

Pleuro-pulmonary findings	No. of findings	Frequency (%)
Pleural effusion	156	41.26
Fibrotic bands	125	33
Non-calcified pulmonary nodule	69	18.2
Pulmonary emphysema	68	17.9
Pleural and pulmonary tuberculosis sequelae	140	36.9
Bronchiectasis	34	8.9
Pneumonia and Bronchopneumonia	28	7.2
tuberculosis	4	1
Lung cancer	9	2.3
Pulmonary infarct	8	2.1
Pulmonary metastases	4	1

A single solid nodule < 6 mm was found in 8 patients (2.1%), with no CT follow-up recommended. A single solid nodule > 8 mm was found in 3 patients (0.7 %), with recommendation for chest CT follow-up after 3 months.

Multiple solid nodules < 6 mm were found in 15 patients (3.9%) (no follow-up was recommended) and with diameters of 6-8 mm in 37 patients (9.7 %) with a recommendation for a further chest CT after 6 months.

Calcified nodules were considered sequelae of tuberculosis, and they don't need follow-up. There was a male predominance associated with calcified pulmonary nodules ($p < 0.01$).

The correlation matrix of diagnosis reveals significant relationships between various diagnoses (fig. 2), highlighting the interconnected nature of pleuro-pulmonary, cardiac, vascular, and malignant conditions.

The study found that: pulmonary emphysema was highly correlated with cardiomegaly (0.96) and valvular calcification (0.88); lung cancer was moderately correlated with pulmonary emphysema (0.33); cardiomegaly was highly correlated with pulmonary emphysema (0.96) and valvular calcifications (0.80), moderately correlated with fibrosis (0.70) and pericardial effusion (0.58); pericardial effusion was highly correlated with dilated pulmonary trunk(0.89), moderately correlated with valvular calcification(0.64) and cardiomegaly (0.58); dilated pulmonary trunk was highly correlated with pericardial effusion 0.89), moderately correlated with pulmonary emphysema (0.74), dilated pulmonary trunk is highly correlated with pericardial effusion (0.89).

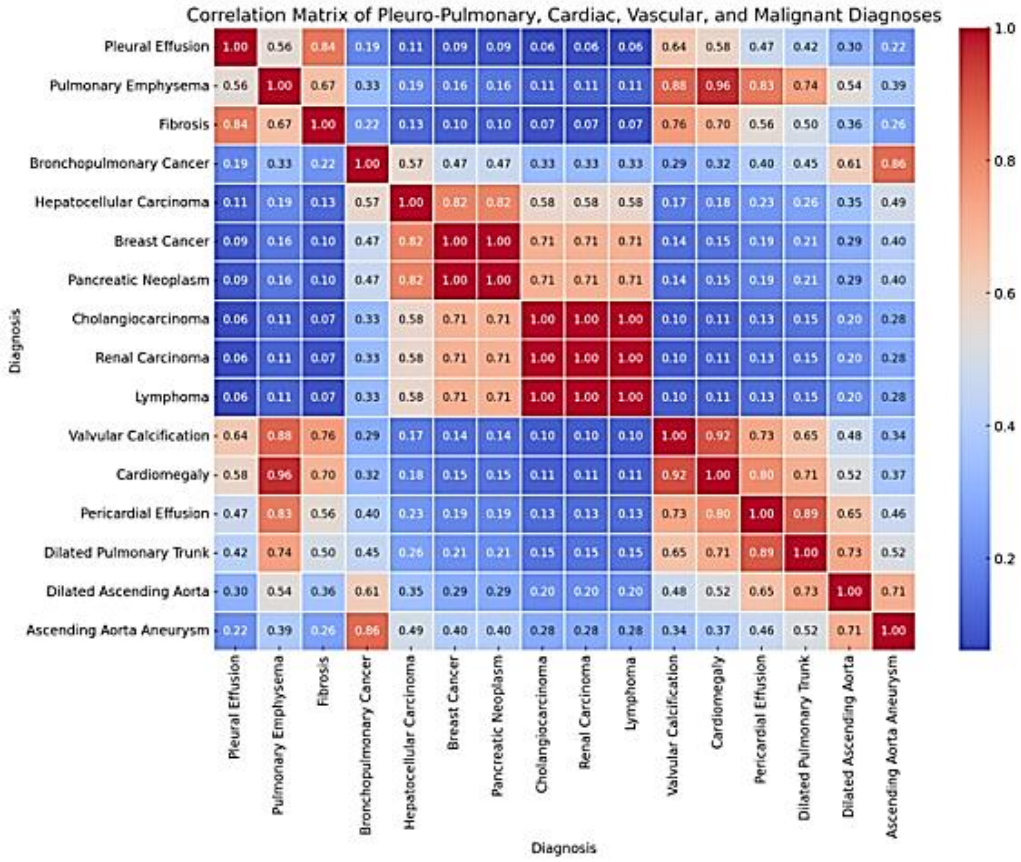

Fig. 2. Correlation matrix revealing significant relationships between various diagnoses

Heart diseases. We found heart diseases in 24.9% patients: valvular calcification (22.2%, n = 84 divided between aortic valve calcification 14.5%, n=55 and mitral valve calcification 7.6%, n = 29), cardiomegaly (19.3%, n=73), pericardial effusion (13.2%, n = 5), pericardial cyst (0.2%, n=1). 21 patients had cardiomegaly and pericardial effusion all together. Cardiomegaly was associated with female gender ($p < 0.05$).

Vascular findings (tab. III) were found in 16.55% of patients. In descending order, we found the fallowing: dilated pulmonary trunk > 33 mm (10.8 %, n=41), dilated ascending aorta > 40 mm (6 %, n=23), descending aorta dissection (0.7%, n=3), ascending aorta aneurism (3.1 %, n=12).

According to studies, main pulmonary artery has normally less than 33 mm. Above this level (with a sensitivity of 58% and a specificity of 95%), pulmonary arterial hypertension should be suspected. There was a significant association between pulmonary hypertension and acute pulmonary embolism, between pulmonary hypertension and pericardial effusion, and between pulmonary hypertension and cardiomegaly ($p < 0.05$).

TABLE III.
Summary of vascular findings

Vascular findings	No. of findings	%
Pulmonary trunk > 33 mm	41	10.8
Dilatation of the ascending aorta (40-50 mm)	23	6.0
Significant Superior mesenteric artery stenosis > 50 %	18	4.7
Ascending aorta aneurism (> 50 mm)	12	3.1
Significant Celiac trunk stenosis >50 %	7	1.8
Descending aorta dissection	3	0.7

Malignant lesions. Among chest malignant lesions (n=19, 6.97%), the most frequent primary tumor in both genders was lung cancer (2.3%, n = 9), followed by breast cancer (1.2%, n=2). Important incidental findings were depicted at the abdominal level and were reported within the limits of the performed protocol. Therefore, we discovered hepatocellular carcinoma (0.79%, n = 3), pancreatic cancer (0.53%, n = 2), cholangiocarcinoma, renal carcinoma, lymphoma (0.2%, n = 1 each). We depicted also secondary lesions with several localizations: liver (1.8%, n = 7), bones (1.5%, n = 6), lungs (1%, n = 4), adrenal glands (1%, n = 4), peritoneum (0.3%, n = 1). Patients with these incidental malignant findings were referred for an oncology consultation to establish disease management (20, 21, 22).

DISCUSSION

This retrospective study on non-traumatic, non-COVID-19-related CT findings in case of patients presenting with acute chest pain demonstrates the critical role of MDCT in diagnosing a wide array of conditions, differentiating the emergencies from non-emergency cases, and establishing an appropriate case management (23, 24).

The mean age in population study was 73.8 years, indicating a significant proportion of old patients. This demographic is particularly relevant because older individuals are more likely to present with multiple comorbidities and complex medical conditions, which can complicate the diagnosis and management of acute chest pain.

This study reveals that pleuropulmonary and vascular findings are prevalent, with pleural effusion and pulmonary embolism being particularly frequent.

Thoracic emergencies were found on only 21.4% of patients. The most frequent one was pulmonary embolism. There were no significant gender differences for most

conditions except pulmonary emphysema, which was more common in males ($p < 0.001$), and cardiomegaly, which was associated with females ($p < 0.05$).

Malignant tumors (25, 26) and cardiovascular disorders (27) were incidental findings which were depicted frequent enough among our patients and were referred for treatment in dedicated departments.

CONCLUSIONS

Given that we encountered mainly elder patients, our findings emphasize the importance of an accurate diagnostic and therapeutic approach in cases presented with chest acute pain in order to ensure rapid and correct identification of a potentially life-threatening condition.

This study is conducted on a limited number of patients, but it can fill a gap in the Romanian healthcare context by providing useful data about the diagnosis depicted by chest CT for patients with acute chest pain, which can be useful for local practitioners.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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RP-HPLC METHOD WITH UV DETECTION FOR THE EVALUATION OF CHITOSAN-GRAFT-B-CYCLODEXTRIN/PVA HYDROGELS AS CARRIERS FOR THE CONTROLLED RELEASE OF VEMURAFENIB

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RP-HPLC METHOD WITH UV DETECTION FOR THE EVALUATION OF CHITOSAN-GRAFT-B-CYCLODEXTRIN/PVA HYDROGELS AS CARRIERS FOR THE CONTROLLED RELEASE OF VEMURAFENIB (Abstract): When developing a topical formulation, it is essential to evaluate it from the perspective of its carrier capacity for the controlled release of the encapsulated drug. The drug should be simply quantified through an accurate and reproducible method. This paper presents a rapid, simple, sensitive, and reproducible high-performance liquid chromatography method with UV detection for evaluating the ability of chitosan-graft-β-cyclodextrin/PVA (CS-g-β-CD/PVA) hydrogels as carriers for the controlled release of vemurafenib (VEM). **Materials and methods:** The chromatographic separation was achieved using a Waters CORTECS C18 column (100 × 2.1 mm ID, 2.7 µm). The mobile phase was a mixture of: A (water/formic acid - 99.9/0.1, v/v), B (acetonitrile), and C (methanol) in the ratio of 40: 55: 5 (v/v/v). The injection sample amount was 10 µL, and the run time was 9 minutes in isocratic mode at a flow rate of 1 mL/min. The analyte was detected using UV absorption at 252 nm. **Results:** The standard calibration curve was linear over the concentration range of 0.78-100 mg/L. The values for LOD and LOQ were 0.5 mg/L and 0.75 mg/L, respectively. The intra- and inter-day precision of measurements were lower than the accepted criteria (RSD ≤ 2%). The high value of recoveries obtained for VEM indicates that the proposed method was found to be accurate. The stability of VEM solutions was assessed, indicating that the drug remained stable under all relevant conditions. **Conclusions:** Finally, the validated method was successfully applied to evaluate the ability of chitosan-graft-β-cyclodextrin/PVA hydrogels to load and sustain release of VEM. The drug entrapment efficiency (DEE%) was between 65±0.08% and 70±0.05%. **Keywords:** VEMURAFENIB, SUSTAINED RELEASE, DRUG DELIVERY SYSTEM, HPLC-UV METHOD, DRUG TARGETING.

INTRODUCTION

Melanoma, the most aggressive variant of skin cancer, exhibits a rising trend in morbidity and mortality indicators worldwide (1). Consequently, it poses a significant threat to global health, characterized

by limited treatment options and potential side effects. The currently available strategies for the treatment of this type of cancer include surgery, radiotherapy, chemotherapy, immunotherapy and targeted therapy (2). Surgical resection of the tumor with adequate margins is the first-line treatment (3), however a significant risk of incomplete removal of the tumor and occurrence of surgical complications still remains. These can cause a series of pathophysiological processes that can subsequently trigger tumor recurrence and the appearance of metastases induced by surgical intervention (4). Yet, for some patients at stages II, III and IV, surgery alone has limited curative potential (5).

Thus, radiotherapy, chemotherapy, immunotherapy or targeted therapy are often used as adjuvant treatments. Melanoma is a relatively radioresistant tumor, therefore, radiotherapy is widely used as a palliative treatment for metastatic melanoma (6, 7). Chemotherapy remains a therapeutic option for the management of melanoma, but it does not show specificity for tumor cells and, consequently, the accumulation of drugs in the tumor microenvironment is low (8). Thus, the therapeutic benefits are limited (9) and the prevalence of side effects is high, necessitating the exploration of novel treatment alternatives. Immunotherapy employing the four classes of monoclonal antibodies aims to stimulate and activate the immune system (10); nevertheless, a large percentage of patients exhibit both innate and acquired resistance resulting in a lack of response to this therapeutic approach.

Targeted therapy is highly effective in treating advanced melanomas when the cancer cells have certain genetic alterations. Half of all melanomas exhibit changes in the BRAF gene. Vemurafenib (VEM) was the first drug approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for metastatic and unresectable melanoma (11) and Erdheim-Chester disease (ECD) associated with $BRAF^{V600}$ mutation (12). Clinically, VEM is administered orally, but it is accompanied by a high rate of side effects as liver injury, kidney failure, changes in heart rhythm, muscle, bone, and connective tissue disease. In addition, the drug must pass through the acidic environment of the stomach and the intestinal mucus barrier to reach its target. Many active substances are denatured or degraded by stomach acid and some drugs, especially hydrophobic ones, can be easily trapped by mucus and, as a result, are rapidly eliminated before they can be absorbed by the intestine (13). To avoid the gastric and intestinal barrier and to reduce toxicity risks, the potential of topical administration of VEM has been explored (14, 15). The skin serves as a suitable route for the topical delivery of drugs intended for localized effects (16). Administration of vemurafenib by skin is challenging due to its low solubility (17) and the presence of stratum corneum (18), which prevents the penetration of macromolecular drugs into the body. The specialized literature mentions the attempt of topical application of VEM in the form of solid-in-oil nanodispersion as an effective and safe way to deliver VEM but only in the early stage of melanoma (19). Hydrogels are one of the common drug delivery systems for the skin (18, 20) and have garnered high interest due to their remarkable characteristics and applications in the biomedical field during the past decade (21–23). Despite their advantages, there are a number of challenges regarding the poten-

tial use of hydrogels in this field. These challenges refer to drug loading, release control, skin permeability efficiency, and long-term stability. The nature of the polymers in the matrix structure plays an important role, as the functional groups on the polymer surface influence the drug loading capacity and their release behavior, specificity, and safety. Also, the hydrogel's composition significantly influences the drug's transdermal permeation. Considering all the aforementioned factors, we considered necessary to develop a novel formulation in the form of a thermosensitive hydrogel loaded with VEM for localized administration in melanoma treatment. For the synthesis of hydrogels, the use of biopolymers is the most common approach, with chitosan and cyclodextrins serving as versatile carriers for antitumor agents (24).

Chitosan (CS) is a biopolymer used due to its ability to form polymer networks that absorb significant quantities of water and biological fluids (25). These properties are useful in loading drug solutions and absorbing skin exudates. Therefore, CS-based formulations are suitable for cutaneous application due to their hydrophilicity and flexibility (26) and for their attractive biological activity, antitumor, antioxidant, and antimicrobial effects (27, 28). Its cationic polymer can act as a penetration enhancer, being correlated with a certain anticancer activity which is also influenced by its molecular weight and degree of deacetylation (29).

Cyclodextrins (CDs) represent an attractive alternative for the development of carriers for anticancer therapeutics. They were used to design hydrogels as carriers and stabilizers for a large number of drug molecules (30, 31). Among them, β-cyclodextrin (β-CD) is highly advantageous owing to its low cost, effective cavity, lack of toxicity and can enhance the solubility and bioavailability of insoluble drugs (32). The low aqueous solubility of many pharmaceutical developments is an area of constant research. VEM exhibits low aqueous solubility, which implies a poor absorption by the human body. Increasing its solubility is an important factor in increasing its efficacy. In addition, CDs used in the development of transdermal formulations positively influenced the release and/or permeability of the drug, stabilized the drug in the formulation or at the site of absorption, reduced local irritation induced by the drug, and supported the release of the drug from the vehicle (33).

Polyvinyl alcohol (PVA) is frequently utilized in drug delivery due to its superior adhesive characteristics, biocompatibility, and nontoxicity (34). PVA has good chemical stability, forming stable complexes with various drugs to ensure their controlled and sustained release (35). The addition of PVA leads to increased hydrogel porosity, significant mechanical stability, and faster drug release, which may have effective anticancer effects on melanoma cells (36).

The use of hydrogels based on CS, β-CD and PVA could represent an interesting approach to improve the skin release of oncology drugs. In this context, we designed VEM-loaded chitosan-graft-β-cyclodextrin/PVA hydrogels for the local treatment of melanoma (37). β-Cyclodextrin-grafted chitosan functions as the structural component of the hydrogel that increases VEM solubility, controls drug release, and ensures biodegradability and compatibility, while polyvinyl alcohol (PVA) enhances mechanical properties. Enhancing the solubility and stability in water, the pharmacokinetic and pharmaco-

dynamic profiles of VEM, along with the possibility of applying these hydrogels at the tumor site, could change the current therapeutic strategy for melanoma. Monitoring VEM from the sustained-release hydrogel is crucial to ascertain the efficacy of this prospective melanoma treatment.

High performance liquid chromatography (HPLC) has proven to be the predominant technology used in pharmaceutical laboratories worldwide to analyze various drug substances, because is a rapid, automated and high-throughput screening method. Various assays have been reported for the determination of VEM in bulk and biological matrices (38-40). The other reported methods are sensitive, but they are quite complex and time consuming, rendering them unsuitable for routine analysis, as they require expensive or sophisticated instruments. Most refer to the coupling of liquid chromatography (LC) with mass spectrometry (MS) (41–43). It is a useful and robust technique; yet it is not easily accessible and cost-effective routine analysis for pharmaceutical products.

To our knowledge only two research regarding HPLC with ultraviolet (UV) detection methods for determination of VEM in plasma have been reported (44, 45). However, HPLC-UV method has not been reported for the determination of vemurafenib in polymer matrix. To monitor the necessary duration of drug availability (long and short term) and its release profile (continuous or pulsatile), accurate methods of analysis are required.

This study aimed to develop and validate a simple, rapid and effective RP-HPLC method with UV detection for the mutated BRAF inhibitor vemurafenib. Full validation was performed, and the validated method was applied to evaluate the ability of chitosan-graft-β-cyclodextrin/PVA hydrogels to load and release sustained VEM.

MATERIALS AND METHODS

1. Materials

Vemurafenib (VEM), the pharmaceutically active substance incorporated into the hydrogels (99.98% purity) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Formic acid, acetonitrile and methanol of HPLC grade were purchased from Merck (Darmstadt, Germany). Ultrapure water was obtained with Milli-Q® Type 1 Ultrapure Water Systems (Merck, PA, USA). All the other chemicals and solvents used were of analytical grade.

2. Equipment

The high-pressure liquid chromatography (HPLC) system used in this study was Shimadzu Nexera LC-40-XR (Shimadzu, Kyoto, Japan). The system was equipped with a serial dual-plunger pump, an autosampler (SIL 40XR), an SPD-40V series UV-Vis, and an RF-20Axs fluorescence detector (Shimadzu, Kyoto, Japan). Data were recorded and the system was controlled using the Lab Solution DB software 6.106SP1.

3. Method development

3.1. Mobile Phase Optimization

Different mobile phase compositions were tested based on previously published articles (46, 47). Some modifications were implemented (38) to increase applicability under laboratory conditions, and we determined that the mobile phase would consist of a mixture of the following solvents: A (water/formic acid - 99.9/0.1, v/v), B (acetonitrile) and C (methanol). By varying the solvent ratios (30: 55: 15 (MP1); 50: 40: 10(MP2); 40: 55: 5 (MP3)), the appropriate

proportions of the three solvents were studied to obtain an optimal resolution.

3.2. Column Optimization

The method was performed with various C18 columns, such as ODS column (4.6 × 200 mm, 10 µm) (Thermo Scientific, Vantaa, Finland), Xterra (4.6 × 100 mm, 5 µm), and Waters Cortecs column (2.1 × 100 mm, 2.7 µm) obtained from Waters, Milford, MA, US.

3.3. Determination of the wavelength corresponding to maximum absorption (λ max)

The standard solutions of VEM were scanned in the range of 200-400 nm to establish the wavelength at which VEM shows a maximum absorbance.

3.4. Determination of the retention time for VEM

To determine the retention time, two solutions with different VEM concentrations (8 and 12 mg/L) were prepared. A volume of 10 µL of each sample was injected and the retention time was determined.

3.5. Determination of the flow rate

To determine the optimal flow rate, VEM samples with known concentrations of 12 mg/L were prepared. A volume of 10 µL was injected, keeping the other parameters constant but varying the flow rate of the mobile phase.

4. Validation

Upon establishing the optimal analysis conditions, the method was validated by adhering to the following parameters: selectivity, linearity, limit of detection (LOD), limit of quantification (LOQ), precision (system precision, method precision, intermediate precision), and accuracy (48, 49).

4.1. Selectivity

Since the hydrogels contain different components, specificity was attained by comparing the maximum retention time of VEM in the formulations with that in the standard solution.

4.2. Linearity

To determine the calibration curve for VEM, a stock solution of VEM was prepared by dissolving 0.002 g of pure drug in 10 mL of acetonitrile, brought into a 10 mL volumetric flask. Successive dilutions were performed in the 0.78125-100 mg/L range of concentrations. Each standard solution was analyzed in triplicate.

4.3. Limit of detection (LOD) and limit of quantification (LOQ)

Limit of detection and limit of quantification were determined based on the standard deviation of the same concentration and calculated by relations (1) and (2):

$$LOD = 3.3 (SD/S) \quad (1)$$

$$LOQ = 10 (SD/S) \quad (2)$$

where, SD - standard deviation of the response of the curve, and S - Slope.

4.4. System Suitability

Chromatographic system suitability study was done by injecting 6 times consecutively 10 mg/L of VEM standard solution. Injection repeatability, reflected in the invariability of both peak area and retention times, defined the accuracy of the system. The capacity factor, tailing factor, and theoretical plates were also evaluated.

4.5. Method precision

It was done by estimating the corresponding responses three times on the same day and on three different days (first, second, and fifth day) for three different VEM concentration levels (40, 50, 60 mg/L). Each concentration level was analyzed in triplicate.

4.6. Accuracy

To determine the accuracy (precision) of the HPLC method for determining VEM,

the addition method was used: in a solution containing a known amount of standard, volumes of VEM solution were introduced to obtain concentrations of 80%, 100% and 120% compared to the concentration of the solution that was tested (50 mg/L). For each concentration level, three samples were prepared and analyzed, experimentally measuring the peak areas. The recovery was determined as a percentage of the theoretical concentration value.

4.7. Robustness

The robustness was studied by testing the influence of small changes in flow rate (±0.2 mL/min), wavelength (±2 nm), and temperature (±2°C).

4.8. Stability study

Freeze-thaw, short-term, and long-term stabilities of vemurafenib were determined according to the FDA guidelines (48,50,51). The stability of the VEM solution was assessed by comparing the area for the standard solution preserved for various time intervals with the freshly prepared standard. For this study the low and high concentration from the calibration curve were used. The responses of VEM from the stock solutions in mobile phase after 24 h at ambient temperature and after 3 months of storage in the freezer (-30°C) were compared in triplicate to fresh stock solutions.

5. The HPLC-UV conditions for sample analysis

The chromatographic separation was carried out using a Waters Cortecs C18 column (2.1 × 100 mm, 2.7 μm). The column temperature was SET at 40°C. The mobile phase was a mixture of: A (water/formic acid - 99.9/0.1, v/v), B (acetonitrile) and C (methanol) in a ratio of 40: 55: 5 (v/v/v). The elution was set at 1 mL/min in isocratic mode. The detection of VEM was performed in UV at λmax = 252 nm. The injection sample amount was 10 μL. The quantitative and qualitative analysis of VEM was based on its retention time and peak areas, respectively.

6. Application to hydrogels' characterization

The validated method was used to evaluate the chitosan-graft-β-cyclodextrin/PVA hydrogels designed as carriers for the controlled release of vemurafenib.

6.1. Sample preparation

Hydrogels were prepared by mixing the active substance solution with the vehicle solution as presented in our previous study (37).

6.2. Drug loading capacity (DL) and drug entrapment efficiency (DEE%) of the formulations.

To quantify VEM content from the carrier, we weighed the hydrogel samples, dissolved them in a 50/50 (v/v) methanol/acetonitrile mixture in 2 mL capacity vials, and centrifuged them for 24 hours. The supernatant was collected and filtered through a 0.22 μm filter. Then a volume of 10 μL of each sample was injected and analyzed using the HPLC method with UV-Vis detection at 252 nm. The DL capacity was calculated using Equation (3) derived from the calibration curve:

$$y = 45988 \cdot x + 11405 \quad (3)$$

The DEE% of VEM from the hydrogel was calculated with Equation (4):

$$DEE = \text{Actual VEM content loaded in the matrix/Theoretical VEM content} \times 100 \quad (4)$$

Determinations were made in triplicate, and results were reported as the mean of three independent experiments ± SD.

6.3. *In vitro* drug release studies and drug release kinetics

Drug release study of pure VEM and

VEM from hydrogel was conducted by the dialysis membrane method. PBS pH = 6.8 was used as the release medium, maintained at 37 ± 0.5°C under continuous stirring at 50 rpm (52). An aliquot of 1 ml was collected at predetermined intervals and replaced with fresh PBS solution. The drug quantification in each withdrawn sample was done by the HPLC method, as aforementioned. The concentration of VEM in each sample was estimated using the equation obtained from the calibration curve. The experiment was conducted three times, and the mean value was calculated. The cumulative quantity that was released was approximated as a percentage using the concentrations that were determined. The release profiles were fitted to various kinetic models for determining the release mechanism.

7. Statistical Analysis

The statistical analysis of all experimental data was conducted using *IBM SPSS Statistics 25 software*. The data were represented as the mean value accompanied by the standard deviation (SD). One-way Analysis of variance (ANOVA) was used, followed by a post hoc test (Tukey's HSD test), to establish the statistical significance of the observed differences. The predetermined threshold of significance was set at $p \leq 0.05$.

RESULTS

1. Optimization of chromatographic conditions

The method development procedure for drug quantification must follow some critical steps in order to be fully completed and accepted. It is important to develop a simple, rapid, reproducible, and sensitive method for VEM quantification. The following parameters were examined for the optimization of HPLC-UV analysis of VEM from the hydrogel: column, mobile phase, flow, retention time, and wavelength (53, 54).

When selecting the mobile phase, its participation in the separation process along with the stationary phase was considered (55). By varying the solvent ratios, convenient proportions of the three solvents, water/formic acid - 99.9/0.1 (v/v), acetonitrile, and methanol, were established to allow for optimal resolution. To further improve the peak shape, formic acid was added.

According to data presented in fig. 1, it was established that the best mobile phase with less retention time and a larger peak area was a mixture of water/formic acid - 99.9/0.1 (v/v), acetonitrile, and methanol in the ratio of 40: 55: 5 (v/v/v). Altering this ratio would result in a reduction in both the area and height of the peak, thereby diminishing the method's sensitivity. The analyte in isocratic elution was controlled by varying the mobile phase composition. The percentage of organic solvent in the mobile phase influenced the retention time of the analyte and significantly impacted selectivity (56). Regarding the mobile phase elution program, an isocratic system was optimal for VEM separation.

The C18 column is recommended for reversed-phase analysis. VEM separation was attempted with C18 columns packed with particles of different sizes. The ODS columns with the particle size of 10 μm did not effectively separate VEM. The C18 column with smaller particle sizes such as Xterra C18 (250 mm × 4 mm, 5 mm) gave partial separation. Waters CORTECS C18 column (2.1 × 100 mm, 2.7 μm) was found to be ideal, providing excellent peak shape and resolution at a flow rate of 1.0 mL/min. Since the retention time of VEM was also adequate, the column was selected as opti-

mal for conducting the experiments. Another HPLC-UV method described by Zheng *et al.* (44) for simultaneous quantification of vemurafenib and erlotinib in plasma used a C8 Xterra® MS column (250 mm×4.6 mm, 5 µm).

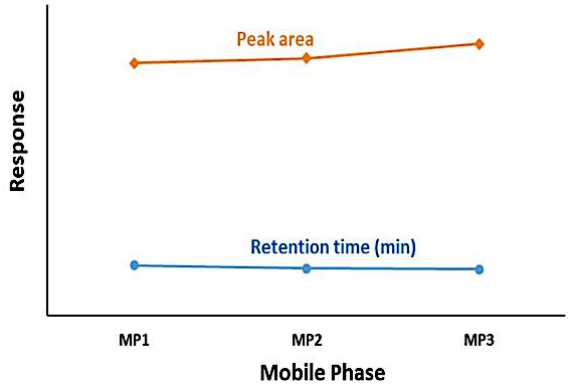

Fig. 1. The effect of mobile phase content to peak area and the retention time of VEM

Based on literature data, we chose 40°C as the working temperature (57). Only one study reported a column temperature of 50°C (44).

Two samples of VEM with different concentrations (8 and 12 mg/L) were analyzed to determine the retention time (T_r). The obtained chromatograms are reproduced in figure 2. The retention time for the analyzed samples was 6 min. The acceptance criteria states that a peak should appear at the retention time, but with different areas depending on the concentration.

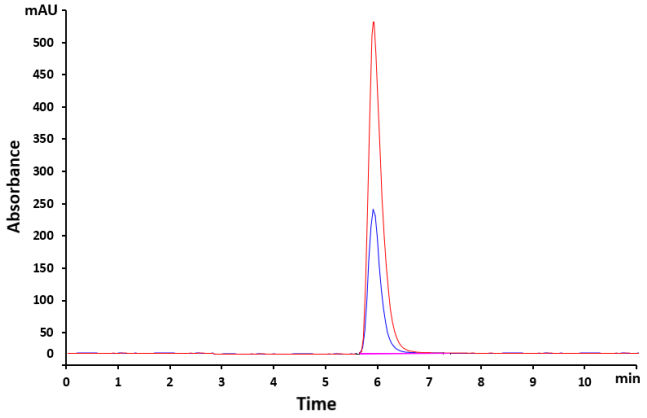

Fig. 2. Overlaid chromatograms of VEM samples with concentrations of 8 and 12 mg/L

The influence of the mobile phase flow rate on peak normalization was studied. To achieve optimal separation and to minimize the total analytical time, flow rates ranging from 0.5 to 2 mL/min were tested. It was observed that at identical concentrations, a

reduction in flow rate resulted in an increased retention time along with a decrease in signal intensity. Conversely, when the flow rate was increased, a decrease in the intensity of the chromatographic peak was observed, although the retention time remained relatively unchanged (fig. 3). All this caused a decrease in the sensitivity of the method. The negative effect would also affect the calculation of LOD and LOQ, which are performance parameters of the method. According to third figure the optimum flow rate was 1mL/min.

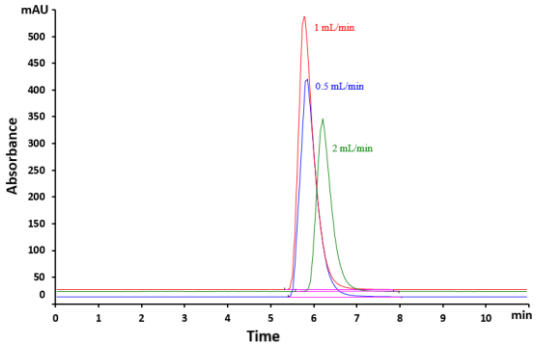

Fig. 3. Overlaid chromatograms of VEM samples with 12 mg/L concentration at different flow rates

We selected the UV detection coupling because, from an analytical perspective, it is a gold standard analytical technology in the pharmaceutical industry (58). The optimal wavelength for VEM was decided after recording the spectrum by the UV detector over the range from 190 to 400 nm. Based on the result (fig. 4), a maximum absorbance for VEM was observed at a wavelength of 252 nm, a finding corroborated by Vakhariya *et al*. (39). This value was used for all validation tests and practical applications. Other studies reported for vemurafenib a maximum absorbance at 307 nm (59), 310 nm (13) and 249 nm (44), respectively.

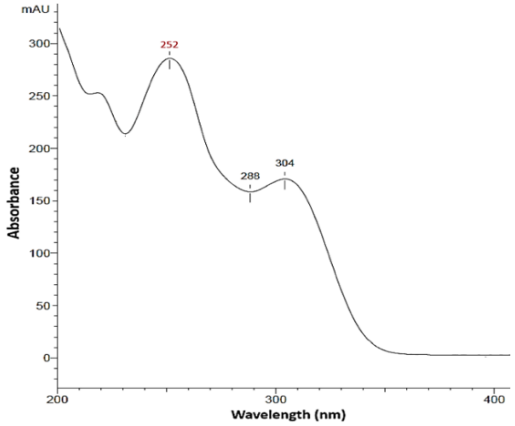

Fig. 4. Absorption spectra of VEM

2. Validation

2.1. Selectivity

The developed method was tested for possible interferences due to the polymer matrix. Six samples were analyzed to investigate the behavior of the matrix components. fig. 5 illustrates that no overlapping peaks were detected at the retention times of the VEM. The retention time of VEM from the sample peak was compared with that of the reference substance. The difference between the two retention times should be ±5% (49). It was found that the retention time of VEM from the sample was 6.02 min, and of VEM for the reference solution was 6.0 min. The proposed method was selective as it effectively separates the peak corresponding to vemurafenib.

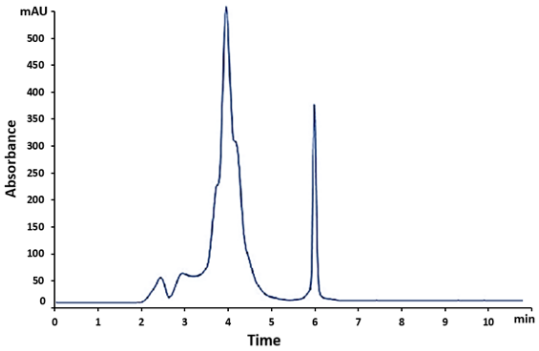

Fig. 5. Sample chromatogram for stability study

2.2. System Suitability

According to the validation guidelines, it is necessary to assess the performance of both the HPLC analytical instrument and the method prior to their application (49). System suitability must be performed before and throughout all regulated assays. System precision was studied by analyzing a number of 6 successive measurements of the same sample (10 mg/L). In this test, the following parameters were investigated: Resolution (R), repeatability (RSD - relative standard deviations - of peak response and retention time), column throughput (N), and tailing factor (T). Considering the values presented in first table, it can be stated that the system was eligible.

TABLE I

System suitability test for VEM

System Suitability Parameter	Acceptance Criteria	Results
Injection Precision for Retention Time (Min)	RSD ≤ 1%	RSD = 0.97
Injection Precision for Peak Area	RSD ≤ 1%	RSD = 0.91
USP Tailing Factor (T)	T ≤ 2.0	1.15*±0.06
Capacity Factor (K)	K ≥ 2.0	7.02*±0.15
Theoretical Plates (N)	N ≥ 2000	5562*±0.02

*Mean of six determinations

2.3. Linearity, LOD, LOQ

To have a robust calibration line, a series of three replicates of each standard was analyzed using the developed method. To study the linear relationship between concentration of VEM (independent variable) and area (dependent variable), we used a least squares method (60). The calibration graph was constructed in the range 0.78-100 mg/L (tab. II and fig. 6). The regression equation was y = 45988·x + 11405, where **y** denotes peak area and **x** the concentration of VEM (mg/L). The linearity of the method was excellent as evidenced by the value of the correlation coefficient (r^2 = 0.9999). The response linearity is verified if the correlation coefficient is 0.99 or greater (54). Also, the Fischer Test showed that $F_{calculated}$ = 19840.115 was greater than $F_{tabulated}$ = 242.98 for a risk of 5% and 11 degrees of freedom, so the correlation ratio between the factorial variable (concentration) and the outcome variable (peak area) is significant. Limit of detection (LOD) and limit of quantification (LOQ) were also performed for VEM with the following results: LOD = 0.5 mg/L and LOQ = 0.75 mg/L. We chose to determine the LOD and LOQ based on the calibration curve because it is more scientifically satisfactory. The LOQ of the present study is 1.66 times lower than that of another previous HPLC-UV method (44) and approximately 4-fold higher than that previously reported with the LC-MS/MS method (38).

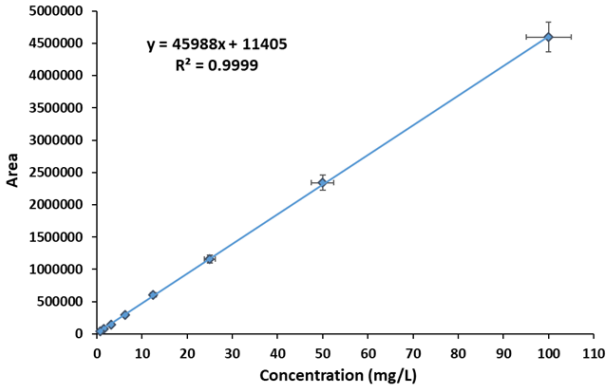

Fig. 6. Calibration graph for VEM over the range of 0.78-100 mg/L (area mean ± SD, n = 3); SD: Standard Deviation

TABLE II

Calibration data for the range 0.78-100 mg/L VEM.

No.	VEM (mg/L)	Area mean ± SD (n = 3)
1.	0.78	37776
2.	1.5625	78257
3.	3.125	146193
4.	6.25	291083
5.	12.5	606229
6.	25	1158865
7.	50	2339810
8.	100	4594650

SD: Standard Deviation

2.4. Intra- and Inter-Day Accuracy and Precision

The obtained accuracy and precision data are summarized in tab. III. The %RSD values found in the precision study (RSD < 2%) showed that the proposed method provides acceptable intra- and inter-day variations for VEM determination. To estimate the accuracy, the recovery was determined for a number of three samples at three different concentration levels. The proposed analysis method was accurate because the maximum value of RSD% was less than 2% and the recovery efficiency across the entire investigation range was within the range of 99.45-100.01%.

TABLE III

Precision and accuracy of VEM determination

Theoretical concentration of VEM (mg/L)	Accuracy		Precision			
	Mean recovered conc. of VEM	Mean % recovery	Intra-day		Inter-day	
			Mean*±SD	%RSD	Mean*±SD	%RSD
40	39.78	99.45	40.98±0.3141	0.71	41.08±0.3401	0.68
50	50.05	100.10	50.12±0.2856	0.67	50.06±0.2536	0.63
75	75.01	100.01	75.08±0.3452	0.78	75.02±0.3452	0.73

*Each value is represented as a mean ± SD of observations;
SD: Standard Deviation; RSD: Relative Standard Deviation.

2.5. Robustness

The data obtained for the robustness study are presented in tab. IV. The results showed that there were no significant changes in the chromatographic pattern when the above modifications were made in the experimental condition (the %RSD values were < 3). Therefore, the method was found to be robust with respect to variability in all robust conditions.

TABLE IV

Robustness results for VEM

Parameter	Variation	Retention time (min)	Theoretical Plates	Tailing Factor
Flow Rate	0.8 mL/min	6.23	5492	1.08
	1 mL/min	6.01	5547	1.12
	1.2 mL/min	5.98	5562	1.12
Wavelength	250 nm	6.03	5649	1.11
	252 nm	6.01	5598	1.10
	254 nm	5.99	5697	1.06
Temperature	38°C	6.02	5789	1.13
	40°C	6.00	5856	1.02
	42°C	6.02	5698	1.03

2.6. VEM solution's stability

The results of the stability study of VEM in mobile phase after different storage procedures are summarized in table V. No losses greater than 6.3% were recorded and the deviations found had low standard deviations. The samples could be submitted to further analysis without suffering degradation, the peak area values for VEM being relatively constant. The stability results can be considered satisfactory for the validation and are consistent with those previously reported for vemurafenib (38, 40, 44).

TABLE V.

Stability data (recovery [%]; ± SD; n = 4) of vemurafenib

Conditions	High concentration	Low concentration
24 hrs. at ambient temperature	101.5 ± 1.3	93.7 ± 0.6
3 free-thaw cycles	105.3 ± 1.6	94.8 ± 2.3
3 month -30°C	102.5 ± 1.8	97.3 ± 3.8

DISCUSSION

To evaluate the performance of the developed method, it was compared with other HPLC methods documented in the literature (tab. VI). Methods coupled with mass spectrometry (38, 41) provide superior sensitivity; nonetheless, they are more complex and require costly and advanced apparatus, rendering them impractical for routine analysis. Thus, it was observed that the linear range was close to other methods (38), and the LOQ had a lower value compared to some data in the literature (44, 57) but it was fairly close to that reported with LC-MS/MS (38, 41). Analysis time was similar to that of some methods (44, 61). Most methods used C18 columns and isocratic elution at a flow rate of 1 mL/min. The mobile phase in most cases was a mixture of solvents represented by water-acetonitrile, water-methanol or methanol-acetonitrile in various proportions. Unlike other methods that determine VEM in pure form and from pharmaceutical and biological products, our method determined the drug substance in a polymer matrix.

TABLE VI.

Comparison of the performed method with other HPLC methods

No.	Stationary phase / chromatographic column	Mobile Phase and flow rate	Detection / T_r	Statistical parameters	Practical application	Ref.
1	Xterra® MS C8 (250 mm × 4.6 mm, 5 µm)	glycine buffer (pH 9.0, 100 mM): acetonitrile (45: 55, v/v) 0.9 mL/min	249 nm 6.3 min	*DL = 1.25-100 mg/L LOQ = 1.25 mg/L r^2 = 0.99 Recovery = 99,1%	mouse plasma	(44)
2	X-Terra RP-18 (250 x 4.60 mm, ID 5 µm)	acetonitrile: water 60: 40 (v/v) 1.0 mL/min	249 nm 6.69 min	DL = 2-10.0 mg/mL LOQ = 0.146 mg/L r^2 = 0.9999 Recovery = 100.1- 102.33%	human urine	(61)

No.	Stationary phase / chromatographic column	Mobile Phase and flow rate	Detection / T_r	Statistical parameters	Practical application	Ref.
3	Acquity UPLC® BEH C18 (30 mm × 2.1 mm, 1.7 µm)	0.1% (v/v) formic acid In water (10%, v/v): water (20%, v/v): methanol (70%, v/v) 0.6 mL/min	MS m/z 490.1→255.05	DL = 0.1-100 mg/L LOQ = 0,1 mg/mL r^2 = 0.9996 Recovery = 99-106%	human and mouse plasma	(38)
4	Symmetry C18 (4.6 mm ×150 mm, 5 µm)	methanol: water (45: 55, v/v) 1.0 mL/min	260 nm 2.379 min	DL = 24-120 mg/L LOQ = 16.7 mg/mL r^2 = 0.998 Recovery = 99.4-99.9%	in pure form and dosage forms	(57)
5	Symmetry ODS C18 (4.6 x 250 mm, 5 µm)	acetonitrile: methanol (80: 20, v/v) 1.0 mL/min	272 nm 3.15 min	DL = 10-50 mg/L LOQ = 3.2 mg/L r^2 = 0.999 Recovery = 98.0-102%	in pure form and dosage forms	(62)
6	Acquity UPLC BEH C18 column (2.1 × 50 mm1.7 mm)	10 mM ammonium acetate in water (A) and methanol (B) with applied phase gradient: 50-80% B (0.0-0.5 min), 80% B (0.5-2.5 min), 80-95% B (2.5-2.6 min), 95% B (2.6-3.6 min), 95-40% B (3.6-3.7 min), 50% B (3.7-7.0 min). 0.25 mL/min	MSm/z 488.2→ 381.0 3.4 min	DL = 1.0 -100.0 mg/L LOQ = 0.1 mg/mL r^2 = 0.9985	human plasma	(41)
7	Waters CORTECS C18 column (2.1 × 100 mm, 2.7 µm)	water/formic acid – (99.9/0.1, v/v): acetonitrile: methanol 40: 55: 5 (v/v/v) 1.0 mL/min	252 nm 6 min	DL = 0.78-100 mg/L LOQ = 0.75 mg/L Recovery = 99,45-100.0% r^2 = 0.9999	hydrogels	The proposed method

*DL = linearity domain

2.7. The capacity of hydrogels in loading and releasing VEM

To show the applicability of the new method after the validation procedure, we investigated the ability of CS-g-β-CD/PVA hydrogels to load and release VEM sustainably. Drug loading has a significant impact on drug release from transdermal systems. The higher drug loading decreases the rate of diffusion to 50%. Lower drug

loading leads to faster drug release.

The drug loading capacity (DL) of the developed formulations, namely CS-g-β-CD/PVA 25/70/5 and CS-g-β-CD/PVA 20/75/5, exhibited 5.7146 μg/mg and 5.7672 μg/mg VEM, respectively. The drug loading process of polymeric networks presented an influence dependent on the composition of hydrogels. It was observed an increase in the networks loading capacity with the decreasing of chitosan content.

The drug entrapment efficiency (DEE%) was between 65±0.08% and 70±0.05%. Those results indicated that both hydrogels exhibited very good DL and DEE% capacity, in line with previous evidence obtained for VEM (13). The exact amounts and loading efficiencies are presented in fig. 7a and 7b. The evaluation of dosage content homogeneity for topical transdermal preparations is mandated by the United States Pharmacopeia, which sets a maximum acceptability value of ±15% (63).

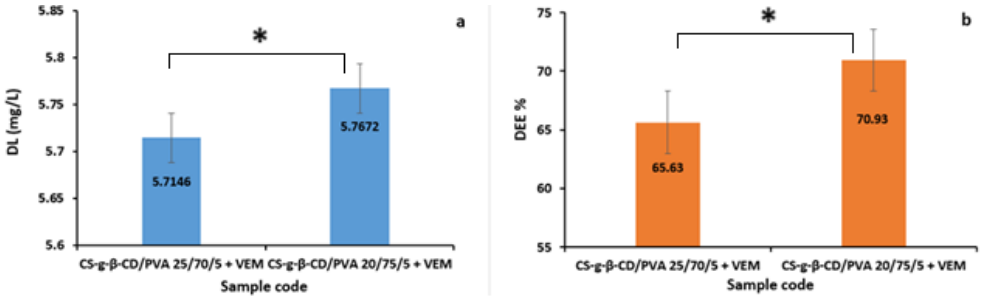

Fig. 7. DL capacity **(a)** and DEE% of hydrogels; **(b)** (mean ± SD, n=3) (* $p < 0.05$)

2.8. In Vitro Drug Release Analysis

CS-g-β-CD/PVA hydrogels with different mass ratios were studied in terms of their VEM release capacity by immersion in the release medium (phosphate buffer solution with pH = 6.8, at 37°C) using the validated HPLC method. The calculation of the amount of drug released was performed based on the equation of the calibration curve. The VEM release profiles under conditions that mimic the biological environment from polymer matrices are represented in fig. 8. The results indicated the prolonged release of VEM from the studied hydrogels when compared to the release of VEM from an industrial product. It was also found that by the increasing the CS concentration in polymer matrix decreased the VEM release rate from the matrix tablets.

According to fig. 8, the release process exhibited a relatively equally intense "burst effect" for the two systems studied. In the first 7 h, CS-g-β-CD/PVA+VEM 20/75/5 releasing around 61% and CS-g-β-CD/PVA+VEM 25/70/5 around 65%. The burst release of VEM might be determined by the presence of drug trapped on the hydrogel surface during the preparation process (64). After that, the release of the drug progressively increases up to 10 hours for both matrices studied, reaching 87% and 91% of the incorporated VEM amount. This stage was followed by a slightly linear sustained release profile with the release of 10% and 12% of the incorporated drug. The slow drug release could be due to poor solubility of drug. At the end of the dissolution test, the amount of VEM in CS-g-β-CD/PVA+VEM 20/75/5 is the lowest.

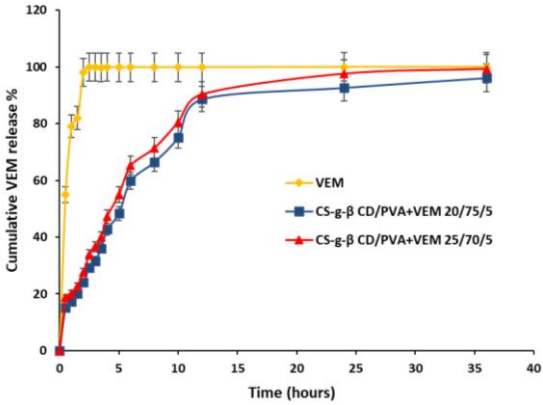

Fig. 8. Drug release of VEM. Data are mean ± SD within ±2 (n = 3)

2.9. Kinetics of in vitro drug release study

To understand the kinetics and the main mechanisms which govern the release of VEM from CH-g-β-CD/PVA hydrogels, the data plotted in fig. 8 were fitted using different kinetic models (65–67). The data are given in tab. VII. The selection of the model that most accurately described the release profile of VEM from the obtained hydrogels was based on the Akaike Information Criterion (AIC) and the r^2 correlation coefficient.

TABLE VII.
Data fitting results of *in vitro* VEM release profile from hydrogels

Kinetic Model	Parameters	Sample	
		CS-g-β-CD/ PVA 25/70/5	CS-g-β-CD/ PVA 20/75/5
Zero order	K_0	4.588	4.302
	r^2	0.668	0.496
	AIC	148.420	144.043
First order	K	0.155	0.156
	r^2	0.895	0.839
	AIC	6.887	14.342
Higuchi	K_H	27.513	29.468
	r^2	0.923	0.865
	AIC	125.549	134.365
Korsmeyer-Peppas	K_P	45.991	48.097
	n	0.372	0.293
	r^2	0.958	0.897
	AIC	103.589	108.123

K_0 - constant of zero order release rate; K - constant of first order release rate; K_H - constant of Higuchi model release rate; K_P - constant of Korsmeyer-Peppas model release rate; n = diffusion exponent.

Values of r^2 and values of AIC range between 0.983-0.991 and 103.589-148.420, respectively (tab. VII). For r^2, the value should be as close as possible to 1 to demonstrate a yield as good as possible for a formulation and the AIC must have minimum values (23, 68). In our study the values of r^2 and AIC indicated that the obtained hydrogels did not follow an ideal zero-order kinetics, nor a first-order kinetics. The r^2 values for the Higuchi and Korsmeyer-Peppas models did not differ significantly. The difference was given by the lower AIC values for the Korsmeyer-Peppas model. This indicated that the release of VEM from hydrogels occurred through the diffusion phenomenon (69). The diffusion mechanisms were indicated by the values of the diffusion exponent (n) in the Korsmeyer-Peppas equation. The values of n indicated a release by Fickian diffusion for VEM.

The choice of the optimal model for drug release could also be determined if there was an initial release of the active substance of 60%. Taking this into account, Korsmeyer-Peppas was the most effective and best-fitting model for release of VEM based on the data collected after 40 h. Over time, the release profile of VEM from different pharmaceutical forms has been associated with the Korsmeyer-Peppas model.

CONCLUSIONS

In the present study, a new, simple, rapid and validated HPLC method with UV detection was developed for vemurafenib determination in polymer matrices.

The advantages of our method include reduced analysis time, a simple procedure for sample preparation, specificity, and the ability to separate the drug from polymeric matrices, without the need of extraction. It also observed that there was no interference from the hydrogel's components for the determination of the VEM. The recovery percentage for the determination of VEM in polymer matrix had a value close to 100% and was similar to other methods documented in the literature. The obtained results during the *in vitro* dissolution test indicated the prolonged release of VEM from the studied hydrogels compared to the release of VEM from an industrial product. The method is suitable for routine analysis of VEM in bulk, in tablet dosage forms or hydrogels and can be also used during *in vitro* studies. The high sensitivity of the method allows its application in further studies of transdermal release or permeability of active ingredients in hydrogels.

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CONFLICT OF INTEREST AND FUNDING

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STRESS DISTRIBUTION IN PERIODONTALLY COMPROMISED MANDIBULAR MOLARS UNDER ASYMMETRIC LOADING: A THREE-DIMENSIONAL FINITE ELEMENT STUDY

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STRESS DISTRIBUTION IN PERIODONTALLY COMPROMISED MANDIBULAR MOLARS UNDER ASYMMETRIC LOADING: A THREE-DIMENSIONAL FINITE ELEMENT STUDY (Abstract): Periodontal disease alters the biomechanical stability of the supporting tissues, predisposing them to damage under occlusal overload. This study aims to use finite element analysis (FEA) to evaluate the effect of traumatic occlusal forces on a mandibular second molar with compromised periodontal support. **Materials and methods**: A three-dimensional FEA model was constructed from CT data and subjected to symmetrical and asymmetrical occlusal loads, ranging from 500 N to 1000 N, with variations in tooth inclination. **Results**: The results showed that axial stress and maximum displacement increased proportionally with force intensity, with the highest values observed under asymmetric loading in mesial inclined teeth with alveolar bone loss. In contrast, symmetrical loading on a well-aligned tooth in normal bone conditions generated minimal stresses. **Conclusions:** These findings highlight the critical role of occlusal force distribution in periodontal biomechanics and confirm that traumatic occlusion may accelerate tissue breakdown in compromised periodontium. **Key words:** FINITE ELEMENT ANALYSIS, TRAUMATIC OCCLUSAL FORCES, PERIODONTAL BIOMECHANICS, MANDIBULAR MOLAR, STRESS DISTRIBUTION.

INTRODUCTION

Finite element analysis (FEA) has emerged as a powerful computational tool to study stress distribution and deformation in biological structures. Unlike conventional experimental models, FEA allows for three-dimensional simulation of complex anatomical geometries and material properties, enabling the investigation of biomechanical behavior under controlled loading conditions (1). In periodontology, FEA has been widely applied to evaluate stress transmission in alveolar bone, periodontal ligament, and restorative materials, offering valuable insights into the interactions between mechanical forces and biological tissues (2).

Applying FEA to the study of traumatized and diseased periodontal tissues offers the opportunity to quantify how occlusal forces contribute to pathological stress concentrations. Such knowledge is critical for clarifying the role of traumatic occlusion as a modifying factor in periodontitis and for guiding clinical decision-making in occlusal adjustment, splinting, and prosthetic rehabilitation (3). The biomechanical analysis of molars provides

insights into dento-periodontal functional patterns during masti-cation and thus allows the evaluation of masticatory function (4).

Occlusal overload has been widely debated as a contributing factor in the progression of periodontal breakdown. While inflammation is the primary etiologic agent in periodontitis, mechanical stresses generated by traumatic occlusal forces can exacerbate tissue destruction, particularly in already compromised periodontal structures (5). Understanding how these forces distribute within the dento-periodontal complex is therefore critical for clarifying the role of occlusal trauma as a modifying factor in periodontal disease progression (6).

To accurately define the stress field of a molar under occlusal loading, it is essential to construct a model that incorporates the geometry of the structure, the properties of the tissues, and the forces acting on the dento-periodontal system (7).

The aim of the present study was to investigate, through finite element analysis, the axial stresses along the direction of force as well as the displacements resulting from the application of occlusal forces of different magnitudes on a mandibular second molar model.

MATERIALS AND METHODS

A three-dimensional finite element analysis model of the mandibular right second molar was constructed based on anatomical parameters described in the literature. The model incorporated the following dimensions: coronal length 7 mm, root length 13 mm, mesiodistal crown diameter 10.5 mm, cervical mesiodistal crown diameter 8 mm, buccolingual crown diameter 10 mm, and cervical buccolingual crown diameter 9 mm. Geometric reconstruction ensured that the tooth and surrounding structures were represented with high fidelity to their anatomical proportions, thereby providing realistic boundary conditions for stress analysis (fig. 1).

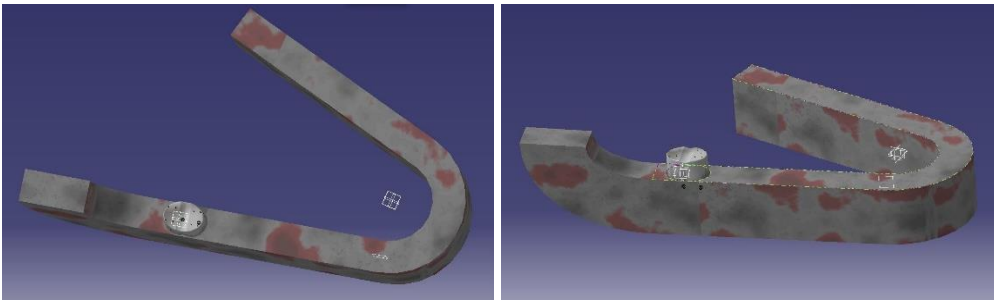

Fig. 1. Three-dimensional finite element analysis model of the mandibular right second molar; superior view (left); latero-superior view (right)

The biomechanical properties of the periodontal tissues were derived from experimental data reported in previous studies, with values assigned to enamel, dentin, pulp, periodontal ligament, and alveolar bone. For the purpose of simplification, and in line with established modeling practice, each tissue was modeled as a homogeneous, isotropic, and linearly elastic material. This approach allows for reliable stress mapping while avoiding unnecessary complexity in material heterogeneity.

The 3D structure was designed and

processed in Catia V5 (Dassault Systèmes, France). The final mesh consisted of 20,875 nodes and 16,616 parabolic prismatic elements, which provided adequate discretization of the model. The element size and density were optimized to achieve a balance between computational efficiency and accuracy of stress prediction. The element dimensions were as follows: mandible - 7.935 mm with a precision of 1.27 mm; periodontal ligament - 0.892 mm with a precision of 0.143 mm; and tooth - 1.27 mm with a precision of 0.203 mm. The mesh refinement was specifically enhanced at the tooth-ligament-bone interfaces, where stress concentrations were anticipated.

Rigid boundary conditions were applied at the base of the alveolar segment to simulate the anchorage of the jawbone. Loading conditions included vertical forces of 500 N, 650 N, 800 N, and 1000 N, applied at occlusal contact areas to represent different intensities of functional and traumatic occlusal loads. The interfaces between dentin and periodontal ligament, periodontal ligament and alveolar bone, enamel and dentin, and dentin and pulp were modeled as perfectly bonded, ensuring no relative displacement between adjacent tissues.

The loading scenarios were designed to simulate both physiological and supraphysiological conditions, enabling comparison between normal mastication forces and potentially traumatic forces. Stress distribution was calculated and expressed as von Mises stress, which is widely used as a criterion to evaluate the biomechanical performance of dental and periodontal structures under complex loading. In addition to stress analysis, displacement vectors were also recorded to evaluate the deformation patterns of the tooth and supporting tissues under different magnitudes of occlusal force.

RESULTS

The application of a symmetrical and uniform occlusal load on a compromised periodontium (simulated bone loss of 5.5 mm) in a non-tilted mandibular second molar produced the following outcomes (tab. I).

TABLE I.

Measurements in bone affected by periodontal disease, simulated as horizontal bone loss of 5.5 mm. The occlusal force was applied symmetrically and uniformly on the occlusal surface of the tooth, without angulation

No.	Occlusal force	*σech* von Mises [mPa]	f (movement) [mm]	*Σdirection of force* [MPa]
1	500 N	324	0.298	139
2	650 N	418	0.388	180
3	800 N	515	0.478	221
4	1000 N	644	0.598	276
$\bar{x}$ ± SD		475.25 ± 136.88	0.441 ± 0.128	204.0 ± 58.52
f = maximal displacement; *σc* = axial stress in the loading direction				

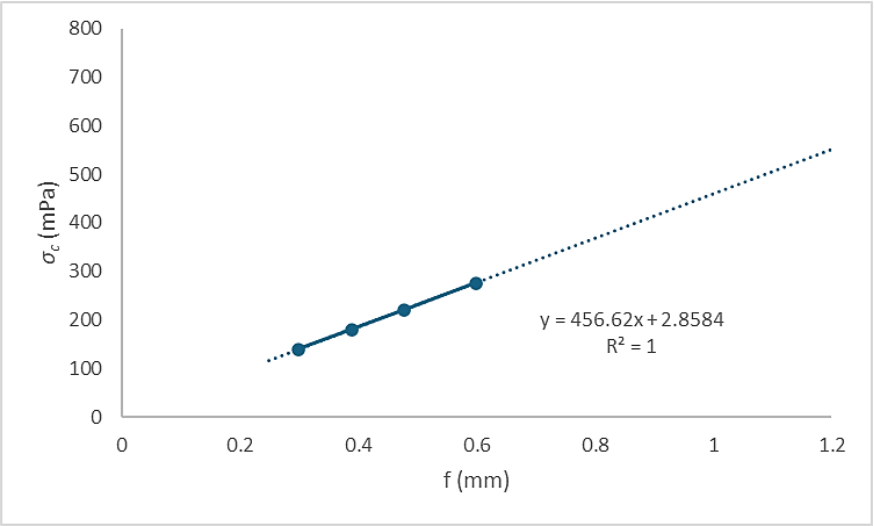

Fig. 2. Correlation between maximum displacement (f) and axial stress (σ_c) in the loading direction in bone affected by periodontal disease.

The axial stress along the direction of loading ranged from 139 to 276 mPa, with a mean value of 204.0 ± 58.52 mPa. A strong positive correlation was observed between occlusal force magnitude and axial stress (r = +0.999; p = 0.001), indicating that stress increased proportionally with the intensity of applied forces. Similarly, axial stress showed a significant correlation with maximum displacement values (r = +0.999; p = 0.001), demonstrating that higher occlusal loads not only increased stress concentration but also amplified tooth movement within the compromised periodontal support. The trendline was linear (R2 = 1), with a slope of 456.62, suggesting a perfect fit of the linear model to the data points (fig. 2).

Simulation of a clinical scenario with 20 degrees mesial inclination of the mandibular second molar, in the context of a compromised periodontium with 5.5 mm horizontal bone loss and the application of unilateral, asymmetric distal forces, yielded the following results (tab. II).

The axial stress in the loading direction ranged from 311 to 622 mPa, with a mean value of 458.75 ± 132.94 mPa. Axial stress in the loading direction increased significantly with the magnitude of the applied occlusal force (r = +0.995; p = 0.006). Axial stress in the loading direction also increased significantly with maximum displacement (r = +0.996; p = 0.006). In this case trendline was also linear (R^2 = 1), but the slope increased to 617.58, suggesting an accentuated displacement in comparison to the perpendicular force action (fig. 2).

The mean level of maximum displacement showed statistically significant differences depending on bone type (p = 0.001). The lowest mean displacement was observed in unaffected bone with a 20 degrees mesial inclined molar (0.027 ± 0.008 mm; range: 0.019 - 0.037 mm). The highest mean displacement was recorded in unaffected bone with a non-tilted molar under symmetrical and uniform distal occlusal loading (9.885 ± 2.854 mm; range: 6.71-13.40 mm).

TABLE II.

Measurements in bone affected by periodontal disease. The occlusal force was asymmetrically applied on the distal surface; molar mesially inclined at 20 degrees

No.	Occlusal force	σ_{ech} von Mises [mPa]	f (movement) [mm]	*Σ direction of force* [MPa]
1	500 N	339	0.506	311
2	650 N	441	0.658	404
3	800 N	542	0.809	498
4	1000 N	678	1.01	622
$\bar{x}$ ± SD		500.0 ± 144.74	0.746 ± 0.215	458.75 ± 132.94
f = maximal displacement; σ_c = axial stress in the loading direction				

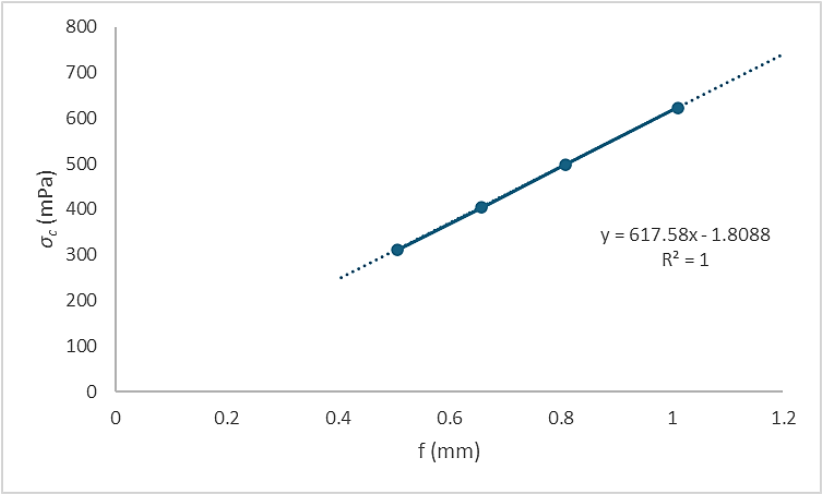

Fig. 3. Correlation between maximum displacement (f) and axial stress (σ_c) in the loading direction in bone affected by periodontal disease; asymmetric occlusal force applied on the distal surface; molar mesially inclined at 20 degrees.

The mean level of axial stress in the loading direction showed statistically significant differences across bone types (p = 0.001). The highest mean value was recorded in bone affected by periodontal disease with 5.5 mm horizontal bone loss, under asymmetric distal occlusal loading and 20 degrees mesial inclination of the molar (458.75±132.94 mPa; range: 311-622 mPa).

The maximum displacements observed under different loading intensities and tooth angulations showed the lowest values for an inclined molar subjected to eccentric force application, and the highest values for a properly aligned tooth with forces distributed across the entire occlusal surface (tab. III).

TABLE III.
Descriptive indicators of maximum displacement (mm) compared across affected bone.

Type of bone	N	Mean	Std. Dev.	Std. Error	Confidence interval 95%		Min	Max
					-95%CI	+95%CI		
Affected bone, non-tilted molar, symmetrical occlusal force	4	0.441	0.128	0.064	0.237	0.644	0.298	0.598
Affected bone, molar mesially inclined at 20°, symmetrical occlusal force.	4	0.746	0.215	0.108	0.403	1.088	0.506	1.010

DISCUSSION

In the present study, a finite element model based on CT images of the mandibular second molar was established to explore the effects of progressively increasing excessive occlusal forces on dental stress. The regions and distribution values of von Mises stress were used to describe the load transfer mechanism. Although this model was developed using CT data from a single volunteer with certain anatomical particularities, the regularities derived from this analysis may be useful for understanding consistent biomechanical patterns of occlusion.

Similar results have been reported in previous studies. Mezzomo *et al*. created a finite element model of a maxillary central incisor and applied a static load of 100 N to the center of the palatal surface (8). Their findings demonstrated that the stress values in the enamel were significantly higher than those in the dentin, with the enamel-cementum junction serving as the boundary (8).

The study conducted by Chen *et al*., using 3D finite element models of different tooth types, investigated the influence of various alveolar bone heights on numerical simulations of tooth mobility and von Mises stress in the presence of periodontitis (9).

The 3D FEA of tooth geometry characteristics and stress relationships under different alveolar bone levels suggested that von Mises stresses may increase as periodontal support decreases. This indicates that teeth are subjected to greater stress when alveolar bone height is reduced, thereby becoming more susceptible to occlusal trauma (9).

Zarrati *et al*. used force sensors to examine differences in tooth mobility between patients with and without periodontal disease (10). They concluded that tooth mobility cannot be attributed solely to the loss of alveolar bone but also results from structural changes within the periodontal apparatus (10). Animal studies reported the simultaneous occurrence of periodontal connective tissue alterations, alveolar bone loss, and increased tooth mobility in animals predisposed to periodontal lesions (11). Animal models have the advantage of experimental inducement of several pathological conditions (12), that could be further evaluated and introduced in simulated mathematical models.

Studies conducted in both humans and

animals have demonstrated an apparent relationship between tooth mobility and periodontitis associated with reduced alveolar bone height. The inflammatory component of periodontal disease represents a critical modifying factor in tissue breakdown and biomechanical stability. Inflammatory mediators such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF-α), and interleukin-1 beta (IL-1β) are upregulated in periodontitis and contribute to connective tissue degradation and alveolar bone resorption (13-15). Low-level laser therapy (LLLT) has emerged as a non-invasive therapeutic modality capable of modulating inflammatory responses in various chronic inflammatory conditions, as emphasized by some researchers from our university, more than a decade ago (16). Meanwhile, the anti-inflammatory mechanisms of photon radiation and the involvement of photobiology in contemporary dentistry became an evocated fact in literature (14).

Wang *et al*. further concluded that there is a strong correlation between the amount of alveolar bone and overall tooth stability (17). However, evidence remains limited regarding the relationship between tooth mobility and variations in alveolar bone height across different tooth types analyzed through 3D FEA simulations (17).

Another variable that could influence the theoretical simulated model is represented by local vascularization, prone to high variability at the facial skeleton level. The recognition of anatomical variations in vascular supply is essential for understanding the biomechanical and biological behavior of oral structures under functional stress. Similar to the documented supernumerary fronto-orbital arteries (18), significant anatomical variability has been observed in the arterial branches supplying the maxillary dentition and periodontal tissues. The infraorbital artery (IOA) and the posterior superior alveolar artery (PSAA), both branches of the maxillary artery, demonstrate considerable variation in their course, diameter, and distribution patterns. In a cone beam computed tomography study, the PSAA was visualized in more than 2/3 of cases, with variable distances from the alveolar crest, directly influencing the vascular supply to maxillary molars, premolars, and the surrounding alveolar bone (19). Furthermore, anatomical surveys have documented anastomoses between PSAA and IOA at the premolar level, forming transverse periosteo-mucosal anastomoses that supply the marginal gingiva, interdental papillae, and periodontal ligament through buccal branches penetrating the interdental septum (20). These vascular networks are critical for maintaining tissue integrity and adaptive responses to mechanical loading. While the present study focused on mandibular second molars and their supporting structures, the documented variability in maxillary arterial supply underscores the need for future finite element models incorporating upper teeth to evaluate the interplay between vascular patterns, periodontal biomechanics, and occlusal stress distribution in the maxilla.

Although it is well established that FEA models cannot provide direct practical predictions regarding the effects of external forces on alveolar bone height or on stress distribution, it is nevertheless reasonable to apply external forces to alveolar bone of varying heights in order to evaluate stress distribution patterns (21).

One of the main advantages of FEA studies is the ability to demonstrate numer-

ical stress variations under normo-occlusal, hypo-occlusal, and hyper-occlusal loading conditions. Normal occlusal loads can be applied according to values reported in the literature, and moreover used in conjunction with artificial intelligence models to increase coverage of different scenarios (22). In contrast, hypo- and hyper-functional loads are hypothetical scenarios designed to estimate possible numerical stress values at specific points of static occlusal loading. In reality, there is wide individual variation in occlusal loading on each tooth, depending on factors such as diet, physiological activity, muscular forces, and parafunctional habits (23).

The determination of stress values is therefore a prerequisite for clarifying the role of occlusion in the health and stability of periodontal tissues. Future directions in this field should focus on understanding biological adaptation mechanisms in order to design reliable methods for evaluating the complex structural and functional interactions that define the dento-periodontal system.

CONCLUSIONS

In the present study, we demonstrated that maximal stress occurs when premature, asymmetric occlusal forces act on a mispositioned tooth already compromised by periodontal disease. In contrast, physiological forces applied uniformly and symmetrically across the occlusal surface of a properly aligned tooth within a normal alveolar bone environment generated minimal stress values.

These findings highlight the critical role of masticatory force distribution under different simulated conditions, showing that traumatic occlusal forces with asymmetric loading can produce severe damage to the supporting periodontal tissues.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding.

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PERSONALITY TRAITS AND MONEY ATTITUDES AMONG SCHOOLTEACHERS: AN EXPLORATORY PSYCHOLOGICAL ANALYSIS

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PERSONALITY TRAITS AND MONEY ATTITUDES AMONG SCHOOLTEACHERS: AN EXPLORATORY PSYCHOLOGICAL ANALYSIS (Abstract). Financial well-being is a multidimensional construct that reflects perceived financial control and security. While it is not directly measured in this study, money attitudes represent an important cognitive-affective component associated with financial well-being. **Materials and methods:** This cross-sectional study examined the relationship between personality traits and money attitude dimensions among 172 schoolteachers from northeastern Romania. The Big Five© plus-short and the Money Attitude Scale (MAS) were applied to assess personality traits and the dimensions of power-prestige, retention-time, distrust and anxiety toward money. **Results:** showed that emotional stability was negatively associated with financial anxiety and distrust, while gender and emotional stability influenced the perception of money as power-prestige. Extraversion, conscientiousness and openness predicted retention-time attitudes. Although the observed correlations were of low intensity, they align with previous research indicating subtle but consistent personality influences on financial cognition. **Conclusions:** These findings suggest that personality traits may shape teachers' money attitudes, with potential relevance for psychological screening and financial awareness programs in educational settings.
Keywords: MONEY ATTITUDE; PERSONALITY TRAITS; TEACHERS; FINANCIAL WELL-BEING; THE BIG FIVE PERSONALITY TRAITS; THE MONEY ATTITUDE SCALE.

INTRODUCTION

Teaching is a profession characterized by complex psychosocial demands that can influence both occupational satisfaction and mental well-being. Teachers often face high workloads, emotional exhaustion, and financial constraints, which may undermine their psychological health and job performance. Among the multiple determinants of well-being, personality traits play a central role in shaping how individuals perceive and cope with work- and finance-related stressors. Financial well-being, although not always explicitly measured, represents a key dimension of overall well-being, reflecting individuals' perceived control, security, and confidence in managing financial demands.

The Five-Factor Model (FFM), or Big Five framework, conceptualizes personality through five broad and stable dimensions: extraversion, agreeableness, conscientious-

ness, emotional stability (the inverse of neuroticism), and openness to experience. These traits have been consistently linked with occupational adjustment, resilience, and mental health outcomes across diverse cultural and professional contexts (1-7). Within educational settings, conscientiousness and emotional stability have been associated with higher job satisfaction and lower burnout (8, 9, 10), while openness and extraversion promote creativity and engagement in teaching.

Beyond the workplace, personality traits also shape financial attitudes and behaviors. Money is not only an economic resource, but a psychosocial symbol linked to power, security, and self-esteem (11). According to Yamauchi and Templer's Money Attitude Scale, four key dimensions capture the cognitive-affective meaning of money: power-prestige, retention-time, distrust, and anxiety (11). Previous research has shown that conscientiousness and openness are associated with prudent financial management, while low emotional stability is related to impulsive spending and financial anxiety (12, 13, 14, 15). However, most evidence derives from general or student populations, with limited attention to teachers—professionals whose financial and psychological well-being are deeply intertwined.

In the educational sector, modest income levels and socio-economic instability can exacerbate stress and anxiety among teachers, especially when combined with emotional or personality-based vulnerabilities. Recent studies have emphasized that financial stress is a significant determinant of teacher mental health, contributing to burnout, absenteeism, and reduced job performance (16, 17, 18, 19). Recent international evidence further confirms that financial strain and economic uncertainty represent significant stressors for teachers, contributing to emotional distress, burnout, and reduced occupational well-being across diverse educational systems (20, 21, 22). These findings highlight the importance of understanding psychological mechanisms, such as personality and money attitudes, that may influence financial well-being and indirectly affect occupational health. Financial well-being should be seen as a psychosocial determinant of mental health (stress, burnout, absenteeism).

Additionally, previous research indicates that financial attitudes vary across the lifespan, reflecting developmental and socio-economic differences. Younger adults often report higher financial anxiety and impulsiveness, whereas older individuals tend to adopt more cautious, future-oriented financial strategies (15, 23, 24). Therefore, age was included in the present study as a control and predictive variable to capture potential generational variations in teachers' financial attitudes.

To our knowledge, no prior study has specifically explored how the Big Five personality traits predict money attitudes among teachers. This research addresses that gap by examining the relationships between personality dimensions, demographic factors, and the four components of money attitudes - power-prestige, retention-time, distrust, and anxiety - in a sample of Romanian teachers. Understanding these associations can inform the development of preventive psychological and financial well-being programs tailored to educational professionals.

The present study aimed to test the following hypotheses:

- (H1) Emotional stability will be negatively associated with power-prestige

- (H2) Extraversion, conscientiousness, and openness will be positively associated with retention-time;
- (H3) Low emotional stability and low conscientiousness will predict higher distrust; and
- (H4) Low emotional stability will predict higher financial anxiety.

Criteria: extraversion, agreeableness, conscientiousness, emotional stability, openness to experience.

MATERIALS AND METHODS

Study design and Participants.

Using the snowball sampling method, a cross-sectional study was conducted among schoolteachers in northeastern Romania. An online questionnaire was created and distributed between February and May 2025 using Google Forms and could only be filled in only after the participants agreed to the informed consent form. The instrument was administered in a research-only, non-clinical context, and participants were clearly informed that the questionnaire did not serve any diagnostic or evaluative purpose. The Big Five© plus-short has been validated for self-administration in online formats in previous Romanian research and was used here in accordance with ethical guidelines for psychological research with non-clinical adult populations. The survey was administered in an anonymous format, ensuring that no personal identifiers were stored, and all responses remained confidential. The teachers were informed about the use of the results and that they could withdraw from study at any time. No incentives were provided to the participants. Continuing to complete the questionnaire indicated the participants' consent to be included in the study. *The inclusion criteria* were teachers who were professionally active in public or private schools and sent questionnaires that were fully filled in. *The exclusion criterion was* the submission of the questionnaires after the deadline. Snowball sampling was selected as a pragmatic approach due to limited centralized access to schoolteacher registries and to facilitate recruitment in a dispersed professional population. Although this non-probability method allowed efficient data collection from active educators, it does not ensure full representativeness of the national teacher population. A total of 172 teachers met the inclusion criteria and were included in the analysis. Of these, 72.1% were female (N = 124) and 27.9% male (N = 48). Participants ranged in age from 19 to 65 years (M = 41.75 ± 12.34), with 36% aged between 19 and 39, 39% between 40 and 50, and 25% between 51 and 65 years. In the Romanian educational system, individuals may begin teaching activities as substitute or trainee teachers from the age of 18-19, particularly in private institutions or in rural schools experiencing staff shortages. Therefore, eligibility to participate was based on *active teaching engagement*, not on formal rank or years of certified teaching service.

Study instruments. An online questionnaire was constructed to assess personality traits and measure money attitudes among schoolteachers. The first part gathered sociodemographic information, such as age and gender. The second part of the questionnaire included several psychological instruments:

1. The Big Five© plus-short (Constantin *et al.*, 2008) was used to assess the five major dimensions of personality: extraversion, agreeableness, conscientiousness, emotional stability, and openness to expe-

rience. (25). The Big Five© plus-short was used to assess the five major dimensions of personality: extraversion, agreeableness, conscientiousness, emotional stability, and openness to experience. The instrument consists of 90 items presented as statements rated on a forced-choice 3-point scale, where 1 = “disagree”, 2 = “partially agree”, and 3 = “agree”. Sample items include: “I enjoy being the center of attention” (extraversion), “I try to maintain harmony in my interactions” (agreeableness), and “I always complete the tasks I start” (conscientiousness). Higher scores reflect a stronger expression of the respective personality trait. The instrument consists of 90 items extracted from the Big Five© plus inventory. The Big –instrument has previously demonstrated satisfactory validity and reliability in Romanian samples, as reported by Constantin *et al.* (2008), confirming its suitability for use in local educational and occupational research. The Cronbach’s alpha internal consistency coefficients obtained for the questionnaire factors were as follows: Extraversion: 0.82; Agreeableness: 0.66; Conscientiousness: 0.75; Emotional stability: 0.85 and Openness to experience: 0.71 (12).

2. The Money Attitude Scale test (MAS; Yamauchi & Templer 1982) was used to identify individuals’ attitudes toward money (11). The scale consists of 29 items designed to evaluate four dimensions of attitudes toward money: power-prestige (9 items; e.g., "Although I should judge people’s success by their actions, I am more influenced by the amount of money they possess"), retention-time (7 items; e.g., "I regularly set money aside for the future"), distrust (7 items; e.g., "It bothers me when I find out I could have got something for a lower price elsewhere"), and anxiety (7 items; e.g., "I spend money to make myself feel better"). Responses were rated on a Likert scale (from 1 = "never" to 7 = "always"). Total scores were calculated for each dimension. The MAS is theoretically grounded in the conceptualization of money as a multidimensional psychological construct associated with power, security, and emotional regulation (11). In addition, research has highlighted the mediating role of money attitudes in the relationship between online behaviors and financial habits (26). Previous international studies have reported acceptable psychometric properties of this scale, and the Cronbach’s alpha coefficients obtained in the present study are consistent with those reported in European samples, supporting its applicability in this context. The Cronbach’s alpha internal consistency coefficients obtained for the scale dimensions are as follows: Power-prestige dimension: 0.71; Retention-time dimension: 0.82; Distrust dimension: 0.70 and Anxiety dimension 0.70.

Statistical analysis. The IBM *Statistical Package for Social Sciences (SPSS) and Statistics for Windows, version 29* (SPSS Inc., Chicago, IL, USA) were used to test the hypotheses (1). The adequacy of the sample size was assessed based on methodological conventions rather than a formal power analysis. For correlational studies in behavioral sciences, sample sizes above 100 are generally considered sufficient to detect medium effect sizes with adequate power (Cohen, 1988; Maxwell, 2000). The present study included 172 participants, exceeding this threshold and thus providing sufficient sensitivity for the intended analyses. Before applying Pearson’s correlation, we inspected the distribution of composite scale scores for skewness and kurtosis and found no severe deviations from

normality. Although the item responses were ordinal, the use of aggregated scale scores is considered acceptable for parametric testing in psychological research, as supported by methodological literature in the field. Therefore, Pearson's correlation was applied, in line with similar studies using the Big Five and MAS scales. Pearson's correlation coefficient was used to test the first four research hypotheses. An exploratory interaction analysis was conducted to test whether the combined effect of personality traits further influences money attitudes (exploratory, no hypothesis pre-registered). The t-test for independent samples was applied to compare group differences. Despite the unequal group sizes, the independent-samples t-test was applied using Levene's correction for unequal variances to ensure the robustness of the gender comparisons. Although no a priori power analysis was conducted, the sample size exceeds the commonly recommended threshold of 30 participants per predictor variable in regression models, suggesting that the study has acceptable power for exploratory analyses. Nevertheless, the statistical power to detect small interaction effects may be limited. To control for potential Type I error due to multiple comparisons, a conservative interpretation threshold was adopted. Only results with $p < 0.01$ were considered meaningful, consistent with exploratory research practices in psychological studies. This approach limits false-positive risk while maintaining reasonable sensitivity to genuine effects. Given the exploratory nature of the study and in line with recommendations for psychological research, associations with theoretical support and $p < 0.01$ were interpreted as robust.

Ethical Approval. The present study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethical Committee, No. 364/ 12.02.2025, Emil Racovita National College, Iași, Romania. Before starting the survey, the respondents were informed about the purpose of the research, the use of the results and confidentiality of the data. Those who agreed to participate filled out the questionnaires distributed online. No incentives were provided to the participants.

Informed Consent Statement.

Informed consent was obtained from all subjects involved in the study.

RESULTS

Pearson's correlation was used to examine the relationship among the research variables. The correlation statistical analysis indicated:

- a positive, low-intensity, and significant association between extraversion and agreeableness ($r = .283$, $p < .001$),
- agreeableness showed positive, low-intensity, and significant correlations with both emotional stability ($r = .193$, $p < .011$) and openness to new experiences ($r = .211$, $p < .005$,
- agreeableness demonstrated a negative, low-intensity, and significant correlation with the power-prestige factor ($r = - .233$, $p < .002$),
- a positive, medium-strength, and significant association ($r = .351$, $p < .001$) was identified between extraversion and openness to new experiences,
- a positive, medium-strength, and significant correlation between conscientiousness and openness to new experiences ($r = .300$, $p < .001$),
- the conscientiousness variable was

positively correlated, with medium intensity and significance, with the retention-time variable, r = .352, p < .001, and had a positive, low-intensity, and significant correlation with the emotional stability variable (r = .206, p < .007),

- a negative correlation of low intensity and significance was obtained between the emotional stability variable and the distrust variable (r = -.299, p < .001),
- a negative correlation of medium intensity and significance was obtained between emotional stability and the anxiety variable (r = - .289, p < .001),
- openness to experience correlates positively, significantly, and with low intensity with the retention-time dimension (r = .179, p < .019),
- the retention-time dimension correlates positively, significantly, and with low intensity with the conscientiousness variable (r = .194, p < .011) and the emotional stability variable (r = .161, p < .035),
- the distrust dimension correlates negatively, with medium intensity and significance with the emotional stability factor (r = -.299, p < .001) and positively with low intensity and significance with the anxiety factor (r = .210, p < .006) (tab. I).

TABLE I.

Correlation analysis

Scale	M(SD[1])	2	3	4	5	6	7	8	9
Extraversion	16.15 (2.91)	0.283**	0.159*	0.153*	0.351**			-0.177*	
Agreeableness	19.19 (1.26)		0.166*	0.193*	0.211**	0.233**			-0.159*
Conscientiousness	17.23 (2.40)			0.206**	0.300**		0.194*		
Emotional stability	16.47 (2.96)						0.161*	-0.299**	-0.289**
Openness to experience	17.42 (2.06)						0.179*		
Power-prestige	8.62 (1.24)								0.348**
Retention-time	10.84 (2.36)							0.153*	
Distrust	8.94 (1.75)								0.210**
Anxiety	7.97 (1.77)					0.348**		0.210**	

[1] Means and Standard deviation (M±) and correlation analysis data

* Correlation is significant at the 0.05 level (2-tailed)/**Correlation is significant at the 0.01 level (2-tailed)

The hypotheses were tested using a stepwise multiple regression analysis. The predictors extraversion, conscientiousness, and openness to new experiences explain 8.1% of the variance in the retention-time variable (F(1.132) = 5.43, p < .021) (fig. 1).

Extraversion and emotional stability accounted for 8.5% of the variance in the distrust dimension measured by the questionnaire on teachers' attitudes toward money (F(1.133) = 11.35, p < .001).

This result suggests that high levels of

extraversion and good emotional stability may contribute to reducing distrust, as individuals who are more open and emotionally balanced may perceive social relationships and professional interactions in a more positive way. Certain personality traits appear to be associated with lower vulnerability to financial anxiety and distrust, suggesting a potential buffering effect (fig. 2).

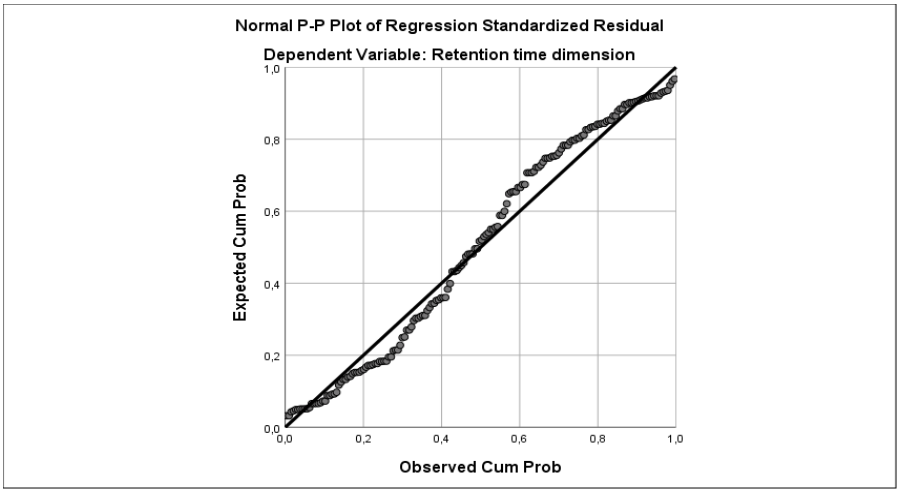

Fig. 1. Graphical representation of regression for the retention-time variable

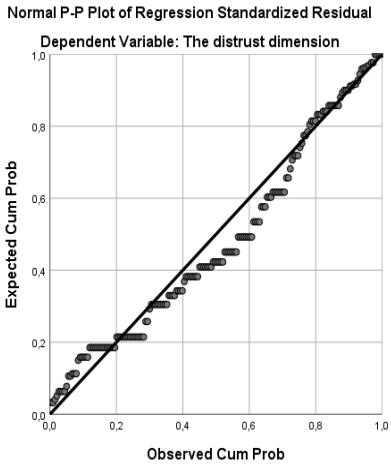

Fig. 2. Graphical representation of regression for the variable distrust

The emotional stability predictor explained 8.6% of the variance in the anxiety variable ($F(1.133) = 10.52$, $p < .001$). The results highlight that the level of emotional stability has a protective role against financial anxiety. Teachers with low emotional stability tend to perceive money-related situations as threatening and stressful,

which amplifies psychological tension and concerns about financial security. In contrast, emotionally balanced individuals are better able to regulate their affective responses and reduce the tendency to view money as a source of anxiety (fig. 3).

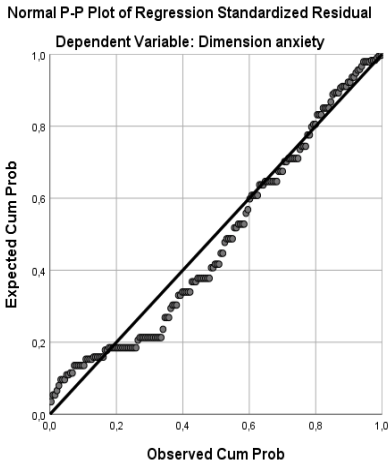

Fig. 3. Graphical representation of regression for variable anxiety

The predictors emotional stability and gender of the subjects explain 14.6% of the variance in the power-prestige variable ($F(1.133) = 18.21$, $p < .001$). The findings show that both emotional stability and gender influence the perception of money as a symbol of status and power. Male teachers, particularly those with a high level of emotional stability, tend to associate money with social prestige and personal success. This attitude reflects the use of financial resources as a way of affirmation and influence in social and professional contexts. (fig. 4).

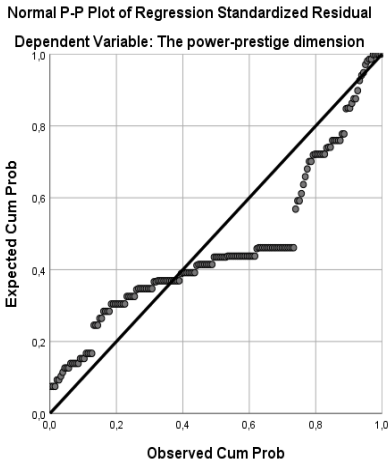

Fig. 4. Graphical representation of regression for power-prestige variable

The application of univariate ANOVA analysis revealed a significant interaction effect between the level of emotional stability and the level of conscientiousness of teachers on the trust dimension (F(1.168) = 2.73, p = .049). Specifically, teachers exhibiting both low conscientiousness and low emotional stability (M = 9.52 ± 1.69) demonstrated a significantly higher degree of distrust (t(108) = 3.13, p < .002), compared their counterparts with low conscientiousness but high emotional stability (M = 8.41 ± 1.91).

These results show that low levels of conscientiousness, combined with fragile emotional stability increase the risk of distrust. Teachers who are less organized and more emotionally vulnerable may be more likely to perceive the financial and professional environment as unpredictable or threatening. Conversely, elevated emotional stability appears to mitigate distrust even when conscientiousness is low, emphasizing the role of emotional regulation mechanisms (fig. 5).

An interaction effect was observed between the independent variables of gender and age of the subjects on the emotional stability factor, F(1.165) = 3.77, p = .025). Detailed results are presented in *Fig. 6*. Specifically, male teachers aged 19-39 (M = 17.11 ± 2.63) exhibited significantly higher levels of emotional stability t(60) = 2.74, p < .010), compared to their female counterparts in the same age group (M = 15.09 ± 2.62).

These results suggest that both gender and age influence contribute to differences in emotional stability among teachers. Young male teachers may demonstrate greater emotional resilience when encountering professional challenges, whereas women within the same age group experienced heightened pressures related to personal and professional responsibilities. (fig. 6).

An interaction effect was identified between the independent variables of gender and age of the subjects on the retention-time dimension, F(1.165) = 3.39, p = .036) (fig. 7).

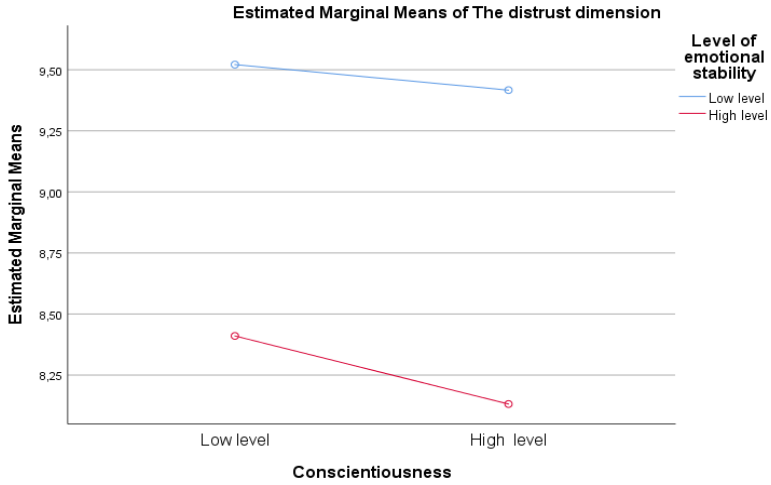

Fig. 5. Graphical representation of the interaction effect between emotional stability and conscientiousness on the dimension of distrust

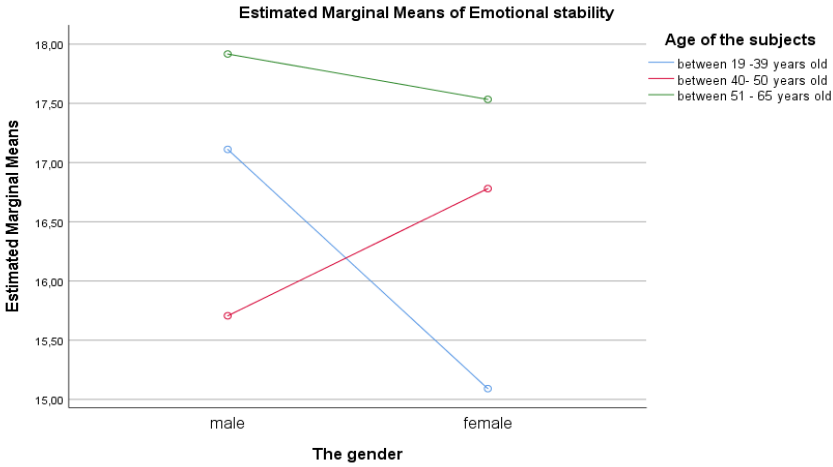

Fig. 6. Graphical representation of the interaction effect between subject gender and subject age on the emotional stability factor

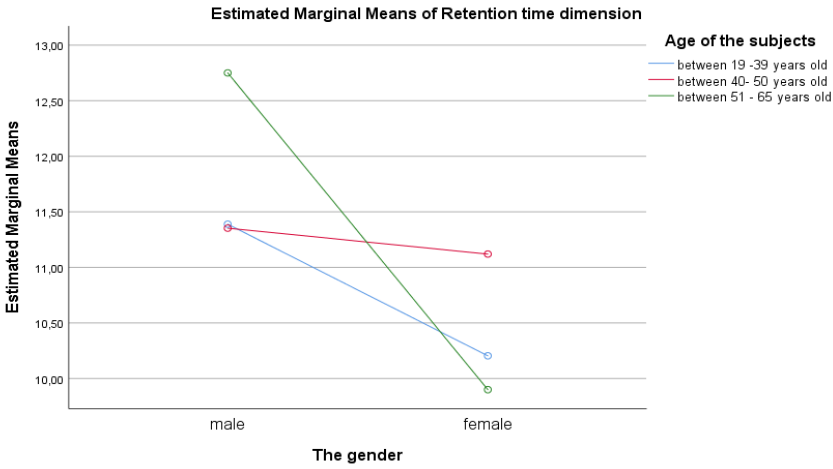

Fig. 7. Graphical representation of the interaction effect between subject gender and subject age on retention-time

Female teachers aged 51 to 65 years (M = 9.90 ± 2.21) exhibited significantly lower level of retention-time ($t(40) = 3.99$, $p < .000$), compared to their male counterparts in the same age group (M = 12.75 ± 1.71).

These results suggest that the aging process, combined with gender differences, may influence cognitive performance. Women in the final stage of their teaching careers appeared to be more vulnerable to a decline in retention-time, possibly due to biological and psychosocial factors, while male teachers in the same age group maintained their cognitive functioning for a longer period (fig. 7).

An interaction effect was identified on

the power-prestige dimension for the independent variables gender and age of the subjects F(1.165) = 3.78, p = .025 (fig. 8).

Thus, teachers aged between 40 and 50 who were male (M = 9.17 ± 1.28) exhibited a significantly higher level of power-prestige t(65) = 3.54, p < .001 than female teachers in the same age group (M = 8.34 ± 0.62).

The power-prestige dimension refers to the perception of money as a symbol and instrument of power, used to impress and influence others, functioning also as an indicator of success, social status, and prestige. Male participants in this age range were more likely to associate financial behavior with status acquisition, whereas female participants tended to show greater interest in the products themselves.

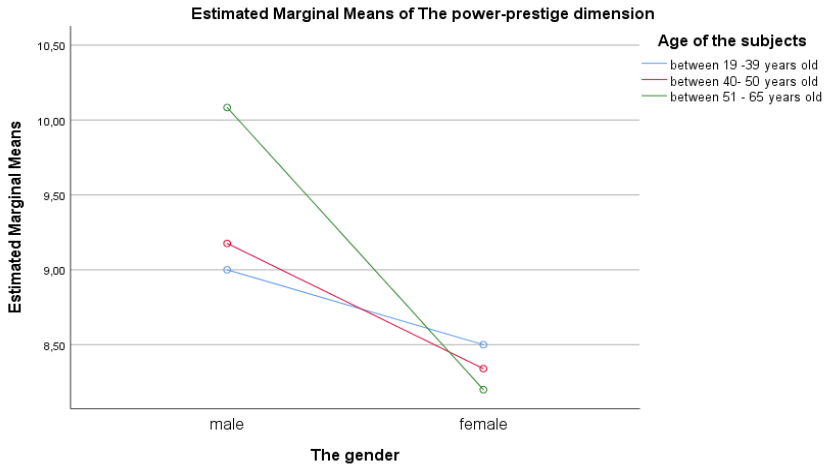

Fig. 8. Graphical representation of the interaction effect between gender and age on power-prestige

Applying the t-test for independent samples revealed significant differences between female and male teachers in terms of agreeableness (M = 19.31 ± 1.06), with results indicating that female teachers scored higher than male teachers in this trait (t(169) = - 2.16, p < .032). Male teachers showed significantly higher scores in the power prestige factor (M = 9.34 ± 1.95, t(169) = 4.85, p < .000) and the retention-time factor (M = 10.50 ± 2.36, t(169) = 3.20, p < .002) compared to female teachers. Previous studies have suggested that, in mid-career stages, men may show stronger status-driven financial orientations, associating financial resources with professional recognition and autonomy (27, 28).

These findings indicate that women exhibited higher levels of agreeableness than men - a trait associated with cooperation, empathy, and effective management of relationships and conflicts in the school environment. From a psychosocial perspective, the difference may reflect both gender socialization patterns and relationship-oriented professional roles (e.g., interacting with students and collaborating with families). Differences in perceived prestige and retention-time should be interpreted in terms of age, seniority, and teaching loads, as these factors may mediate or moderate

the link between agreeableness and professional outcomes.

Statistical analysis of the age variable revealed significant differences in emotional stability among teachers. Specifically, those aged between 40 and 50 years demonstrated a significantly higher level of emotional stability compared to their colleagues aged 19-39 ($M = 16.41 \pm 2.82$, $t(127) = -2.30$, $p < .023$). Similarly, teachers aged 51-65 exhibited a greater degree of emotional stability compared to those aged 19 to 39 ($M = 17.58 \pm 2.80$, $t(103) = -3.45$, $p < .001$). These differences suggest that accumulated professional experience and psychological maturation may contribute to enhanced emotional stability in older teachers. Mid-career teachers have developed more effective strategies for managing stress and adapting to the demands of the profession, while younger teachers are still in the process of developing these mechanisms.

Teachers aged between 19 and 39 showed a significantly higher level of distrust compared to their colleagues aged between 50 and 65 ($M = 9.35 \pm 1.89$, $t(103) = 3.15$, $p < .002$). This result suggests that distrust is more pronounced among young teachers, possibly due to financial instability and lack of professional experience. Conversely, older teachers, who typically possess more established careers and greater economic security, tend to perceive external threats less intensely, resulting in lower levels of distrust.

DISCUSSION

The present study investigated the associations between the Big Five personality traits and money attitudes among Romanian schoolteachers. Overall, the results partially supported the proposed hypotheses, indicating that personality traits influence the way teachers cognitively and emotionally relate to money. Although the effect sizes were modest, these findings align with prior research emphasizing that personality exerts subtle yet consistent effects on financial cognition and behavior (29, 30, 31, 32).

Consistent with previous literature, emotional stability was statistically associated with lower levels of financial distrust and anxiety. This pattern suggests a potential buffering relationship between emotional stability and money-related stress, rather than a direct or causal effect. Teachers with higher emotional stability were less likely to experience worry or insecurity regarding financial matters, suggesting that effective emotional regulation may buffer against money-related stress. Similarly, the combination of low conscientiousness and low emotional stability predicted greater financial distrust, supporting the notion that self-discipline and emotional resilience jointly contribute to confidence in financial decision-making. These results extend earlier studies showing that neuroticism and impulsivity are linked to financial anxiety and poorer financial management (12, 15, 33). Similar patterns linking financial stress to anxiety, depression, and burnout among teachers have been reported in recent large-scale studies conducted in different cultural contexts, highlighting the global relevance of financial well-being as a component of occupational mental health (34, 35).

Regarding the retention-time dimension, extraversion, conscientiousness, and openness to experience were positive predictors. Teachers who were more sociable, organized, and intellectually curious tended to plan ahead and adopt a future-oriented

financial perspective. This finding is consistent with previous evidence linking conscientiousness and openness to financial prudence and long-term goal setting (36-38)

In contrast, the power-prestige dimension was influenced by both emotional stability and gender, indicating that male teachers with higher emotional stability were more likely to associate money with status and social recognition. Similar gender-based distinctions in financial attitudes have been reported in other studies, where men perceive money as an indicator of achievement, while women exhibit greater relational and emotional orientations toward spending (39, 40). It should be noted that the gender-related findings should be interpreted cautiously. The sample was unbalanced, with a higher proportion of female participants, which may have influenced the observed mean differences. Moreover, socio-cultural norms regarding gender roles and financial behavior in the Romanian context could partly explain these results, rather than reflecting intrinsic personality-based distinctions. This result aligns with prior studies suggesting that men in mid-career stages tend to associate financial success with professional recognition and autonomy. Such attitudes may reflect culturally reinforced norms linking financial resources with social achievement and status, especially among individuals with stable employment and family responsibilities

Age-related analyses revealed that older teachers displayed higher emotional stability and lower financial distrust, suggesting that professional experience and psychological maturity contribute to greater financial confidence. Younger teachers, facing early-career instability and lower financial security, tended to experience higher anxiety and distrust-patterns observed across various populations (41, 42, 43, 44). These results highlight the interplay between demographic factors and personality in shaping financial perceptions, which may have implications for teacher support and retention programs.

From a psychological and occupational health perspective, the findings underscore the importance of addressing financial well-being as part of comprehensive mental health initiatives for educators. Personality-informed interventions - such as financial coaching integrated with emotional regulation training - could help teachers develop healthier attitudes toward money, thereby reducing stress and improving well-being . Incorporating such programs within school health policies may contribute to mitigating burnout and enhancing job satisfaction. In line with personality-based financial models, individual differences in Big Five traits have also been shown to relate to long-term financial outcomes and perceived financial success, supporting the relevance of personality-informed approaches to financial well-being interventions (45, 46, 47, 48).

Strengths and limitations. This study fills a gap in the literature by examining the intersection of personality and financial attitudes in a professional group often neglected in financial psychology research. The use of validated instruments (Big Five© plus-short, MAS) and inclusion of both personality and sociodemographic variables strengthen the findings. However, the study's cross-sectional design limits causal inference, and the sample - composed of teachers from a single Romanian region - reduces generalizability. The reliance on self-reported data may also introduce response biases. Future studies should

replicate these findings using larger and more diverse samples, and consider longitudinal or experimental designs to explore causal mechanisms.

Health Policy and Practical Implications. At the institutional level, financial well-being should be recognized as an integral component of teachers' occupational health. Educational and health authorities could integrate financial literacy and psychological resilience training into teacher development programs. Early identification of individuals with high financial anxiety or distrust could guide targeted interventions to prevent stress-related outcomes. Embedding such measures in school health frameworks aligns with *Health Care*'s focus on mental well-being and sustainable workforce health.

Personality traits - particularly emotional stability, conscientiousness, and extraversion - play a modest but meaningful role in shaping teachers' financial attitudes. Understanding these psychological underpinnings offers valuable insights for developing financial awareness and mental health support programs tailored to educators. Promoting financial confidence and emotional balance may indirectly enhance teachers' occupational functioning, job satisfaction, and overall well-being.

CONCLUSIONS

The study demonstrates that personality traits play a protective role in how teachers relate to financial confidence and trust.

Teachers who are less organized and more emotionally vulnerable may perceive the financial and professional environment as unpredictable or threatening. Women showed higher levels of agreeableness than men - a trait associated with cooperation, empathy, and effective management of relationships and conflicts in the school environment.

Moreover, professional experience and psychological maturity appear to contribute to greater emotional stability, suggesting that early-career support and mentoring could help novice teachers adapt more effectively to workplace demands.

Although the observed correlations were modest, they are consistent with previous evidence indicating that personality-related effects on financial cognition are subtle but meaningful over time. From a practical standpoint, these findings suggest that financial awareness and well-being programs for teachers should incorporate personality-informed approaches. Interventions aimed at reducing financial anxiety could include emotional regulation strategies for individuals with lower emotional stability, whereas financial planning workshops might focus on enhancing retention - time attitudes among teachers with lower conscientiousness.

At a broader level, educational policymakers and school management structures may consider integrating light psychological screening for financial attitudes as part of teacher support and mentoring initiatives. Such measures would not serve diagnostic purposes but could guide preventive and supportive actions, helping teachers develop healthier cognitive and emotional relationships with money-ultimately contributing to occupational well-being, mental health, and long-term retention in the profession.

CONFLICTS OF INTEREST AND FUNDING

The authors declare no conflicts of interest and this research received no external funding.

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LIFE QUALITY IMPROVEMENT IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMAS USING TOTAL DENTURES

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LIFE QUALITY IMPROVEMENT IN PATIENTS WITH ORAL SQUAMOUS CELL CARCINOMAS USING TOTAL DENTURES(Abstract**) Aim:** Treatment of oral cancer patients is followed by the occurrence of significant functional and aesthetic alterations. Dental rehabilitation is the key factor for post-op life quality. **Material and methods:** The study included 22 patients treated for squamous cell carcinoma: tongue (11 cases), floor of mouth (10 cases) and lower lip (1 case). The study group included only edentulous patients with appropriate mandibular bone height. The group was further divided into three subgroups: patients requiring floor-of-mouth lowering (9 cases), patients who needed lower labial vestibuloplasty (8 cases) and patients who required a vestibule and a floor-of-mouth extension procedure (5 cases). Healing was by second intention in most patients by using an acrylic splint fixed by circummandibular wire and maintained in place 4 weeks (16 cases). Eight patients underwent vestibuloplasty: in 3 using total skin grafts, 3 - jugular mucosa and in 2 cases palate mucosa. The acrylic splint was made for two patients out of eight. **Results:** Secondary tissue retraction did not allow the proper retention of the mandibular removable prostheses in 3 patients who delayed their appointments. Prosthesis was made without any inconvenience in all other patients. Thereby 19 out of 22 patients had significant improvement in all functional aspects (mastication and swallowing), as well as facial aesthetics (restoring the vertical dimension of the lower face). **Conclusions**: Using less traumatic and non-expensive surgical techniques, quality of life patients with oral SCC can be greatly increased, allowing the social reintegration of these individuals. **Keywords:** ORAL CANCER, MANDIBULECTOMY, GLOSECTOMY, VESTIBULOPLASTY, PREPROSTHETIC SURGERY.

The sixth most common cancer is the oral and oropharyngeal cancer and in 90 % of the cases the squamous cell carcinoma (SCC) is involved with or without the presence of the metastasis in the cervical lymph nodes. The most frequent localizations are the tongue and the floor of the mouth (fig. 1).

The literature showed that the incidence of the oral cancer is in direct relationship with the chronic use of alcohol and/or tobacco. The treatment of choice for this type of oral cancer is radical primary surgery, with reconstruction by free tissue transfer where indicated (1, 2, 3).

Oral cancer treatment can affect secondary the quality of the patient's life by producing complications such as disfig-

urement, impaired speech, masticatory dysfunction, dysphagia, or dysarthria. The dysfunctionalities that occur after the oral cancer resection have a significant impact on the patients' lives. Some experience difficulties in eating and speaking (4, 5) others can remain with psychosocial sequelae that can change the relationship and the acceptance of the society making the head and neck cancer patients distressed (6).

Fig. 1. SCC left floor of mouth

To obtain a quality life as high as possible for a head and neck cancer patient we must find a way to rehabilitate the postsurgical defect from dental point of view. Rehabilitation of mandibular defects associated with tumor resection present a significant challenge for prosthodontic rehabilitation (7). Reestablishing occlusion and optimizing tongue mobility are important to post-operative oral function (8). There are many cases where periprosthetic surgery is needed, to obtain a stable prosthetic field (fig. 2).

This study concentrates on the possibilities for dental rehabilitation of oral cancer treated patients.

Fig. 2 Post-op intraoral aspect after treatment of carcinoma by direct suture, with the disappearance of the paramandibular trench

MATERIAL AND METHODS

A total of 22 patients admitted to the Oral and Maxillofacial Surgery Clinic of the Iasi "Sf. Spiridon" County Clinical Emergency Hospital were recruited after at least one relapse-free year post surgery. The subjects underwent a glossopelvectomy, associated or not with marginal mandibulectomy; in one patient was performed a lower lip resection in the study hospital between January 2013 and August 2017. All patients were males, aged 47 to 72 years at the time of surgery. Eleven patients (50%) were diagnosed with tongue cancer, 6 (27.2%) with cancer of the lateral floor of the mouth, 4 (18.1%) with cancer of the anterior floor of the mouth and 1 patient (4.5%) with lower lip cancer. The reconstruction techniques used at the primary surgery were: direct suture 13 cases (59%), cicatrization / healing *"per secundam"* 4 cases (18.18%), free split-thickness skin grafts 2 cases (9%), radial forearm free flap 2 cases (9%), and Camille Bernard technique for lower lip reconstruction 1 case (4.5%).

The study group containing only edentulous patients was further divided into three subgroups: (1) patients who needed floor-of-mouth lowering (9 cases), (2) patients who needed lower labial vestibuloplasty (8 cases) and (3) patients who needed vestibule and floor-of-mouth extension procedure (5 cases). In all patients included in the study, the orthopantomography highlighted appropriate mandibular crest height for total denture restauration.

Floor-of-mouth lowering technique. The technique used in lowering the floor of the mouth involved detaching the mylohyoid muscles from the mylohyoid ridge area and repositioning the muscle inferiorly, (effectively deepening the floor of the mouth area) and relieving the influence of the mylohyoid muscle on the alveolar crest. After the intervention, we applied an occlusal splint secured to the mandible with circummandibular wires for 4 weeks. Healing was by second intention (fig. 3).

Fig. 3 Post-op lowering floor of mouth

Vestibuloplasty technique. Vestibuloplasty technique implied the deepening of the vestibule by lowering the connection of the mucosa and muscle. In this study, we used three techniques: (1) skin graft technique, (2) mucosal graft technique (jugal and palatal mucosa), and (3) dental split technique with healing by second intention.

Full-thickness grafts were used over the denuded area after the apical extension of the vestibule in 3 cases (13.63%) (including a patient with vestibule and floor-of-mouth extension procedure). The skin donor site was obtained from the supraclavicular area. Fixation of the graft was performed by surgical sutures.

We used two types of mucosal grafts: jugal mucosal grafts in 3 cases (13.63%) (including a patient with vestibule and floor-of-mouth extension procedure) and palatal mucosal grafts in 2 cases (9%).

Vestibuloplasty using dental split technique with healing by second intention was performed in 2 patients (9%).

Vestibule and floor-of-mouth extension technique. This technique implied a simultaneous procedure for the deepening the vestibule and lowering the floor of the mouth, where the denuded area remained uncovered. Healing was by second intention, using a dental split secured to the mandible with circummandibular wires for 4 weeks (fig. 4).

Fig. 4. Splint. After the deepening of the paramandibular shafts, an acrylic collar was used to protect the postoperative wound and to guide the secondary scarring

RESULTS

Initially, in all patients we obtained a good depth of the paramandibular trenches. Three of the patients (13.63%) in whom we used the *"per secundam"* healing technique, did not show up to their appointment to have the dentures made. The first surgical outcome was lost, and the prostheses did not have adequate stability. None of these patients ever wanted a corrective secondary intervention. In the remaining patients the prosthetic dressing was performed without any difficulties, 11 patients (50%) of whom were entrusted to dental practitioners.

In all patients in whom dentures were made, the functional results were excellent increasing the quality of mastication and swallowing (fig. 5).

Fig. 5. Dentures in a post-op lowering floor of mouth patient

Restoring lower facial height contributed to the improvement of facial aesthetics (fig. 6).

Fig. 6. Post-op view (front and profile) with dentures

DISCUSSION

After oral cancer surgery, tissue defects can cause deformity, limited mobility, complicating many essential functions and leading to a decreased quality of life (5). The prosthetic treatment in oral cancer

treated patients can be difficult and challenging, but it is an important step in improving the quality of life. In our study, at the extraoral examination, all patients presented with decrease of the lower facial third in relation to the middle third and the accentuation of the natural folds (fig. 7).

The main prosthetic problems consist in the loss of movable mucosa in the buccal and lingual part, changes in jaw direction toward the affected side and the loss of the masticatory mucosa (5). The aim of the preprosthetic surgery of the mandible is to increase the stability of the prosthesis (9, 10) fact that can be achieved by deepening the sulcus on the buccal/lingual side or on both (buccal and lingual) sides of the mandible.

Fig. 7. Post-op view (front and profile) without dentures

The principle of vestibuloplasty is that the vestibulum is extended apically by surgically shifting the tissue apically and the denuded open exposed soft tissue area is left for secondary epithelization. The thought behind this is that the denture-bearing area can be extended. However, the extension of the vestibulum results in rapid relapse due to scar contraction. To avoid this, attempts have been made to cover the denuded area by a graft (11). Free grafts can be classified into skin grafts and mucosal grafts (palatal mucosa, buccal mucosa).

Skin grafts have been used as good alternatives since they encounter no problems with shrinkage and scars, which are unavoidable in the secondary epithelialization process. Studies show a high level of satisfaction in patients with vestibuloplasty with partial thickness skin graft. When used in the mouth, skin has associated problems that lead to patient dissatisfaction: it is a different color and texture from normal mouth epithelium; in the first years after grafting, there is a bad taste or odor, probably resulting from the presence of

hair and sebaceous glands in the graft and insufficient oral hygiene; and the development of scars and discomfort in the donor site (10).

Palatal tissue offers the potential advantages of providing a firm, resilient tissue, with minimal contraction of the grafted area. Although palatal tissue is relatively easy to obtain at the time of surgery, the limited amount of tissue and the discomfort associated with donor site harvesting are the primary drawbacks. In areas where only a small localized graft is required, palatal tissue is usually adequate (12).

Full-thickness jugal mucosa harvested from the inner aspect of the cheek provides advantages like those of palatal tissue. This mucosa does not become keratinized, and often results in an inadequate denture-bearing surface in case of inadequate technique of suturing at the periostea (12).

The combination procedure of vestibule and floor-of-mouth extension technique effectively eliminated the dislodging forces of the mucosa and muscle attachments and provides a broad base of fixed keratinized tissue on the primary denture-bearing area.

The prosthetic field we obtained was very easy to dent even by the dentists who are not familiar to difficult scar tissue situations. Using less traumatic and non-expensive surgical techniques it is possible to have a nice prosthetic field. This field is then easily covered with total dentures thereby improving all functionally aspects (mastication, deglutition, speech and language). At the same time the, restoring the lower face vertical dimension, the facial aesthetic was improved.

Improving quality of life may be as important as eradicating the cancer and should be identified as a final treatment goal. Studies showed that even if oral function was not restored sufficiently, oral health-related quality of life improved with the placement of a maxillofacial prosthesis (5).

CONCLUSIONS

In our study we used less traumatic and non-expensive surgical techniques. The life quality of patients with oral SCC can be greatly increased using these techniques. Restoring occlusion with removable prosthesis, extraoral aspect and all the functions as mastication, phonation and phonation are improved. So, this allows the social reintegration of these individuals.

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NEWS

NEW FINDINGS ON HUMAN PAPILLOMA VIRUS VACCINATION

Human papilloma virus (HPV) is an oncogenic virus, 13 of the 100 types being responsible for cancer of various sites: cervix, vulva, vagina, anus, penis, tonsils, and base of the tongue. Cervical cancer is the fourth most common cancer in women worldwide and is most commonly caused by HPV types 16 and 18. According to the World Health Organization, 58 countries have introduced anti-HPV vaccination into national immunization programs. Two vaccines containing purified viral proteins for HPV are currently available: the bivalent vaccine (types 16 and 18) and the quadrivalent vaccine (types 6, 11, 16 and 18). A recent study conducted by Huh WK and collaborators evaluated the efficacy of a new ninevalent vaccine that includes the types HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58. The study group included 14 215 participants of whom 7106 were vaccinated with ninevalent vaccine and 7109 were immunized with quadrivalent vaccine. The results showed that the ninevalent vaccine had more than 97% efficacy against types 31, 33, 45, 52, and 58. Both vaccines have demonstrated similar efficacy against types 6, 11, 16 and 18. These results support the inclusion of the ninevalent vaccine in national immunization programs. This measure could increase to 90% prevention in cervical cancer (Huh WK, Joura EA, Giuliano AR, Iversen OE, de Andrade RP, Ault KA, Bartholomew D, Cestero RM, Fedrizzi EN, Hirschberg AL, Mayrand MH, Ruiz-Sternberg AM, Stapleton JT, Wiley DJ, Ferenczy A, Kurman R, Ronnett BM, Stoler MH, Cuzick J, Garland SM, Kjaer SK, Bautista OM, Haupt R, Moeller E, Ritter M, Roberts CC, Shields C, Luxembourg A. Final efficacy, immunogenicity, and safety analyses of a nine-valent human papillomavirus vaccine in women aged 16-26 years: a randomised, double-blind trial. *Lancet*. 2017 Sep 5. pii: S0140-6736(17)31821-4. doi: 10.1016/S0140-6736(17)31821-4. [Epub ahead of print])

Cătălina Luncă

USING OF MULTIPLE CHROMOSOMAL AND MOLECULAR ANALYSES TO ELUCIDATE THE ETIOLOGY OF PLURIMALFORMATIVE SYNDROMES

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USING OF MULTIPLE CHROMOSOMAL AND MOLECULAR ANALYSES TO ELUCIDATE THE ETIOLOGY OF PLURIMALFORMATIVE SYNDROMES (Abstract): Congenital anomalies are structural or functional abnormalities occurring during intrauterine life in 3% of all pregnancies, representing an important public health problem through frequency, morbidity and mortality. In 6% of cases they are determined by unbalanced chromosomal abnormalities and their cytogenetic diagnosis is a prerequisite for proper management including proper genetic counseling. **Material and methods**: The retrospective study included 331 patients who were investigated by clinical and cytogenetic (conventional or molecular) methods at the Genetics Pathology Center in Iaşi, from 1 January 2015 to 31 December 2016. **Results**: The study group was divided into four subgroups: patients with plurimalformative syndromes or patients with association of multiple congenital anomalies (108 cases of which in 25 patients were confirmed with an unbalanced structural or numerical chromosomal abnormalities), patients with craniofacial dysmorphism (88 cases - in five cases cytogenetic abnormalities were confirmed), patients with Down syndrome (96 cases - 90 being cytogenetically confirmed), patients with Turner syndrome (39 cases of which 16 were cytogenetically confirmed). In 24 patients Fluorescence in Situ Hybridization (FISH) was applied with probes for the chromosomal regions involved in clinically suspected microdeletion syndromes and in 12 cases these were confirmed. In the other 12 cases (not confirmed by karyotype / FISH) we demonstrated that the syndrome is generated by another type of molecular mechanism using Multiplex ligation dependent probes amplification (MLPA). In 11 cases with complex chromosomal abnormalities, the investigation continued and the array CGH investigation is still in progress. **Conclusions**: Elucidation of the chromosomal etiology of congenital anomalies is important for providing appropriate genetic counseling and identifying other people with high genetic risk in their family. **Keywords**: CONGENITAL ANOMALIES, UNBALANCED CHROMOSOMAL ANOMALIES, KARYOTYPE, FLUORESCENCE IN SITU HYBRIDIZATION, MICRODELETION SYNDROME.

Congenital anomalies (CA) are structural or functional abnormalities that occur during intrauterine life and can be identified prenatally, at birth or later in life (1).

They affect about 3% of all pregnancies and 10-20% of cases are identifiable syndromes (2). Etiology is heterogeneous: 45% have genetic causes, 5% non-genetic and, in approximately 50% of cases, a causal factor cannot be established (3, 4). Among the genetic causes we mention: unbalanced chromosomal abnormalities (6% of total CA), monogenic mutations (7.5% of isolated CA), multifactorial hereditary diseases (20-30%) (5).

CA are a major public health problem because they are frequent and severe birth defects, representing an important cause of intrauterine death and infant morbidity and mortality (6,7). The EUROCAT Statistical Monitoring Report-2012, which aimed at pan-European analysis of births from the period 2003-2012, revealed that there is an ascending trend for chromosomal abnormalities due to the increase in the prevalence of Down and Edwards syndrome determined by the increase in maternal age in Europe (8).

For the detection of unbalanced chromosomal abnormalities involved in CA pathogenesis, chromosomal analysis is required. The cytogenetic techniques used are *conventional cytogenetic techniques* (karyotype) and *molecular cytogenetic techniques* (FISH, M-FISH, MLPA, QF-PCR, CGH, DNA microarrays). Depending on the number of abnormalities identified, there are pangenomic and targeted techniques.

Pangenomic techniques (karyotype, M-FISH, DNA microarrays) can reveal several anomalies that may be of interest to all chromosomes. Target techniques (FISH) can reveal fewer anomalies that only interest certain chromosomes.

MATERIAL AND METHODS

Our study was based on conventional banding cytogenetic, FISH analyses and MLPA. In all cases, the chromosomal analysis was performed on a short time T lymphocyte culture stimulated by phytohemagglutinin. The chromosomes were G-banded using trypsin and Giemsa solution according to standard techniques. The FISH and MLPA methods were applied only in selected cases when a microdeletion syndrome was suspected. The results of conventional banding cytogenetic analysis and FISH were elaborated in accordance with the guidelines of the International System of Human Cytogenetic Nomenclature (ISCN) (2013) (9).

The study group consisted of 331 patients clinically investigated at the Genetics Pathology Center in Iaşi. Conventional and molecular cytogenetic diagnostic investigations were applied in the Cytogenetics Laboratory of the University of Medicine and Pharmacy "Grigore" T. Popa "Iasi, from 1 January 2015 to 31 December 2016.

The study group was subdivided into four subgroups depending on clinical suspicion:

- Subgroup A. Patients with plurimalformative syndromes or with association of multiple congenital anomalies
- Subgroup B: Patients with craniofacial dysmorphism
- Subgroup C. Patients with clinical suspicion of Down syndrome
- Subgroup D. Patients with clinical suspicion of Turner Syndrome

RESULTS

The four subgroups had the following composition:

- Subgroup A –108 cases, of which 54 males and 54 females;
- Subgroup B - 88 cases, of which 32 males and 56 females;
- Subgroup C - 96 cases, of which 40 males and 56 females;
- Subgroup D - 39 cases.

Investigations of the patients from subgroup A (pluriformative syndromes) and subgroup B (craniofacial dysmorphism) by conventional chromosomal analysis - G-banded karyotype – confirmed the presence of unbalanced numerical or structural chromosomal abnormalities in 28 cases (table I).

In other 24 patients from the subgroup A or B, the clinical phenotype was suggestive for a plurimalformative syndrome generated by a chromosomal microdeletion: Williams syndrome, Wolf-Hirschhorn syndrome, velocardiofacial syndrome, Prader-Willi syndrome, Angelman syndrome, *cri du chat* syndrome, Miller-Dicker syndrome. In these cases, we applied FISH method using appropriate DNA probes from Metasystem®, Cytocell® and Kreatech®:

- For Williams syndrome, there was only one case confirmed by the FISH method using specific 7q11.23 probes.
- Wolf-Hirschhorn syndrome clinical suspicion was in seven cases; four of these were not confirmed by FISH method, using specific 4p16.3 probes and neither by MLPA; in other 3 cases, the FISH and MLPA confirmed the presence of 4p16.3 microdeletion.
- In the case of velocardiofacial syndrome, there were seven cases, four of which were confirmed by the FISH method using specific 22q11.2 probes.
- For Prader-Willi Syndrome there were four cases, one with a 15q11-13 deletion detected on karyotype and confirmed by the FISH method, using 15q11-13 probes, one with 15q11-13 microdeletion not detected on karyotype, but confirmed by the FISH method, using 15q11-13 probes and one without cytogenetic changes (neither microscopic or infra-microscopic) but with an imprinting defect in 15q11 - 13 region; the last case was infirmed both by FISH and MLPA.
- In Angelman syndrome there was only one case, not confirmed by the FISH method, using 15q11-13 probes.
- In case of *cri du chat* syndrome, there was only one case, seen on karyotype and confirmed by the FISH method, using 5p15.3 probes.
- The only case with Miller-Dieker syndrome was confirmed by FISH method using probe for 17p13.3 region
- The main data on cases with plurimalformative syndromes and craniofacial dysmorphism with unbalanced chromosomal abnormalities are presented in Table I.
- In the subgroup C we had 96 patients with clinical suspicion of Down syndrome. Six of these cases were not confirmed by karyotype (five girls and one boy). Most of the patients had free and homogeneous trisomy 21, with karyotype 47,XX,+21, in 47 of the 51 girls with Down syndrome, respectively 47,XY,+21 in 35 of the 39 boys with Down syndrome. We identified three cases of mosaic (47,XX,+21/46,XX two cases and 47,XY,+21/46,XY – one case).

TABLE I.

Patients with plurimalformative syndromes and craniofacial dysmorphism with unbalanced chromosomal abnormalities

Crt.	No. case	Name	Age	Clinical suspicion	Result of karyotype	Molecular analysis
	9	TR	3 m	PMS	46,XX,add(1)(pter)	MLPA add 6p and del(1)(q36)
	20	CDN	7 y	MOS	46,XX,del(1)(p36.2→pter)	Array CGH in progress
	23	T ♂	nb	VCFS	46,XY	FISH del(22)(q11.2)
	25	LA	3 y	PMS	46,XY,del(11)(q23.1→qter)	MLPA del 11q Array CGH in progress
	29	PMC	3 y	VCFS	46,XY	FISH + MLPA del(22)(q11.2)
	37	HV	2 y	CFD	46,XY	MLPA del 18p + dup 18q
	41	P ♂	nb	PMS	47,XY,+18	
	54	NL	6 w	PMS	46,XY,9qh+,inv(9)(p12q13)	
	56	PAP	5 y	CFD	46,XX,add(16)(p13.3)	Array CGH in progress
	60	ZA	nb	PMS	46,XX	MLPA del 10q
	61	MD	5 m	PMS	46,XY	MLPA del 10q
	64	HM	nb	PMS	47,XY,+21/48,XY,+21,+mar [77]/[3]	
	71	CE	4 y	PMS	46,XX,1qh+/ 46,XX,1qh+,t(2;9)(q37.3;q12) [30]/[8]	Array CGH in progress
	72	CM	3 m	CCS	46,XY,del(5)(p15.1→pter)	FISH + MLPA del(5)(p15.3)
	75	M ♀	nb	PMS	46,XX/47,XX,+18 [47]/[3]	FISH trisomy 18
	76	MAM	1 y	CFD	46,XX,der(9)inv(9)(p13.3q21.13), del(9) (p13.1p13.3)	Array CGH in progress
	82	C ♀	nb	PMS	47,XX,+18	
	83	B ♂	nb	PMS	46,X,Yqh+,1qh+, der(18)t(13;18)(q12;p11.2)	Confirmed by FISH, C and NOR banding
	100	SI	8 y	VCFS	46,XX	FISH + MLPA del(22)(q11.2)
	106	CO	5 y	CLS	46,XX,add(3)(p25)	MLPA dup 3q Array CGH in progress
	114	RRP	2 w	PMS	46,XY	MLPA dup 2p and del 1q
	121	A ♀	2 w	PMS	46,XX/47,XX,+20	
	123	CAM	17 y	PMS	46,X,del(X)(qter→p11.2)/ 45,X [29]/[3]	
	125	PGC ♀	21 y	MS	46,XY	
	126	PAM ♀	9 y	MS	46,XY	
	137	GE	nb	PWS	46,XX,del(15)(q11.2q12)	FISH + MLPA del(15)(q11-13)
	144	FŞ	5 y	WS	46,XX	FISH + MLPA del(7)(q11.23)
	148	LNA	nb	PWS	46,XY	MLPA –15q11-13 Im printing defect
	152	R ♂	nb	VCFS	46,XY	FISH + MLPA del(22)(q11.2)
	173	MSR	13 y	PMS	46,XX	MLPA del 2q
	181	BMI	14 y	PMS	48,XXXY	-
	278	ADI	nb	CFD	46,XX	MLPA – del 1p + dup 4q
	283	SS	6 m	CFD	46,XX,inv(9)(p12q13),add(10)(qter)	-
	284	BLA	6 m	PMS	46,XY,der(10)(10pter→ 10q26.2::4q26→4qter)	Array CGH in progress
	285	BMA*	32 y	-	46,XY	
	286	BAE**	28 y	-	46,XX,t(4;10)(q26;q26.2)	-
	296	CA	10 y	MDS	46,XY	FISH del 17p13.3 Array CGH in progress
	300	I	nb	WHS	46,XY,r(4)(p15.1q35)/45,XY,-4/ 46,XY,-4,+mar/ 47,XY, r(4)(p15.1q35),+mar/	FISH + MLPA del 4p Array CGH in progress

Crt.	No. case	Name	Age	Clinical suspicion	Result of karyotype	Molecular analysis
					47,XY,der(4),+mar [53]/[6]/[3]/[2]/[2]	
	303	BDA	nb	PMS	46,XY[30]/45,X[5]	FISH – X monosomy
	308	IA	5 y	CFD	47,XXY	-
	318	BSD	nb	PS	47,XX,+13	-
	325	SDG	1 y	WHS	46,XY	FISH + MLPA del 4p
	326	GSM	nb	WHS	46,XX,del(4)(p16→pter)	FISH + MLPA del 4p

PMS – plurimalformative syndrome; DS – Down syndrome; MOS – Moebius syndrome; CFD – craniofacial dysmorphism; VCFS – velocardiofacial syndrome; CCS – cri du chat syndrome; CLS – Cornelia de Lange syndrome; MS – Morris syndrome; PWS – Prader-Willi syndrome; WS – Williams syndrome; WHS – Wolf-Hirschhorn syndrome; MDS – Miller-Dieker syndrome; PS – Patau syndrome; * -father of case 284; ** - mother of case 284

We discovered two cases of Down syndrome with robertsonian translocation between chromosomes 14 and 21: 46,XY,rob(14;21)(q10;q10).

Excepting the typical chromosomal formulas, in subgroup of Down syndrome patients we found some particular karyotype: 46,XX,dic(14;22)(p11.2;p11.2),+21, 46,XY, ins(21;16) (q22.1;p12.1p13.13) and 47,XX,1qh+,16qh+,+der(21)del(21)(q11.2q 22.1).

In clinical suspicion of Turner syndrome (subgroup D), 23 cases were not confirmed and 16 cases were confirmed, with different abnormal chromosomal formulas (tab. II). In six cases, a homogenous anomaly was present: X monosomy (five cases) and a Xq isochromosome (one case). In the other ten cases, we identified different types of mosaics with two or three cell lines. In two cases we discovered aneuploid mosaics: 45,X/46,XX and 45,X/47,XXX. In other cases, we found mosaics that had an association between a complete X monosomy and a partial X monosomy produced by an unbalanced structural chromosomal anomaly (Xq isochromosome – 5 cases; X ring chromosome – 1 case; deletion on the long arm of X chromosome – 1 case; dicentric X chromosome – 1 case).

TABLE II.

Turner syndrome cases confirmed by classical chromosomal analysis

Crt.	No. case	Name	Age	Karyotype result
1	33	NM	10 y	45,X[23]/46,X,del(X)(q25→qter)[12]
2	39	FA	4 m	45,X,1qh+
3	48	CRI	10 y	46,X,i(X)(q10q10)
4	57	BAM	13 y	45,X
5	70	VAE	16 y	45,X[33]/46,X,i(X)(q10q10)[18]
6	119	LM	6 y	46,XX[22]/45,X[20]
7	140	HA	5 y	46,X,i(X)(q10;q10)[38]/47,X,i(X)(q10;q10),i(X)(q10;q10)[5]
8	172	IAI	17 y	45,X
9	219	FA	21 y	45,X[17]/46,X,i(X)(q10q10)[11]
10	239	BEG	16 y	45,X
11	259	ANA	11 y	45,X[37]/46,X,r(X)(p11.3q21)[12]/46,X,+mar[11]/46,XX[2]
12	268	BEM	16 y	45,X
13	291	CL	18 y	46,X,i(X)(q10q10)[24]/45,X[19]
14	301	SII	6 y	45,X[23]/47,XXX[13]
15	327	CAM*	20 y	45,X[77]/46,X,idic(X)(pter→q26::q26→pter)[43]/46,X,+mar[3]
16	328	CS	15 y	46,X,i(X)(q10q10),1qh+[24]/45,X,1qh+[19]

* array-CGH in progress

Of the cases where chromosomal analysis has detected abnormal chromosomal formulas we present a case to illustrate sequential genetic diagnosis.

Clinical case 83

Patient B, male, from rural area, was investigate in Medical Genetics Center at the age of 3 days due to the presence of a plurimalformative syndrome.

Birth occurred naturally, after 35 weeks of amenorrhea. Ultrasound monitoring of pregnancy in this case was not made. The child was born by a 16-year-old minor unmarried mother.

Newborn anthropometric data showed intrauterine growth retardation: weight – 2100 g; length - 44 cm; head circumference - 29 cm; APGAR score- 5.

The clinical examination revealed the existence of a plurimalformative syndrome with craniofacial dysmorphism (microcephaly, dolichocephaly, broad and flat nasal root, micrognathia, dysplastic ears) associated with abnormalities in the hallux position and shape, micropenis and atrioventricular defect (fig. 1).

Fig. 1. Patient B phenotype ♂

Due to the clinical aspect, peripheral blood was harvested and T-lymphocytes were cultured in vitro. The karyotype revealed the presence of a derivative chromosome, resulting from the union of segments from chromosomes 13 and 18 – chromosomal formula: 46,X,Yqh+,1qh+,der(18)t(13;18)(q12;p11.) (fig. 2).

To verify whether the derivative chromosome was monocentric or dicentric, a C-banding technique was applied, which established that this abnormal chromosome had a single centromere (fig. 3).

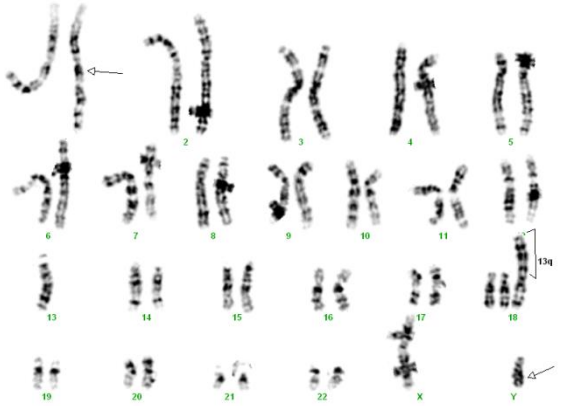

Fig. 2. G banding – karyotype 46,X,Yqh+,1qh+,der(18)t(13;18)(q12;p11.2)

Fig. 3. C banding - karyotype 46,X,Yqh+,1qh+,der(18)t(13;18)(q12;p11.2)

To identify the origin of the centromeric fragment of this chromosome, the FISH assay was performed using Metasystem® probes corresponding to chromosomes 13, 18 and 21 (commonly used for the prenatal diagnosis of aneuploidies); this analysis established that this derivative chromosome has a centromere derived from chromosome 18 (fig. 4).

To complete the investigation, the FISH test was also applied using subtelomeric probes from Cytocell® corresponding to the

long arm of chromosome 18 and the short arm of chromosome 18, which confirmed the association of a partial trisomy 13 (containing almost all the long arm of this chromosome) with a small partial monosomy (determined by the absence of the terminal end of the short arm of chromosome 18) (fig. 5).

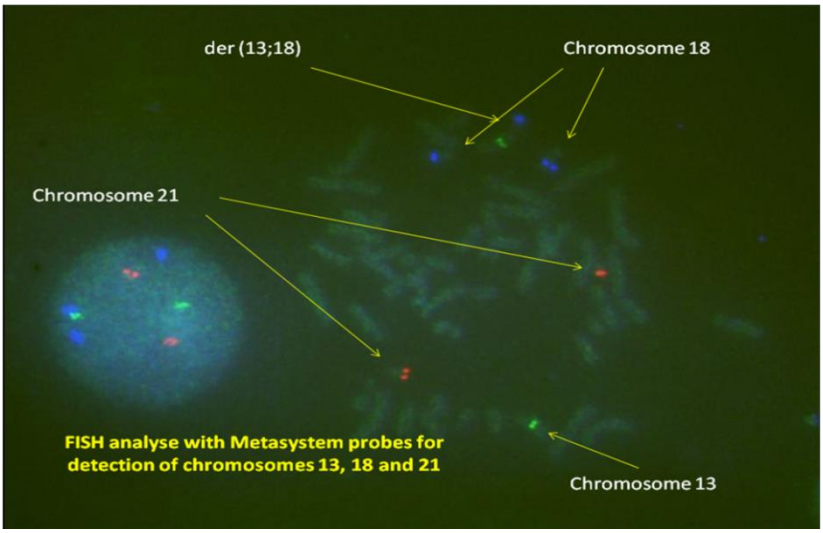

Fig. 4. FISH analysis with Metasystem® probes for chromosomes 13 (green) 18 (blue) and 21 (red)

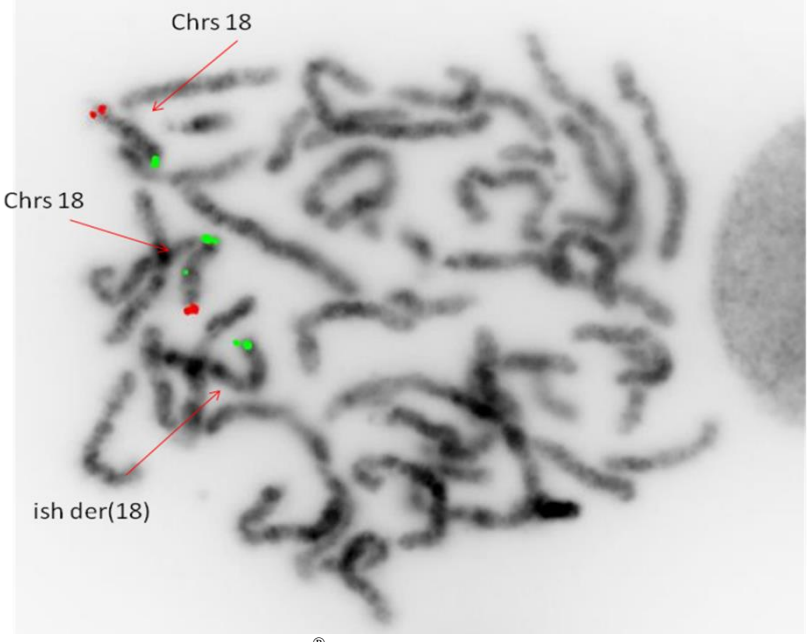

Fig. 5. FISH analysis with Cytocell® probes corresponding to the subtelomeric region of chromosome 18 red signals 18p tel – 74G18; green signals – 18q tel – dJ964M9

DISCUSSION

In our investigations of patients with congenital anomalies we used a sequential diagnostic strategy, increasing the resolution at each stage. We started with conventional karyotype (GTG banding), followed using molecular cytogenetic methods like FISH or MLPA. In cases with chromosomal anomalies to increase the precision of diagnosis we applied DNA microarrays, but definitive results are not completed.

Conventional chromosomal analysis has some disadvantages: relatively long *duration* of analysis 4-10 days; relatively limited *resolution* to 5-10 Mb; requires specialized technicians to analyze GTG banding karyotype, all these meaning high costs and organizational difficulties in small laboratories. But these types of analysis are the only genomic analysis that could identify a balanced structural chromosomal abnormality. In addition, such analysis can identify the position of a genomic imbalance in the cases with partial trisomy or partial monosomy. Thus, GTG banding karyotype continues to be a part of protocol investigation of chromosomal diseases (3, 10, 11).

In cases with a suggestive phenotype for a microdeletion syndrome / microduplication syndrome, some protocols indicate the use first of FISH or MLPA technique with locus - specific probes bound to a region of a chromosome. Even in these cases, the karyotype must be made because in some cases a derivative chromosome could be present. If the diagnosis is not confirmed by FISH or MLPA technique, the diagnostic strategy imposes a pan-genome analysis (array-CGH) able to identify subtle genomic imbalances (3, 6, 10).

Microdeletion / microduplication syndromes are produced by the loss / gain of small chromosomal fragments (1-4 Mb) impossible to be detected by conventional cytogenetic methods. The detection of such genomic imbalances requires the use of a molecular technique: FISH, MLPA or array-CGH. In a minority of cases when the chromosomal fragment involved is large, it may also be highlighted by conventional G-banded chromosome analysis. The selection of cases for the FISH test is done using clinical diagnostic scores. There must be a rigorous selection, as the analysis is expensive, laborious and targeted ("we will find only what we are looking for") (3,12).

Genomic techniques based on DNA microarrays (array-CGH) increased the resolution to 50-100 kb. The major disadvantage of this technique is the possibility of discovery of a CNV (copy number variation). The CNVs represents about 12% of the genome, and the classification in pathogen or not pathogen is difficult. If the CNV was already associated with a human disease, it is considered pathogen. If the CNV contains a high number of genes and its size is big, the CNV is considered potential pathogen. If the CNV is also present in one of the patient's parents, the CNV is benign. In the other situation when the anomaly is *de novo* and it is not associated with a specific phenotype, the CNV is considered as VOUS (variants of uncertain significance) (13-16).

Fourteen of the cases with clinical suspicion of a microdeletion syndrome were confirmed by molecular techniques (FISH and MLPA). We consider that the confirmation of approximately 60% of such cases is a proof of good clinical diagnosis. The absence of confirmation in other cases could have different explanations. In some diseases, it is present a genetic heterogeneity. In other cases, the genomic imbalance is

very small and its identification requires the use of array-CGH technique.

For example, in Wolf-Hirschhorn syndrome different types of abnormalities were described. About 55% of patients have a "pure deletion" identifiable by conventional G-banded chromosome analysis or FISH technique. In 40-45% of cases, the phenotype is the consequence of an unbalanced translocation resulting in chromosome 4p distal segment deletion. The rest of the cases were generated by complex rearrangements. (17). The FISH test detects 95% of WHS cases using a WHSCR critical region specific-probes. A false negative result can be obtained when the probe hybridizes outside of the critical region. The array-CGH technique has a good detection rate (95%) and for this reason it is recommended for the diagnosis of complex rearrangements involving the 4p16.3 critical region (18,19).

Most of cases (90-95%) with velocardiofacial syndrome present a small genomic imbalance of 22q11.2 region. For this reason, the karyotype is not useful for diagnosis. The commercial FISH probes used for the detection of abnormalities in the critical region for the velocardiofacial syndrome are TUPLE1 and N25. The FISH technique detection rate of deletions at this level is approximately 95%, the two probes being equivalent to the detection rate, but if the deletion is less than 40 kb then it cannot be highlighted. In the 22q11.2 region six duplicate segments, named from A to F, were identified. Most patients with velocardiofacial syndrome (85%) present a deletion that include the interval between segment A and D. The TUPLE1 and N25 probes are in the region between segmental duplications A and B, and for this reason the cases with other type of deletion will be not detected with 22q11.2 commercial FISH probes. In such cases, the use of array-CGH technique represents the best solution (20,21).

Prader-Willi syndrome is caused by genomic imbalances in 15q11-q13 region. Molecular classes of PWS are the following: Ia (deletion of 5-6 Mb, 65-75%), Ib (chromosomal rearrangements, <1%), II a (maternal 15q11-q13 UPD, 20-30%), IIb (maternal UPD with predisposition to parental translocation/chromosome marker, <1%), III a (imprinting defect with imprinting center deletion), III b (epimutation: imprinting defect without imprinting center deletion). Approximately 70% of individuals with PWS have a deletion involving the 15q11-q13 region, so that the proportion of cases detected by FISH method is 65-75%. If the deletions are small or atypical the FISH test result may be false negative. A very good detection rate (99%) has MLPA because it can detect both microdeletions and methylation status (uniparental disomy and imprinting defect). In approximately 1% of cases, both FISH and MLPA methods do not confirm the clinical suspicion of Prader-Willi syndrome, and the identification of genomic abnormality requires the application of array-CGH technique (22, 23).

Trisomy 21 being the most common chromosomal disease (1/650 - 1/800 newborns), requires epidemiological surveillance mainly through three types of actions: monitoring the impact of intellectual disability, screening programs evaluation and adapting medical services to associated comorbidities (24-27).

The results of chromosomal analysis in subgroup of Down syndrome are concordant with other studies. Thus, most of cases (92-95%) present a free homogenous trisomy 21. The rest of cases have a mosaic

trisomy 21 or a trisomy 21 due to a Robertsonian translocation. In the last case, the most frequent translocation is rob (14q;21q), that is concordant with our data. In less than 0,1% of cases, Down syndrome is generated by a partial trisomy 21q, chromosomal anomaly being an unbalanced reciprocal translocation, duplication, dicentric chromosome 21, marker chromosome 21 etc. (6, 24)

The clinical suspicion of Down syndrome was not confirmed in approximately 6.31% of investigated cases. This result is concordant with data previously presented by Gorduza *et al.* (2007) that indicated a 5.77% of cases with clinical suspicion of Down syndrome that are not confirmed by karyotype. A possible explanation is genetic heterogeneity, but also could be take in consideration the presence of somatic mosaicism or a mosaic trisomy 21 with a small number of abnormal cells (24, 28).

Identifying the real phenomenon could be the premises for initiating prophylactic actions. These prenatal screening and diagnosis programs are applied also in Romania, but are only partially funded by health insurance. Diagnosis of a Down syndrome patient is the starting point of an adequate management process that involves investigating possible associated comorbidities and their management on the one hand, and on the other hand the obligation of genetic counseling adapted to the case for the identification of other people with genetic risk in the proband family (29).

The cytogenetic results in patients with Turner syndrome (subgroup D) are relatively concordant with literature data. We found a high rate of X monosomy (complete or partial) mosaicism – 10/16 cases (62.5%) that are like data presented by Abulhasan *et al.* (1999) that discovered 22 cases with X monosomy mosaicism from 37 cases with Turner syndrome cytogenetically confirmed (59.45%) (30). Other reports that used large cohorts of patients with Turner syndrome presented different results, with an approximately frequency of X homogenous complete monosomy of 45% (31, 32). We consider that confirmation of clinical diagnosis in only 16 from 39 patients with Turner syndrome suspicion is correlated with the age of patients. Most of our patients were children and during childhood the only major symptom of Turner syndrome is the growth delay.

Our study showed the importance of use of different chromosomal analysis for confirmation of etiology of congenital anomalies. In 148 from 331 cases (44.71%) we discovered a chromosomal abnormality using only the karyotype or using the association of karyotype and FISH or MLPA. We consider that the use of karyotype remains the better approach for identification of chromosomal abnormality in aneuploid syndromes and in plurimalformative syndrome without a specific clinical pattern. If the patient has the characteristic features of a microdeletion/microduplication syndrome the first-choice analysis must be FISH or MLPA with specific probes and the use of karyotype is reserved to cases with possible derivative chromosomes. The array-CGH is a good method for characterization of genomic imbalances, but the high cost per analysis limits its use in practical medicine.

CONCLUSIONS

Elucidation of the chromosomal etiology of congenital anomalies is an important objective in their management, due to different recurrence risks depending on the chromosomal abnormality, and for identifying other individuals in the family at risk of

carrying a balanced chromosomal abnormality. The strategy of genetic testing is sequential, and usually must be started with the most simple and inexpensive technique that remains, the karyotype. In cases with clinical suspicion of a syndrome caused by a microdeletion, it is necessary to perform FISH or MLPA test for confirmation. If karyotype and/or FISH (MLPA) method does not identify a genomic imbalance it is important to apply array-CGH method that is able to confirm small genomic imbalances. However, because some congenital anomalies are produced by other factors (environmental, monogenic, polygenic) the application of all cytogenetic techniques – conventional cytogenetic methods (karyotype) molecular techniques (FISH, MLPA, array-CGH)–could remain without results and supplementary investigations are necessary.

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EVALUATION OF THE EFFICACY OF SURGICAL RESECTION OF MALIGNANT GLIOMAS USING 5-AMINO-LEVULINIC ACID

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EVALUATION OF THE EFFICACY OF SURGICAL RESECTION OF MALIGNANT GLIOMAS USING 5-AMINO-LEVULINIC ACID (Abstract): **Aim:** To evaluate the efficacy of a modern therapeutic procedure, surgical resection of malignant gliomas using 5-Amino-Levulinic Acid (5-ALA) compared with tumor resection associated with radiotherapy and/or antineoplastic medication. **Material and methods:** The investigation was performed on 127 patients diagnosed with malignant glioma, aged 20 to 80 years, admitted to the Neurosurgical Department of the Iasi "Prof. Dr. N. Oblu" Hospital between 2009-2015. Of these patients, 17 underwent surgical resection and 5-ALA (Gliolan®) treatment. 5-ALA was administered orally, 3 hours before the induction of general anesthesia, under the strict supervision of an anesthetist. The study protocol was approved by the Hospital Ethics Committee and informed consent was obtained from all patients enrolled in this study. A Kaplan-Meier analysis was used for the evaluation of therapeutic efficiency and assessment of the surviving subjects for a certain period of time after treatment. **Results:** Survival rate was significantly higher in the Gliolan® group compared to the group treated by classic methods at 6 months, 1 year, but especially after 2 years of treatment (0.65 vs 0.48). **Conclusions:** 5-ALA is a relevant intraoperative marker for direct visualization of the tumor and infiltrative tissue, providing the basis for a feasible future in the therapeutic management of glioblastoma and promising an increase in the survival rate compared to classic surgical method. **Keywords:** MALIGNANT, GLIOMA, 5-ALA.

In adults, malignant brain tumors account for approximately 2% of all neoplasms, their incidence increasing with age and declining in elderly patients aged over 75 (1, 2). Despite their relative rarity, primary central nervous system tumors are an important source of morbidity and mortality and still represent a major health problem (3). Researches on the etiology and the causes that contribute to the occurrence of gliomas are underway, but the relative rarity of the disease and the rapid death of the patients diagnosed with aggressive subtypes hamper these processes (4). Therefore, to date no causal factors are known and it is only assumed that exposure to therapeutic doses of ionizing radiation for the treatment of cancer results in an increased risk of glioma, as well as of meningioma, and nerve sheath tumors (5, 6). Different hereditary disorders or genetic mutations are also known to be involved in

the etiopathogenesis of gliomas (7).

In the literature, a link between cerebral trauma and brain tumors as a risk factor has been suggested. However, so far there is no conclusive evidence to support this assumption (3). On the other hand, it is considered that exposure to insecticides, pesticides, petrochemicals, organic solvents, lead compounds and nitro-derivatives increases the possibility of developing glioblastoma (8). Malignant brain gliomas are locally invasive tumors that inevitably lead to death within 1-2 years after diagnosis. These tumors remain associated with poor prognosis despite the maximum surgical treatment, completed with radiotherapy and chemotherapy. The degree of tumor resection is accepted as important for optimal surgical treatment, and recent studies provide evidence for a maximal cytological reduction in the management of newly diagnosed malignant gliomas (9, 10).

Patients with brain tumors may have different symptoms and clinical signs depending on their location, growth rate and presence of intracranial hypertension. The symptomatology associated with brain tumors is mainly determined by the tumor mass, adjacent edema, or normal brain tissue infiltration and destruction (11). Tumors positioned in functional areas of the cortex or along the ventricular system, with a high rate of growth, are clinically manifest shortly after onset, unlike those located in nonspecific areas, with late-onset manifestations (12). Among the most common symptoms are: headache, convulsions, sensory disturbances, motor or language deficits, confusion, personality changes, memory loss and visual deficits (11, 13).

Currently, treatment of malignant gliomas involves both surgical resection and multimodal treatment (radiotherapy, chemotherapy), considering that the best treatment option is to remove as much tumor volume as possible, while maintaining morbidity at a minimum level (14). There is increasing evidence that a maximum surgical cytoreduction (more than 98% of the initial tumor mass) is an important prognostic factor for patient survival, but this is not easy to achieve due to the infiltrative nature of these tumors (15). Despite the indisputable advances in imaging-guided surgical technique (neuronavigation), targeted radiotherapy and new chemotherapies (e.g. temozolamide, carmustine), the prognosis of patients diagnosed with malignant gliomas is currently gloomy, so it is imperative to develop new antitumor strategies (16, 17, 18). Remarkable discoveries in molecular biology and biotechnology, and particularly a better understanding of tumor biology (angiogenesis, apoptosis, immune response) open major possibilities for improving the prognosis of these patients. Biotech and especially biologic progress will open new attractive therapeutic targets to decrease the proliferation process of malignant brain tumors (14).

In the present study we **aimed** at evaluating the efficacy of a modern therapeutic procedure, surgical resection of malignant gliomas using 5-Amino-Levulinic Acid (5-ALA).

MATERIAL AND METHODS

We conducted a study of 127 patients diagnosed with malignant glioma, aged 20 to 80 years, admitted to Neurosurgical clinics (I, II, III) of the Iasi "Prof. Dr. N. Oblu" Hospital, between 2009 and 2015. Of these patients, 17 underwent surgical resection and 5-ALA (Gliolan®) treatment. 5-ALA was administered orally, 3 hours before the induction of general anesthesia,

under the strict supervision of the anesthetist. It is considered to be a relevant biomarker for the gross total resection of glioblastomas which has received approval for use in Europe since 2008 (19). The experimental protocol has been approved by the Hospital Ethics Committee, in accordance with the international regulations regarding the clinical trials and use of medical data, and the informed consent was obtained from all the enrolled patients. Inclusion criteria were: age over 18 years, diagnosis of malignant glioma confirmed by imagining methods. Exclusion criteria were: refusal of subjects or their family to be included in the clinical study, history of allergy to any of the 5-ALA compounds, porphyria, chronic liver disease, mental illness, non-cooperating patients. The following data were collected from all patients: socio-demographic data, clinical manifestations, biochemical parameters, tumor imaging and histopathologic appearance, therapeutic method used, efficacy of treatment over time, survival rate, relapses.

Evaluating the therapeutic efficiency and assessing the surviving subjects for a certain period of time after treatment was performed using the Kaplan-Meier analysis (20). This method (21) estimates data regarding the survival of patients with various diseases under a specific treatment, at different times during the investigation (especially when not all subjects continue the study). It also provides the comparative analysis of the efficacy of different applied therapeutic methods. In clinical trials, the effect of a therapeutic procedure is measured by counting the number of subjects surviving or being rescued at a specified time after applying the treatment. This type of analysis involves calculating the probability of occurrence of an event at a set time point (20).

The probability of survival at a given time point in the study is calculated using the following formula: (number of subjects living at the beginning of the study – number of deceased subjects) / number of subjects living at the beginning of the study (20).

Data were expressed as +/- standard deviation and significance was tested using un-paired t-test and Fisher`s method. P-values below 0.05 were considered statistically significant.

RESULTS

Kaplan-Meier's analysis revealed that patients with glioma who underwent surgery associated with Gliolan® treatment had a higher survival rate at 6 months, 1 year and 2 years, respectively, compared to the group without Gliolan®, treated using classic procedures (tumor resection associated with radiotherapy and/or antineoplastic chemotherapy). The survival rate was significantly higher in the Gliolan ® group (0.65), compared with the group treated by classic methods (0.48), especially after 2 years of treatment (fig. 1).

Statistical data analysis revealed that all 17 patients who were treated with Gliolan® survived 6 months after surgery.

Long-term monitoring of the subjects included in the study showed that: at the 6-month follow-up the disease recurred in 2 of the subjects; 15 of the patients survived 1 year after therapy (1patient with relapse); 11 of the patients treated with Gliolan® survived at 2 years (in 9 of them the disease recurred) (fig. 2, 3). Statistical data processing revealed that all 110 glioma patients treated by classic methods (radiotherapy and/or antineoplastic chemotherapy) survived 3 months after surgical intervention (fig. 4, 5).

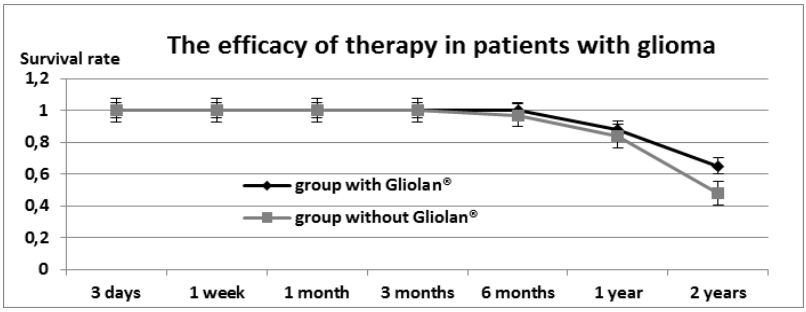

Fig. 1. Survival rate of patients with glioma according to the administered treatment

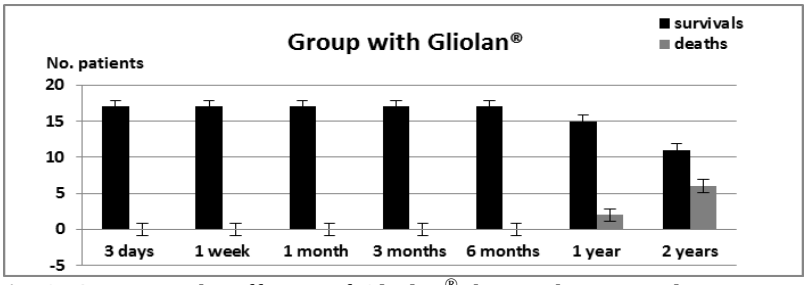

Fig. 2. Assessing the efficacy of Gliolan® therapy by survival rate in time

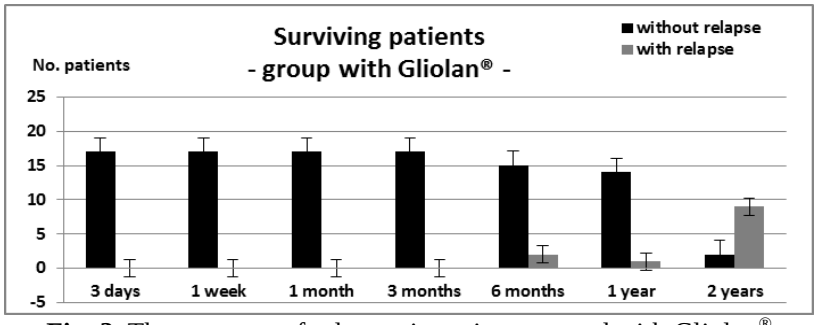

Fig. 3. The presence of relapses in patients treated with Gliolan®

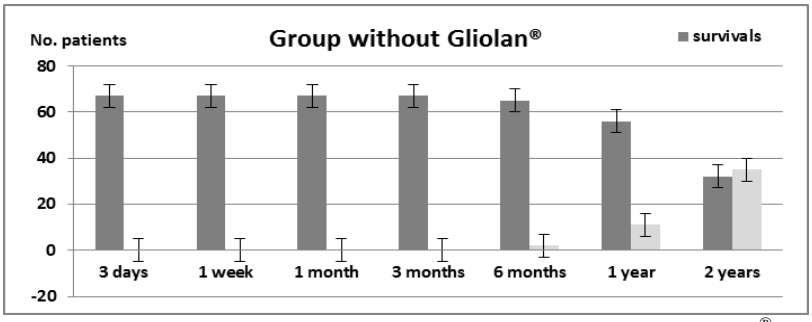

Fig. 4. Assessing the efficacy of classic therapy (without Gliolan®) by survival rate at 6 months, 1 year and 2 years after treatment

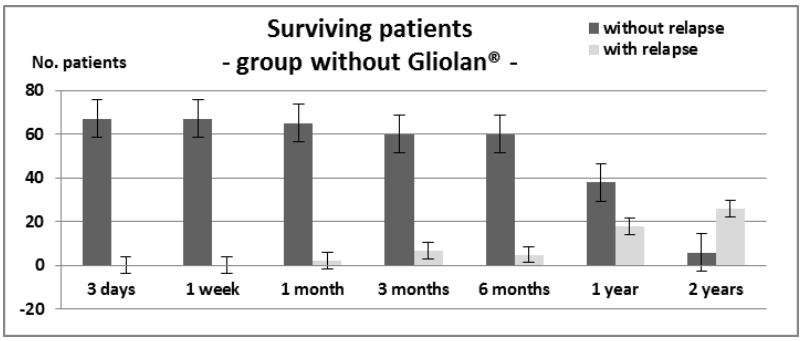

Fig. 5. The presence of relapses in patients without Gliolan®

The long-term assessment of the subjects included in the study showed that: one month after surgery the disease recurred in 2 patients; at the 3-month follow-up, it was found that the disease relapsed in 7 of the investigated patients; 65 patients survived 6 months after therapy (5 with relapses); 56 patients survived 1 year after therapy (in 18 the disease recurred); 32 patients survived at 2 years (but 26 presented disease relapse).

DISCUSSION

Over the past six decades, a variety of innovative techniques have been developed to help neurosurgeons and to increase the effectiveness of malignant brain tumor resections. Among these methods that have contributed to both improved and increased life expectancy, we mention: intraoperative electro-physiological monitoring, neuronavigations techniques, intraoperative ultrasound and magnetic resonance, and also fluorescein-guided cytoreductive surgery, involving the use of 5-aminolevulinic acid (9, 10).

Since its approval by the US Food and Drug Administration (FDA) in 2007, numerous studies have reported the use of 5-aminolevulinic acid in various brain tumors such as: meningioma, metastases or gliomas (19, 20). 5-Aminolevulinic acid is a natural compound produced in hemoglobin metabolism pathways, and its exogenous administration allows it to penetrate the blood-brain barrier and to be captured in neoplastic cells from both tumor and infiltration zones. By photodynamic action it induces apoptosis and tumor cell death, thus allowing maximum resection (23).

The steps taken to approve the use of this substance began after several clinical trials conducted in the neurosurgery departments of various university clinics which showed the benefits of maximal resection, with an improvement of the survival rate. In 2001, Lacroix M. (24) demonstrated an increase in the 5-month survival interval in patients with glioblastoma after maximum resection and treatment with Gliolan®. Four years later, Van den Bentet MJ (25) substantiated an increase in survival rate of 18.3 months in patients treated with Gliolan® compared with 13.5 months in those treated by the classical method, result which supports the efficacy of this substance.

CONCLUSIONS

In our clinical investigation we proved that intraoperative use of the navigation system has been of real help taking into account the changes that may occur, reach-

ing an accuracy of less than 1 cm, without altering the actual location of the tumor. This study showed that the survival rate was significantly higher in the Gliolan® group compared to the group treated by classic procedures, particularly 2 years after tumor resection. Based on the observations that 5-ALA is an important intraoperative marker for direct visualization of the tumor and infiltrative tissue, we can support the idea that it provides the basis for a feasible future in glioblastoma therapeutic management, promising an increase in survival rate compared to classic surgical method, by achieving a maximum resection.

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NEWS

SYNTHESIS OF HIERARCHICAL CuO NANOSTRUCTURES: BIOCOMPATIBLE ANTIBACTERIAL AGENTS FOR GRAM-POSITIVE AND GRAM-NEGATIVE BACTERIA

Copper Oxide is one of the important materials among other antimicrobial agents such as silver, zinc oxide, or titanium dioxide. CuO nanostructures have stimulated great interest in the field of environmental remediation because of their important characteristics which include: high stability, structural properties, antimicrobial activity. The novelty of this study is the biocompatibiliy determination of nanostructured CuO. The findings of the present work proved that the prepared CuO nanostructures are highly stable in aqueous solution and biocompatible in blood. They also exhibited pronounced bactericidal activity towards both Gram positive and Gram negative bacteria (Sonia S, Jayasudha R, Jayram ND, et al. Synthesis of hierarchical CuO nanostructures: Biocompatible antibacterial agents for Gram-positive and Gram-negative bacteria. *Current Applied Physics* 2016; 16: 914-921).

Oana Dragostin

HIDDED BEHIND AN EARLY REPOLARIZATION SYNDROME - CASE REPORT

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HIDDED BEHIND AN EARLY REPOLARIZATION SYNDROME-CASE REPORT (Abstract): The electrocardiographic (ECG) pattern of "early repolarization syndrome" is no longer considered benign, mainly due to life-threatening rhythm disorders and sudden cardiac death sometimes associated to this syndrome. We report the case of a 55-year old man who was hospitalized after an episode of chest discomfort, emphasizing an aspect of early repolarization syndrome on electrocardiogram and diffuse ST segment depression at submaximal cycle ergometer test. However, the coronarography revealed severe distal occlusion of the left main artery including the origin of left anterior descendent and circumflex arteries. The revascularization treatment consisted of two drug eluting stents placed on left main artery, with good clinical and angiographic results. This association of severe coronary lesion hidden behind a benign ECG aspect has been rarely mentioned in the literature. **Keywords:** EARLY REPOLARIZATION, CORONARY ARTERY, LEFT MAIN, J POINT.

The "benign" label attached to early repolarization syndrome (ERS), defined as an electrocardiographic (ECG) pattern characterized by a prominent J-point and ST-segment elevation, notching or slurring (pseudo delta wave or late delta wave) of the end portion of the R wave with upward concavity ending in a positive high amplitude symmetric T-wave in most ECG leads, has been removed by Haïssaguerre et al. in 2008 (1). Recent evidences argue that ERS can be considered as part of a continuous spectrum of similar molecular, ionic and cellular mechanism expression, which may be generally referred to as "J-wave syndromes" (2). J wave syndromes are associated with amplification of the transient outward current (Ito)-mediated J waves that can initiate life-threatening ventricular arrhythmias (3). On the other hand, as cause of ST segment abnormality in Emergency Department, 12% of patients with chest pain have ERS on their ECG, thereby becoming a common diagnostic challenge for clinicians (4). ERS can mask the presence of acute ischemia, while lower R-wave amplitude, a higher ST-segment elevation and a longer QTc duration are arguments in favor of a subtle anterior STEMI (5). The common opinion is that between

ERS and ischemia there is no a direct causal relationship. Nonetheless, ERS was tagged in general conception as essentially benign. We report a case of severe ischemia generated by the presence of a tight left main stenosis appeared on an initial ECG as ERS.

CASE REPORT

A 55-year-old Caucasian male, construction worker, current smoker, chronic drinker (gamma glutamyl trans peptidase 35 UI/ml), without a personal or hereditary history of cardiovascular diseases comes to the Emergency Department and was referred to the Cardiology Clinic for a new onset of chest discomfort occurring at effort, disappearing at rest, radiating to the left arm, lasting for more than 20 minutes, that was progressively intensified in the last week. At admission, he was hemodynamically stable, with blood pressure of 140/90 mmHg, without significant tensional difference between the right and left arms, heart rate of 55 bpm and normal peripheral pulse. Further physical examination showed normal weight patient (BMI 24.5 kg/m²) and temperature 36.8^0 C, without any pathological findings or neurological deficits. His standard 12-lead electrocardiogram (ECG) (fig. 1) shows a sinus bradycardia 55 bpm, concave ST elevation in the precordial leads (V_2-V_6), with notching and slurring at the J-point, prominent, slightly asymmetrical T-waves that are concordant with the QRS complexes. No previous ECG of the patient was available to see whether ERS was present before.

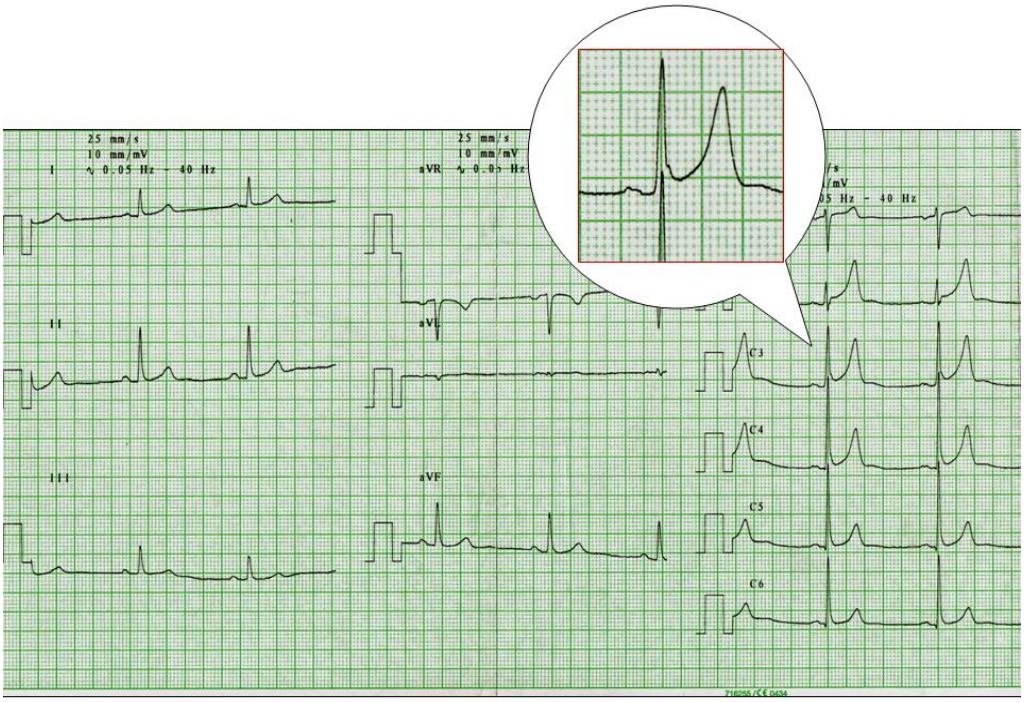

Fig. 1. 12-leads ECG: sinus bradycardia 55 bpm, high R wave in V_2, concave ST elevation in the precordial leads (V_2-V_6), with notching and slurring at the J-point, suggestive for an ERS.

The degree of ST elevation is modest compared to the T-wave amplitude. ST elevation is below 2 mm in the precordial leads. No reciprocal ST depression to suggest STEMI. Should be noted that, per literature, this ERS pattern (ER followed by rapidly upsloping ST segments after the J point) is not associated with an increased risk for arrhythmic death (6), contrary to ERS associated with horizontal or descending ST segments after the J point. ST changes were relatively stable over time, with no progression on serial ECG tracings. Laboratory analysis showed dyslipidemia (serum cholesterol 306 mg/dL, HDL-C 43 mg/dL, LDL-C 233 mg%, triglycerides 152 mg/dL) and negative cardiac biomarkers (creatine phosphokinase 78 ui/l, creatine phosphokinase-MB 15 ui/l, troponin I 0.001 ng/ml), with normal liver (serum glutamate-pyruvate transaminase 25 ui/l and serum glutamate-oxaloacetate transaminase 16 ui/L) and renal (BUN 37 mg/dL, serum creatinine 0.7 mg%) function, without anemia (hemoglobin 15.2 g%, hematocrit 45.7%). Chest discomfort was quickly removed after nitroglycerin spray (0.4 mg) administration. Thereafter, the patient was admitted as unstable angina and received loading doses of clopidogrel and aspirin upon admission continued with aspirin 75 mg o.d., clopidogrel 75 mg o.d., enoxaparin 60 mg bid, nebivolol 5 mg o.d., perindopril 10 mg o.d. and atorvastatin 80 mg o.d..

The rapid control of chest pain in a patient with cardiovascular risk factors (chronic smoker with hyperlipoproteinemia) with ECG changes suggestive of early repolarization syndrome and considering that programming for coronary angiography implies registration on a long waiting list has led us performing, for shaping the diagnosis, the cycle ergometer exercise test (fig. 2). Nebivolol was not discontinued prior to performance of the stress test. Exercise stress testing electrocardiogram showed ST-segment depression in eight leads with elevation in leads aVR and V_1, suggestive for subendocardial ischemia related to three-vessel or left main coronary artery disease: positive submaximal exercise test with the occurrence of chest pain associated with a ST-segment horizontally depression (1-2 mm) at 60 W in leads V_4-V_6, then V_3-V_6, sharp changes in the recovery period, ST segment depression in inferior leads and ST elevation in aVR and V_1. Maximum BP was 160/90 mmHg, HR maximum 92 bpm.

The patient was urgently directed to a PCI center where angiography was performed and revealed: distal sub occlusion 99% of the left main coronary artery (LMCA) including the origin of left anterior descendent (LAD) and circumflex (Cx) arteries, dominance of the left coronary bed, a 60% plaque of the first marginal artery and 30% plaque on the right coronary artery (fig. 3). The interventional cardiologist has decided to perform within the same session the coronary angioplasty.

The percutaneous coronary intervention procedure included (fig. 4) the predilatation of the distal left main artery toward LAD and placement of a Promus dug eluting stent (DES) (PROMUS™ Everolimus-Eluting Coronary Stent, Boston Scientific Corporation). The post-dilatation was performed by using kissing balloon technique toward LAD (3.5/15 mm, 8 atm) and toward Cx (2.5/20 mm, 12 atm) arteries and a Taxus DES (TAXUS™ Element™ Paclitaxel-Eluting Coronary Stent System, Boston Scientific Corporation) implantation in the ostium of Cx (3.0/16 mm, 14 atm). The result was very good. A 7-day double-dose clopidogrel regimen after

procedure was administered according to CURRENT trial (7) where in patients undergoing PCI for acute coronary syndromes this regimen was associated with a reduction in cardiovascular events and stent thrombosis compared with the standard dose. Subsequently, the patient was recommended clopidogrel 75 mg o.d. for 24 months after stenting and aspirin 75 mg o.d. lifelong.

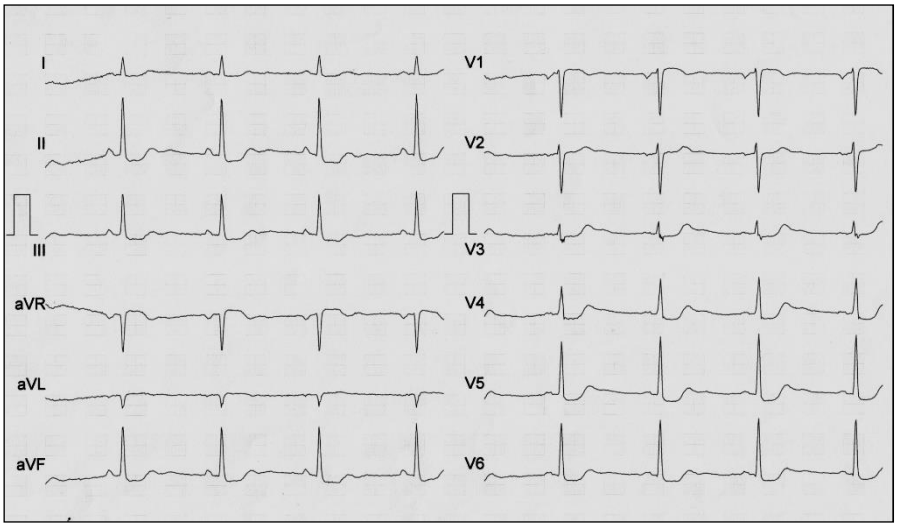

Fig. 2. Exercise stress testing electrocardiogram of the patient with angina without elevated cardiac biomarkers: ST-segment depression in eight leads with elevation in leads aVR and V_1 suggestive for subendocardial ischemia related to three-vessel or left main coronary artery disease.

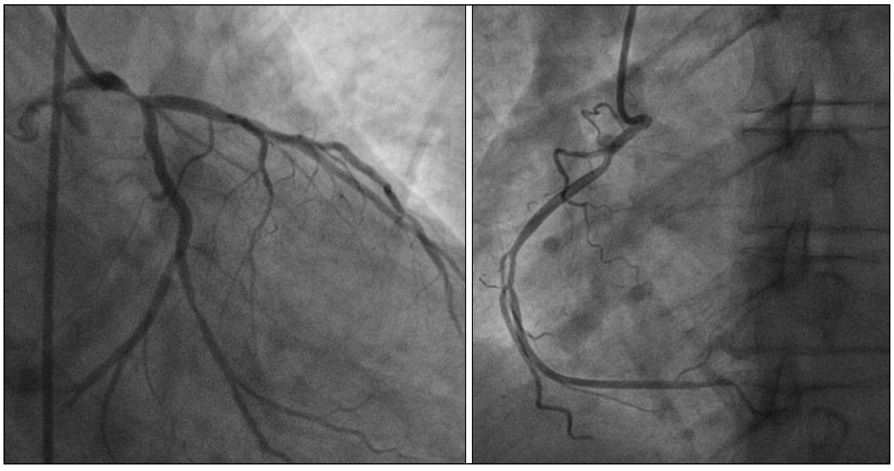

Fig. 3. Coronary angiography: distal sub occlusion 99% of the left main artery (LMCA) including the origin of left anterior descendent (LAD) and circumflex (Cx) arteries. Right coronary artery with no significant plaques.

Fig. 4. Coronary angioplasty: pre-dilatation of LMCA and a DES placement; post-dilatation using kissing balloon technique toward LAD and Cx arteries with a DES implantation in the ostium of Cx.

DISCUSSION

The relationship between ERS and fatal arrhythmias, that once seemed a heresy, nowadays is accepted and intensively studied: the assuasive electrocardiographic pattern was acknowledged to be a marker of hidden risk. Probably, it would worth being reviewed the relationship between J wave syndromes and myocardial ischemia. Currently, the following assertions may be formulated about ERS:

Firstly, ERS is an ECG pattern quite frequently encountered: Tikkanen et al, in a study involving 10.864 middLe-aged subjects, found a prevalence of ERP of 5.8% (8).

Secondly, there are some recent attempts to redefine the ERS definition on much stricter criteria (9) established with the peak of an end-QRS notch and/or the onset of an end-QRS slur (Jp) that must be determined when an ERS is suspected. To certify its presence, the presence of a Jp ≥ 0.1 mV is mandatory, while ST-segment elevation is no more a required criterion.

Thirdly, there was a debate whether J wave syndromes are repolarization or depolarization/conduction abnormalities (10). J waves as repolarization components are commonly seen in young males with structurally normal hearts, while depolarization/conduction abnormalities are commonly found in elderly with structural heart diseases. Nonetheless, Antzelevitch et al. tried to solve this issue (11).

Fourthly, a relationship between ventricular fibrillation and J wave with ERS was demonstrated in a relatively large number of patients (1). Left precordial terminal QRS notching is prevalent in ma-

lignant variants of ERS and could be used as a tool for risk stratification (12). ERS in the inferior leads was at borderline significance in predicting cardiac and death of all-cause (8). Moreover, J-wave dynamics can be sometimes involved in the generation of ventricular fibrillation (12).

Fifthly, referring to the relationship between ERS and ischemia, the available data are often unclear. The association, coincidentally or not, between ERS and ischemia has few reports. Yan and Antzelevitch point out that myocardial ischemia is known to reduce inward currents and to increase the outward potassium currents particularly by intensifying I_{to} especially in the epicardium, thus possibly unmasking J waves (13). A relationship between ERS and myocardial ischemia has been mentioned cases of coronary spasm (14, 15) which can cause fatal ventricular arrhythmias or sudden cardiac death. The augmentation, development and altered J-wave dynamics may be related to the heterogeneous distribution of transmural ventricular repolarization in patients with myocardial ischemia induced by provoked coronary spasms (14). There are arguments in favor of the assumption that a type of acquired J wave syndrome is "ischemia-induced J wave syndrome", as shown by a series of studies published in China (16, 17). The mechanisms underlying ischemia-induced J wave must be better understood because sudden arrhythmic death is the major cause for mortality associated with acute coronary syndrome (3). On the other hand, the importance of differentiating between acute ischemic changes and ERS true benign pattern must be underlined: in benign ERS reciprocal ST segment changes are possible only in lead aVR in contrast to the idiopathic ventricular fibrillation and acute coronary syndrome where reciprocal ST segment changes can be observed in several leads (18). Moreover, in benign ERS the ST segment and T wave patterns have a relative temporal stability (18).

In our reported case, ERS changes were diffuse (inferior and precordial leads). Perhaps the cycle ergometer exercise test was a bit bold in this case, but the positivity led to further invasive investigations in emergency. Moreover, the interventional cardiologist has decided to perform a complex percutaneous intervention which was prohibited a couple of years before. The immediate and long-term outcomes after stenting the LMCA stenosis in patients with normal left ventricular (LV) function have been demonstrated to be excellent (19), evolution like our case. It is worth mentioning that the initial ECG aspect has not changed after PCI.

It is possible that some cases of sudden death are due to ERS associated to severe ischemic substrate, such as LMCA lesions. The hypothesis that a diffuse distribution of ERS-like pattern is associated with left main or tri-vessels lesions, especially in patients with positive exercise stress test, must be validated by further studies.

CONCLUSIONS

Our case report shows that a seemingly benign ECG pattern can hide a serious coronary lesion, especially in patients with chest pain, in whom it is important to complete the usual diagnostic algorithm with at least an exercise stress test. Reflecting on the link between ERS and ischemia it is worth wondering whether some of the cases of sudden death might have occurred in the context of such so-called "benign" ECG pattern that could have appeared on a severe ischemic substrate, such as left main lesion.

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COMPARATIVE STUDY OF HYSTEROSCOPY AND 3D ULTRASOUND FOR DIAGNOSING UTERINE CAVITY ABNORMALITIES

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COMPARATIVE STUDY OF HYSTEROSCOPY AND 3D ULTRASOUND FOR DIAGNOSING UTERINE CAVITY ABNORMALITIES (Abstract) **Objective**: Hysteroscopy is the most accurate method for diagnosing intrauterine pathologies related to abnormal bleeding and infertility. The accuracy of three-dimensional (3D) ultrasonography and hysteroscopy were compared in identifying uterine cavity abnormalities. **Material and methods**: A total of 139 cases of abnormal uterine bleeding or infertility first had two-dimensional and 3D ultrasound performed before hysteroscopy. Findings on 3D ultrasound and hysteroscopy were compared, and sensitivity, specificity, positive predictive value, negative predictive value, and likelihood ratio were calculated. **Results**: Mean patients' age was 36.5 (+/- SD 9.04). Three-dimensional ultrasound had a sensitivity of 88%, specificity of 94%, a positive predictive value of 96%, negative predictive value of 84%, likely ratio of 5,5, and accuracy of 90% in diagnosing uterine cavity abnormalities. Three-dimensional ultrasound had a high sensitivity and specificity for polyps (97% and 97%, respectively), congenital uterine malformations (100% and 99%, respectively) and submucous myoma (87% and 100%, respectively), but a low sensitivity and high specificity for uterine synechia (41% and 99%, respectively). **Conclusions**: For diagnosing uterine cavity anomalies, 3D ultrasound is a useful tool and could replace diagnostic hysteroscopy for certain conditions, such as congenital uterine anomalies. **Keyword**: HYSTEROSCOPY, THREE-DIMENSIONAL ULTRASOUND, UTERINE CAVITY ABNORMALITIES.

Abnormal uterine bleeding and infertility are two of the most common complaints in gynecologic practice. To properly diagnose and manage both conditions, assessing the uterine cavity for possible abnormalities is essential. Transvaginal ultrasonography is the standard method for screening for possible endometrial or uterine cavity abnormalities. When indicated, saline/gel infusion sonography, hysterosalpingography, or hysteroscopy may also be used to evaluate the uterine cavity lining. Hysteroscopy is widely accepted to be the most accurate method for investigating the uterine cavity (1, 2).

Three-dimensional (3D) ultrasound is a new technology that allows us to examine pelvic structure and their volumes with great accuracy. The main advantage 3D ultrasound confers is the ability to obtain a view of the coronal plane of the uterus (3), which, in turn, may be used to locate the

position of intrauterine pathology. Therefore, 3D volume ultrasound is superior to two-dimensional (2D) sonography in identifying uterine cavity abnormalities.

Our study aimed to compare 3D ultrasound with hysteroscopy in assessing the uterine cavity and to find a place for this technology in gynecologic practice.

MATERIAL METHODS

Our study included 139 women attending our university hospital for hysteroscopy. The main indications for hysteroscopy were abnormal uterine bleeding and infertility. Before hysteroscopy was scheduled, transvaginal 2D and 3D ultrasound examinations were performed for each patient. For both examinations, we used 3D endovaginal ultrasound probes (RIC6-12D, 4-8Mhz, Voluson E8 and RIC 5-9H Voluson 730 Pro, GE Healthcare). The ultrasound examination began with a conventional 2D ultrasound. First, the uterus was localized and its position was noted, then the uterus was measured in three planes (longitudinal, coronal, transverse). Endometrial thickness was measured in the longitudinal plane, including the two layers at its maximum thickness. Then, the adnexal region was scanned to identify the ovaries, fallopian tubes, or adnexal pathology. The 3D volume was obtained longitudinally along the length of the uterus. The coronal plane of the uterus was obtained using the Abuhamad technique (4). For patients with infertility, the ultrasound examinations were scheduled in the follicular phase of their menstrual cycles (Days 3-10). Informed consent was obtained from all patients.

A 5-mm outer-diameter continuous flow Bettocchi hysteroscopy was used with a 30° direction of view (Karl Storz Endoscopy). Normal saline solution was used for distension of the uterine cavity at a pressure of 20-50 mm Hg. The hysteroscopy procedures were performed under local and/or general anesthesia. Systematic inspection of the uterine cavity was done, and internal os and cervical canal were visualized. The hysteroscopically detected intrauterine abnormalities were defined as endometrial polyps, submucous myomas, endometrial hyperplasia, intrauterine adhesions, or uterine congenital anomalies, such as arcuate or septate uterus. Treatment consisted of removal of polyps, myomas, adhesions, or septa using scissors, grasping forceps, or a resectoscope (Karl Storz Endoscopy). After the intervention, a detailed record was completed with information about patient tolerance, diagnostic findings, and treatment.

Statistical methods

Data was statistically represented in terms of range and mean standard deviation (+/- SD) and percentages. Accuracy was represented using the terms of specificity, sensitivity, positive predictive value, negative predictive value, likely ratio, and overall accuracy. The statistical data were calculated using the Microsoft Excel (Microsoft Corporation, NY, USA). Diagnostic accuracy of hysteroscopy and 3D ultrasound for different intrauterine pathologies were compared using tables.

RESULTS

A total of 139 patients with abnormal uterine bleeding, primary infertility, or secondary infertility were included in the study (Table 1). The patients' mean age was 36,5 +/- 9,04 years old. Abnormal uterine bleeding included cases of menorrhagia, metrorrhagia, hypomenorrhea, and postmenopausal bleeding (Table 2). A

comparison of the uterine anomalies detected using 3D ultrasound and hysteroscopy are shown in Table 3.

In diagnosing uterine cavity abnormalities, 3D ultrasound has a sensitivity of 88%, specificity of 94%, a positive predictive value of 96%, negative predictive value of 84%, likely ratio of 5,5 and accuracy of 90%. When we analyzed different uterine cavity pathologies, we observed a high sensitivity and specificity of 3D ultrasound for polyps (97% and 97%, respectively), myoma (87% and 100%, respectively), and uterine congenital abnormalities (100% and 99%, respectively), but a low sensitivity for uterine synechia (41%) (tab. IV).

TABLE I

Patients' Indications for Hysteroscopy

Indications for hysteroscopy	Number of cases	Percentage
Primary Infertility	51	36,71%
Secondary infertility	42	30,21%
Abnormal uterine bleeding	46	33%
Total	139	100%

TABLE II

Type of abnormal uterine bleeding

Symptoms	Number of cases	Percentage
Metrorrhagia	8	17,3%
Menorrhagia	20	43,47%
Menorrhagia and Metrorrhagia	9	19,57%
Postmenopausal bleeding	9	19,57%
Total	46	100%

TABLE III

Anomalies Detected using 3D Ultrasound and Hysteroscopy

Type of Anomaly	3D Ultrasound	Hysteroscopy
	No. Cases	No. Cases
Endometrial polyp	45	44
Endometrial hyperplasia	8	7
Submucous myoma	7	8
Uterine congenital anomalies	7	6
Osseous metaplasia	2	2
Uterine synechia	8	17
Uterine neoplasm	1	1
Uterus normal	61	54

TABLE IV
Sensitivity, specificity, positive predictive value and negative predictive of 3D ultrasound value for different uterine cavity pathologies.

Uterine anomaly	Sensitivity	Specificity	PPV	NNV
Polyp	97%	95%	95%	98%
Myoma	85%	99%	100%	99%
Congenital anomaly	100%	99%	85%	100%
Synechia	44%	100%	87%	92%

DISCUSSION

Abnormal uterine bleeding and infertility are two of the most common complaints in gynecology. Their management depends on diagnostic accuracy, and the first step is evaluating the uterine cavity. The basic work-up consists of transvaginal sonography possibly followed by hysterosonography, hysterosalpingography, or hysteroscopy to directly assess the uterine cavity.

Hysteroscopy is generally considered to be the gold standard in diagnosing intrauterine pathologies, including endometrial polyps, submucous myomas, intrauterine adhesions, and uterine septa (5). Compared to histopathology or hysterectomy findings, hysteroscopy is very accurate for diagnosing intrauterine abnormalities (6). One advantage of hysteroscopy is the possibility of performing a directed biopsy or a small surgical intervention during the same session (5). Also, in more recent years, hysteroscopy has been increasingly performed in an outpatient setting, which is highly acceptable and popular with patients. Office hysteroscopy has increasingly been recommended as a routine procedure in infertility evaluation (7, 8, 9, 10). After repeated implantation failure during in vitro fertilization cycles, the uterine cavity should be reevaluated using hysteroscopy; this practice has been demonstrated to improve pregnancy rates (6, 11, 12). However, hysteroscopy is an invasive procedure, and complications, such as uterine perforation and ascending genito-urinary infections, although very rare, can still occur (13, 14).

Because transvaginal ultrasound is a common and noninvasive procedure, its role in diagnosing uterine cavity anomalies should not be neglected. Although hysteroscopy is the gold standard, 2D transvaginal ultrasound has a sensitivity of 78.15% and specificity of 44.4% in detecting cavity abnormalities (15). However, 2D sonography cannot confidently diagnose submucosal fibroids in a uterus with multiple fibroids, distinguish between a hyperplastic endometrium and a large polyp, nor differentiate between an arcuate and a septate uterus (16, 17). These limitations can be overcome by 3D ultrasound, which has become more important in recent years in gynecology and obstetrics.

First introduced in the 1980s, 3D ultrasound has taken some time to be adopted. In 1993, a transvaginal application was developed, which allowed the acquisition of 3D surface images of the uterus and ovaries (18). The main advantage of 3D ultrasound is the ease in obtaining the coronal view with the entire endometrial cavity, the relationship of the endometrium to myometrium, and the uterine serosa. Compared to using 2D slices, the ability to ob-

tain and navigate through the 3D coronal views of the uterus allows the clinician to visualize the cavity more precisely and accurately. Three-dimensional ultrasound with surface- and transparent-mode reconstructions of the uterus has reported advantages over conventional 2D scanning. In experienced hands, a sensitivity of 93% and a specificity of 100% have been achieved (19).

In our study, compared to hysteroscopy in diagnosing uterine cavity abnormalities, 3D ultrasound has a sensitivity of 88%, specificity of 94%, a positive predictive value of 96%, negative predictive value of 84%, likely ratio of 5,5, and accuracy of 90%. Using transvaginal 2D sonography or hysterosalpingography, Sylvestre et al. studied 209 subfertile patients who were thought to have an intrauterine lesion (20). Using saline infusion sonography with 2D and then 3D sonography, 92 patients were subsequently identified as having a variety of intrauterine lesions, suggesting a sensitivity and specificity of 97% and 11%, respectively, for 2D sonography, 87% and 45%, respectively, for 3D sonography, and 98% and 100%, respectively, for 2D saline infusion sonography.

In our study, when analyzing different uterine cavity pathologies, 3D ultrasound had a high sensitivity and specificity for identifying polyps (97% and 97%, respectively), myoma (87% and 100%, respectively), and congenital uterine abnormalities (100% and 99%, respectively) (fig. 1, 2). In cases with synechia, 3D ultrasound had a low sensitivity (41%). The low sensitivity for synechia could be explained by the timing of the ultrasound. The ultrasound was performed during the follicular phase of the menstrual cycle when the endometrium was thin. Performing transvaginal sonography at midcycle (three-layer endometrium) rather than after menstrual cessation when endometrial thickness is less than 3 mm enables better imaging of small intrauterine adhesions (17). Also, in cases with synechia that were not detected by ultrasound, the adherence was very fine and minimal; this could be another reason why they were misdiagnosed during ultrasound examination.

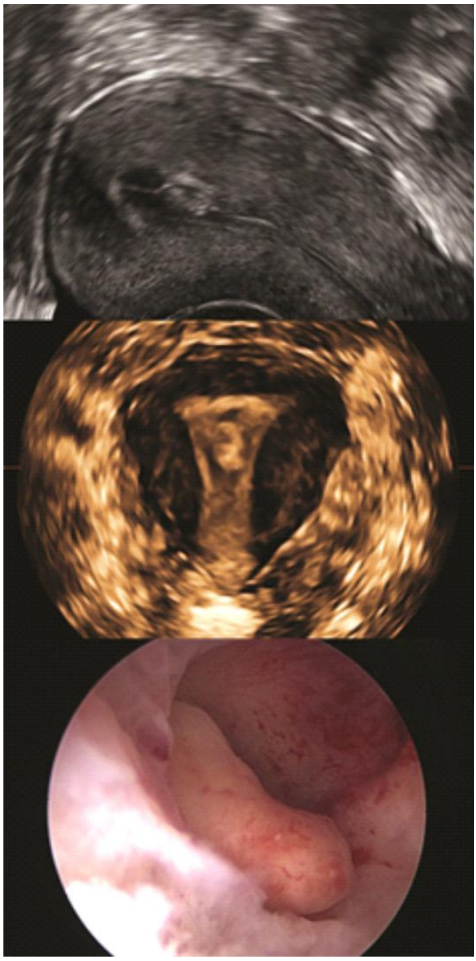

Fig. 1. Endometrial polyp.
a. 2D longitudinal image of the uterus.
b. 3D coronal image of the uterus.
c. Hysteroscopic image of the polyp.

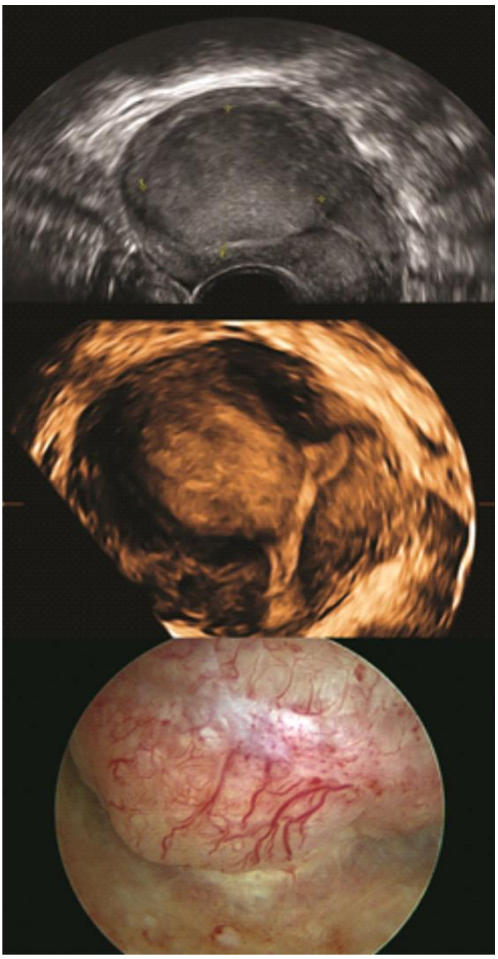

Fig. 2. Intramural and submucous myoma.
a. 2D longitudinal image of the uterus.
b. 3D coronal image of the uterus.
c. Hysteroscopic image of the myoma.

La Torre et al. compared 3D sonography with conventional 2D imaging with and without saline contrast in their study of 23 patients in whom subsequent hysteroscopy revealed the presence of 16 endometrial polyps (21). Standard 2D sonography demonstrated a relatively poor specificity of only 69.5%, suggesting the presence of polyps in 23 patients. In contrast, 3D sonography diagnosed 18 polyps, with a specificity of 88.8%, and 3D hystero-sonosalpingography diagnosed 16 polyps, per hysteroscopic and histologic findings, with a specificity of 100%. Another prospective study of 70 patients that compared 3D ultrasound with hysteroscopy in premenopausal bleeding found that 3D ultrasound had a sensitivity of 63.2%, specificity of 80.8%, positive predictive value of 54.6%, negative predictive value of 85.7%, and accuracy of 76.1% (22).

In our study, 3D ultrasound proved to be very accurate for diagnosing uterine congenital anomalies, in agreement with the literature. Ghi et al. consider 3D ultrasound to be extremely accurate for the diagnosis and classification of congenital uterine anomalies and suggest that it may become the only mandatory step in the assessing the uterine cavity in patients with a history of recurrent miscarriage (23). The advantage of 3D ultrasound over hysteroscopy is that it can show the external contour of the uterus and can differentiate septate uterus from bicornuate uterus. In addition, 3D ultrasound is superior to 2D views in identifying the exact location of endometrial polyps and the degree of protrusion of a submucous fibroid into the cavity, as well as the amount of myometrium remaining outside of the submucous fibroid. In recent years, hysteroscopic resection of submucous fibroids has become an accepted method for treating premenopausal women with a history of abnormal uterine bleeding. The success and safety of this operation depends largely on the accurate preoperative assessment of the position of the submucous fibroid in relation to the uterine cavity (24). Before planning surgery for uterine myoma, 3D ultrasound could be an essential tool because it can provide precise information regarding the size of the myoma and the depth of myometrial exten-

sion. Moreover, 3D is useful also during post-surgical management.

CONCLUSIONS

Hysteroscopy remains the gold standard for diagnosing uterine cavity anomalies. However, 3D ultrasound has high sensitivity and specificity for uterine cavity anomalies and should be used a primary screening tool. Although 3D ultrasound has a low specificity for uterine synechia, it has a high specificity for uterine congenital anomalies. Further, 3D ultrasound is useful before planning an intervention on the uterine cavity, especially in cases of congenital anomalies and uterine fibroma.

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NEWS

CLOPIDOGREL AND THE ENDOTHELIAL FUNCTION – BEFORE AND AFTER

The conclusion of a recent article published in the Journal of American Heart Association is that the administration of Clopidogrel in healthy people with high platelet aggregation improved the microcirculatory endothelial function and it apperas to have additional vascular benefits. The researchers evaluated the platelet activity of the healthy persons, a group of 287/241 Old Order Amish, before and after the administration of Clopidogrel for 7 days, quantifying it by laser Doppler flowmetry (LDF) mediated by thermal hyperemia and postoclussive reactive hyperemia. The results showed that Clopidogrel influenced only LDF mediated by thermal hyperemia, having a bigger impact on women than men; also, the evaluation before Clopidogrel showed that women have a higher platelet aggregation (Salimi S., Lewis J.P. et al. Clopidogrel Improves Skin Microcirculatory Endothelial Function in Persons With Heightened Platelet Aggregation. *J Am Heart Assoc.* 2016; 5:e003751).

Măriuca Balan

EXPERIMENTAL MODEL OF MIXED INTESTINAL INFARCTION IN RABBIT

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EXPERIMENTAL MODEL OF MIXED INTESTINAL INFARCTION IN RABBIT (Abstract): The article presents an experimental model of the mixed intestinal infarction obtained by ligaturing of the cranial mesenteric artery (CMA) and of the cranial mesenteric vein (CMV) in rabbit. **Keywords**: RABBIT, INTESTINAL INFARCTION, EXPERIMENTAL MODEL

Acute mesenteric ischemia (AMI) consists of interruption of the blood flow in the irrigation area of the superior or inferior mesenteric artery, that results in intestinal infarction (1).

The first description of mesenteric vascular occlusion was attributed to Antonio Beniviene from Florence in the latter part of the fifteenth century (2). Little progress was made until 1895, when Elliot reported the first patient recover following resection of infarcted intestine (3).

In 1926, Cokkinissaid: „*Occlusion of the mesenteric vessels is apt to be regarded as one of those condition of which* […] *the diagnosis is impossible, the prognosis hopeless and the treatment almost useless*" (4). However, the progress in understanding of the pathophysiology and diagnosis, has made it possible to save sometimes the lives of patients with mesenteric ischemia.

For the physio pathological study of intestinal ischemia/ perfusion injury, the following animals are mainly used in experimental models: mice (5), rat (6-7), rabbit (8-9), pig (10).

In this study, the mixed intestinal infarction was reproduced in rabbits by ligaturing simultaneously the cranial mesenteric artery (CMA) near the emergence and by ligaturing the cranial mesenteric vein (CMV) near its pouring point in the portal vein. The abdominal topography of the rabbit presents some particularities: the cecum occupies a considerable part of the dextroventral half of the abdominal cavity, the spleen is positioned on the caudomedial surface of the stomach, and the kidneys are asymmetrically positioned in the retroperitoneal space – the right kidney lays between the planes through the 10-11 intercostal space and through the first lumbar

vertebra, the left kidney is between the 2nd and the 4th lumbar vertebra (11). CMV is direct constant affluent of the portal vein, that helps its marking (12).

In humans, the vascular mixed mesenteric obstruction is realized rather by an extrinsic compressive mechanism: tumors from near surrounding structures, of the mesenteric roots, of the duodenojejunal flexure (13), incarcerated trans mesenteric intestinal hernia (14), aortic aneurysm situated in the pouring point of the mesenteric artery. The double occurrence in artery and vein through intrinsic mechanism is rarer found (in mesenteric vasculitis (15-16)). Some authors consider the mixed form an evolutive stage of the arterial or venous infarction (17).

MATERIAL AND METHOD

All experimental procedures were performed in the Experimental Medicine Center, "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca, with approval of the Ethical Commission of the university. Seven adult Californian rabbits (*Oryctolagus cuniculus sp.*) in two lots: A–experiment (five rabbits) and B–control (two rabbits, which were undertaking the same preoperative procedures and anesthetics like the rabbits from group A).

In order to eliminate from the study, the animals with abdominal pathology or vascular malformation of the cranial mesenteric package, the preoperative exam included veterinarian clinical examination, abdominal x-ray (fig. 1) and abdominal ultrasound. For the ultrasonography, we used a GE Logiq S7 machine, with a 6-15 MHz matrix transducer. We used the B mode to identify and assess the morphology of the vessels (fig. 2). We used color Doppler flow mode to visualize the direction and the laminar nature of the blood flow and we measured the velocities and flow indexes using spectral Doppler (fig. 3-4).

A

B

Fig. 1. Abdominal X-ray A - rabbit in orthostatic position, AP incidence: normal aspect; B - rabbit in quadrupedal position, LL incidence: normal aspect.

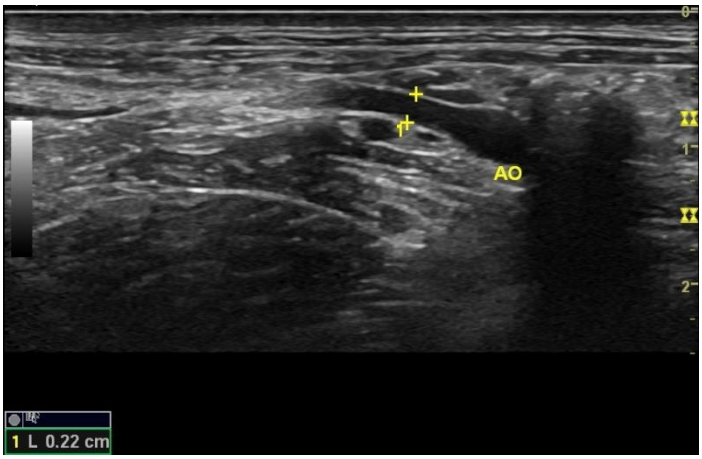


Fig. 2. Ultrasound identification of CMA emerge and measuring of its diameter.

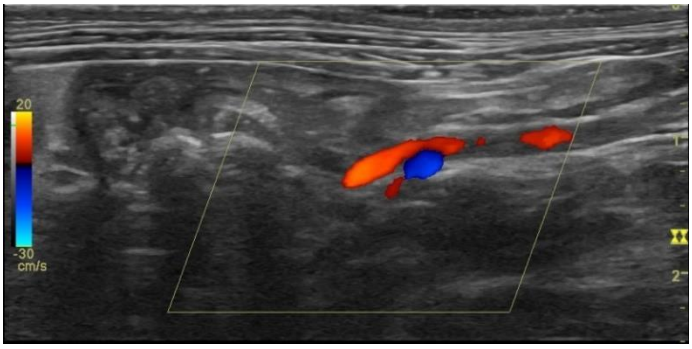


Fig. 3. Doppler ultrasound identification of the cranial mesenteric vascular package – CMA (red), CMV(blue).

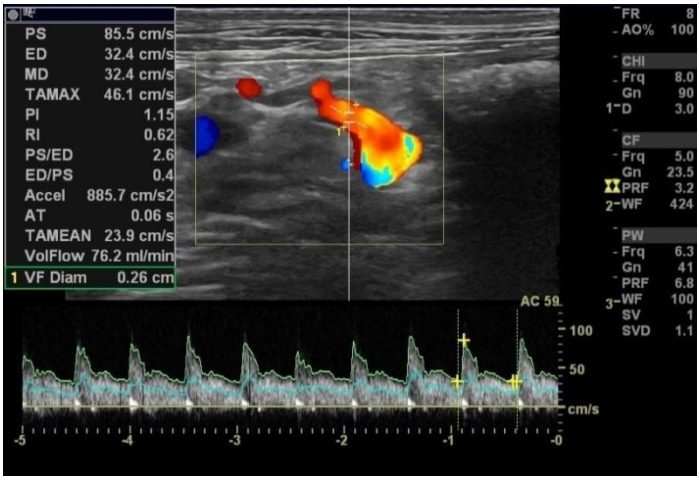


Fig. 4. Doppler ultrasound measuring of hemodynamic parameters in CMA.

Preoperative diet consisted in food deprivation for 24 hours, with hydration.

Preoperative, abdominal fur was shaved off.

Induction of anesthesia was performed with Ketamine 100 mg/ml, 0,35 ml/kg body weight and Xylazine 20 mg/ml, 0,15 ml/kg body weight, after which the animal was bound on the operating table in a dorsal decubitus position (fig. 5).

Fig. 5. The animal on the operating table in a dorsal decubitus position.

The surgical instrument set was made out of cutting and stinging instruments (scalps, electrocautery, straight and curved scissors), hemostatic forceps, dissecting forceps, anatomical forceps, surgical forceps, microsurgical instruments, Farabeuf retractor, instruments for sewing tissues. Preoperative, the instruments were sterilized through boiling for 60 minutes at 110°C in an electric boiler.

After preparing and isolating the surgical field, laparotomy was preformed through midline incision.

While exploring the abdominal cavity, the aspect and the viability of the intestine, the presence of the peristalsis was followed. After the exteriorization of the intestine through the surgical incision and it's flapping towards the right side, the left kidney was looked for as a reference point and the retroperitoneal adipose tissue was penetrated. Following the left renal artery, path the aorta was localized and through its discovery, overlying the emerge of the left renal artery, the CMA was identified, easily mobilized by the movement of the mesentery and intestine. After the discovery of the CMA for a length of ca. 5mm from the emergence (fig. 6), the CMA was ligatured with a non-absorbable 4.0 (fig. 7) and the absence of the pulse distal to the ligature was verified.

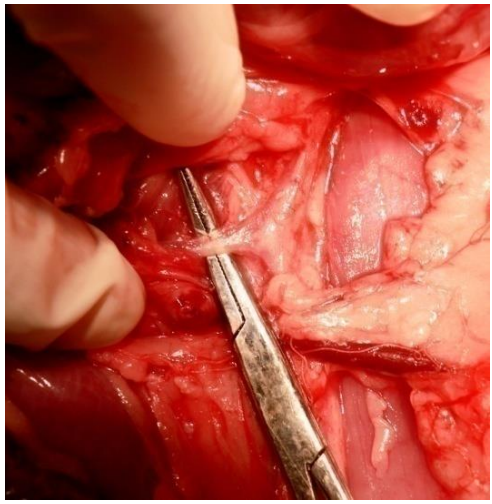


Fig. 6. Discovery of CMA, mobilization through light pulling of mesentery.

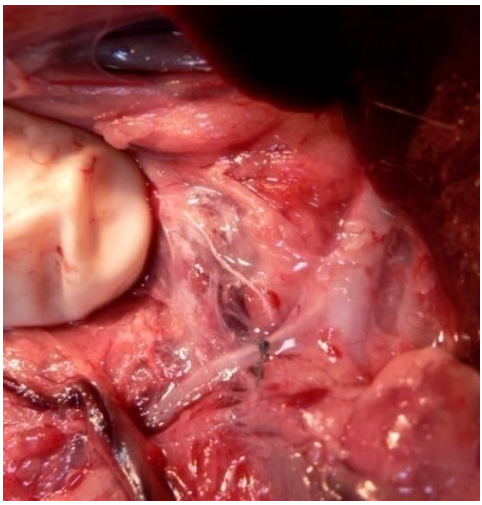


Fig. 7. Ligaturing of CMA.

After flapping the intestine on the left side, near to the CMS, the CMV was identified. After its isolation, CMV was ligatured with non-resorbable 4.0 (fig. 8).

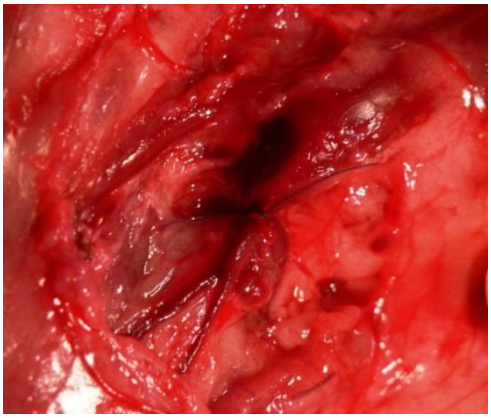


Fig. 8. The CMV ligature close to its entry into the portal vein.

During the intervention the intestine was covered by sterile compresses soaked in physiologic salt solution.

After ligaturing, the abdominal cavity was washed with physiological salt solution which was warmed to 37°C. The laparoraphy was performed in anatomical layers with non-resorbable 2.0.

Post operatory, the rabbits were put into recently cleaned and disinfected cages.

RESULTS

Intraoperatory and post operatory observations. The clinical state of the animals was permanently supervised intraoperatory. Short periods of tachypnea-tachycardia (<1min) were observed, not observed in group B – control during the anesthesia.

Approximately 5 minutes after ligaturing the vessels, the intestinal loops presented hyperperistalsis, afterwards they turned pale.

After approximately 6-7 hours the animal presents respiratory perturbations and death occurs.

Necropsy and histopathological evaluation. The necropsies were effectuated in the scheduled interval of 1-3 hours after death occurred.

While the external examination, the corpses showed the signs of real death. In three of the five cases, the outflow of a serohemorrahic liquid through the suture's fibers was observed.

While opening the abdominal cavity, in three of the five cases from group A, approximate 3 ml serohemorrahic ascites were stated. The cytological examination revealed rarely flaked mesothelial cells, rarely erythrocytes and leukocytes.

Signs of peritonitis were not observed.

The intestine is relaxed, with parietal edema, and thrombosis in the mesenteric vascular arcades (fig. 9).

On the surface, the duodenum presents the color pink-cyanotic and an accentuation of the parietal vascular drawing (fig. 9), the microscope shows the necrosis of the duodenal villosity and stasis is stated (fig. 10).

Concluding that the duodenum presents incipient ischemic modifications.

On the surface, the proximal jejunum presents the color Bordeaux and thrombosis in the adjacent mesenteric vessels. The distal jejunum and the ileum presents strongly cyanosed loops with the color purple-black (fig. 11); the mucosa is intensely red colored. Thrombosis in the adjacent mesenteric vessels is remarked. Microscopically, massive necrosis of the intestinal mucosa and submucosa is stated, necrotic-hemorrhagic content in the lumen. Thrombosis in the mesentery (fig. 12).

On the level of the appendix, no ischemic modifications are stated.

The proximal cecum presents a purple-black color whereas the distal cecum and the proximal colon presents alternations of purple and light grey areas (fig. 9). Microscopically, this segment of the digestive tube presents partial necrosis of the mucosa, areas where the epithelium of the surface is ulcerated, stasis and thrombosis in mucosa, alternating with areas of normal aspects (fig. 13, fig. 14). The distal colon presents incipient ischemic modifications or has a normal aspect.

The mesentery presents a hemorrhagic infiltration.

The liver presents stasis and multiple Zahn infarcts (fig. 15). The spleen presents stasis and thrombosis in the splenic vein (fig. 16). The stomach presents stasis and, in two of the five cases, hemorrhagic shock ulcerations (fig. 17).

The pancreas presents stasis and necrotic areas (fig. 18). The paravertebral ganglia present stasis (fig. 19).

The microscopically control of the ligature reveals the complete halting of the bloody flux in the cranial mesenteric package (fig. 20).

Fig. 9. Small and large intestine with infarction

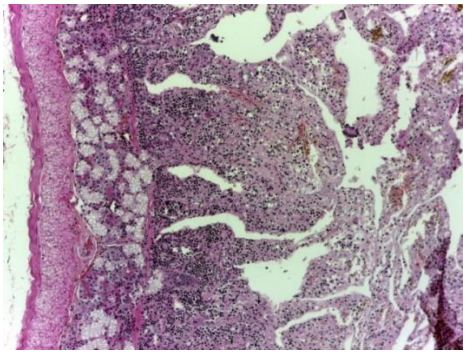
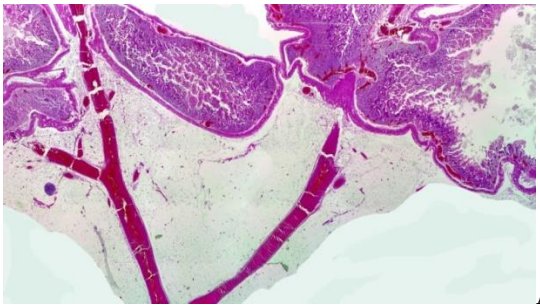


Fig. 10. Duodenum, HE, 40x: necrosis of villosity, stasis.

Fig. 11. Ileal loop with infarction and thrombosis of mesenteric arcade.


A

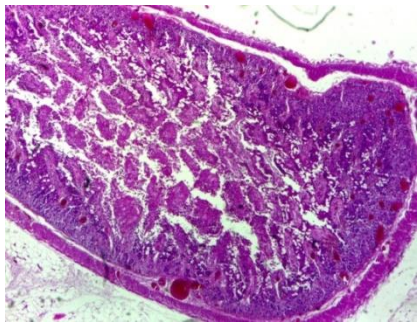

B

Fig. 12. A - Jejunum, HE, 10x: necrosis of mucosa, thrombosis in mesenter; B - Jejunum, HE, 40x: necrosis of mucosa.

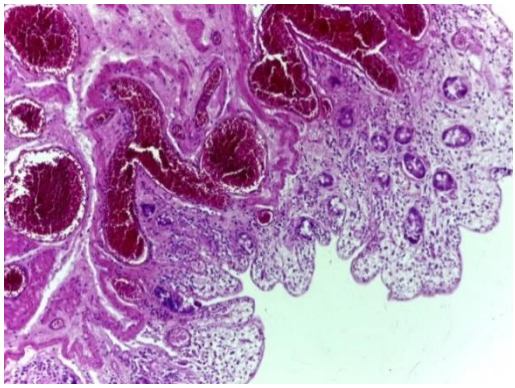


Fig. 13. Cecum, HE, 100X: severe stasis in mucosa and detail with epithelium of surface absence

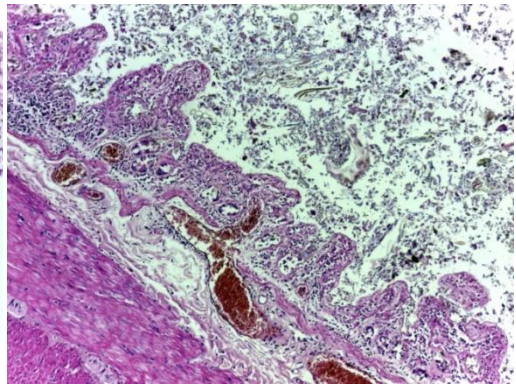


Fig. 14. Colon, HE, 100x: partial necrosis of mucosa.

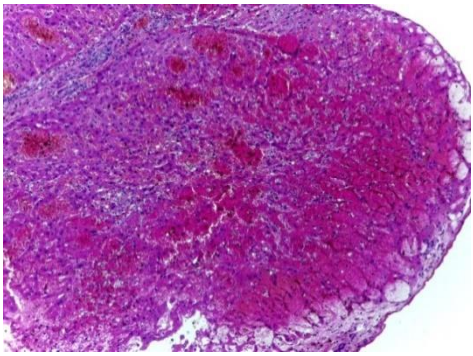


Fig. 15. Liver, HE, 40x: Zahn infarct.

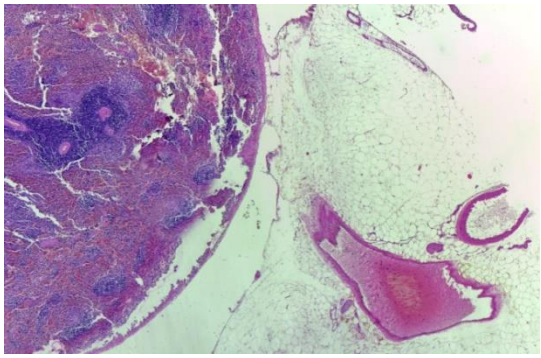


Fig. 16. Spleen, HE, 40x: stasis; thrombosis in splenic vein.

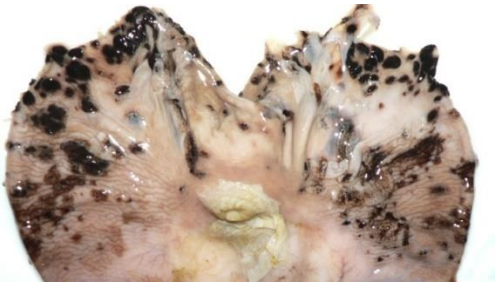


Fig. 17. Stomach: hemorrhagic shock ulcerations.

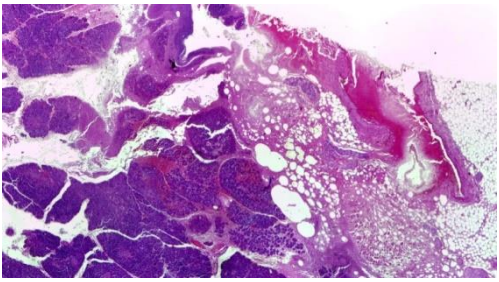


Fig. 18. HE, 40x: thrombosis of CMV, necrosis of pancreas and adipose tissue.

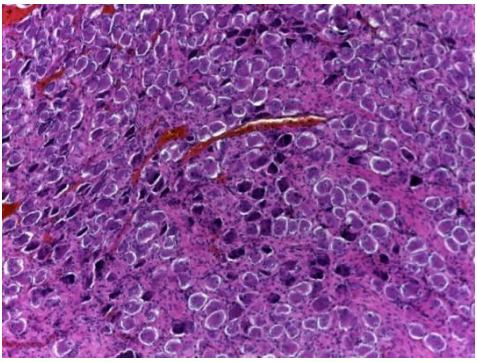
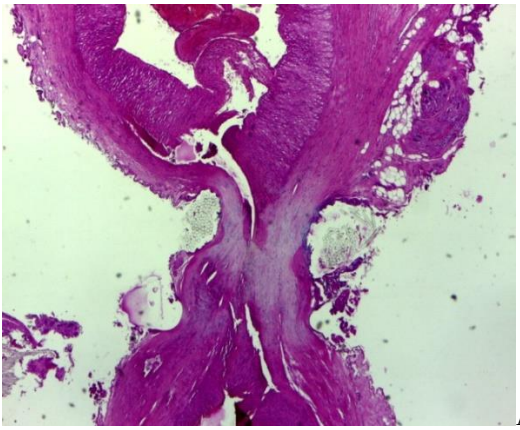
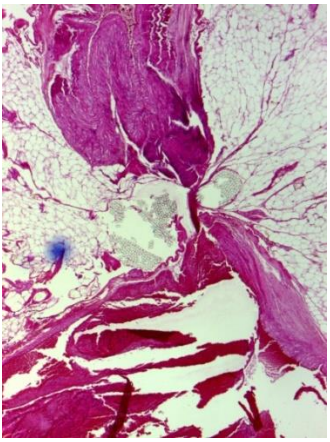


Fig. 19. Sympathetic ganglions, HE, 200x: stasis.



A



B

Fig. 20. A - CMA, HE, ob. 40X: detail of vascular ligature;
B - CMV, HE, 40x: detail of vascular ligature.

DISCUSSION

The splanchnic circulation receives approximately 25% of the resting and 35% of the postprandial cardiac output (18-19). 70% of the mesenteric blood flow is directed to the mucosa and submucosa of the

bowel, with the remainder supplying the muscularis and serosal layers. Multiple major elements interact to provide the intestinal tract with an appropriate share of the blood supply, including the intrinsic (metabolic and myogenic) and the extrinsic (neural and humoral) regulatory systems (19-20).

Pressure-flow autoregulation, reactive hyperemia, and hypoxic vasodilation are considered intrinsic controls. In the metabolic theory, oxygen delivery rather than blood flow causes adaptive changes in splanchnic circulation. An imbalance between tissue oxygen supply and demand will raise the concentration of local metabolites (hydrogen, potassium, carbon dioxide, and adenosine), resulting in vasodilation and hyperemia. The myogenic theory suggests that arteriolar tension receptors act to regulate vascular resistance in proportion to transmural pressure. An acute decrease in perfusion pressure is compensated for by a reduction in arteriolar wall tension, thereby maintaining splanchnic blood flow (21).

The vascular network of the submucosa prevents the installation of ischemia when the irrigation's debit of the extramural vessels sinks significantly (22).

In the first place, in physiological conditions, only 20% of the mesenteries capillaries are opened; when one of the mesenteric arteries is obstructed, a reduction of pressure takes place in the distal arterial bed, which triggers the opening of the capillaries of submucosa. After a couple of hours, this compensatory mechanism is overstrained and the distal arteries enter vasoconstriction. Initially, the vasoconstriction is reversible, but after a period of time it can become irreversible, even if the trigger of the ischemia was identified and corrected (23).

In the second place, when the O_2 contribution sinks, the extraction capacity grows (once the bloody flux sinks under 30ml/min/100g tissue or the systemic blood pressure drops under 50mmHg, the O_2 extraction becomes dependent on the flux (24)).

In this way, the intestine could endure a sinking of 75% of the irrigation debit up to 12 hours without causing cellular injury (25).

Through the sudden and complete installation of the vascular obstruction during the CMA ligature, the compensatory mechanisms are rapidly overstrained and it cannot be talked about the capacity of collateral circulation. The obtained results of our team are similar to those obtained by experimentally reproducing the mixed infarction in rabbits (the bowel became pale 5 to 10 minutes after vessel ligation, 3 hours after the ligation, the necrosis of the small bowel was evident, 6 hours after the ligation, the animals had extensive patchy necrosis and dilation throughout the small bowel with free hemorrhagic intraperitoneal fluid, 9 hours after the ligation, the small bowel was dilated and black, most of the animals died 9 hours after vessels ligation (26)) or in dogs (30 minutes after ligaturing, the loops are lightly cyanotic, without peristalsis; after 1h the loops are relaxed, Bordeaux colored, with parietal edema, the mesenteric vascular arcades are turgescent, ascites is present; after 1,5 hours the loops are strongly cyanotic, ascites in great amount, after 2,5 hours the loops are black, in some places perforated, the mesentery strongly infiltrated, all the vascular arcades are black, rigid, after 3,5 hours the animal dies (17)).

The fact that the proximal duodenum

and jejunum presents incipient, respectively middle ischemic modifications are due to the anastomotic paths given by the celiac trunk through the right pancreatico-duodenal arteries, which form an anastomosis with the left pancreatico-duodenal, the first collateral of the CMA. The distal colon presents minor or absent ischemic modifications due to the vascularization which gets through the anastomosis of the caudal mesenteric artery with the Riolan arcade and through the collaterals from the caudal mesenteric artery.

The lack of an adequate oxygen supply leads to tissue ischemia, which, in turn, causes cellular dysfunction and cell death (27). Intestinal injury causes significant damage to the intestine, particularly to the mucosa and neurons. The epithelial surface of the mucosa can be lost, which allows bacteria and toxins to enter the gut wall, compromises the ordered absorption of nutrients, and disturbs regulated water and electrolyte transport (28-30). The basic electrical rhythm of the intestine is known to decrease during mesenteric ischemia (31-32).

The most abundant constituents of epithelial cellular membranes are phospholipids. After ischemia, the oxygen radicals, which are the products of this oxidative stress, destroys lipid via lipid peroxidation (33). Consequently, intestinal permeability increases, lysosomal hydrolases are released and proteolysis is enhanced, and intestinal barrier fails through destruction of endothelial and epithelial cells. Finally, indigenous enteric bacteria translocates to extra intestinal sites, induces sepsis and multiple organ system failure (34-37).

On the other hand, 20-25% of the cardiac debit stays sequestrated after ligaturing the mesenteric vessels, thus the animal suffers a volume depletion.

The fact that multiple abdominal organs present stasis, attests the cardio-circulatory insufficiency installed post operatory.

CONCLUSIONS

By ligaturing of the CMA and of the CMV, we obtained an experimental model of the mixed intestinal infarction in rabbits.

Necropsy and histopathological evaluation showed: the duodenum presents incipient ischemic modifications, the distal jejunum and the ileum presents massive necrosis of the mucosa and submucosa, the cecum and the proximal colon presents partial necrosis of the mucosa, areas where the epithelium of the surface is ulcerated, stasis and thrombosis in mucosa, alternating with areas of normal aspects; the distal colon presents incipient ischemic modifications or has a normal aspect.

On the level of the appendix, no ischemic modifications are stated.

The mesentery presents hemorrhagic infiltration.

Multiple abdominal organs present stasis (liver, spleen, sympathetic ganglions) or shock signs (stomach, pancreas).

The rabbits died by the cardio-circulatory insufficiency and multiple organ system failure.

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NEWS

A NOVEL ONCE-WEEKLY DPP-4 INHIBITOR IN THE CARE OF TYPE 2 DIABETES

Omarigliptin is a novel onceweekly (q.w.) dipeptidyl peptidase IV (DPP-4) inhibitor for the treatment of patients with type 2 diabetes. This study aimed to evaluate the long-term safety of optimal dose. In a multicenter, double-blind, 12-week, dose-range finding study, 685 oral antihyperglycemic agent-native or washed-out subjects with type 2 diabetes were randomized to one of five once-weekly doses of omarigliptin (0.25 mg, 1 mg, 3 mg, 10 mg, or 25mg) or placebo. Analysis included all patients who received at least one dose of the study medication. At week 12, the omarigliptin 25-mg dose provided the greatest glycemic efficacy. Subjects who completed the base study were eligible to enter a 66-week extension study. Omarigliptin 25 mg q.w., compared with placebo, provided significant glucose lowering and was generally well tolerated throughout the base and for up to 78 weeks in patients with type 2 diabetes (Sheu WHH, Gantz I, Chen M et al. Safety and efficacy of Omarigliptin (MK-3102), a novel once-weekly DPP-4 inhibitor for the treatment of patients with type 2 diabetes. *Diabetes Care.* 2016;38:2106–2114 | DOI: 10.2337/dc15-0109).

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EARLY COMPLICATIONS FOLLOWING ANATOMIC LUNG RESECTIONS - EXPERIENCE OF A THORACIC SURGICAL ONCOLOGY UNIT

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EARLY COMPLICATIONS FOLLOWING ANATOMIC LUNG RESECTIONS- EXPERIENCE OF A THORACIC SURGICAL ONCOLOGY UNIT (Abstract). **Aim:** To analyze the medical and socioeconomic impact of the complications of anatomic lung resections performed at the Thoracic Surgery Unit of the Iasi Regional Cancer Institute. **Methods:** This retrospective study included patients who underwent anatomic lung resections between January 2013 and August 2015**. Results**: Over this interval a total of 172 major lung resections were performed: 31 (18.02%) pneumonectomies, 5 (2.91%) bilobectomies, and 136 (79.06%) lobectomies. Complications occurred in 36 patients, including bronchial stump fistula in 7 patients (4.06%), bronchopneumonia (9/5.23%), pleural empyema without bronchoscopically documented bronchial stump fistula (1/0.58%), chylothorax (1/0.58%), postoperative arrhythmia (3/1.74%), early postoperative stroke (1/0.58%), prolonged air leak (requiring hospital stay longer than 14 days) (11/6.38%), and postoperative hemothorax requiring reintervention (3/1.74%). Secondary to complications, a number of 3 (1.74%) patients died early postoperatively. **Conclusions:** The incidence of immediate and early postoperative complications is comparable to those reported by other authors. **Keywords:** ANATOMIC LUNG RESECTIONS, EARLY COMPLICATIONS, LOBECTOMY, BILOBECTOMY, PNEUMONECTOMY.

Lung cancer surgery is associated, according to the literature (1, 2), with a wide range of complications that can occur in the immediate postoperative period. The most frequent are supraventricular arrhythmias, prolonged air leaks, atelectasis of the remaining parenchyma and hemorrhages, while pneumonia, empyema, bronchopleural fistulas, chylothorax, wound complications, lobar torsion and nerve lesions are less common (1, 2). Most of these complications can be treated conservatively, but have an important impact on quality of life and patient survival by prolonging the time interval from surgery to initiation of adjuvant therapy, especially considering the favorable outcomes of neoplastic bronchopulmonary lesions with adjuvant therapy even in elderly patients with good performance status (3).

The paper aims to analyze the medical and socioeconomic impact of immediate complications occurring in patients who have undergone major surgery in the Thoracic Surgery Unit of the IInd Surgical Clinic of the Iasi Regional Cancer Institute.

MATERIAL AND METHODS

This retrospective study was conducted on a group of 172 patients who underwent anatomic lung resections and aimed at conducting an audit of the early complications following this type of resection. The data for this study were collected from the medical records of patients who underwent anatomic lung resections between January 2013 and August 2015 in the Surgery Clinic II of the Iasi Regional Cancer Institute. In this study we used the term anatomic lung resections to define surgeries such as pneumonectomies, bilobectomies and lobectomies.

The data collected from medical records included: gender, area of residence, main and secondary diagnoses, type of surgery performed and histopathology report, reported complication, duration of the surgical procedure, time between surgery and complications and length of hospital stay.

RESULTS

Clinical data. Over the 32 months study interval 172 major lung resections have been performed, of which 31 (18.02%) pneumonectomies, 5 (2.91%) bilobectomies and 136 (79.06%) lobectomies. 36 (20.93%) of these patients experiencing complications: 28 following lobectomies, 7 following pneumonectomies, and 1 following bilobectomy.

The comparative analysis by gender, age, duration of surgery and length of hospital stay between all patients who underwent major lung resections and those who developed complications showed significant differences in incidence (tab. I)

TABLE I

Distribution of patients by sex, age, mean duration of surgery and mean length of hospital stay

Characteristics of the group	Group of patients who underwent anatomic lung resections	Group of patients who experienced complications
Male/female	131/41	32/4
Age (years)	62.52 ± 9.29	62.7 ± 9.46
Length of hospital stay (days)	14.54 ± 8.16	20.27 ± 13.39
Duration of surgery (minutes)	154.1 ± 43.94	163.46 ± 42.95

Histological features. In the subgroup of patients who experienced complications 52.7% underwent surgery for bronchopulmonary adenocarcinoma. The distribution of patients experiencing complications by histological type of the lesion that required surgery showed: 19 (52.7%) adenocarcinomas, 10 (27.77%) squamous cell carcinomas, 2 (5.55%) small cell carcinomas and one case each (2.77%) of neuroendocrine carcinoma, pleomorphic sarcomatoid carcinoma, cystic adenomatoid malformation, bronchogenic cyst and aspergilloma.

Analysis of complications. Analysis of medical records allowed the classification of complications into pleuropulmonary and extrapulmonary (tab. II). In the analyzed study group 3 (1.74%) deaths secondary to complications of major lung resections were recorded. One of these patients required reoperation for hemothorax 3 hours after left pneumonectomy and death occurred as a result of nosocomial pneumonia in the remaining lung. The second death was caused by cardiorespiratory arrest secondary to pneumonia in the remaining parenchyma following right pneumonectomy. The third death was due to respiratory failure unresponsive to therapy following a right superior lobectomy.

TABLE II
Distribution of patients by type of complications

General complications			Pleuropulmonary complications		
No.	*Percentage (%)*	*Complication type*	*No.*	*Percentage (%)*	*Complication type*
3	1.74	arrhythmia	11	6.38	prolonged air leaks
1	0.58	stroke	9	5.23	pneumonia
			7	4.06	bronchial stump fistula
			3	1.74	hemothorax
			1	0.58	empyema without documented bronchial fistula
			1	0.58	chylothorax

DISCUSSION

Analyzing the obtained results, the general complications in the study group included 3 (1.74%) cases with postoperative arrhythmias and 1 (0.58%) patient with postoperative stroke. Comparatively, Mitsudomi et al. (4) reported a rate of postoperative arrhythmias of up to 35%. In our study there were no cases of pulmonary embolism, redoubtable complication of lung resection surgery as indicated in their works by Dent et al. (5) and Ziomek et al. (6).

As to the postoperative pleuropulmonary complications, other authors mention such complications requiring reintervention as hemothorax, bronchial stump fistulas and pleural empyema (7). According to these authors hemothorax is present in 52-75% of cases (8, 9). Currently it is estimated that the rate of hemorrhagic complications of lung resection surgery is of about 3% due to technical progress in the design of surgical instruments and surgical techniques in a multidisciplinary approach context (7). In our study postoperative hemothorax was present in 1.74% of cases.

Bronchial stump fistulas occurred in 7 (4.06%) of our study patients. Three (1.74%) of these 7 cases required reoperation consisting in completion pneumonectomy, repair of the bronchial stump followed by closure of the remaining stump and plasty of the suture using serratus muscle flap. In 4 (2.32%) patients the fistula was closed bronchoscopically with biological glue. Other authors (7, 8, 9) reported the presence of this complications in 17.8% -25.5% of the cases. Pleural empyema without documented bronchopleural fistula was present in 0.58% of the patients in our study, compared with 2-12% reported by Vallieres et al. (10). Lobar torsion was found by Wong et al. in 0.5% of cases (11). In our study no patient developed this complication, the explanation being the fixation of the middle lobe to the inferior lobe after all right upper lobectomies.

Prolonged air leaks, reported by other authors as a frequent problem with an incidence of up to 50% of patients (10) were found in 6.38% of our study patients. Along with bronchial stump fistulas they are associated with a significant increase in length of hospital stay with important economic implications, explaining the particular interest of other studies describing the development of predictive scores for persistent air leaks, such as IPAL (Index of Prolonged Air Leak) (10). A particular case in our study and rarely reported by other authors (12, 13, 14) was the occurrence of chylothorax following the surgical treat-

ment of a Stocker type II cystic adenomatoid malformation.

The mean length of hospital stay in the group of patients with complications was about 5 days longer compared to the entire cohort of patients, demonstrating the negative impact of delayed initiation of adjuvant therapy on patient survival. The difference of about 10 minutes between the average duration of surgery for the entire study group and for the patients with complications suggests a possible relationship between case difficulty and complications.

CONCLUSIONS

Our findings on immediate and early complications after major lung resections are comparable to those in the literature. The impact of these complications on the quality of life and survival of patients due to the delayed initiation of adjuvant therapy remains a topic of major interest, and the periodic follow-up data of the patients in the study will be published later.

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CONCENTRATIONS OF VITREAL CYTOKINES IN RHEGMATOGENOUS RETINAL DETACHMENT

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CONCENTRATIONS OF VITREAL CYTOKINES IN RHEGMATOGENOUS RETINAL DETACHMENT (Abstract): Proliferative vitreoretinopathy (PVR) is one of the most frequent causes of failure of rhegmatogenous retinal detachment (RRD) surgery. **Aim:** To measure the vitreous levels of granulocyte colony stimulating factor (G-CSF) and monocyte chemoattractant protein 1 (MCP-1) in eyes with RRD and in a control group. **Material and methods:** A prospective study of 40 patients operated for RRD (study group) and 20 patients with epiretinal membrane or macular holes (selected as control group since they needed vitrectomy but had attached retinas). Vitreous samples were collected during vitrectomy and were assessed for the presence of cytokines using a fluorescent bead-based multiplex assay. **Results:** The concentration of G-CSF (8.59 pg/ml) and MCP-1 (1615.2 pg/ml) were significantly increased in the study group, when compared to the control group (0 and 469.13 pg/ml, respectively). MCP-1 was also significantly increased in the subgroup of patients with PVR compared to the patients with uncomplicated RRD. **Conclusions:** The levels of these biomarkers support the idea that proliferative vitreoretinopathy has an inflammatory component. **Keywords:** RHEGMATOGENOUS RETINAL DETACHMENT, PROLIFERATIVE VITREORETINOPATHY, VITREOUS CYTOKINES.

INTRODUCTION

Proliferative vitreoretinopathy (PVR) is a complication of rhegmatogenous retinal detachment (RRD) characterized by fibrocellular, contractile, epiretinal or subretinal membranes that prevent retinal reattachment. It is one of the most frequent causes of RRD surgery failure (1). Retinal break and subsequent retinal detachment are believed to allow macrophages, retinal pigment epithelial cells, glial cells and fibroblasts to migrate into the vitreous, where they proliferate, form extracellular matrix and assemble into membranes (2).

Several models of the pathogenesis of this disease have been described, some models focusing on the role of inflammatory cells, others on the role of growth factors and cytokines (2). Numerous authors have identified elevated levels of a variety of growth factors, cytokines and chemokines in the vitreous of eyes with PVR (3).

Cytokines serve as signals between cells and are involved in cell proliferation and

migration, inflammation, angiogenesis and fibrosis. Chemokines are mediators that may promote recruitment of leukocytes to the sites of inflammation, enhance immune responses, and also participate in angiogenesis and fibrosis (4).

Multiplex bead-based flow cytometric assays have the major advantage of assessing the presence of numerous different biomarkers in a small quantity of biological fluid. Several authors believe that multiplex immunoassays show similar performance to ELISA in terms of accuracy and reproducibility and have superior sensitivity. (5).

In the present study we have measured the vitreous concentrations of granulocyte colony stimulating factor (G-CSF) and monocyte chemoattractant protein-1 (MCP-1) in eyes with RRD and in a control group, using a multiplex fluorescent bead-based flow cytometric assay.

MATERIAL AND METHODS

The design of this study was prospective, controlled and observational. The study group consisted of 40 eyes of 40 patients with RRD who underwent 23-G pars plana vitrectomy in the Ophthalmology Department of the Iasi University Hospital, Romania, between March and September 2015. All patients were aged over 18 years and had a primary RRD. There were 30 patients with uncomplicated RD and 10 patients with RD and PVR (4 stage B, 5 stage C and 1 stage D, according to the 1983 Retina Society classification) (6). The exclusion criteria were: history of intraocular surgery in the past 6 months, history of intraocular inflammation, diabetic retinopathy or retinal vascular occlusions, general or intraocular steroids or anti-vascular endothelial growth factor in the past 3 months, impossibility to visualize the causative retinal break preoperatively.

The control group consisted of 20 patients with macular hole or epiretinal membrane who also underwent pars plana vitrectomy. We have selected these conditions because the retinas are attached but they also require a surgical approach, thus providing an opportunity for the sampling of vitreous.

All patients and controls underwent an ophthalmological examination by the recruiting surgeon, including measurement of visual acuity and intraocular pressure, biomicroscopy of the anterior segment of the eye, biomicroscopy or indirect ophthalmoscopy of the posterior segment.

The study was conducted according to the principles of the Declaration of Helsinki and was approved by the Ethics Committee of the Iasi University of Medicine and Pharmacy. Informed consent was obtained from each patient prior to study initiation.

Surgeries were performed by a single surgeon (C.D.), using local anesthesia, 3-port transconjunctival vitrectomy system (Alcon Constellation, Alcon Laboratories, Alcon, Fort Worth, Texas, USA) and a wide-angle viewing system (EIBOS, Moeller-Wedel GmbH & Co. KG, Wedel, Germany).

The vitreous samples were obtained undiluted by manual suction in a sterile syringe connected to the vitreous cutter, before opening the infusion line. If suction became impossible because of blocking with vitreous gel, the cutter function was activated and the suction continued. The content of the syringe was immediately transferred into labelled 1.5 ml Eppendorf Protein LoBind tubes and frozen at -20° C. The tubes were then stored at -150° C. In all cases, the infusion line was opened

immediately after sample collection and vitrectomy was carried on uneventfully.

All vitreous samples were thawed and centrifuged in September 2015. The concentrations of biomarkers (cytokines, chemokines and growth factors) were measured using a multiplex microbead-based immunoassay designed for cytometry. (Cytometric Bead Array®, BD Biosciences, New Jersey, USA). The capture beads corresponding to each analyte to be measured were mixed together in wash buffer, centrifuged, the supernatant was discarded and the beads were resuspended in diluent to a final concentration of 50 µl/test. 50 µl of the supernatant from the centrifuged samples were incubated in labelled tubes with 50 µl mixed capture beads for 1 hour at room temperature. 50 µl of phycoerythrin (PE) detection reagent were added in each tube and then incubated for 2 hours. After washing away the unbound PE and resuspension in wash buffer, the samples were acquired on a FACSAria™ flow cytometer platform. Each bead population has distinct fluorescence intensity, permitting simultaneous measurements of multiple protein concentrations. The median fluorescence intensity of each biomarker was correlated with the dilution of standard biomarkers, the resulting correlation enabling us to quantify the concentration of biomarkers in the study samples. The sensitivity range of each assay was 0-2500 pg/ml.

The statistical analysis was performed using MedCalc (MedCalc Software bvba, Ostend, Belgium). The significance of the difference in concentration of biomarkers between groups was assessed by the Wilcoxon test. A p value of less than 0.05 was considered significant.

RESULTS

The mean granulocyte colony stimulating factor (G-CSF) concentration in the study group was 8.59 ± 28.9 pg/ml (between 0-161.9 pg/ml), whereas all thye eyes in the control group had undetectable levels of G-CSF (0 pg/ml). Since the found values did not have normal distribution, a Wilcoxon test was used to study the difference between groups. The difference was statistically significant, p=0.02.

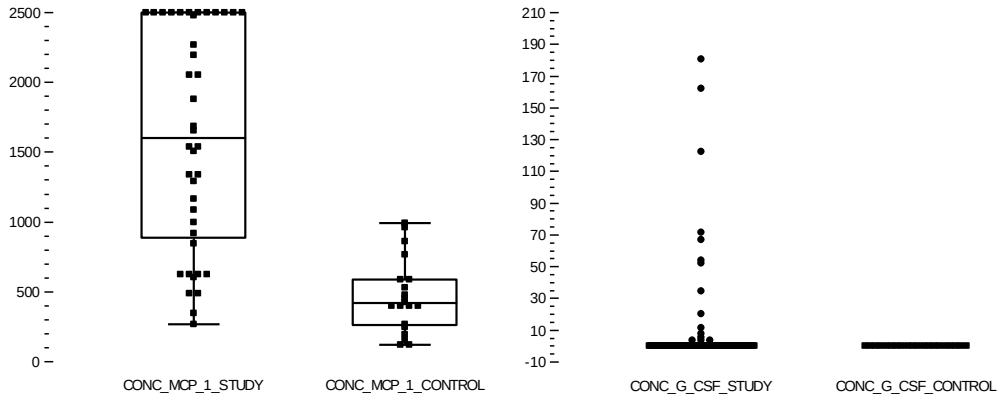

Fig 1. Concentrations of cytokines in the study and control group (pg/ml)

The mean monocyte chemoattractant protein-1 (MCP-1) concentrations in the study and control group were 1615.2 ± 790.8 pg/ml (range 270.1- 2500) and 469.13 ± 263.08 pg/ml (range 118.48-990.49), respectively. The Wilcoxon test showed a highly significant difference, p=0.0001 (fig. 1).

When comparing the concentrations of MCP-1 and G-CSF between the PVR and non-PVR group we found a significant increase of MCP-1 in the PVR group (but not of G-CSF). (tab. I)

TABLE I

Concentration of G-CSG and MCP-1 in retinal detachment – comparison between eyes with or without proliferative vitreoretinopathy.

	Mean concentration, RRD without PVR (pg/ml)	Mean concentration, RRD with PVR (pg/ml)	Wilcoxon test p value
G-CSF	4.09 ± 14.31 (0-71)	22.12 ± 51.92 (0-161.9)	0.6
MCP-1	1451.22 ± 795.15 (270.16-2500)	2107.46 ± 560.4 (597.3-2500)	0.029

DISCUSSION

The course of PVR is the result of the balance (or disruption of balance) between the destructive and protective mechanisms triggered by the occurrence of a retinal break.

The clinical features of PVR corresponds to the histopathological findings, represented by fibrocellular (and, in evolution, contractile) membranes on the anterior or posterior retinal surface (3).

The primordial element seems to be partial de-differentiation, migration and proliferation of retinal pigment epithelial (RPE) cells, creating areas of hyperplasia, first at the limit between detached and attached retina and at the margins of retinal breaks. This process may start 3 days after retinal detachment begins. The next step is the activation of glial cells, with proliferation of astrocytes, Muller cells, microglia and capillary endothelial cells (1).

The role of growth factors, cytokines and chemokines is highlighted by an ever-growing body of literature, the sampling of vitreous and subretinal fluid being relatively easy during modern vitreoretinal surgery. The measuring of different factors is also made easier by tests that use minute quantities of biological material.

In our study group of eyes with rhegmatogenous retinal detachments we found a significant increase in the concentrations of G-CSF and MCP-1 (when compared with a control group of eyes with macular holes or idiopathic epiretinal membranes).

Our findings support the data published by Kunikata et al. (8) who have also found that MCP-1/CCL2 and G-CSF were significantly elevated in eyes with RRD, while TNF was not. They have reported that intravitreal triamcinolone is able to suppress elevated levels of intraocular MCP-1 in eyes with RRD. MCP-MCP-1 (also known as C-C motif ligand 2) is a chemokine that recruits monocytes, memory T cells and dendritic cells to sites of tissue injury (4). In animal models of retinal detachment, it is expressed in the Muller glia and macrophage/microglia. It may contribute to monocyte recruitment in areas of RRD, and appears to be implicated in the pathogenesis of photoreceptor degeneration and apoptosis following retinal detachment (9).

Yoshimura et al. (4) have also reported that MCP-1 was significantly elevated in

eyes with diabetic macular edema, proliferative diabetic retinopathy, vein occlusion or rhegmatogenous retinal detachment. They have found that MCP-1, IL6 as well as IL8 are increased.

G-CSF is a cytokine that stimulates the survival, proliferation and function of neutrophil precursors and mature neutrophils. In an experimental setting, vitreous from eyes with proliferative vitreoretinopathy stimulated G-CSF and IL-17A production by ARPE-19 cells (10). G-CSF has been shown to have a neuroprotective effect after retinal ganglion cell axotomy (11)

In a retrospective case-control study, Ricker et al. (12) have compared the concentration of 50 biomarkers in the vitreous of patients with uncomplicated RRD and patients who developed PVR within the first 3 months after surgery. They have found a significant increase of IL2, MCP-1/CCl2 and other 15 biomarkers in the vitreous of patients that developed PVR. They found no differences for the values of IL4, IL10, IL12p70, VEGF, bFGF, TNF and other 17 biomarkers (however, they did not provide values for the biomarkers that did not differ between the study and control group). Banerjee et al. (13) promoted the idea that the detectable levels of interleukins and chemokines support the role of an inflammatory response in PVR.

CONCLUSIONS

In our study group of patients with rhegmatogenous retinal detachment, the concentrations of G-CSF and MCP-1/ CCL2 are significantly increased when compared with a control group. A significant increase of MCP-1 in eyes with proliferative vitreoretinopathy compared to eyes with uncomplicated retinal detachments was also found, supporting the hypothesis of the implication of this soluble factor in the pathogeny of PVR.

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NEWS

DREAM OR REALITY? VIDEO HEAD IMPULSE TEST READY TO BE USED

Looking in the past, over the history of vestibulogy, the aim was always to measure the function of all the vestibular receptors. Vestibular evoked miogenic potentials (cervical and ocular) show the activity of the saccule and the utricle. Now, using video Head Impulse Test is possible quantify the semicircular canals function by measuring the eye rotation response to an abrupt head rotation in the plane of the canal. The main measure of canal adequacy is the ratio of the eye movement response to the head movement stimulus i.e. the gain of the vestibulo-ocular reflex (VOR). However there is a need for normative data about how VOR gain is affected by age and also by head velocity, to allow the response of any particular patient to be compared to response of healthy subjects in their age range. This study held on helthy subjects divided in decade age groups. VOR gain decreased at high head velocities, but was largely unaffected by age (McGarvie LA, MacDougall HG, Halmagyi GM, Burgess AM, Weber KP, Curthoys IS. The Video Head Impulse Test (vHIT) of Semicircular Canal Function – Age-Dependent Normative Values of VOR Gain in Healthy Subjects. *Frontiers in Neurology* 07/2015; 6:154.)

Maria Cristina Hera

VACCINATION – COLLECTIVE RESPONSIBILITY OR VIOLATION OF RIGHTS?

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VACCINATION – COLLECTIVE RESPONSIBILITY OR VIOLATION OF RIGHTS? (Abstract) Vaccination is considered to be the most effective and the cheapest medical intervention through which individual and collective immunisation is achieved. Statistics show that, at present, immunisation annually saves 400 million lives and protects approximately 750,000 children against disabilities of varying degrees. Approximately 80% of worldwide children are vaccinated against diphtheria, tetanus, pertussis, polio, measles, etc.; these diseases used to be considered incurable in the past. Vaccines help the body to produce antibodies; they help the immune system to detect germs and inactivate their cells. The immunological protection is installed after a variable period of time following the inoculation and is long lasting. Immunisations can be achieved in several ways: through national immunisation campaigns with general recommendation – they may be compulsory, optional or prophylactic (for the diseases for which a vaccine is available); vaccinations not included in the compulsory immunisation programmes; they may also be targeted to the contagious infectious outbreaks or to groups of population in certain situations. There is no guarantee that a vaccine will provide 100% protection. However, it will significantly reduce the risk of getting an infection. Vaccines have side effects which can be divided into reactions triggered by the vaccine or reactions exacerbated by it, without a causal relationship to the vaccine. **Keywords**: VACCINE, IMMUNISATION, BCG, VPI.

According to a survey conducted in March this year, one in ten Romanians has no confidence in vaccinations, and one in three is uncertain about immunisation. Despite the statistics, vaccination still remains, according to experts, the most important means of preventing diseases, particularly those with an epidemic evolution (1).

Throughout the world history, there have been laws which imposed immunisations on people and, at the same time, social movements which opposed them. Such examples can be found Britain, where the Vaccination Act of 1853 made the vaccina-

tion against smallpox compulsory for children aged up to 3 months. Later, in 1867, the law was changed and the vaccination against smallpox became compulsory for children under 14 years old. Over time, people have objected to vaccines in multiple ways. Firstly, they were bothered by the mandatory character of immunisations, as they considered it as a human rights violation (1, 2). The Leicester Demonstration March of 1885 defines the anti-vaccination movement of those years. Almost 100,000 people went out in the streets with banners and branded dolls of Edward Jenner. All these movements shortly led to the establishment of the first British commission to study the impact of vaccination on the public health. In 1896, the Commission concluded that the vaccine was effective against smallpox. At the same time, however, the same commission suggested removing sanctions for non-vaccination (2).

In 1905, the US Supreme Court ruled that states were entitled to coerce vaccination to protect its citizens in case of infectious diseases. It was the first decision of the Supreme Court related to the issue of public health. Today, in Belgium, parents who do not vaccinate their children against polio are fined and sentenced to up to five months in prison. In Australia, parents and GPs receive material rewards for vaccination. In the UK, material rewards are given to doctors only when they achieve the immunisation targets set by the government (2).

HISTORY

Vaccination is a means of prophylactic active immunisation against diseases by inoculating a biological formula with antigenic properties, which triggers the occurrence of an immune response in the body subject to vaccination. Immunological protection is installed after a variable period of time after inoculation (weeks, months), depending on the vaccine, and is active for a long period of time (years). Some vaccines are administered in maternity, while others are administered soon after birth. Using special techniques, harmful components that can trigger the disease mechanism are removed from the extremely complex structure of the virus, and only structures useful for stimulating the immune response are used (2).

Edward Jenner, the one who gave the name of "vaccine" to the immunisation process, noticed that patients who developed cowpox (an infectious disease specific to cows that is transmissible to humans and is characterised by cutaneous rash) were protected against smallpox. A cowpox epidemic outbreak on a farm in the spring of 1796 gave him the opportunity to experiment and to broaden his knowledge and research. In May 1796, he made an incision on an 8-year-old boy's arm, where he applied pus collected from a pustule of a milkmaid infected with cowpox. A pustule appeared at the incision site, which healed shortly thereafter. In July 1796, Jenner voluntarily and consciously infected this boy with smallpox, but it turned out that the child was immune to the disease. This made the doctor repeat the procedure on 8 more children, including his son. In all cases, it turned out that patients had become immune to smallpox. Jenner gave the name of "vaccine" to the process, a word derived from the Latin "*vacca*" – cow, and the method was going to expand rapidly in Europe and beyond (3).

IMPORTANCE

Poliomyelitis

Vaccination is considered the most ef-

fective and the cheapest medical intervention, through which the individual and collective immunisation is achieved, which prevents the pathogen from spreading. Statistics show that, at present, immunisation annually saves 400 million lives and protects approximately 750,000 children against disabilities of varying degrees. Also, approximately 80% of worldwide children are vaccinated against diphtheria, tetanus, pertussis, polio, measles, etc.; these diseases used to be considered incurable in the past (2, 3).Vaccines help the body produce antibodies; they help the immune system to detect germs and inactivate their cells. The immunological protection is installed after a variable period of time following the inoculation and is long lasting (years). Immunisations can be achieved in several ways: 1) through national immunisation campaigns with general recommendation – they may be compulsory, optional, prophylactic (for the diseases for which a vaccine is available); 2) optionally, for the prevention of diseases for which a vaccine is available – these vaccines are not included in the compulsory immunisation programmes; 3) they may also be targeted to specific contagious infectious outbreaks or to groups of population in certain situations (2, 4). There is no guarantee that a vaccine will provide 100% protection, but it can significantly reduce the risk of getting an infection. Over time, there have been people who were vaccinated but developed the infection that should have been prevented by the vaccine; yet, it is considered that immunisations used at present do not trigger the disease they are supposed to prevent. Vaccines have side effects which can be divided into reactions triggered by the vaccine, connected to the properties of the vaccine and to the individual response of the body, and which are unlikely to occur in the absence of the vaccine; on the other hand, there are reactions exacerbated by the vaccination, which are present in the absence of the vaccine, but which are enhanced by the vaccination or by manufacturing errors and which have no causal relationship to the vaccine (2, 5).

In Romania, vaccine production began in July 1921 when the government established by decree of King Ferdinand I the Serums and Vaccines Institute, the current Cantacuzino Institute. An important role was played by scientist Ioan Cantacuzino, who prepared the serum anti-dysenteric, the anti-cholera serum, the anti-tetanus serum, the anti-typhoid serum and other serums in the institution he founded and due to which, in our country, many extensive campaigns aiming to eradicate contagious diseases have been conducted. The specialists of the Institute have been in charge of diagnosis, research, public health and education ever since its establishment (2, 4).

The first outbreak was reported in Europe in the early 19th century and in the US in 1843. In the next 100 years there were polio epidemics recorded in the developed countries in the northern hemisphere every summer and autumn. They became increasingly serious and the average age of the people affected increased. Polio reached a very high threshold in the United States in 1952, with over 21,000 cases of paralysis. After the introduction of vaccines, the incidence of polio decreased abruptly. The last case of wild polio virus in the United States was recorded in 1979. In the next 10 years, polio was globally eradicated. A polio eradication programme conducted by the Pan American Health Organization managed to eliminate polio in the Western Hemisphere in 1991 (3, 6).

At a worldwide level, the effort of eradicating polio has taken significant steps since the launch of the Global Polio Eradication Initiative in 1988. At that time, about 350,000 children became paralyzed every year. In 2008, there were only 1,655 reported cases of confirmed polio in the world. In the last 25 years, the number of cases has been reduced by more than 97%; consequently, in 2013 there were 223 cases of polio, which was endemic in only three countries: Afghanistan, Nigeria and Pakistan. In 2013, there was another major epidemic with 174 victims in Somalia (5, 6, 7).

In Romania, there were several epidemic episodes: in 1927, 1955, 1980 – 1982 (221 cases caused by wild polio virus type 2 and 3), as well as between November 11, 1990 – April 25, 1992 (13 cases caused by wild polio virus type 1, which involved unvaccinated children, especially Rrom children, many of them being also infected with the human immunodeficiency virus HIV). WHO official reports show that our country recorded 18 cases in 1992. For the following years, the figures are as follows: 1993 – 10 cases, 1994 – 8 cases and 1995 – 5 cases. 1995 was the last year when our country recorded cases of polio (2, 7, 8).

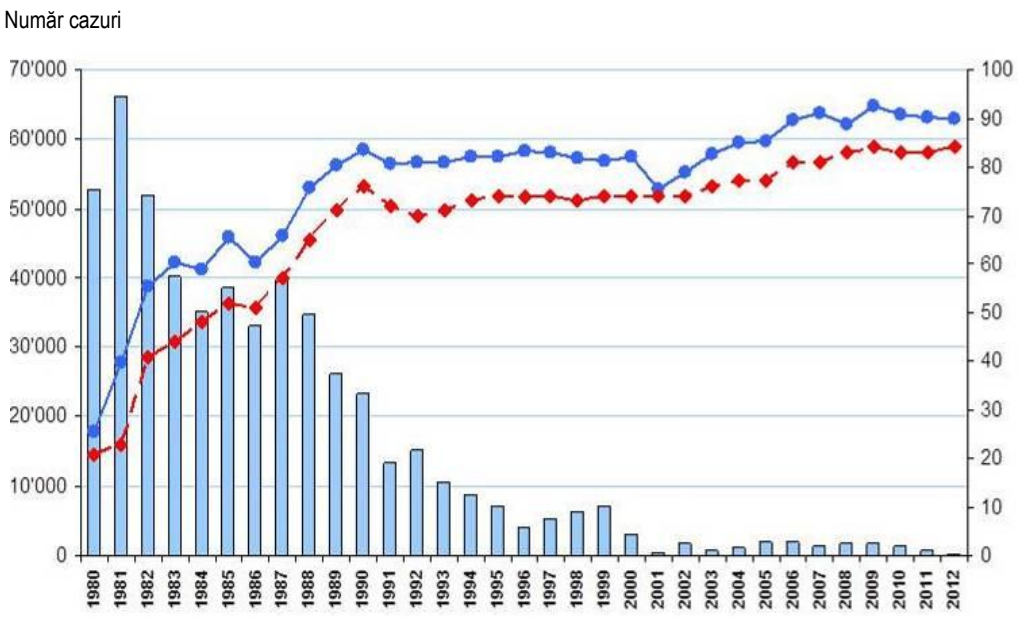

Fig. 1. Number of worldwide annual cases of polio and official immunisation rate / WHO/UNICEF estimates (July, 2013). *Source*: WHO

In Romania, two types of vaccines have been available and used. One of them is the inactivated polio vaccine (IPV) – the first polio vaccine, recorded in 1955 in the US. The second one is the oral polio vaccine (OPV), approved in 1961 as a monovalent vaccine and in 1963 as a trivalent polio vaccine. After the introduction of the OPV vaccination at a global scale, the cases of polio associated to the vaccination (VAPP) started to occur. The World Health Organization concluded, with regard to the safety

of the polio oral vaccine, that there were 1,003 cases of paralysis associated with polio vaccination (VAPP) reported in 13 countries during 1970-1984, out of which 470 cases (47%) were recorded in Romania. After 1995, with the introduction of IPV in the immunisation of children in orphanages and the wide access to oral antibiotics, the incidence of vaccine-associated polio cases has reduced to a case / year on average; in other words, the risk is comparable to that found in other regions where polio immunisation is almost exclusively done with OPV (2, 3, 9).

Inactivated polio vaccine (IPV) is highly effective in producing immunity to polio virus and protection against paralytic forms. Over 90% of vaccinated individuals develop antibodies against all three types of polio virus after two doses and at least 99% are immune after the following three doses. Protection against paralytic polio correlates with the presence of antibodies (6, 7).

IPV produces less local gastrointestinal immunity than OPV, thus the individuals who receive IPV are more easily infected with the wild polio virus than those who are vaccinated with OPV. The duration of immunity following the IPV vaccination is not known with certainty, although the vaccine is likely to provide protection for many years after a full series of vaccinations has been completed (2, 7, 8).

Oral polio vaccine (OPV) is highly effective in producing immunity against the polio virus. A single dose of OPV produces immunity to all three viruses in the vaccine in approximately 50% of vaccinated individuals. Three doses of vaccine develop immunity to all three types of polio virus in 95% of vaccinated subjects. As with other live virus vaccines, the immunity obtained following the vaccination with an oral polio vaccine is expected to last a lifetime. OPV produces excellent intestinal immunity, which helps to prevent infection with a wild virus (6, 7).

Inactivated polio vaccine (IPV) is highly effective in producing immunity to polio virus and ensuring protection against paralytic polio. Over 90% of vaccinated individuals develop antibodies against all three types of polio virus after two doses and at least 99% are immune after the following three doses. Protection against paralytic polio correlates with the presence of antibodies (8, 9). IPV produces less local gastrointestinal immunity than OPV, thus the individuals who receive IPV are more easily infected with the wild polio virus than those who are vaccinated with OPV (7, 9). The duration of immunity following the IPV vaccination is not known with certainty, although the vaccine is likely to provide protection for many years after a full series of vaccinations has been completed.

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type 3 vaccine virus (up to 85% in one study). A fourth dose – most studies use OPV for the fourth dose – usually produces seroconversion rates similar to those of the three doses of IPV or OPV (2, 7, 9). OPV trivalent vaccine was chosen in the United States (and in most countries) on its approval in 1963. The almost exclusive use of OPV has eliminated wild polio virus in the United States in less than 20 years. However, with every 2-3 million doses of OPV administered, there is a case of VAPP (vaccine-associated paralytic poliomyelitis), which has resulted in about 10 cases of VAPP in the US every year. In the period 1980-1999, VAPP was responsible for 95% of reported cases of paralytic polio in the United States. In 1996, an increase in the use of IPV was recommended, as part of an IPV/OPV sequential immunization schedule. This recommendation was intended to reduce the occurrence of paralytic polio associated with the vaccine (8, 9, 10). The sequential programme was intended to eliminate VAPP in vaccinated people as a result of the production of humoral immunity to the polio viruses in the vaccine with inactivated polio vaccine administered prior to the exposure to the live virus in the vaccine (2, 5, 9). As VPO was still used for the third and fourth dose in the polio vaccination programme, the risk of VAPP continued to exist among the close contacts of the vaccinated individuals, who were exposed to the vaccine live virus in the stool of the vaccinated people. The IPV/OPV sequential immunization schedule was widely accepted both by suppliers and by parents. In 1998 and 1999, fewer cases of VAPP were reported, proving the result of the increased usage of VPI. However, only a complete elimination of the OPV will lead to the elimination of VAPP. To emphasize the goal of completely eliminating the paralytic polio in the United States, ACIP recommended in July 1999 that inactivated polio vaccine be used exclusively in the United States starting from 2000. OPV is no longer available in the US in routine programmes. The exclusive use of IPV prevented the vaccine live virus from spreading as well as the occurrence of any case of indigenous VAPP (7, 9, 10).

Tuberculosis

According to estimates, one third of the world population is infected with *M. tuberculosis*, and about 90% of infected people show latent asymptomatic infections; in their case, there is a 10% probability that during their lifetime, latent infection might progress to the stage of active tuberculosis (4, 8).

In 2007, 13.7 million chronic active cases were estimated worldwide. In 2010, nearly 8.8 million new cases were recorded as well as 1.5 million TB-related deaths, of which a fourth in patients also infected with HIV, mostly in countries in course of development. It is believed that the number of new cases of tuberculosis worldwide has been declining since 2002. Approximately 80% of the population of Asian and African countries test positive in tuberculin, compared to 10% of the US population. However, in developing countries, many people contract tuberculosis because of low immunity levels, in many cases as a side effect of HIV infection (2, 4).

The only available vaccine is BCG, which is effective against the spread of the disease in childhood, but with a varying degree of protection against pulmonary tuberculosis (therefore controversial). Despite this, over 90% of children are still vaccinated with BCG. Vaccination at an

early age provides immunisation for a period of about 10 years, but cases have been reported where false positive results were determined in the tuberculin skin test, which makes the test unreliable in identifying the disease (2, 11).

It has been shown that the effectiveness of BCG varies geographically. Thus, in India and Malawi it is not effective against tuberculosis (although it helps against leprosy) – the average efficiency is 50% and deaths are reduced by 70%; the protection against TB and miliary (disseminated) meningitis is 75-86%; the protection against pneumonia is 40%; the protection against febrile diseases is 18%. The protective effect lasts for at least 15 years. In many cases, it is assumed that the BCG vaccine alone is enough, and that a second vaccination does not provide extra protection (11, 12).

Tuberculosis occurs more frequently in Romania than in other European countries – the 6th position, according to a WHO ranking of reporting European countries – over 27,000 patients are treated annually for TB, of which more than 15,000 are new cases. It is estimated that an average of 130 cases occur per 100,000 inhabitants, compared to the European average of 30 patients per hundred thousand inhabitants. Of the 15,000 patients diagnosed with tuberculosis annually, 2,000 are relapses, and more than 500 cases develop a severe form, unresponsive to conventional therapy. A national strategy for TB control was drafted in Romania for the period 2015 - 2020, which aims at eliminating TB as a public health problem in Romania by 2020; in other words, TB incidence must decrease by 10% annually during this period (2).

The legal principles of public health assistance are: society's responsibility for public health; focus on population groups and primary prevention; concern for health determinants: social, environmental, behavioural and health services; multidisciplinary and intersectoral approach; active partnership with the population and with central and local public authorities; decisions based on the best scientific evidence available at the time (evidence-based public health); under specific conditions, fundamental decisions based on the precautionary principle (1,2).

CONCLUSIONS

The most important roles of public health care refers to: developing policies, strategies and programmes regarding public health; monitoring and analysing the health of the population; public health planning; epidemiological surveillance, disease prevention and control; strategic management and marketing of public health services; prevention of epidemics, including the establishment of epidemiological state of alertness; protecting the population against environmental risks; informing, educating and communicating to promote health; ensuring the means for responding to disasters or threats to life and health, including the introduction of restrictions on the movement of people and goods (2, 12).

The main areas of intervention of public health assistance are: prevention, surveillance and control of transmissible and non-transmissible diseases by: providing immunisations; control of epidemics; disease surveillance; surveillance of behavioural risk factors; accident prevention; health monitoring, evaluation of the needs of the population in terms of public health services; health promotion and health education by information-education-communication campaigns (1, 2).

In this context, another important aspect needs to be emphasised – the close connection between the right to health and other fundamental rights: the right to life and physical and mental integrity; the right to respect for private and family life; the right to information and the right to a healthy environment.

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BILATERAL DACRYOCYSTOCELE CONDUCT AND PRENATAL DIAGNOSIS

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BILATERAL DACRYOCYSTOCELE CONDUCT AND PRENATAL DIAGNOSIS (Abstract): Dacryocystocele develops during fetal life as a result of the obstruction of the nasolacrimal canal. The disease can be both unilateral and, very seldom, bilateral and it is characterized by the appearance of a cystic blue mass over the area of the lacrimal sac after birth. Prenatal ultrasonography can diagnose dacryocystocele and differentiate between dacryocystocele and other birth defects. We are describing a case of bilateral dacryocystocele observed at 34 weeks of gestation, clearly visualized using 2D ultrasonography. It is very important for the mother to be informed about the treatment, evolution and the potential complications of the disease. It is also necessary to reconfirm the fact that dacryocystocele is not a facial malformation, which could be a major cause of concern and distress for the mother before birth. **Keywords:** Bilaterale dacryocystocele, prenatal diagnosis, treatment.

Dacryocystocele (mucocele, amniocele) is the occlusion of lacrimal drainage system distally and proximally during the fetal life (1).When the lacrimal sac is filled with mucus it is called mucocele, when it is filled with amnion it is called an amniocele (2, 3). The valve of Hasner is the most frequent site of a distal obstruction due to incomplete canalization, a process that starts in the third fetal month and is completed by the sixth month (4, 5). A congenital dacryocystocele accompanies nasolacrimal duct obstruction in approximately 0.1% of infants (6).

The median age of presentation is approximately 7 days of life and it is frequently unilateral in 60% (7) - 100% (5,8-11) but may also be bilateral (12).

Clinically speaking, it appears as a bluish cystic mass located below the medial canthus. Prenatal diagnosis is important because bilateral dacryocystocele extend-

ing intranasally may cause neonatal nasal obstruction (1, 13, 14). Prenatal ultrasound can differentiate dacryocystocele from other birth defects, thus being essential in the early diagnosis, starting from 24 weeks'gestation (15-18).

CASE REPORT

We describe a case of bilateral dacryocystocele seen at 34 weeks of gestation, with clear visualization of this defect using 2D ultrasonography. Examination revealed a two hypoechogenic cystic mass (1 cm), located medially to the eye sockets (fig.1, 2). No anomalies of the intracranial or facial bony structures were found. The amniotic fluid volume was normal for 34 weeks gestation and the orbits appeared symmetrical. Postpartum examination revealed two bluish masses in the left and right medial canthus. The bilateral cyst drained spontaneously three days after birth.

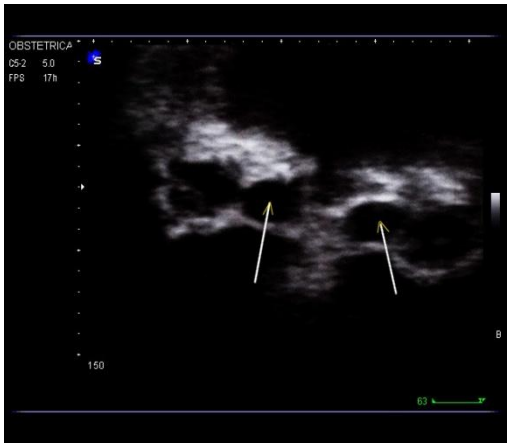


Fig. 1. Ultrasonography-transversal axial section that shows bilateral dacryocystocele (arrows) (Personal collection of PhD A. Cărăuleanu)

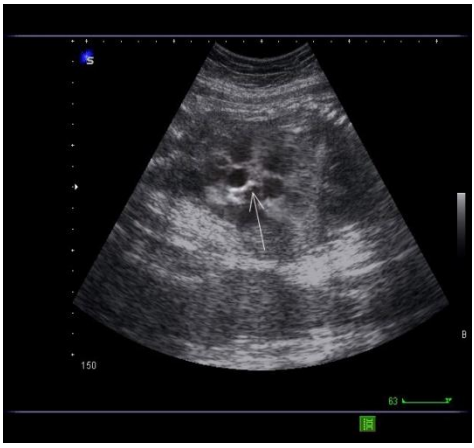


Fig. 2. Ultrasonography-frontal section that shows bilateral dacryocystocele (arrow) (Personal collection of PhD A.Cărăuleanu)

Prenatal diagnosis of dacryocystocele is simple due to its specific appearance and position.

Diagnostic tests: Routine obstetrical ultrasound may identify a specific hypoechogenic mass located inferomedially to the orbit. If this diagnosis is not set before birth, physical examination can provide enough evidence for its recognition, after birth (1, 5).

Associated anomalies:

- no associated anomalies are usually present, but the cyst may cause septal deviation
- the occurrence of hyperteleorism is rarely, due to the extra soft tissue at eye level.

Differential diagnosis includes:

- meningo-encephalocele (11)
- hemangioma
- dermoid cyst
- nasal glioma (2).

Prognosis: excellent, dacryocystocele being a benign tumor, there is no predisposition to developing a facial anomaly. Once corrected spontaneously, medically or surgically the infant will have a normally

functioning nasolacrimal drainage system.

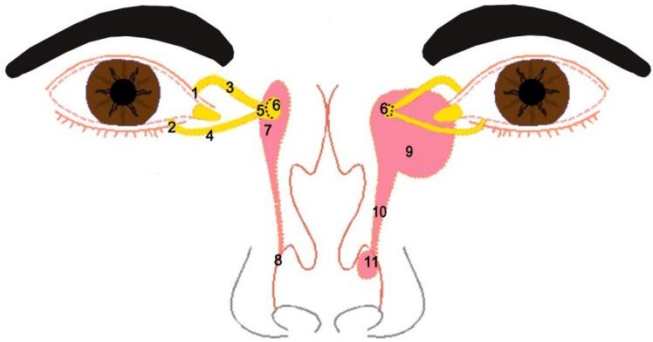
Management:

- it can resolve spontaneously
- external digital pressure on the cyst may allow the contents to decompress through the nose
- if conservative treatment fails, probing of the nasolacrimal duct is performed
- recurrence is possible following probing the nasolacrimal duct
- rarely dacryocystorhinostomy may be required.

DISCUSSION

Congenital dacryocystocele is relatively common in newborns and infants (19). This clinical entity is a congenital obstruction of the nasolacrimal duct resulting from the upper obstruction of the Rosenmuller valve and lower obstruction of the Hasner valve, and it usually develops in first weeks after birth (2, 4, 11, 20, 21).

The frequency of congenital dacryocystocele reported by different authors ranges from 1.25% to 12.5% (22). Numerous studies have shown that this disease is more common in women because they have a narrower nasolacrimal duct than men (23). The mechanism for dacryocystocele formation is represented by the filling of the lacrimal system with mucoid secretions, amniotic fluid, tears and bacterial colonization (19) resulting in the distension of the lacrimal system and occurrence of a bluish cystic formation located upper and below the medial canthus.



1. Upper punctum
2. Lower punctum
3. Upper canaliculus
4. Lower canaliculus
5. Common canaliculus
6. Valve of Rosenmuller
7. Lacrimal sac & duct
8. Valve of Hasner
9. Dacryocystocele
10. Enlarged lacrimal duct
11. Nasal extension

Fig. 3. Normal anatomy compared to dacryocystocele (After Tardiff A, *et al*.,1991) (24)

The correct diagnosis of this clinical entity is important as it can often be mistaken for an encephalocele, hemangioma, dermoid or sebaceous cyst (23).

Dacryocystocele can be diagnosed prenatally by ultrasound scanning, which is a non-invasive method, and can be confirmed after birth by CT, MRI and surgical maneuvers for probing the nasolacrimal duct.

Rand (1989) (25) and Walsh (1994) (26) diagnosed dacryocystocele by ultrasound scanning at 30-36 weeks gestation.

Sharony *et al*. (1999) (27) reported an early diagnosis at 27 weeks gestation, describing 6 cases of dacryocystocele. In our case the bilateral dacryocystocele was diagnosed at 34 weeks gestation.

The sonographic appearance of dacryocystocele is described as a hypoechogenic mass located inferomedially to the orbit that may be seen in coronal or parasagittal view including the nose or medial angle of the orbit. Dermoid cyst is a hyperechoic entity located superolaterally to the eyeball, with areas of calcification. Hemangioma is located in the neck or head and is a solid tumor mass diagnosed by using Doppler ultrasound scanning. Anterior encephalocele is a defect located on the midline and is associated with hydrocephalus (1).

After birth, it is important to make a conclusive clinical diagnosis of meningoencephalocele: biopsy is contraindicated due to subsequent cerebrospinal fluid leakage and risk of meningitis (11, 28).

Shields (2004) mentioned that differentiation with non-inflamed dacryocystocele can be difficult because it characteristically appears at, or shortly after, birth as a pulsating swelling at the medial canthus. Examination with CT imaging usually highlights the bone defect that causes protrusion of the brain tissue (28).

As patients with dacryocystocele may have difficulty breathing and breastfeeding and risk of infection on lesion side (23) early diagnosis is essential.

The difficulties mentioned above may also lead to the occurrence of reactive psychopathological manifestations in the parents, especially in the mother. Due to their lack of experience, the primiparae are the most exposed to emotional manifestations. Any literature review reveals that over 40% of the women suffer an emotional imbalance after giving birth, even when their baby is in good health. The most commons manifestations are anxious disorders. Being already worried about their inexperience, their continuously stressful and unidirectional focus on the youngest member of their family may be sufficiently consuming to trigger an imbalance, which may variably affect their ability to care for their young and even their own health state. Although it is easily detectable and treatable, if adequate therapeutic measures are not taken, this suffering may have much more serious medical consequences than it seemed initially.

The treatment of dacryocystocele is most often or initially conservative, including lacrimal sac massage for 7-14 days to facilitate drainage of the contents into the nasal cavity, administration of topical antibiotics, warm compresses and the result is a 76% cure rate (23). Mansour *et al.*,and Harris showed dacryocystocele cases that resolved spontaneously before surgical intervention with and without a reflux of material on compression of the dacryocystocele (8,29). In our case the bilateral dacryocystocele drained spontaneously three days after birth.

There are controversial opinions on the time of surgery for congenital dacryocystocele (19). When conservative treatment fails, surgical intervention consisting of lacrimal duct probing is required. Probing reduces the risk of dacryocystitis, improving success rate (10). In the absence of treatment there is an increased risk of infection and permanent closure of the nasolacrimal duct, requiring dacryocystorhinostomy (27, 30).

CONCLUSIONS

The prenatal diagnosis of dacryocystocele is made by means of ultrasound

starting with the third trimester of pregnancy. It is essential to inform the mother about the management and prognosis of a dacryocystocele in the newborn.

This inexpensive method of investigation has an important role in reconfirming the fact that dacryocystocele is not a facial malformation, which could be a major cause of concern and distress for the mother before birth.

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NEWS

IDIOPATHIC DILATED CARDIOMYOPATHY: COMPUTERIZED ANATOMIC STUDY OF RELASHIONSHIP BETWEEN SEPTAL AND FREE LEFT VENTRICLE WALL

Dilated cardiomyopathy (DCM) is the most common among the primary diseases of the myocardium and is featured, in special, by dilation of one or both ventricles and systolic dysfunction. This is a very interesting model inside a current context of major interest in studies that correlate shape and function of the cardiac pump. The geometrical alterations due to dilation observed in DCM can cause severe functional impairment. Several authors have studied the effects of these alterations in the left ventricle and developed theories that could explain the reasons for this functional impact. Through exams such as magnetic resonance, for example, verified the influence of geometrical alteration, tending to sphericity, affecting the ventricular torsion during systole or even the ventricular relaxation globally decreasing the cardiac pump performance. However, few studies have evaluated the ventricular chambers splitting them into segments, or observing the changes in size in different places inside the chambers, thus seeking for understanding the real complexity of the geometrical changes in the ill heart. These can give support to the deepening of studies about the influence of the geometrical deformation on cardiac performance as well as the development of more accurate techniques to provide a reverse remodeling. The study aimed to evaluate if, in different regions, increased transverse perimeter of left ventricular chamber in idiopathic dilated cardiomyopathy occurs proportionally between the septal and non-septal segment (free wall), in order to confirm the hypothesis that dilation occurs proportionally between these segments (Juliani PS, da Costa EF, Correia AT, et al. Idiopathic dilated cardiomyopathy: computerized anatomic study of relashionship between septal and free left ventricle wall. Rev Bras Cir Cardiovasc 2014;29 (3): 338-43).

Doina Butcovan

MANAGEMENT OF TRAUMATIC LIVER LESIONS

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MANAGEMENT OF TRAUMATIC LIVER LESIONS (Abstract): **Aim:** To determine the correct therapeutic approach to the different grades of liver trauma. **Material and methods:** The study is based on a retrospective analysis of treatment outcomes in 56 patients with abdominal trauma admitted over a 9-year period to in the IIIrd Surgical Clinic of the Iasi "Sf. Spiridon" Hospital. It is focused on operative or non-operative management of liver trauma, surgical technique used, morbidity and postoperative mortality. Data were collected from electronic medical records and observation sheets and processed and interpreted using Microsoft Excel statistical functions. **Results:** In the interval May 26, 2005 – April 19, 2013 56 cases of abdominal trauma were recorded, 31 (55.35%) residing in urban areas, and 25 (44.64%) in rural areas. The mean age was 39 years, range 18-83 years old. The male/female ratio was 2.5/1 and the group consisted of 40 (71.42%) male patients and 16 (28.57%) female patients. The causes of abdominal trauma were: car accident in 29 (51%) cases, fall from different heights in 6 (10%) patients, workplace-related accidents in 8 patients (14%) and direct hit injury in 12 patients (12%). In our cohort, 51 (91%) patients with abdominal trauma have been emergency admitted, 3 patients (5%) were transferred from different medical units, and 2 patients (4%) were referred by a specialist doctor. Two or more simultaneous lesions were diagnosed in 53 (96%) cases. Of the 45 patients with traumatic liver injuries diagnosed on admission, 32 (71%) required surgical intervention. In the remaining 13 (29%) patients, the therapeutic management was conservative. **Conclusions:** Hepatic traumas are often severe, and frequently associated with multiple injuries. The non-operative management is indicated in liver lesions grade I, II and III according to the American Association for the Surgery of Trauma (AAST), if abdominal cavity organs are not injured. Higher grade liver lesions (over IV) in which the hemorrhagic risk persists or reappears require surgical intervention as soon as possible, and according to the type of lesion, the right procedure should be chosen. **Keywords**: TRAUMATISM, SURGICAL, LIVER, TREATMENT.

Nowadays abdominal trauma is one of the most important parts of emergency surgery. The abdominal region is widely exposed to traumatic agents acting under a lot of circumstances: car accidents, sports, workplace accidents, violence. The management of the patient with hepatic trauma has significantly evolved over the last years partly due to the changing attitude of the medical specialists from the "interventional" one at the beginning of 60's towards the conservative (non-surgical) one in the last

20 years. Imaging analysis of liver lesions favors the decision-making regarding the therapeutic approach to be adopted, to operate or not, based on all parameters.

Liver injury is one of the most common traumatic emergency, on one hand due to the large size of the liver and fragility of the parenchyma, on the other hand due to its location in relation to the chest wall and spine, all these factors exposing it to the action of different agents which act at the level of the thoracic wall and abdomen (1-3). The management of patients with abdominal trauma and consequently of those with hepatic trauma underwent major changes in approach and concepts over the last years. These patients, victims of a true "traumatic epidemic" are usually active persons, aged 15 to 50 years. The probability of trauma-related death in this age segment is three times higher than from other causes (4).

The modern liver surgery developed in parallel with the understanding of liver anatomy and physiology, initiated by Couinaud in 1957 and later by Bismuth in 1982.

This study deals with the diagnosis, treatment, and outcome of patients with trauma to the liver (5-8).

MATERIAL AND METHODS

The inclusion criterion was traumatic liver lesion confirmed by clinical examination and laboratory investigations. The study was based on a retrospective analysis of treatment outcomes in 56 patients with abdominal trauma admitted over a 9-year interval to the IIIrd Surgical Clinic of the Iasi "Sf. Spiridon" Hospital, being focused on the operative or non-operative management of liver trauma, surgical technique used, morbidity and postoperative mortality. Ninety-one percent of patients have been admitted, investigated and treated in the Surgical Clinic, the remaining being transferred from medical units where they received primary care immediately after the accident (8%).

The term "trauma" is defined as an acute severe state following an impact of a different nature: mechanical, thermal, chemical or electric, being characterized by the presence of a physical injury which affects isolated or concomitant organs from one, two or more anatomical regions of the body (9-11). "Polytrauma patients" were the patient who sustained two or more injuries to organ systems, one of which or the combination of some lesions being life-threatening, with a severity score >16 on ISS scale, according to the definition accepted by the stuff of Floreasca Emergency University Department, Romania (12,13).

RESULTS

Diagnosis. The diagnosis of liver injury was made by history, complete physical examination, laboratory tests and imaging investigations. Abdominal ultrasound scan was performed in all patients. In case of diagnostic uncertainty or for a greater diagnostic accuracy a CT scan or abdominal MRI and/or angiography were performed. Liver function was assessed by determining the hepatic enzymes, bilirubin, cholesterol, serum iron, coagulation tests, serum proteins and albuminemia. In case of biliary obstruction, to determine its localization, extent, and cause the following were also performed: percutaneous cholangiography, ERCP (endoscopic retrograde cholangiopancreatography) and/or MRCP (magnetic resonance cholangiopancreatography).

Distribution. Analyzing the gathered data, in the interval May 26, 2005 – April 19, 2013 56 cases of abdominal trauma were recorded. Of these, 31 (55.35%) resided in

urban areas and 25 (44.64%) in rural areas.

In the study group, the mean age at injury was 39 years, range 18 - 83 years.

The male/female ratio was 2.5/1, the study group consisting of 40 (71.42%) male patients and 16 (28.57%) female patients (fig. 1). Most trauma patients were males, accounted for by the nature of activities performed by them (workplace accidents, agricultural or domestic activities), or incidents they were involved in (car accidents, conflicts and violence, sports).

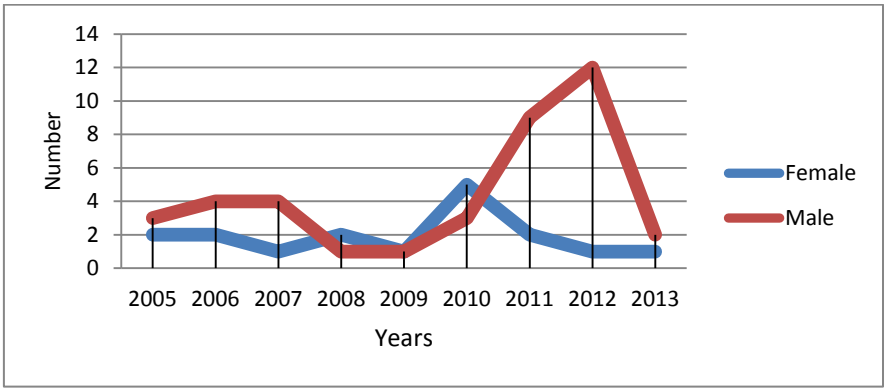

Fig. 1. The distribution of abdominal trauma patients based on sex

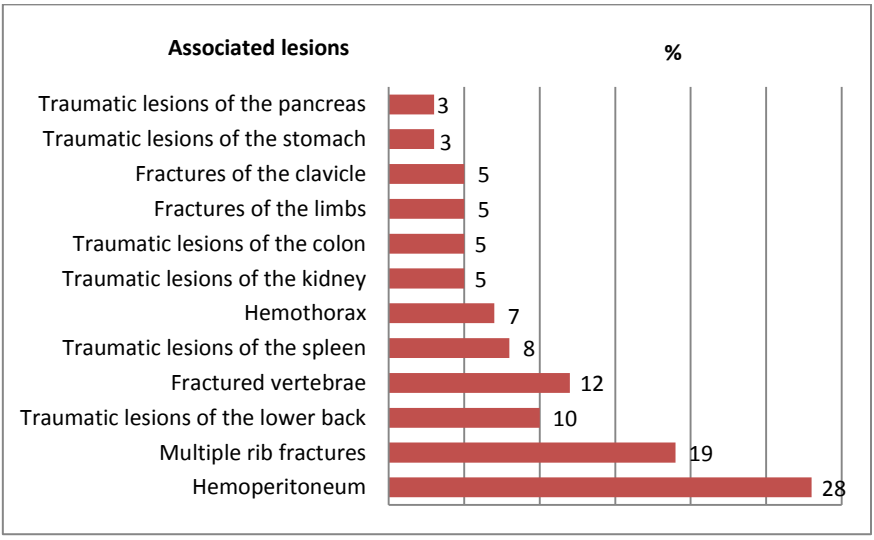

Fig. 2. Main associated lesions

Etiology. Abdominal trauma, especially that caused by car accidents which were present in 29 (51%) of our cases was the most common cause of liver injury being found in 26 (89%) of the 29 cases. Fall from different heights caused trauma in 6 cases (10%), workplace-related accidents led to abdominal trauma in 8 (14%) cases, and direct hit injury in 12 (12%) cases.

Admission type and associated lesions.

In our study group, 51 patients (91%) with abdominal trauma were emergency admissions, 3 (5%) patients were transferred from different medical units, and 2 (4%) were referred by a specialist. Of the 45 patients with liver trauma (from the total of 56 abdominal trauma patients), the great majority were polytraumatized (40 cases – 89%). Isolated liver lesions were found in 5 (11%) cases, a significantly lower value compared to frequency of liver lesions in associated trauma.

The frequency of associated lesions appears to be significant, very often two or more simultaneous lesions being diagnosed. In 53 (96%) of the cases there were concomitant lesions, their nature being detailed below (fig. 2). Hemoperitoneum was most frequently associated with abdominal trauma (16 cases/28%). Multiple rib fractures were identified in 11 (19%) patients, fractures of the vertebrae in 7 (12%) patients and of the clavicle in 3 (5%) patients. The most frequently concomitantly injured retroperitoneal organ was the spleen, 5 (8%) cases.

Traumatic lesions to the liver and gallbladder were diagnosed in 15 (34%) cases, contusion and hematoma in 18 (40%) cases, minor laceration in 7 (16%) patients, moderate laceration in 1(2%) patient, major laceration in 3 (6%) patients, and liver abscess in 1 (2%) patient (fig. 3).

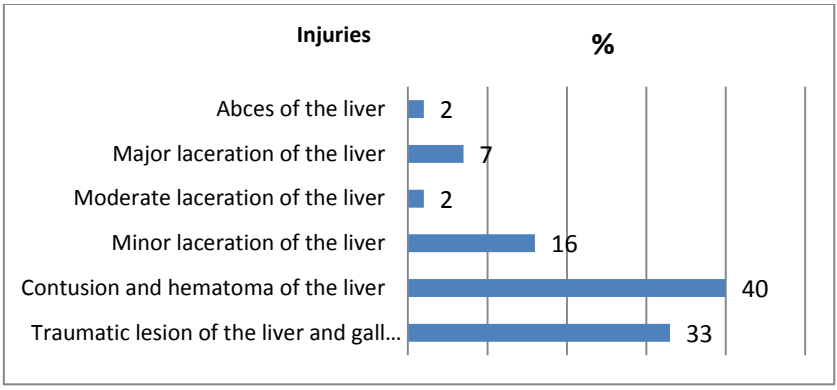

Fig. 3. Liver injuries diagnosed on admission

Type of treatment received. Of the 45 patients with traumatic liver injuries diagnosed on admission, in 32 (71%) cases a surgical intervention was required. In the remaining 13 (29%) patients, the therapeutic approach was conservative. Of the 15 patients with injuries to the liver and gallbladder, 5 (33%) patients were treated conservatively, and a surgical intervention was required in 10 (67%). As to the patients diagnosed with contusion and hematoma of the liver, in 5 (28%) cases the conservative treatment was preferred, and in the remaining 13 (72%) cases surgical intervention was required. The minor liver laceration was treated conservatively in 2 (29%) cases and surgically in 5 (71%) cases. The only case of moderate liver laceration received conservative treatment, while for major liver laceration surgery was required in all 3 patients. The patient diagnosed with a liver abscess was also surgically treated.

Patient discharge status. It should be mentioned that in 23 (51%) patients the

discharge diagnosis was cured, in 10 (22%) improved health status, in 4 (9%) patients stationary health status, and 8 (18%) patients died (fig. 4). Analyzing the number of deceased patients, we concluded that 8 of 9 patients died due to the severity of liver trauma and associated lesions.

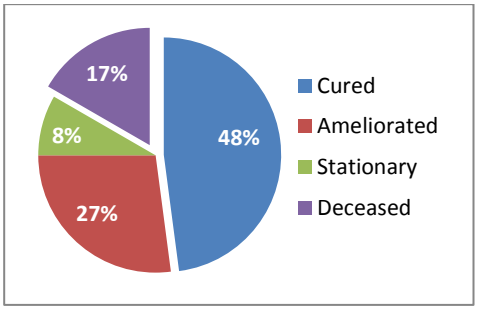

Fig. 4. Medical condition at discharge

DISCUSSION

In the last years, the frequency of liver trauma has increased considerably, and the incidence is much higher among young males aged 21-30 years and 31-40 years, thus demonstrating the social importance of the problem.

Abdominal trauma, especially that caused by car accidents identified in 29 (51%) of the patients is the most frequent cause of liver injury: 26 (89%) of 29 patients with abdominal trauma were diagnosed with liver injuries.

The failure of non-operative treatment (conservative) is influenced only by the severity of the parenchymatous organ injury associated with hemodynamic instability on admission (3).

As to mortality, none of the 8 deaths were caused by the liver trauma itself, but by the association with other severe lesions.

US and CT screening are compulsory for the correct diagnosis, stadialization of lesions, adequate treatment and prevention of possible complications (8).

CONCLUSIONS

Liver traumas are often severe, and frequently associated with multiple injuries (in 96% of the cases concomitant lesions being identified). In the vast majority of cases, the patients were admitted in emergency (91%).

The non-operative approach is indicated in liver lesions grade I, II and III according to the American Association for the Surgery of Trauma (AAST), if abdominal cavity organs are not injured. In 29% of the patients the therapeutic approach was conservative.

High-grade liver lesions (over grade IV), in which the hemorrhagic risk persists or may reappear, surgical intervention is necessary as soon as possible, and the procedure adequate to the type of lesion should be chosen. Surgery was required in 71% of cases.

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NEWS

PRIMARY CLEAR CELL SARCOMA OF THE TONGUE

Clear cell sarcoma shares features with melanoma, but frequently shows EWSR1 rearrangements. It is an aggressive tumor typically occurring in the soft tissues of the extremities, with a gastrointestinal variant with less consistent melanocytic differentiation. It is extremely rare in the head and neck region, with no reported cases in the oral cavity. The authors report a case of an 82-year-old woman with a clear cell sarcoma arising in the tongue, with cervical lymph node metastases. Histologically, the tumor showed some features of gastrointestinal clear cell sarcoma. No osteoclast-type giant cells were present. The tumor cells were positive for S100 protein and negative for other melanocytic markers. Fluorescence in situ hybridization showed rearrangements of EWSR1 and ATF1. This case expands the spectrum of clear cell sarcoma with a gastrointestinal-like variant in a novel site, emphasizing the need to consider it as a differential diagnosis to melanoma in mucosal sites. This report raises the question of whether CCS of head and neck mucosal sites is a rare distinct subtype within the GI CCS spectrum, or whether this case is merely a ‘‘typical’’CCS arising in an unusual site. The morphologic and immunophenotypic features of this case support the former interpretation, but additional cases will be necessary to confirm this interpretation. Given the morphologic and immunohistochemical overlap with malignant melanoma, it is also possible that such cases were previously diagnosed as melanoma. In agreement with that, a recently published study performed on cases from the GI tract that were diagnosed as melanoma showed that a small fraction in fact were CCS. While future studies may answer this question, the practicing pathologist should be aware of CCS when encountering an S100 protein–positive epithelioid and spindle cell neoplasm arising in an unusual site without diagnostic features of melanoma, such as an in situ component, and should have a low threshold for performing additional molecular studies to further investigate this possibility (Stefan Kraft, Cristina R. Antonescu, Andrew E. Rosenberg, Daniel G. Deschler, G. Petur Nielsen, Primary Clear Cell Sarcoma of the Tongue. Arch Pathol Lab Med. 013;137:1680–1683).

Doina Butcovan

PREGNANCY AND RECURRENCE RATES IN INFERTILE PATIENTS OPERATED FOR OVARIAN ENDOMETRIOSIS

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PREGNANCY AND RECURRENCE RATES IN INFERTILE PATIENTS OPERATED FOR OVARIAN ENDOMETRIOSIS (Abstract): **Aim:** The study deals with the preoperative ultrasound diagnosis of ovarian endometriosis, postoperative ultrasound reassessment, laparoscopic surgical resolution of ovarian endometriosis, estimation of recurrence risk 12 months after surgery by ultrasound, reappearance of clinical symptoms (such as pain) or second-look laparoscopy, and pregnancy rate 2 years after surgery. **Material and methods:** 140 patients with endometriosis and infertility admitted to the Iasi "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology between the years 2009-2011 were included in the study. The patients were divided into 2 groups: group 1 - 59 cases that refused in vitro fertilization procedures, did not afford in vitro fertilization (IVF) or had minimal endometriosis and young ages and thus a possibility of delaying treatment, and group 2 - 62 cases which underwent IVF procedures immediately after surgery. **Results:** No significant differences in the chance of becoming pregnant were found between the two groups (χ^2=2.06, p=0.0891, 95% CI); in group 1, 11.86% pregnancies were obtained while in group 2 the pregnancy rate was 11.29%. Based on the nonparametric method of analysis of contingency tables we could estimate the odds of becoming pregnant in the study groups, obtaining an odds ratio (OR=1.16, CI: 1.04-2.23, 95% CI). This result indicated that in group 1 the odds of becoming pregnant was not significantly higher, and the estimation was made for a confidence interval of 95%. The study had in view the assessment of pregnancies obtained in the study groups and the time interval (number of weeks) from the time of intervention until the occurrence of pregnancy. The Kaplan-Meier analysis enabled the assessment of the mean value and the median value of the number of weeks until becoming pregnant, and these values did not show significant differences (χ^2=1.55, p=0.212, 95% CI). **Conclusions:** For endometriosis associated with infertility, hormonal suppression does not improve fertility, and therefore surgery followed by controlled ovarian hyperstimulation and intrauterine insemination (IUI), provided the anatomy of the pelvis is preserved in early cases or *in vitro* fertilization in severe cases is preferred. **Keywords:** ENDOMETRIOSIS, INFERTILITY, PELVIC PAIN, LAPAROSCOPY.

Endometriosis is a benign disease characterized by the growth of the endometrium outside the uterus under the influence of estrogen. This cyclical growth followed by bleeding in the surrounding tissue determines symptoms such as pain and infertili-

ty. The only form of endometriosis diagnosed preoperatively by noninvasive means is the ovarian endometrium, which is the formation of a cyst full of blood inside the ovary. The most common forms are the intraperitoneal ones, but distance localizations are not excluded. Optimal treatment is surgical laparoscopy to remove the endometrial tissue by ovarian stripping or hormonal suppression. In most cases it is followed by relapses.

Endometriosis is a hormone-dependent progressive disease, which can be responsible for symptoms like: dysmenorrhea, dyspareunia, chronic pelvic pain and infertility. After 150 years of research, when multiple hypotheses were issued, the etiology of endometriosis remains unknown. No theory can fully explain the clinical manifestations; therefore, endometriosis can rather be viewed as a syndrome. The diagnosis is often established macroscopically by laparoscopy; histological confirmation is essential because in several cases the endometrial origin of these lesions is invalidated. In the literature, the prevalence of endometriosis varies significantly according to the method of diagnosis: 2-78% in infertility, 0.7-43% in celioscopy for tubal ligation and 4.5-82% in patients who underwent surgery for pain (1). In teenagers with severe dysmenorrhea or pelvic pain, endometriosis is diagnosed by celioscopy in 50% of the cases (2). There are no relevant epidemiological data regarding the incidence of endometriosis in the general population.

The study dealt with the preoperative ultrasound diagnosis of ovarian endometriosis, ultrasound reassessment after surgical treatment, laparoscopic surgical resolution of ovarian endometriosis, estimation of recurrence risk 12 months postoperatively by ultrasound, reoccurrence of clinical symptoms such as pain or by second-look laparoscopy, and the pregnancy rate 2 years after surgery. The aim was to highlight the role of conservative surgery in the treatment of endometriosis-related infertility and the increase of recurrence rate following ovarian hyperstimulation.

MATERIAL AND METHODS

One hundred and forty cases of endometriosis and infertility admitted to the Iaşi "Cuza−Vodă" Hospital of Obstetrics and Gynecoogy between the years 2009-2011 were included in this study. The age distribution showed that 25% of the patients were older than 33 years (quartile interval Q75-33 years) with a mean age of 29.7 years ±4.9SD, while 50% of these were younger than 26 years (median – 26 years). We did not use the R-AFS classification because of its known subjectivity, and the cases were classified according to the size of the endometriomas: 100 cases of unilateral or bilateral endometriomas less than 5 cm in size and 40 cases with endometrial cysts over 5 cm.

The patients included in the study had a ultrasound diagnosis of unilateral or bilateral ovarian endometriosis histologically confirmed after laparoscopy. The conventional ultrasound diagnostic criteria were: presence of ovarian cysts with characteristic appearance, with a non-homogenous "ground glass" appearance and/or hyperechogenic wall foci with absence of vascularization on power Doppler. The cyst size ranged from 2 cm to 11 cm. The ultrasound signs of deep endometriosis were not looked for. Doctors explained to all patients the pathophysiology of the disease and its repercussions on fertility and quality of life. Patients who refused any intervention or did not come back for consultation were excluded from the study.

Inclusion criteria: age above 18 years, endometrioma over 3 cm and infertility, absence of pregnancy, absence of associated pathologies that do not indicate laparoscopic intervention, the patient's expressed wish for immediate diagnosis, history of previous IVF failure, association of other causes of infertility. *Exclusion criteria:* pregnancy, patients who opted for hysterectomy with bilateral adnexectomy, patients with endometriomas not associated with infertility or who did not want children, endometriomas less than 2 cm in size (oral birth control pills were recommended), relapse after a new intervention.

Informed consent for the participation in this clinical study was obtained from all patients after the diagnosis of ovarian endometriosis was made. The consent for laparoscopy was obtained upon admission for the surgical intervention.

All participants in the study were included in a database where the following were recorded: age, weight, menarche, last menstruation, duration of cycles and interval between cycles, previous hormonal treatments, duration of symptoms, ultrasound cyst size, unilaterality or bilaterality, presence or absence of hydrosalpinx, clinical stability or mobility of cysts, previous admissions, preoperative CA125 levels and possibly preoperative AMH levels, and type of surgery. The pre-anesthesia consultation included the laboratory exam, the cardiologic consultation and the chest X-ray.

Of the 140 patients with ultrasonographically suspected endometriosis, only 121 cases (86.43%) were operated of which 7 opted (5%) for hysterectomy with bilateral adnexectomy and 12 patients (8.57%) had other diagnoses (dermoid cyst: 4, hemorrhagic cyst: 8). These 121 patients who remained in the study were then divided into two groups depending on a series of factors which influenced decision making in the performance of laparoscopic surgery: history of infertility and other possible causes (male sterility); size of the endometrioma and the anatomic distortion of the pelvis; unilateral or bilateral hydrosalpinx; history of tubal or ovarian surgery; age, presence of the pelvic pain syndrome, other coexisting factors influencing the couple fertility, the patient's wish, if the patient can afford the costs knowing that in Romania the fertilization procedures are not reimbursed by the Health Insurance Fund.

Group 1 - 59 patients who did not want IVF, did not afford IVF or had minimal endometriosis and young ages with possibility of delaying the treatment. Characteristics of the group: infertility without other associated causes: adherences, hydrosalpinx, preservation of pelvic anatomy, bilaterally positive methylene blue test, no history of inflammatory disease.

Group 2 - 62 patients who underwent IVF procedures immediately after surgery. Characteristics of the group: infertility, changes of pelvic anatomy by adherences or unilateral or bilateral hydrosalpinx, other associated causes of infertility. The group consisted of: 59 patients (95.16%) aged between 25 and 39 years with primary infertility and 2 cases (3.22%) with primary infertility and male infertility. The mean duration of infertility was 4 years. Tubal infertility was associated with unilateral or bilateral hydrosalpinx in 10 cases (16.13%), previous surgery in 3 cases (4.84%) and history of failed IVF in 6 cases (9.68%).

The 2 groups were compared with respect to the recurrence of endometriosis one year postoperatively (based on MRI, ultrasound, repeat laparoscopy, or reoccurrence of pain) and pregnancy rate following various treatment methods.

RESULTS

We conducted a comparative study of two homogenously distributed groups of patients with endometriosis and infertility. The 121 patients (86.43%) who had infertility and unilateral or bilateral ovarian endometriosis were advised to try to conceive as soon as possible after surgery, either naturally or by IVF. In the 62 patients (51.23%) in group 2, bilateral hydrosalpinx followed by tubal coagulation, older age, large cysts with multiple adherences, low preoperative AMH level or patient's desire for fertility were the main indications for recommending reproductive medicine immediately after interventional laparoscopy. In milder cases, with unilateral endometriomas of small sizes, preserved tubal permeability at the methyl blue test, younger age under 30 years, the patient's financial situation or lack of desire for immediate fertility, were indications for getting pregnant naturally.

During laparoscopy, the cysts were removed by ovarian stripping, the endometrial implants were coagulated and the adhesions were totally or partially lysed; in case of hydrosalpinx, the tubes were cauterized or even removed by salpingectomy for successful IVF. In 3 instances laparoscopy was re-converted to laparotomy: in the first case, for large bilateral cysts, with multiple adhesions, in the second case, for suspicion of ovarian neoplasm, confirmed by extemporaneous exam, and in the third case, for difficult hemostasis. The excision of endometriomas was made with observing the ovarian vascularization, without excessive coagulation and with observing the remaining ovarian cortex to preserve the follicular reserve. Tubal permeability was tested in all cases in order to make a decision regarding the possibility of becoming pregnant by natural way after the removal of endometriosis. All cases with proximal obstruction were counselled for immediate reproductive medicine. The study had in view particularly the chances of becoming pregnant and the occurrence of recurrences (fig. 1).

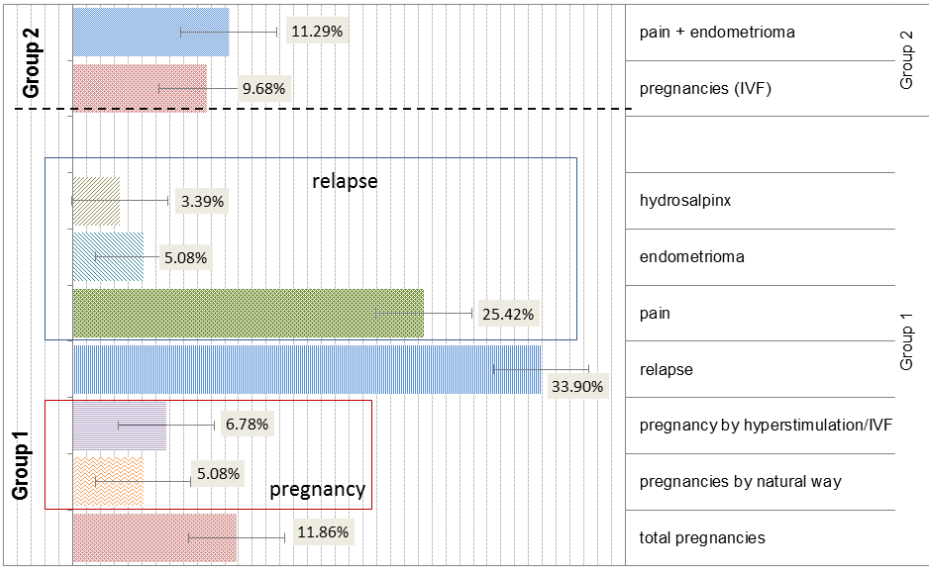

Fig. 1. Pregnancies and recurrence rate

In group 1, 3 (5.08%) natural pregnancies were obtained. The expectancy for pregnancy was 1 year. At the end of the year, the patients were called for ultrasound and clinical re-assessment and reconsidering the therapeutic decision. Pain reoccurred after 1 year in 15 cases (25.42%), and endometrioma in 3 cases (5.08%), but in all 3 cases its size was under 2 cm. The hydrosalpinx occurred postoperatively in 2 cases (3.39%) probably as a result of adhesion formation and change in pelvic anatomy. The remaining 56 patients were counselled for reproductive medicine, 2 pregnancies being obtained following hyperstimulation with *clomiphene*, without intrauterine insemination (IUI), 1 pregnancy following hyperstimulation with gonal and IUI and only one pregnancy following 17 IVF procedures performed in 10 patients. The total number of obtained pregnancies was 7 (of 59 patients - 11.86%). Clinically or ultrasonically detected recurrence occurred in 3 cases (5.08%).

In group 2, 240 IUIs were performed of which only 20 by natural cycle without ovarian hyperstimulation and only 2 pregnancies were obtained, and 4 twin pregnancies were obtained from 80 IVFs; therefore, the pregnancy rate was 6 of 61. Recurrences occurred in 7 cases by reoccurrence of pelvic pain and ovarian endometriosis. A laparoscopic reintervention for adhesions lysis was performed in all 7 cases. In the 2 cases with recurrence of endometriosis stripping was difficult because of adhesions and postoperative fibrosis. In the 7 re-operated cases IVF was performed, but only one pregnancy was obtained. Patients older than 35 years with recurrence did not get pregnant (the patient who got pregnant was 28 years old).

From the point of view of becoming pregnant, no significant differences between the two groups were found (χ^2=2.06, p=0.0891, 95% CI); in group 1, 11.86% pregnancies were obtained while in group 2 the pregnancy rate was 11.29%. Based on the nonparametric method of analysis of contingency tables we could estimate the odds of becoming pregnant in the study groups, obtaining an odds ratio (OR=1.16, CI: 1.04-2.23, 95% CI).This result indicates that the odds of becoming pregnant in group 1 was not significantly higher, and the estimation was made for a confidence interval of 95%.

TABLE I

Estimated parameters in the Kaplan-Meier analysis of pregnancy rate in the two study groups

Means and Medians for pregnancies

	Mean[a]				Median			
	Estimate (weeks)	Std. Error	95% Confidence Interval		Estimate (Weeks)	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound			Lower Bound	Upper Bound
Group 1	27.663	1.345	25.028	30.299	24.000	.571	22.880	25.120
Group 2	30.951	1.652	27.714	34.188	24.000	1.171	21.706	26.294
Overall	29.572	1.113	27.391	31.754	24.000	.446	23.127	24.873

a. Estimation is limited to the largest pregnancies time if it is censored.

The study had in view the assessment of pregnancies obtained in the study groups and therewith, the time interval (number of weeks) from the time of intervention until the occurrence of the pregnancy. The Kaplan-Meier analysis (fig. 2) enabled the assessment of the mean value and the median value of the number of weeks until

becoming pregnant, and these values did not show significant differences (χ^2=1.55, p=0.212, 95% CI).

The mean time interval (weeks) between intervention and becoming pregnant was 29.5 weeks, and the median valued indicated that 50% of cases got pregnant in the first 24 weeks after the intervention (table II). The Kaplan-Meier curves clearly showed an absence of significant differences between the pregnancy rates in the two study groups (fig. 2).

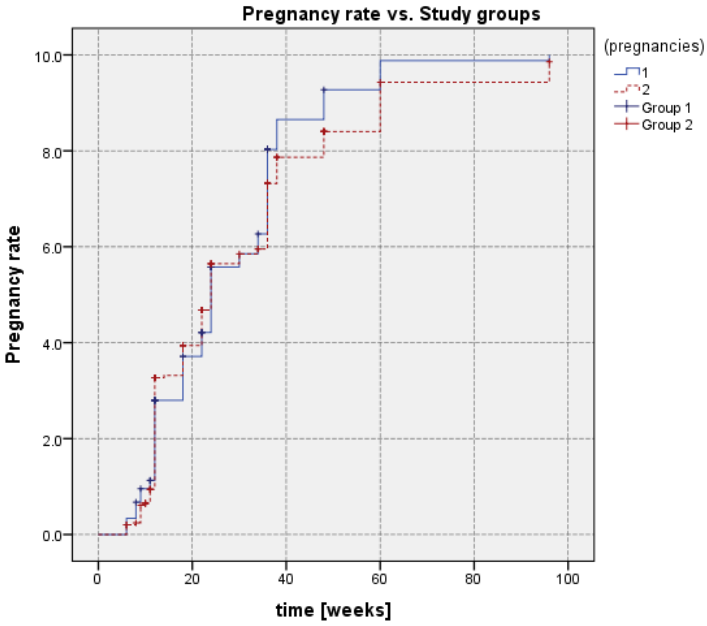

Fig.2. Kaplan-Meier curves of pregnancy rate in the two study groups

TABLE II

Result of comparison tests regarding the rate of becoming pregnant

Overall Comparisons

	Chi-Square	df	Sig.
Log Rank (Mantel-Cox)	1.555	1	.212
Breslow (Generalized Wilcoxon)	.025	1	.874
Tarone-Ware	.326	1	.568

DISCUSSION

In two cohort prospective studies on infertile women with moderate and severe endometriosis (AFS classification) who underwent laparoscopic surgery, the pregnancy rate was 57–69% (moderate endometriosis) and 52–68% (severe endometriosis) (3, 4). In our study, the pregnancy rate was much lower (maximum 11.86%), but we did not use the R-AFS classification.

Data in the literature suggest that cystectomy increases the pregnancy rate compared to drainage and electrocoagulation of the cyst (5). In our study, following cystectomy 7 patients got pregnant although they had a history of infertility of 2 to 16 years

(mean 4 years) and of drained endometriomas without cystectomy. One patient who was infertile for 16 years and had bilateral endometrial cyst got spontaneously pregnant 3 months after the surgical intervention. Both surgical techniques, ablation and drainage, can affect the ovarian reserve, but it appears that pregnancy rate is higher after cystectomy (6).

There are no comparative studies on pregnancy rate between the expectant management after laparoscopic surgery and the active management by human assisted reproduction techniques. In our case, there were no significant differences in pregnancy rate between the two groups (6.78% group 1 *vs.* 9.68% group 2). A bias would be related to the fact that no intracytoplasmic sperm injection (ICSI) was performed in any of the patients and another one by the fact that patients with an AMH lower than 1 ng/ml were not accepted for IVF. A meta-analysis on 22 studies (7) demonstrated that women with endometriosis have a pregnancy rate below 35%. Our study confirmed this finding.

The therapeutic approach depended on the desire and individual characteristics of the patient: age, number of previous interventions, failure or success of previous therapies, endometrioma size, tubal damage, and exclusion of occult malignancies

Human assisted reproduction as a first choice before laparoscopy has a low success rate and high costs, being the first choice only in women older than 35 years (8, 9). Postoperator terapeutic management can be used to delay surgery until fertility is desired (10).In Romania the reported endometriosis rate has increased alarmingly, either because of the advances in diagnosis (ultrasound diagnostic laparoscopy) or because more women who have chronic pelvic pain or infertility seek medical advice (11). Therefore, gynecologists should be prepared to counsel such patients. It is the doctor's duty to make a diagnosis as early as possible and to inform the patient about the possibility of disease recurrence and the associated infertility. After making the diagnosis, which most of the time is late, it is necessary to establish a treatment protocol, which most of the time does not consider the Hippocratic principle *primum non nocere*, because the disease is so invalidating through the chronic pelvic pain that the only therapeutic option is total hysterectomy. Affected women are usually nulliparous, and the recommendation of a radical surgical procedure for removing all endometrial fragments, which practically would lead to the relief of pain caused by the endometrial implants, would put the patient in the impossibility of conceiving a child in the future (12,13,14).

CONCLUSIONS

In our study, in spite of a strong relation of causality between endometriosis and infertility, it is clear that treatment of endometriosis may improve fertility in some cases. Expectant management can by a reasonable approach in younger patients with less severe disease and a shorter period of infertility.

Laparoscopic surgery seems a better approach compared to medical therapy in endometriosis increasing the odds of spontaneous pregnancies by reconstructing the pelvic anatomy after adhesiolysis.

The pregnancy rate after immediate post-cystectomy procedures is equal to that of patients on postoperative expectant management. Expectant management should be indicated only in younger patients with perfect pelvic anatomy and

without associated causes for obtaining a spontaneous pregnancy. Recurrence risk is equally high after expectant management or human assisted reproduction.

In women with minimal to mild endometriosis, IUI with controlled ovarian stimulation may be effective in increasing live birth rate, compared with expectant management. Furthermore, IUI with controlled ovarian stimulation may be more effective in increasing pregnancy rate than IUI alone, and may be as effective in women with minimal or mild endometriosis within 6 months of surgical treatment as in women with unexplained infertility.

Based on the conducted study we can recommend controlled ovarian hyperstimulation treatments followed by intrauterine insemination which are better, cheaper and easier options than IVF, only if the surgical anatomy of the pelvis is corrected by the lysis of adhesions, removal of cysts and positive bilateral dye test. Postoperative active intervention through procedures of assisted reproductive medicine increases the pregnancy rate (11.86%) regardless of the size of the endometrioma compared to the passive management.

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LAPAROSCOPIC TREATMENT OF A LARGE SYMPTOMATIC LEIOMYOMA OF THE ROUND LIGAMENT

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LAPAROSCOPIC TREATMENT OF A LARGE SYMPTOMATIC LEIOMYOMA OF THE ROUND LIGAMENT (Abstract): Round ligament leiomyoma are very rare benign tumors. They can appear on the extraperitoneal part of the round ligament (more common) or on the intraperitoneal round ligament (very rare). We present a case of 48-year-old women diagnosed with a large (122/108/98 mm) intraperitoneal leiomyoma of the left round ligament that was managed by laparoscopy. Laparoscopy proved to be a highly useful method, overcoming the limitation of ultrasound or CT and providing a safe and minimally invasive treatment method. **Keywords**: ROUND LIGAMENT LEIOMYOMA, LAPAROSCOPY, ROUND LIGAMENT TUMOUR.

Although uterine myoma is one of the most common benign gynecologic pathology, extra-uterine myoma are quite rare. Extra-uterine myoma usually appears in the genito-urinary tract (vulva, broad ligament, ovaries, round ligament, urethra). Round ligament leiomyoma are very rare benign tumors. The exact incidence of round ligament myoma is not known, specialized literature presenting several cases, most of them case report or mini-series. Usually they appear in the extraperitoneal round ligament and rarer in the intraperitoneal round ligament. The myoma of the left intraperitoneal ligament are considered extremely rare (1, 2). This is the case of a large leiomyoma of the round ligament situated in the left intraperitoneal round ligament, initially diagnosed as pedunculated uterine myoma, and managed by laparoscopy.

CASE REPORT

A 48-year-old pregnant female, came to gynecology for abdominal pain for several months, treated intermittently with non-inflammatory steroids. Her past medical history was unremarkable. Speculum examination revealed normal cervix and vagina. On vaginal examination, the uterus was deviated to the right, normal in size, with a large non-tender mobile solid lobulated mass, palpated in the left iliac fossa. Abdominal and endovaginal ultrasound revealed a myoma-like tumor 100/90/100 mm situated to the left side of the uterus (fig.

1,2). Computed tomography (CT) scan confirmed the presence and the size of the tumor mass (122/109/98mm) localized in the left iliac fossa. The established diagnosis was a pedunculated uterine myoma. A laparoscopy was scheduled after careful explanation and informed consent from the patient. Intraoperatory it was observed a large myoma-like mass originating in the left round ligament of the uterus (fig. 3,4). The uterus, both ovary and tubes were normal and well delineated from the tumor. The mass was removed entirely and histopathology result confirmed the diagnosis of leiomyoma. The post-operative course was otherwise uneventful and patient was discharged the second day after the operation.

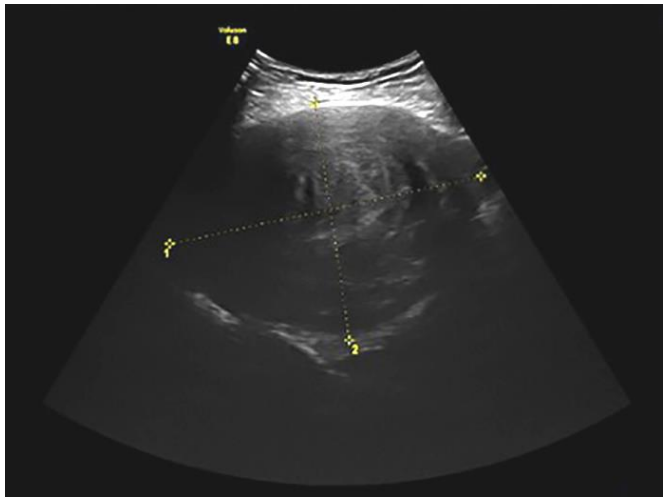

Fig. 1. Ultrasound image: longitudinal view of the lower abdomen showing a mass with echogenic characteristics of uterine fibroid in the left adnexal area

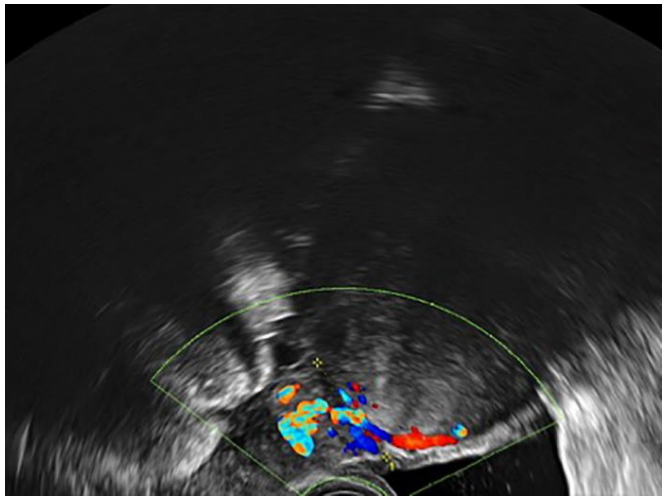

Fig. 2. Ultrasound image: the tumor is situated near the uterus

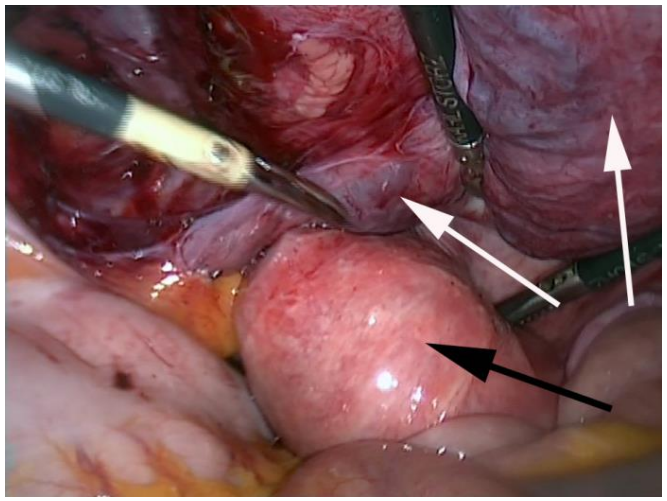

Fig. 3. Intraoperatory image: large left round ligament leiomyoma (white arrows); the uterus (white arrow)

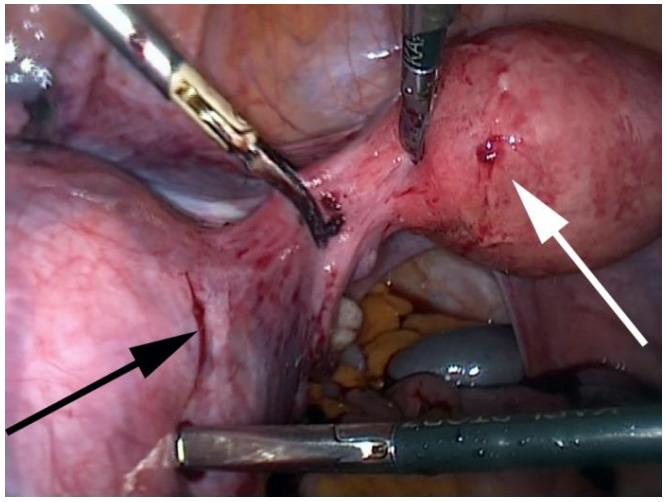

Fig. 4. Intraoperatory image: black arrow-large tumor of left round ligament leiomyoma, white arrow the uterus

DISCUSSION

The round ligament is a fibrous band attached to the uterus and extends laterally and anteriorly, hooks around the inferior epigastric artery, traverses the inguinal canal, and terminates in the *labium majus*. It is responsible for the descent of the ovary from the posterior abdominal wall to the uterus. The round ligament is accompanied in the fetus, and occasionally in the adult, by a process of peritoneum, the processes vaginalis. The round ligaments consist principally of muscular tissue, prolonged from the uterus and fibrous and areolar tissue, besides blood vessels, lymphatics and nerves.

Tumors of the round ligament are rare, the exact incidence it is not known. Among these tumors, leiomyomas are the most frequent, followed by endometriotic tumors. Various very rare tumors can also be found. Transformation of muscular structure of the female genital tract to leiomyoma involves somatic mutations of normal smooth muscle and complex interactions between sex steroids and local growth factors (3). Smith et al (4) demonstrated that both estrogen and progesterone receptors are found in round ligament. The presence of these receptors could explain why the majority of cases described in the literature were diagnosed during perimenopause. There are also several cases describes during pregnancy or menopause (2, 3, 5, 6).

Approximately one half to two-third of round ligament leiomyomas appear in the extraperitoneal part of the round ligament and for unknown reason are more common on the right side (7). Exceptionally a tumor can have a both abdominal and extraperitoneal development from the inguinal insertion (8). When they appear in the inguinal canal they can be misdiagnosed as incarcerated hernia, cyst of the Nuck canal, thrombosis of varicose veins around round ligament. They also can be diagnosed as complications by incarceration in the inguinal canal (9). Tumors of the intraperitoneal cavity can be misdiagnosed as ovarian tumors or pedunculated uterine myoma, like in our case. Therefore fibroids should always be kept in mind during the differential diagnosis of any pelvic mass. The size can vary widely from 2-3 cm when they can be discovered incidentally to more than 10-20 cm, cases that are usually symptomatic.

A useful tool for diagnosis is the ultrasound examination where typically a myoma has whorled pattern appearance with variable echogenicity, depending on the presence of degeneration and calcification. In some cases cystic degeneration with extensive edema forming cystic, fluid-filled spaces can appear and the diagnosis can be more difficult to establish. Magnetic resonance imaging (MRI) is the best diagnostic modality, as it can make the difference from round ligament fibroids, ovarian masses or broad ligament mass. But even in MRI confusion can persist in presence of degenerative changes like hyalinization or cystic degeneration. Another pitfall for the diagnosis is the fact that sometimes, round ligament fibroids can mimic a pseudo-Meig's syndrome with elevated CA-125 pointing towards metastatic ovarian carcinoma (10). In these cases differentiating round ligament fibroids from ovarian masses before surgery is a diagnostic challenge. As we see, imaging techniques can sometimes misdiagnose these tumors. In these difficult cases, laparoscopy is a valuable tool not only for diagnosis but also for its possibility to excise these tumors.

When the tumor is situated in the inguinal canal, the surgical approach is inguinal and some authors recommend a formal inguinal hernia repair technique, performed preferably with mesh (9). However, other types of hernia repair without mesh could also be considered depending on surgeons' preference. When the tumor is intraperitoneal the management depends on the age of the patient, the desire to preserve fertility or other associated condition (like uterine myoma). A laparoscopic conservative approach represented by laparoscopic myomectomy is feasible in young patients who desire to preserve fertility.

We confirm with our case the highly valuable role of laparoscopy, which not only overcomes the limitation of imaging, ultrasound or CT, but also provides a safe and minimally invasive treatment method.

CONCLUSIONS

In our study we observed that a large round ligament fibroid is a rare entity and can be easily mistaken for a uterine pedunculated myoma when it develops in the intraperitoneal ligament. Leiomyoma of the round ligament must be retained as a possible etiology of inguinal mass or pelvic tumor. Laparoscopy overcomes the limitation of imaging, ultrasound or CT, but also provides a safe and minimally invasive treatment method even in a large myoma.

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TREATMENT OF PROXIMAL HUMERAL FRACTURES USING "TELEGRAPH" ROD: RETROSPECTIVE STUDY OF 47 CASES

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TREATMENT OF PROXIMAL HUMERAL FRACTURES USING THE "TELEGRAPH" ROD: RETROSPECTIVE STUDY OF 47 CASES (Abstract**): Aim:** To assess the early results observed in a series of patients treated with a first-intention Telegraph rod. **Material and methods:** In the interval 2011-2013 forty-seven patients with fractures of the upper extremity of the humerus were assessed both clinically and radiologically. Mean follow-up was 8.9 months. The median age was 61 years. The study group included 39 women and 8 men, the left shoulder being most commonly affected (33 cases). Cause of injury was accidental fall in 39 cases fall, sports injury in 6 cases, and car crash injury in 2 cases. Neer classification was used to assess the type of fracture. For qualitative analysis, we used the functional *Constant-Murley score* in comparison with the contralateral side. The analysis of fracture reduction was based on plain X-rays in anterior-posterior plane and measurement of humeral head tilt relative to diaphyseal shaft. **Results:** No case showed infection, humeral head necrosis occurred in 2 cases, loss of reduction in 3 cases which required repeat reduction, and a subacromial impingement syndrome in 2 cases. Average *Constant-Murley score* was 70.33 points for the injured and 86.4 points for the healthy shoulder. Mean value of reduction angle of the humeral head relative to diaphyseal shaft was 33.75 degrees (range 3 to 81 degrees). **Conclusions:** Telegraph rods provide a secure fit in extra-articular and simple articular fractures of the proximal humerus. When performing a closed anatomical reduction, the method provides good functional results in most patients. This option should be considered in young patients and even in the elderly, the results being comparable to those obtained by hemiarthroplasty. **Keywords:** HUMERAL FRACTURES, RETROSPECTIVE STUDY, TELEGRAPH ROD

The incidence of proximal humeral fractures is increasing accounting for 5.7% of all fractures (1). This increase occurs mainly in the elderly, osteoporotic people (1-5). Treatment options range from orthopedic treatment to intra medullary rods, open reduction with internal fixation or arthroplasty. Fractures without/with minimal displacement should usually be treated conservatively, a good functional outcome for this group of fractures being reported (6). Fortunately, 70-80% of these fractures can be treated conservatively. On the other hand, there is a consensus that displaced for four-part fractures and/or comminuted fractures, fractures-dislocations in the elderly should be treated with primary hemi arthroplasty (7).

Minimally invasive techniques with closed reduction began to be supported in order to preserve the soft tissue, vascularization of the humeral head and fracture

fragments. Therefore, many surgeons choose to use intramedullary rods suitable for fixing the fracture, while others tend to favor the use of locked plates (8-11).

AIM

The purpose of this study was to evaluate the early results observed in a number of patients treated with a first intention Telegraph rod.

MATERIAL AND METHODS

In the interval 2011-2013, 47 patients with fractures of the upper extremity of the humerus were assessed clinically and radiological. Mean follow-up was 8.9 months (range 7 -12 months). The median age was 61 years (range 29-87 years). The study group included 39 women and 8 men, the left shoulder being most commonly affected (33 cases). Cause of injury was accidental fall in 39 cases (82.97%), sports injury in 6 cases (12.76%) and car crash injury in 2 cases (4.25%). To assess the type of fracture we used Neer classification (group A - fractures with two fragments, 31/47 cases (65.95%), group B - 3 fracture fragments, 13/47 cases (27,65%), group C - 4 fracture fragments, 3/47 cases (6,38%).

The surgical technique consisted of: loco-regional anesthesia, the patient in supine position, closed/open reduction, incision of about 4 cm above the acromion, based on the greater tuberosity, transdeltoidian. Site of rod insertion was at the edge of the articular surface between it and the greater tuberosity, in the middle of the humeral head. The rod was inserted with gentle movements of rotation. It was positioned in the center of the humeral head under Rx-TV control and fixed with screws. Distally the rod was fixed with one or two screws.

The average time of surgery was 30 minutes (range 25-47 minutes). Postoperatively the affected upper limb was immobilized in a scarf for two weeks and physiotherapy was initiated after 2-4 weeks depending on fracture characteristics. Clinical and radiological follow up was performed at 1, 3, 6, 12 months (fig. 1, 2).

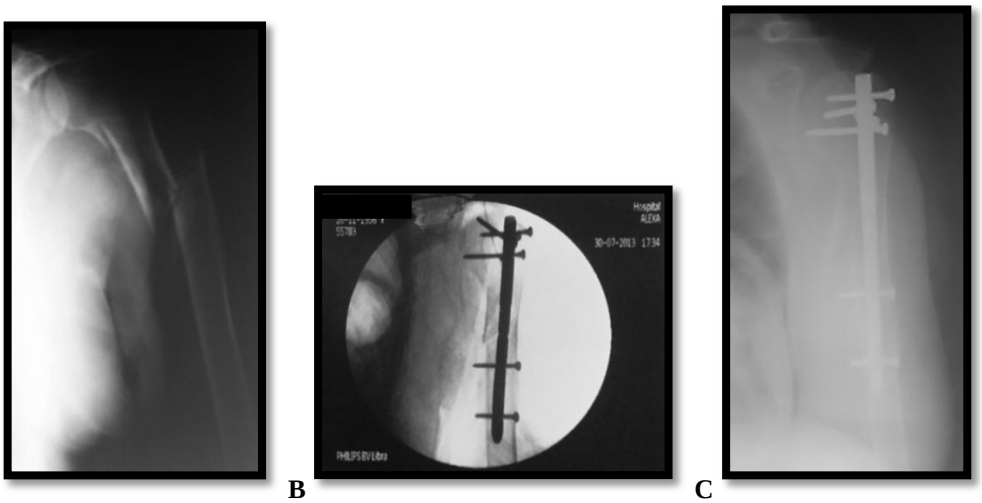

Fig. 1. Patient M.R., M, 46 years old: **A –** bifocal fracture of the proximal humeral extremity, **B -** fluoroscopic control immediately after surgery, **C –** X-ray control at 3 months.

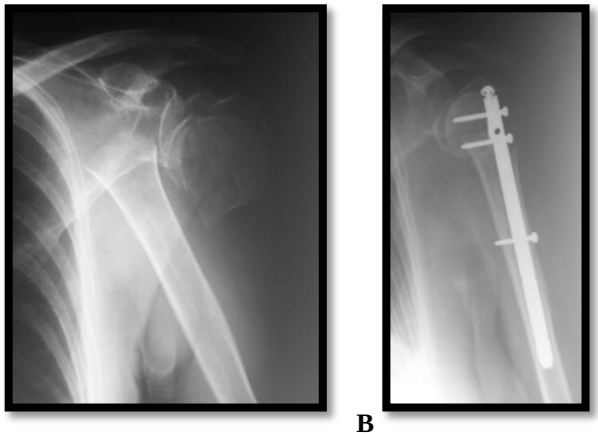

Fig. 2. Patient A.E., F, 59 years old: **A –** fracture-dislocation of the proximal humeral extremity Neer IV, **B –** X-ray control at 1 month.

All fractures healed within 1 to months. For qualitative analysis we used the functional *Constant-Murley score* in comparison with the contralateral side. For the analysis of fracture reduction we used plane X-rays in anterior-posterior view and measured the humeral head tilt relative to diaphyseal shaft. Normally this angle is 45 degrees. Reduction was considered adequate for an angle between 30 degrees and 60 degrees.

RESULTS

No case showed infection, humeral head necrosis occurred in 2 cases, loss of reduction in 3 cases requiring repeat reduction, and subacromial impingement syndrome in 2 cases that led to rod removal at 6 months (fig. 3).

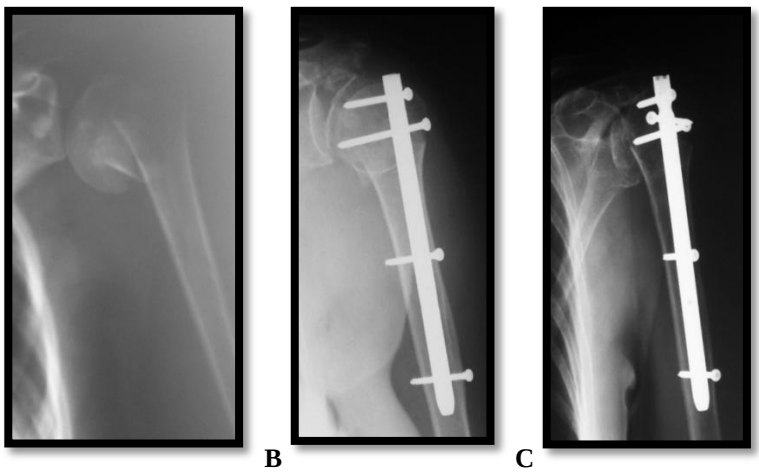

Fig. 3. Patient R.I., F, 65 years old: **A –**fracture of the proximal humeral extremity Neer III, **B -** X-ray control at 1 month, **C –** X-ray control at 3 months - loss of reduction with subacromial impingement syndrome.

Average *Constant-Murley score* was 70.33 points (range 39-98 points) for the injured and 86.4 points the healthy shoulder. Average *Constant-Murley score* for group A was 78.5 points (range 59-98 points), group B - 70.5 points (range 36-82 points), and group C - 62 points (range 49-75 points).

The mean head-shaft angle reduction 33.75 degrees (range 3 to 81 degrees). In group A reduction was considered good in 24 cases (77.41%) and poor in 7 cases (22.58%), in group B the reduction was considered good in 7 cases (53.84%) and poor in 5 cases (38.46%), and in group C reduction was considered good in 2 cases (66.66%)and poor in 1 case (33.33%).

DISCUSSION

A good surgical fixation should include anatomic reduction of the tuberosities, restoration of length, alignment and rotation of fragments. This should be done with minimal trauma to soft tissue, preserving the medial periosteal hinge and blood supply. To prevent impingement, the amount of hardware to the cortical surface should be minimal. In a biomechanical cadaver study, intramedullary fixation proved to be more stable and more resistant than traditional plate or plate locked with screws (12). This allows for stable fixation through a small incision with minimal soft tissue damage, facilitating early mobilization and functional recovery of the shoulder.

Cuny et al. (13) followed the results obtained after using 64 telegraph rods. They were favorable, including patients with complex fractures involving three or four pieces. In addition to providing a stable anatomic reconstruction, Telegraph rod has the advantage of allowing early mobilization of the shoulder joint, this method being considered a useful alternative to prosthetic reconstruction for traumatic fractures of the proximal humerus.

Blum et al. (14) treated 151 unstable fractures of the proximal humerus with two and three fragments, and observed as complications: articular surface piercing (n= 8), pain related to the implant (n=10), secondary displacement (n=2), nonunion (n=2), necrosis of the humeral head (n= 3) and superficial infection (n=1). Functional results obtained were good, *Constant-Murley score* having an average value of 75.3 compared with 89.9 in the healthy shoulder. Cuny et al. (15) retrospectively analyzed 67 patients with proximal humerus fractures treated with telegraph rod and noticed that this technique provides a reproducible and satisfactory surgical result for cervical and valgus impacted fractures, the result being less satisfactory for unstable fractures or fracture-dislocations joint (avascular necrosis - 37.5%, secondary displacement in all 6 cases tuberosity fractures with four sides, subacromial impingement in 8 cases).

Popescu et al. (16), based on the results obtained after treating 21 fractures of the proximal humerus through osteosynthesis with T2 PHN, indicate that it is a safe and reliable method in the treatment of two- and three-parts proximal humeral fractures. Proximal fixation mechanism decreases the rate of screw slippage, a common complication described in the literature. Good functional results were obtained in patients younger than 70 years, but these were not statistically significant.

Thomazeau et al. (17), treating 51 patients with unstable 3-part or 4-part proximal humerus fractures, concluded that telegraph rod fixation can be recommended for 3-part fractures, because osteonecrosis

is uncommon and well tolerated in this subgroup. However, this method was not more beneficial than other techniques of internal fixation to prevent osteonecrosis or malunion in patients with 4-part fractures.

CONCLUSIONS

In our study, telegraph rods provides a secure fit in extra-articular and simple articular fractures of the proximal humerus. When performing a closed anatomical reduction, the method provides good functional results in most patients. This option should be considered in young patients and even in the elderly, the results being comparable to those obtained by hemiarthroplasty.

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NEWS

HISTOLOGIC AND IMMUNOHISTOCHEMICAL ANALYSES OF ENDOMETRIAL CARCINOMAS

Endometrial cancer is one of the most common malignancies of the gynecologic tract. Endometrial cancers are classified into 2 broad histologic types, defined as type I and type II. Type I consists of endometrioid carcinoma (EMC) and its histologic variants; type II endometrial cancer includes serous carcinoma, clear cell carcinoma, and carcino sarcoma. Uterine serous carcinoma (ESC), type II, accounts for approximately 10% of cases, whereas EMC, type I, encompasses bout 80% to 85% of cases. Clear cell carcinoma and other rare forms of endometrial carcinoma comprise the few remaining cases. Type I and type II endometrial carcinomas are considered biologically distinct disease entities, characterized by their divergent histology, molecular genetics, tumor genesis, and clinical behavior. Different patterns of molecular alterations are now thought to underlie the pathogenesis and/or progression of type I and type II uterine carcinomas. For example, the most common genetic alterations in EMC include microsatellite instability, mutations of PTEN, b-catenin, PIK3CA, and K-ras. In contrast, in the case of ESC, p53 mutations are common and ERBB2 (formerly HER2) overexpression or amplification is also frequently seen. Clinically, ESC has a higher propensity for lymphovascular invasion and intraperitoneal as well as extra-abdominal spread than EMC. During presentation, approximately 40% to 70% of women with ESC will have disease beyond the uterus. Accurate diagnosis of tumor type in endometrial biopsies/curetting specimens is critical for patient management. Tumor classification in endometrial adenocarcinoma based on histology and immunohistochemistry has some limitations, particularly in lesions with mixed epithelial differentiation or vague morphology. Moving forward, development of molecular biomarkers may be the next step in refining our classification of these tumors in general and on a case-by-case basis (Wei JJ, Painta Al, Keh P. Histologic and Immunohistochemical Analyses of Endometrial Carcinomas. *Arch Pathol Lab Med*. 2013; 137: 1574-1583).

Doina Butcovan

PAIN AS A LATE EMOTIONAL REACTION. CLINICAL RESEARCH EVALUATING THE PSYCHOSOCIAL EXPRESSION OF PAIN DISORDER IN PREOPERATIVE AND POSTOPERATIVE PERIOD

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PAIN AS A LATE EMOTIONAL REACTION - CLINICAL RESEARCH EVALUATING THE PSYCHOSOCIAL EXPRESSION OF PAIN DISORDER IN PREOPERATIVE AND POSTOPERATIVE PERIOD (Abstract) **Aim:** A comparative analysis of the influence of psychosocial factors on pain perception in preoperative and postoperative period in patients scheduled for abdominal surgery. **Material and methods:** A psychological assessment was performed in 50 patients without psychiatric history scheduled for abdominal surgery. The verbal expression of pain, intensity of anxiety, catastrophizing level, and the interpersonal relationships were assessed. **Results:** Surgery has a powerful effect on the perception of pain intensity. Postoperatively patients perceive significantly lower pain than in the preoperative period (p=0.001). In the postoperative period the patients mainly use emotional and evaluative verbal expressions (p=0.000 and p=0.000, respectively), have a high index of anxiety (p=0.000), adopt a higher level of coping self-statements (p=0.002), and have a higher level of perception of others (family, friends) (p=0.005). **Conclusions:** In patients undergoing surgery, surgery affects their psyche, triggering a series of events that can have psychological effects on pain intensity, doctor-patient relationship, postoperative complications, and shortening of recovery time. **Keywords:** ALGIC DISORDER, PAIN PERCEPTION, PSYCHOSOCIAL FACTORS, PREOPERATIVE PHASE, POSTOPERATIVE PERIOD

Surgery is the most fascinating and dramatic specialty of medicine, with a large emotional charge for both the surgeon and patient, often at the edge of survival. Surgical act depends not only on lucid thinking and unerringly movement of surgeon hand, but also on how the patient reacts to the surgical act, or later on how he recovers from the body changes (even if they are lifesaving) caused by surgical maneuvers. More and more studies on patients with various types of surgery show that psychical factors and psychological implications play an important role in the dynamics of doctor-patient relationship and in postoperative course (1, 2, 3, 4).

There are many opinions in favor of replacing the technicist-somatization mentality with psychosomatics, recognizing that psychological characteristics are present in most surgeries: the novelty of the situation created by the presence of lesion or dysfunction to be corrected by the surgeon (with special effects on the surgeon) and anxiety (extreme for patient, but great for surgeon) generated by the surgical risks that often target patient survival. The aim of this research was to study how psycho-

logical factors influence the surgical act and patient mental state.

MATERIAL AND METHODS

Patients. This research was carried out in 50 inpatients scheduled for abdominal surgery, was approved by the Ethics Committee of the Iaşi „Dr. Iacob Czihac" Emergency Military Hospital, and was conducted in conformity to the Declaration of Helsinki.

The inpatients enrolled in this research had predominantly abdominal pain, and were scheduled for abdominal surgery. Exclusion criteria included prior abdominal surgery and psychiatric history.

Patient examination. All patients underwent a clinical interview: the McGill Pain Questionnaire (5), Coping Strategies Questionnaire (6), Anxiety Symptom Questionnaire (7), Pain Catastrophizing Scale (8) and West Haven-Yale Multidimensional Pain Inventory (9).

The clinical interview was designed to highlight: non-verbal indicators of pain, family history, description, evaluation, frequency and persistence of present pain, memory of pain, pain behavior, perceived attitudes of others, self-assessment of pain tolerance, types of pain therapy, indicating the body areas affected by pain so far, beliefs about pain, involvement in daily activities, family relationships, medical information about pain.

The McGill Pain Questionnaire is a self-report questionnaire that allows individuals to give their doctor a good description of the quality and intensity of pain that they are experiencing. The McGill Pain Questionnaire consists primarily of 3 major classes of word descriptors – sensory, affective and evaluative – that are used by patients to specify subjective pain experience. It also contains an intensity scale and other items to determine the properties of pain experience. The 3 major measures are: 1. pain rating index, based on two types of numerical values that can be assigned to each word descriptor, 2. number of words chosen; and 3. present pain intensity based on a 1-5 intensity scale.

The Coping Strategies Questionnaire (CSQ) consists of 50 items assessing patient self rated use of cognitive and behavioral strategies to cope with pain. It comprises six subscales for cognitive strategies (ignoring pain, reinterpretation of pain, diverting attention, coping self statements, catastrophizing, praying/hoping) and two subscales for behavioral strategies (increasing activity levels and increasing pain behaviors). Each coping strategy subscale consists of six items measured with a numerical rating scale ranging from 0 (never do that) to 6 (always do that) indicating how frequently the strategy is used to cope with pain. An additional two single item questions each with a scoring range of 0–6 are used as effectiveness ratings of control over pain and ability to decrease pain.

The Anxiety Symptom Questionnaire (LWASQ), developed to measure the level of anxiety, consists of 36 items, which include somatic, cognitive and behavioral components of anxiety: 16 somatic items (physical manifestation of anxiety), 9 behavioral items (avoidance of social situations), and 11 cognitive items (the tendency to worry and ruminate). The scale ranges from zero (never) to eight (extremely often).

The Pain Catastrophizing Scale (PCS) is a 13 item scale, with each item rated on a 5–point scale: 0 (not at all) to 4 (all the time), broken into three subscales: magnification, rumination, and helplessness. The scale was developed as a self-report measurement tool that provided a valid index of

catastrophizing in clinical and non-clinical populations, a reliable and valid measurement tool for catastrophizing. From a clinical perspective, the PCS is useful in identifying individuals that may be more susceptible to high distress responses from aversive medical procedures such as chemotherapy or surgery.

The West Haven-Yale Multidimensional Pain Inventory (WHYMPI) is designed to provide a brief, psychometrically-sound, and comprehensive assessment of the important components of the chronic pain experience. The WHYMPI is a 52-item, 12-scale inventory that is divided into three parts. Part I includes five scales designed to measure important dimensions of the chronic pain experience including; 1. perceived interference of pain in vocational, social/recreational, and family/marital functioning, 2. support or concern from spouse or significant other, 3. pain severity, 4. perceived life control, and 5. affective distress. Part II assesses patients' perceptions of the degree to which spouses or significant others display Solicitous, Distracting or Negative responses to their pain behaviors and complaints. Part III assesses patients' report of the frequency with which they engage in four categories of common everyday activities; Household Chores, Outdoor Work, Activities Away from Home, and Social Activities. In addition to the individual scale scores, a General Activity scale score, obtained from the combination of all four activity scale scores, has been recommended for some purposes. Patient responses to WHYMPI items are given using a 7-point numeric scale.

Follow-up. Psychological evaluation of patients was performed at surgeon's request, being focused on their behavior during surgical preparation. Postoperative assessment was done one month after surgery, the mandatory postoperative follow-up (randomized study).

Main outcome measures. The primary outcome measure was emphasizing pain perception: sensory, affective and evaluative pain experience; pain rating index; the number of words chosen; and the present pain intensity. The secondary outcome measures included determination of: anxiety levels, catastrophizing levels, coping types, and components of chronic pain experience.

Statistical analysis. The continuous variables were tested using Student's tests (normally distributed data). The statistical analysis was performed using *SPSS 13.0* statistical software.

RESULTS

Regarding the perception of pain by patients in preoperative and postoperative periods, this research did not show statistically significant gender, age and education differences in pain threshold $p < 0.05$.

The total index measuring patient pain after surgery was significantly lower ($p=0.001$) than the total index measuring patient pain before surgery. The sensory component of patient pain after surgery was significantly lower ($p=0.000$) than the sensory component of the patient pain prior to surgery. The affective component of patient pain after surgery was significantly higher ($p=0.000$) than the affective component of patient pain before surgery. The evaluative component of the patients pain after surgery was significantly higher ($p=0.000$) than the evaluative component of patient pain before surgery. Generalized anxiety level of patients after surgery is significantly higher ($p=0,000$) than the level of generalized anxiety in patients prior to surgery. The generalized anxiety level of patients after surgery was significantly higher ($p=0.000$) than the level of

generalized anxiety prior to surgery. The level of reactions of others (family, friends) to patient complaints of post-surgery pain was significantly higher (p=0.000) than the level of reactions of others (family, friends) to patient complaints of preoperative pain.

DISCUSSION

This research is relevant because it demonstrates how verbal expression of pain, emotional disposal, cognitions and interpersonal relationships vary by surgery time. A comparative analysis of preoperative and postoperative pain perception indicates a significantly less overall postoperative pain. This result is inconsistent with studies that claim that post-surgical pain intensity index increases (10, 11, 12). The lower intensity of the perceived post-surgery pain can be interpreted psychologically by the quasi-objective relationship between pain caused by somatic disease, surgery, perceptual environment (due to anesthesia and analgesics the patient does not experience any pain) and expectations of healing - the patient thinks he regains health.

Postoperatively patients describe less sensory pain than preoperatively. The sensory discriminative dimension reflects the somatic aspect of pain. Preoperative pain is considered an acute pain which initiates protective physiological mechanisms in response to somatic injury. Postoperatively patients do not feel threat to the body, and therefore describe less sensory pain than in the preoperative phase. Surgery produces changes in bodily sensations and mental state; pain is interpreted by the patient as having different clinical significances, as an indicator of disease or of healing, and therefore patients describe less sensory pain postoperatively than preoperatively.

Patient language is an indicator of mental state; thus post-surgery patient is dominant in affective and evaluative verbal expressions.

Postoperatively patients perceive more emotional pain than preoperatively, describing emotional pain one month after surgery indicates that patients still live under the "threat" of the disease, and that the pace of health progress, the recovery, is inconsistent with the expectations.

In postoperative period patients perceive more evaluative pain than patients in preoperative phase. Surgical act exposing the body to a traumatic situation is mind-boggling in patients with no previous surgical experience, and although accepted as necessary, it triggers attitudes, associations, thoughts, and significances about the meaning of pain, disease, and even life. In the preoperative period the patient cannot categorize and analyze the significance and consequences of surgery (13). After surgery, the information is organized, analyzed and has an effect on patient depending on the differences between preoperative and postoperative expectations. Assessment of pain perception in the preoperative and postoperative periods and of how the intensity and quality of pain is described may be indicators of post-traumatic stress disorder.

Generalized anxiety level in post-surgery patients is significantly higher, because of physical discomfort and possible functional impotence, but also because the patient recalls the dangers he went through and the fear that these, or other similar ones, will happen again.

If in the preoperative period anxiety is sometimes controlled pharmacologically or psychotherapeutically by the intervention team, the postoperative patient is a "solved problem" and, therefore, many patients return to hospital or continue clinically investigations, becoming likely to have iatrogenic stress.

The level of coping self-statements is significantly higher after than before surgery (14). Repressed fear and emotions before surgery are expressed postoperatively, and the use on this method of coping signifies the need for a patient to control pain, as coping self-statements are active coping, "everything is OK". Post-surgical patient adopts an active coping and is responsive to how "pain" will influence family relationships. This active coping has a variable degree of stability and qualitative value likely to resist or succumb to the action of psychological and traumatic factors on social and family life.

The level of reactions of others (family, friends) to patient complaints of pain after surgery is significantly higher than the level of reactions of others (family, friends) to patient complaints of pain before surgery (15, 16). Family behavioral and attitudinal reactions and support provided during post-surgery favor recovery and reintegration in the social and professional life.

CONCLUSIONS

Surgery affects the psyche of the patient undergoing surgery, triggering a series of events that can have psychological effects on pain intensity, doctor-patient relationship, post-surgery complications, and shortening of recovery time.

These results suggest that the integration of the psychosomatic concept in surgical practice, without denying the professionalism of the surgeon, can contribute to increased surgery success rate; good knowledge of psychological elements involved in the dynamics of surgery can be used for a successful surgical optimization.

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NEWS

IMAGING EVALUATION OF JOINTS PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS

A group of researchers conducted a study that assessed the prevalence of joint involvement in patients with systemic lupus erythematosus (SLE) by means of clinical assessment, joint US and MRI and evaluated the sensitivity and specificity of physician evaluation of joint involvement. At enrolment, patients underwent a complete physical examination including a 44-joint count, and hand deformities were scored. On the day of enrolment, each patient underwent a non-dominant hand-wrist ultrasound (US) examination and a non-dominant hand-wrist MRI study without contrast injection. By physician examination hand or wrist involvement was diagnosed in 23.5%. At least one pathological finding was revealed by US examination at wrist and/or hand joints in 55%. They found a low sensitivity (46.5%) with high specificity (93.2%) of the physician assessment for the evaluation of joint involvement. The MRI imaging showed at least one erosion in 47.3% patients at the hand and in 98.9% at the wrist. The authors concluded that the physicians tend to underestimate the severity of joint involvement in SLE, the US assessment showed a high prevalence of joint and tendon involvement, and the MRI evaluation showed a high prevalence of damage, suggesting that joint involvement in SLE could be more severe than expected. (Mosca M, Tani C, Carli L et al. The role of imaging in the evaluation of joint involvement in 102 consecutive patients with systemic lupus erythematosus *Autoimmun Rev*. 2014 Aug 23.[Epub ahead of print]. PMI 2518324

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GONIOSCOPIC FINDINGS IN PATIENTS WITH ACUTE CENTRAL/HEMICENTRAL RETINAL VEIN OCCLUSIONS

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GONIOSCOPIC FINDINGS IN PATIENTS WITH ACUTE CENTRAL/HEMICENTRAL RETINAL VEIN OCCLUSIONS (Abstract) **Obiectives:** To evaluate prospectively the gonioscopic findings and their changes during a 3-year follow-up period in patients with acute central/hemicentral retinal vein occlusions (C/HRVOs). **Material and methods:** A comprehensive ophthalmological examination of both eyes including static and dynamic gonioscopy as well as ocular biometric measurements was performed in 57 patients with acute C/HRVOs. The angle configuration and its changes, the axial length of the globe, the anterior chamber depth and the corneal thickness were assessed. **Results:** 12 C/HRVO patients presented with narrow drainage angles (≤ 20º) and 45 had normal angles (>20º). Ocular globes with narrow angles had axial length and anterior chamber depth significantly smaller as well as cornea thickness significantly greater than the eyes with normal angles. Out of the 12 patients with narrow angles, 6 cases had primary angle closure suspect (PACS), 5 cases had primary angle closure (PAC) and one case primary angle closure glaucoma (PACG). 3 of the 6 PACS patients progressed to PAC in the 6th, 10th, and 18th months of the follow-up period. From the 8 PAC cases existing during the whole study interval, one case progressed to PACG during the 24th month of the folow-up interval. **Conclusions:** In the context of an ocular globe having its size significantly smaller than the normal eye, narrow angle may represent a local risk factor predisposing to C/HRVO. Intermittent episodes of angle closure may contribute by increasing the intraocular pressure to the occurrence of C/HRVO as well to the progression of the gonioscopic configuration from PACS to PAC and from PAC to PACG.
Keywords: CENNTRAL/HEMICENTRAL RETINAL VEIN OCCLUSION, PRIMARY ANGEL CLOSURE SUSPECT, PRIMARY ANGLE CLOSURE, PRIMARY ANGLE CLOSURE GLAUCOMA

Gonioscopy is considered the current reference-standard examination whose purpose is to visualize the anterior chamber angle as completely as possible. It enables identification of the iridotrabecular contact and is the only examination method that is able to differentiate appositional from synechial angle closure (1, 2).

Closure of the anterior chamber angle in central retinal vein occlusion (CRVO) may appear as a primary (in predisposed eyes) or secondary event; the latter case is caused

either by neovascularization of the angle (3) or by a marked anterior displacement of the lens-iris diaphragm due to abnormal accumulation of blood or edema fluid in the posterior segment of the eye, a non-rubeotic state having some similarities to malignant glaucoma (4, 5).

The aim of this study is to evaluate prospectively the gonioscopic findings and their changes during a 3-year follow-up period in patients with acute central/hemicentral retinal vein occlusions (C/HRVOs).

MATERIAL AND METHODS

Patient population. The study was carried out in 57 unilateral acute C/HRVO patients, was approved by the University "Grigore T. Popa" Iaşi Ethics Committee and conformed to the tenets of the Declaration of Helsinki.

The patients enrolled in the study had a unilateral CRVO or hemicentral retinal vein occlusion (HRVO) with duration of symptoms of venous occlusive event of < 1 month. Exclusion criteria included prior tratament (filtering surgery, corneal transplantation, pars plana vitrectomy, or photocoagulation), aphakia or pseudophakia and any vascular retinal disorders in the study eye or age-related macular degeneration (e.g. drusen, geographic atrophy and neovascular form) in any of the 2 eyes.

Patient examination. All patients underwent a comprehensive baseline ophthalmological examination of both eyes. Best corrected visual acuity (BCVA) score was determined using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol (Snellen equivalent plus ETDRS letters) (6). Careful testing of the visual field was performed by means of the Goldmann perimeter using I2e, I4e and V4e isopters. In case of suspicion of concomitant primary glaucoma, the Humphrey standard automatic perimetry (Humphrey Instruments, Inc, San Leandro, California; model 720i; programme SITA FAST, 30-2) was used. Biomicroscopic examination was carried out with dilated and nondilated pupil. Thorough evaluation of the ocular fundus was performed with direct and indirect ophthalmoscopy and, if needed, using a contact lens. Fluorescein angiography was focused on both disc and macula during early examination times, on middle retinal periphery of each quadrant during intermediate phases and again on disc and macula in the late examination times. Intraocular pressure (IOP) was determined by using the Perkins applanotomometer and was adjusted for the corneal thickness. A scan ultrasonic ocular biometry was undertaken with Alcon Ultrascan Imaging System (Alcon, Inc, Forth Worth, Tx). The examination included following determinations: the axial length of the eye, the anterior chamber depth and the lens thickness. Optical Coherence Tomography (Stratus OCT; Carl Zeiss Meditec, Dublin, CA, USA) was used for evaluating the macular morphology and thickness, the optic disc and the retinal nerve fiber layer (RNFL). Gonioscopy was performed with the patient sitting at a slit-lamp, at high magnification (16 x) in a dark room using a two-mirror Goldmann gonioscopic lens. Beside the usual technique of the static gonioscopy, we used also dynamic gonioscopy.

Treatment and follow-up. The treatment applied to acute C/HRVO patients consisted of intravitreal bevacizumab (IVB) (Avastin; Genentech Inc., San Francisco, CA) injections in a dose of 2.5 mgr per injection until a stabilization of the BCVA score, lasting at least 6 months was

achieved. Patient follow-up was carried out every two months during the first year and every 6 months thereafter within the next two years.

Diagnostic criteria. A) Normal (not occludable) anterior chamber angle (> 20 grade) (7): The posterior third of the trabecular meshwork (usually pigmented) is visible for more than 180 degrees, on static gonioscopy with the eye in the primary position. This configuration involves both eyes. B) Narrow drainage angle (≤ 20 grade) (2, 8, 9): Non-visibility of the posterior trabecular meshwork for ≥ 180 degrees of the angle circumference on non-indentation gonioscopy with the eye in the primary position. It has two variants, namely, moderately narrow angle (angular width 15-20º) and extremely narrow angle (≤ 10º). This configuration involves both eyes. C) Primary angle closure suspect [PACS] (1, 7), namely an eye in which appositional contacts between the peripheral iris and the posterior trabecular meshwork is considered possible/probable: -Narrow drainage angle, IOP below 22 mmHg, no peripheral anterior synechiae (PAS) in the angle, and without glaucomatous optic neuropathy (GON); -Absence of the ocular pathology, that can induce PAS formation (uveitis, neovascularization, trauma, surgery). D) Primary angle closure [PAC] (7, 9, 10, 11, 12, 13): -Narrow drainage angle; -Excluded were eyes in which narrow angles or shallow anterior chambers were thought to be secondary to the other ocular conditions, such as, lens abnormalities, chronic uveitis, trauma, rubeosis iridis or retinopathy of prematurity; -Optic disc and visual field definitely considered as nonglaucomatous; -The presence of at least one of the following features indicating that trabecular obstruction by the peripheral iris occurred: a) PAS; b) IOP > 21 mmHg; c) Excessive pigment deposition on the trabecular meshwork surface, especially superior; d) Ischemic sequelae of acutely raised IOP (distorsion of the radially orientated iris musculature, iris stromal atrophy, dilated nonresponsive pupil, focal necrosis of lens epithelium causing glaucomfleken) (1) ; e) Clear history of clinical signs or symptoms such as headaches, congestion, blurred or halo vision, corneal edema or a mid-dilated pupil, consistent with sudden IOP rise; f) Evidence of a surgical peripheral iridotomy; g) Dark room provocation test giving a rise in IOP of ≥ 8 mmHg from baseline. E) Primary angle closure glaucoma [PACG] (1, 7, 9, 13) :- Presence of PAC; - Consistent visual morbidity; - Typical GON (1,10.14) is characterized by irreversible structural and/or functional glaucomatous lesion, indistinguishable from the primary open angle glaucoma; structural glaucomatous damage includes aquired characteristic progressive optic neuropathy (eg optic disc cupping; narrowing the area of neuroretinal rim; localized “notch”; disc hemorrhages; newly appeared asymmetry in the cup/disc ratio ≥ 0.2 between both eyes; change in the cup/disc ratio > 0.2) that could be documented alongside the follow-up, and/or changes of the RNFL (diffuse or localized defects); functional glaucomatous damage comprises reproducible characteristic changes of the visual field (retinal nerve fiber bundle defects) corresponding to the optic disc lesion; - Atypical GON (1) occurs after an acute symptomatic episode of angle closure and presents with a pale but flat optic disc, suggesting an anterior ischemic optic neuropathy.

Main outcome measures. The primary outcome measure was emphasizing the gonioscopic configuration of the anterior

chamber angle in patients with C/HRVOs. The secondary outcome measures included: determination of the axial length of the eye, the anterior chamber depth as well as the lens and the cornea thicknesses; the cumulative prevalences of the PACS, the PAC and the PACG; the progression rates from PACS to PAC as well as from PAC to PACG.

Statistical analysis. The continuous variables were tested using Student's tests (normal distributed data) and both the Man Whitney U and the Wilcoxon tests for non-parametric distributions. Categorical data were analyzed by means of the Person chi-square test and the Fisher exact test. Cumulative prevalence calculation was made by using the Kaplan-Meier estimate. The statistical analysis was performed using *SPSS* statistical software (version 16.0) and *Epi-Info 2000*.

RESULTS

From total, 12 C/HRVO patients presented with narrow anterior chamber angles (≤ 20º) and 45 patients had normal drainage angles (>20º) (tab. I). Venous occlusions in patients with narrow angles occurred at an age significantly greater (p=0.001) than those in patients with normal angles.

TABLE I

Demographic and clinical baseline characteristics in 57 patients with central / hemicentral retinal vein occlusion

	Anterior chamber drainage angle			**Total**
Variables	**Narrow (n = 12)**	**Normal (n = 45)**	**p-value**	**(n = 57)**
Age (years)				
Mean ± SD	72.08 ± 9.66	56.37 ± 13.12	0.001	59.68±13.97
Median (range)	72.5 (58-85)	59 (21-75)	0.019	62 (21-85)
Stratification n (%)				
≤ 55	0 (0)	20 (44.4)	0.005	20 (35.1)
> 55	12 (100)	25 (55.6)	0.005	37 (64.9)
Gender n (%)				
Male	9 (75)	26 (57.8)	0.335	35 (61.4)
Female	3 (25)	19 (42,2)	0.335	22 (38,6)
Occlusion type n (%)				
CRVO	11(91.7)	43 (93.3)	0.623	53 (93)
HRVO	1 (8.3)	3 (6.7)	0.623	4 (7)
Occlusion form n (%)				
Ischemic	12 (100)	9 (20)	0.001	21 (36.8)
Nonischemic	0 (0)	36 (80)	0.001	36 (63.2)

CRVO = central retinal vein occlusion; HRVO = hemicentral retinal vein occlusion

Biometric measurements emphasized an axial length of the ocular globe significantly smaller (21.69±0.93 mm; p=0.004) and an anterior chamber depth significantly lower (1.8±0.09 mm; p=0.0009) in patients with C/HRVOs having a gonioscopic configuration of narrow angle compared with those with a normal angle configuration (22.69±0.94 mm and 2.58±0.55 mm, respectively). The cornea thickness was significantly great (p=0.0009) in patients with narrow angles (0.6 ± 0.02 mm) *vs.*

those with normal angles (0.54 ± 0.02 mm).

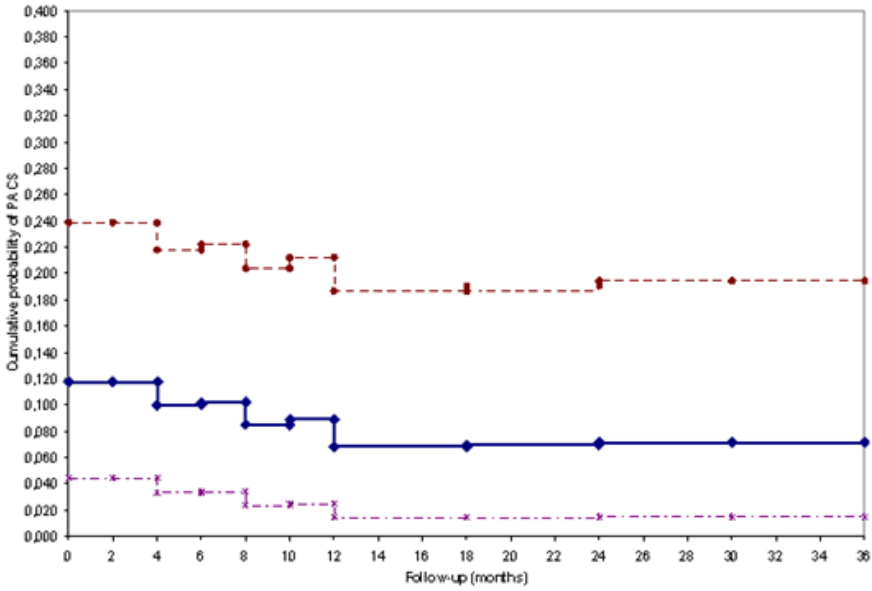
Three possible clinical forms of angle closure status existed at the beginning of the study in the 12 patients with venous occlusions having narrow angles (tab. II), namely PACS in 6 cases, PAC in 5 cases and PACG in one case.

TABLE II

Clinical forms of angle-closure status in 12 patients with central/hemicentral retinal vein occlusion having narrow drainage angles

Angle-closure status	PACS	PAC	PACG	Total
Baseline (n)	6	5	1	12
Final (n)	3	7	2	12

PACS = primary angle closure suspect; PAC = primary angle closure; PACG = primary angle closure glaucoma

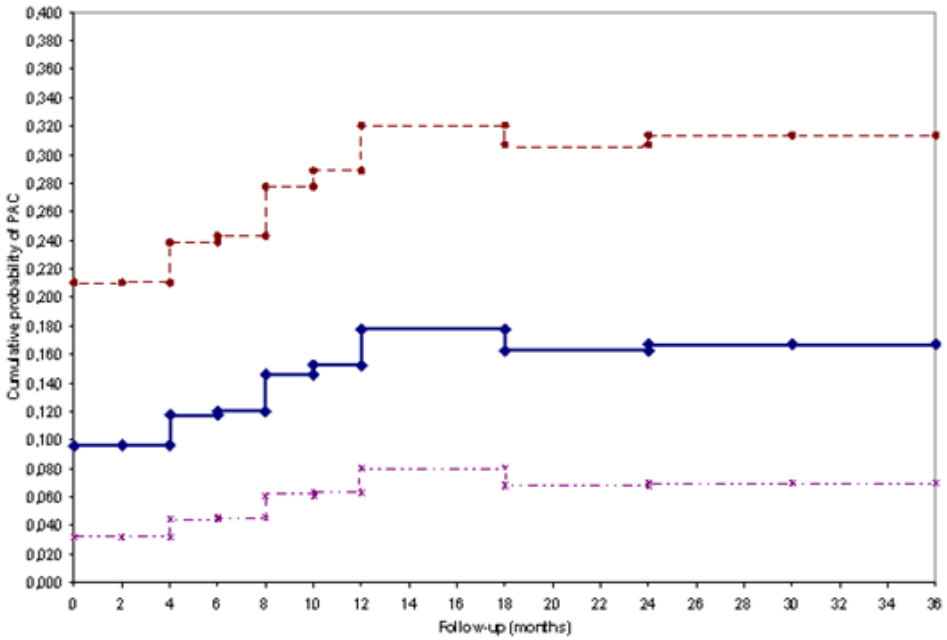


Follow-up	2	4	6	8	10	12	18	24	30	36
No at risk	51	51	50	49	47	45	44	43	42	42
PACS	6	0	-1	0	-1	0	-1	0	0	0
Censored	0	0	0	1	1	2	0	1	1	0

Fig. 1. The Kaplan-Meier estimate of the cumulative prevalence of the primary angle closure suspect over the entire course of the study in 57 central/hemicentral retinal vein occlusion patients. Patients at risk are those who did no present primary angle closure suspect at the beginning of the study. Patients who were lost before the end of the study were censored from the last completed visit. The dotted lines represent the upper and lower bounds of the 95% confidence interval.

Peripheral laser iridotomy was performed in 4 out of the five patients with PAC and trabeculectomy in the patient with PACG. One patient with PAC refused laser

intervention. 3 of the 6 PACS patients (50%) progressed to PAC in the 6-th, 10-th and 18th months of the follow-up period (16.6% per year), following some asymptomatic episodes of angle closure (unilateral progression in the occlusion eye). Peripheral laser iridotomy was performed in all three patients. So, the cumulative prevalence of PACSs decreased from 11.76% (95% confidence interval (CI), 4.44-23.87) to 7.14% (95% CI 1.5-19.43) (fig. 1) while the cumulative prevalence of PAC increased from 9,6% (95% CI, 3.2-21.03) to 16.6% (95% CI, 6.97-31.36) (fig. 2).

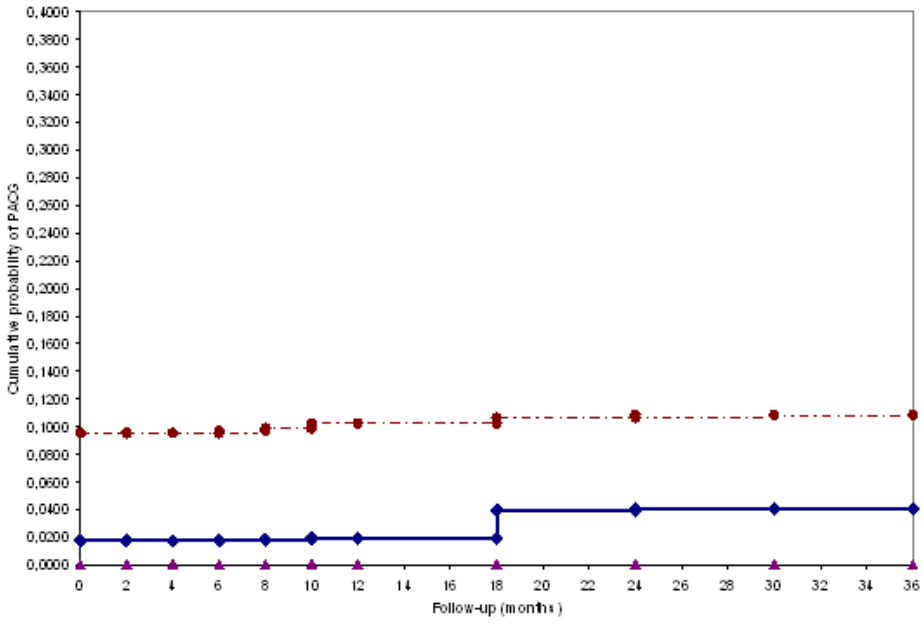


Follow-up	2	4	6	8	10	12	18	24	30	36
No at risk	52	52	51	50	48	46	45	43	42	42
PAC	5	0	1	0	1	0	1	-1	0	0
Censored	0	0	0	1	1	2	0	1	1	0

Fig. 2. The Kaplan-Meier estimate of the cumulative prevalence of the primary angle closure over the entire course of the study in 57 central/hemicentral retinal vein occlusion patients. Patients at risk are those who did not present primary angle closure at the beginning of the study period. Patients who were lost before the ends of the study were censored from the last completed visit and those who died were censored at their date of death. The dotted lines represent the upper and lower bounds of the 95% confidence interval.

From the 8 cases with PAC existing during the whole study period, only one (12.5%) progressed to PACG during the 24th month of the follow-up period (4.16% per year) follow-

ing an acute episode of angle closure (unilateral progression in the occlusion eye). This patient (a female aged 74 years) is the one who refused laser treatment during the PAC status and who declined again the surgery during PACG status. She exhibited a pale but flat optic disc with altitudinal visual field defects closely resembling anterior ischemic optic neuropathy. So, the cumulative prevalence of PACG increased from 1.78% (95% CI, 0.045-9.552) to 4.08% (95% CI, 0.05-10.85) (fig. 3).



Follow-up	2	4	6	8	10	12	18	24	30	36
No at risk	56	56	56	55	54	52	52	50	49	49
PACG	1	0	0	0	0	0	0	1	0	0
Censored	0	0	0	1	1	2	0	1	1	0

Fig. 3. The Kaplan-Meier estimate of the cumulative prevalence of the primary angle closure glaucoma over the entire course of the study in 57 central/hemicentral retinal vein occlusion patients. Patients at risk are those who did not present primary angle closure glaucoma at the beginning of the study period. Patients who were lost before the ends of the study were censored from the last completed visit and those who died were censored at their date of death. The dotted lines represent the upper and lower bounds of the 95% confidence interval.

DISCUSSION

An important ascertainement of this study is the great incidence (12 cases; 21%) of the narrow angles (≤ 20º) in patients with C/HRVOs, namely moderately narrow angles (15-20º) in 7 cases and extremely narrow angles (≤ 10º) in 5 cases. This frequency is evidently greater compared to that existing in the general population [between 2% (15) and 14.8% (16)].

The statistically significant difference between the mean age of the venous occlusion patients with narrow and normal angles (tab. I) caused us to suspect the possible role played by an advanced age in the occurrence of such high percentage of the narrow angles. The narrowing of the anterior chamber angle appears to be a function of normal aging (11). With advancing in age, an angle may become occludable.

In the eyes with narrow angles of our series, the ocular biometric measurements were totally different from those existing in the ocular globes with normal angles (> 20º). So, the axial length of the globe and the anterior chamber depth were significantly smaller and the cornea thickness was significantly greater in eyes with narrow angles compared to eyes with normal angles. This configuration of the ocular globe having the size of the anterior segment smaller than the average may represent a local risk factor predisposing to CRVO, especially for an ischemic form of the venous occlusions. The changes mentioned are not the result of the occlusion but an expression of the constitutional factors with inherited characteristics. Shorter axial length may also indicate crowded posterior segment. In such eyes, retinal vein and artery that share the same adventitial sheath are more crowded and impaired as they pass through the *lamina cribrosa*; this status may narrow the lumen of the vein with all its subsequent consequences, namely, decrease of the blood flow, increase of the blood viscosity and local turbulence that could cause thrombosis (17).

At the beginning of the study, 6 cases of CVROs with narrow angles were diagnosed as having closure of the angle, namely 5 cases with PAC and one case with PACG (tab. II). These patients were experiencing a history of symptoms of intermittent IOP rises as a consequence of the angle closure. After reversing such an episode, patients complained about the persistence of blurred vision, which determined them to have an ophthalmological examination. On this occasion the ophthalmologist ascertained the existence of the venous occlusion. In these cases, the episodes of ocular hypertension due to angle closure may have triggered the occurrence of the venous occlusions (18). Increasing the IOP induce compression, stretching, deformation, and backward displacement of the lamina cribrosa that secondarily may distort the vein (19). The collapse of the retinal vein due to compression and subsequent endothelial lesion with intimal proliferation caused the vein to occlude at the level of the lamina cribrosa.

Progression from the PACS to PAC occurred in 3 eyes with CRVOs out of the 6 cases with PACS being present at the entry (50%; 16.6% per year). This incidence per year of progression from PACS to PAC is greater than that existing in the general population (22%; 4.4% per year) (7), which plead in favor of the fact that CRVO may represent a risk factor for the progression. The progression from PAC to PACG appeared only in a single eye with CRVO out of the 8 PAC cases existing during the whole study period (12.5%; 4.16% per year); so, the incidence per year of progression from PAC to PACG is consistent with that being present in the general population (28.57%; 5.7% per year) (13).

The successive episodes of angle closure after venous occlusion occurrence may account for the progressions ascertained. Increased thickness of the lens accompanied by a change in its shape due to slackening of the zonules causing the lens to

move forward (15), represent the main growth and dynamic factors that, in the presence of the constitutional ones with inherited characteristics, determine decreasing the angular width as the age advances (7, 15, 20). All of these factors combine to hasten the closure of the angle with advancing in age.

The main limitation of the study was the lack of a control group recruited from the general population of the same location in which all the determinations should have been done, namely the evaluation of the incidence of different angle configurations as well as of the cumulative prevalence of the varied clinical forms of the angle closure and their progressions.

CONCLUSIONS

From our study we can conclude that the narrow angle in the context of an ocular globe having its size significantly smaller than the normal eye may represent a local risk factor predisposing to C/HRVO. Intermittent episodes of angle closure may contribute to the occurrence of the C/HRVO as well as to the progression of the gonioscopic changes.

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NEWS

ELECTRONIC MEDICAL RECORDS - AN IMPERFECT SYSTEM WITH GOOD RESULTS FOR IDENTIFYING PATIENTS WITH A COMMON PHENOTYPE

The objective of this review was to summarize literature describing approaches aimed at automatically identifying patients with a common phenotype. The authors performed a review of studies describing systems or reporting techniques developed for identifying cohorts of patients with specific phenotypes. Every full text article published in (1) *Journal of American Medical Informatics Association*, (2) *Journal of Biomedical Informatics*, (3) *Proceedings of the Annual American Medical Informatics Association Symposium*, and (4) *Proceedings of Clinical Research Informatics Conference* within the past 3 years was assessed for inclusion in the review. Only articles using automated techniques were included. The identification of patients who satisfy predefined criteria from a large population in an institution has numerous use cases, including clinical trial recruitment, outcome prediction, survival analysis, and other kinds of retrospective studies. However, the process of distinguishing these patients on the basis of their patient records can be extremely time-consuming and challenging depending on the complexity of the criteria. This is because the data matching these criteria are buried within multiple documents and across multiple data points in the electronic health record (EHR) of a patient. Some data, such as laboratory results, medications, and diagnoses, have a structured format. The ability to extract meaningful pieces of information from the EHR and consolidate them into a coherent structure would provide great value for automatically identifying patient cohorts that satisfy complex criteria. Studies in this review addressed different diagnoses as the phenotype of interest. A large number of papers focused on diabetes, cancer, heart failure, rheumatoid arthritis, or cataract. A few studies described generic methods that could be applied to multiple diagnoses. Several papers addressed identification of adverse drug events. Some studies also analyzed genomic data of a predetermined phenotype to gain insight into other phenotypic properties of the same cohort. Authors have developed a top of the first 10 phenotypes of interest. Although cancer has been reported as a generic phenotype, there were 12 types of cancer (with breast cancer being most prevalent) studied in seven articles. Congestive heart failure and heart failure were counted as the same phenotype. Similarly, hypertension and resistant hypertension were considered to be one phenotype. Some articles also considered other observable characteristics such as smoking status and obesity among patients. Pneumonia and a variety of other infectious diseases were studied. This review is not without limitations, especially for the vagueness of the term phenotyping, meaning that there is no exhaustive search strategy for retrieving appropriate articles across the entire available body of biomedical literature. However, because of the venues considered and the recent popularity of this topic, this review is representative of past and present efforts in this domain. (Shivade C, Raghavan P, Fosler-Lussier E, Embi PJ, Elhadad N, Johnson SB, Lai AM. A review of approaches to identifying patient phenotype cohorts using electronic health records *J Am Med Inform Assoc* 2014; 21: 221-230 doi:10.1136/amiajnl-2013-001935)

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RAD51 OVEREXPRESSION AND RESISTANCE TO GENOTOXIC AGENTS. A STUDY IN THE FISSION YEAST *SCHIZOSACCHAROMYCES POMBE*

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RAD51 OVEREXPRESSION AND RESISTANCE TO GENOTOXIC AGENTS. A STUDY IN THE FISSION YEAST SCHIZOSACCHAROMYCES POMBE **(Abstract): Background:** Many cancer cell lines have been found to overexpress the recombinase Rad51. The overexpression is associated with increased invasive potential and resistance to DNA-damaging therapeutic agents. This has been attributed to an increased capacity of cells over-expressing Rad51 to repair DNA lesions or to a genetic stabilization of the genome. **Aim:** As the explanations are somewhat controversial, we attempted to reproduce overexpression in the unicellular eukaryote *Schizosaccharomyces pombe* to have a simpler tool to study the problem of Rad51 overexpression and its induced resistance to DNA-damaging agents. **Methods:** We used the *nmt1* promoter inserted upstream of *rad51*gene to induce its overexpression and studied the phenotype of the transformed strain, especially its sensitivity to camptothecin and hydroxyurea. **Results:** We found that overexpression induced sensitivity to the two drugs even when it was associated with the deletion of a recombination mediator *rad22/rad52* gene. However, when overexpression was associated with the deletion of the helicase-encoding *fbh1* gene, the sensitivity to camptothecin was diminished. **Keywords**: RAD51 OVEREXPRESSION, HOMOLOGOUS RECOMBINATION, RAD22, FBH1.

Rad51 protein is a central player involved in repair of the DNA double-strand breaks by homologous recombination. Rad51 is a component of the recombination nucleo-protein filament: a DNA single stranded overhang (resulting from the end processing of a double-stranded end) wrapped around a Rad51 polymer in a helical manner. The nucleofilament invades the double helix of a sister chromatid or a homologous chromosome forming the so called D loop, a structure that initiates homologous recombination (1). Rad51 is essential in mammalian cells; mouse embryos carrying deletion of the *rad51* gene are unviable (2).

Publications issued after 2002 have reported a Rad51 overexpression in many (over 50%) of human cancers (3). The overexpression exceeds about 2-7 fold the level of the expression in normal parental cells, in terms of the Rad51 protein amount. It is due to an excessive transcription of the gene (4). Some activating influence over the genes promoter occurs. In a series of 13 cancer cell lines, the activity of the *rad51* promoter was tested with a different gene reporter (luciferase) and a dramatically increased level of the reporter protein occurred (several hundred fold) in cancer cells when compared to normal fibroblasts (5). In the same cell lines the

Rad51 mRNA and protein were increased only about six fold, which means that a limiting action on the transcript's level also occurs. The overexpression confers either increased invasive potential and/or increased resistance to genotoxic agents used for cancer therapy (4,6).

Overexpression has *causes* and *consequences*. Both causes and consequences are important to be known for cancer prevention and therapy. A way of discovering them is to look for genome modifications accompanying the Rad51 overexpression. Some of these are the ones leading to losses of function of p53, BRCA1, BRCA2 and gain of function of the oncoprotein BCR/ABL (3).

p53 is a well known tumor suppressor. It is a transcription factor important for the regulation of cell cycle. p53 is a negative regulator of the *rad51*gene expression by directly inhibiting its transcription (6). BCR/ABL is an activator of the *rad51* expression through STAT5-dependent transcription of *rad51* and inhibition of Rad51 protein cleavage by caspase-3 (7). BRCA1 and 2 are proteins involved in homologous recombination like Rad51 itself (8). Their loss-of-function mutant alleles may be involved in Rad51 overexpression through their deficiency in repairing DNA lesions, which is deleterious for the cell viability and favor selection of cells overexpressing Rad51 to compensate for their own defect. In this one vision, the BCR/ABS gain-of-function and p53, BRCA1 and BRCA2 lss-of-function are causes of Rad51 overexpression. However, a reverse scenario is also possible: Overexpression of Rad51 could be the cause of genetic instability (also deleterious for the cell growth) and then selection of mutations to compensate for it in the genes coding for either p53 or BRCA1 or BRCA2 would occur as a consequence (9). The main support of this hypothesis is that Rad51 overexpression is deleterious in normal cells but it is beneficial for cells with BRCA1 or BRCA2 loss-of-function (10).

The resistance of cancer cells overexpressing Rad51 to DNA damaging agents (MMS, camptothecin, cisplatin) is of utmost interest for cancer chemotherapy (11), but it is questionable whether this is due to overexpression itself or to the accompanying mutations.

We assumed that the use of simple eukaryotic organisms such as yeasts could shed some light on the problem of Rad51 overexpression. It would permit a better differentiation between causes and consequences of this Rad51 status. Rad51 can be overexpressed by putting its gene under the control of an artificial active promoter and testing of the genomic modified strain for resistance/sensitivity to DNA-damaging agents can be performed. In this way, the overexpression being unaccompanied by other mutations, its sole influence on the resistance/sensitivity can be assessed. We carried out the genomic modification and studied its effects in the yeast *Schizosaccharomyces pombe*.

A previous study (12) has shown that overexpression of Rad51/Rhp51 in *Schizosaccharomyces pombe* had deleterious effects on the strain which carried it. Cells had reduced viability and a defect in chromosome segregation at mitosis due to disruption of microtubule attachment to the chromosomes. In this study we confirm loose of viability (to a lesser degree than reported previously (12) and we also report an increased sensitivity to camptothecin and hydroxyurea of transformed cells. However, in strains lacking the *fbh1* gene

(coding for a helicase/translocase which opposes the nucleofilament formation) (13) overexpression of Rad51 conferred lesser sensitivity compared to the strains with normal activity of Fbh1.

MATERIAL AND METHODS

The *S. pombe* strains used in this study, their complete genotype, and their source are presented in tab 1.

Strains AMC 504, AMC60 and 272 were kind gifts from A.M. Carr, University of Sussex UK. The other strains were constructed by us as follows: The *S. pombe* active promoter *nmt1* was inserted upstream of rad51 as described (14, 15). The strain carrying *E-rad22* was obtained similarly but accidental mispriming resulted in a hypomorphic allele (with partial loss-of-function) of rad22. The other strains were derived by standard crosses of 251 (nmt1-rad51) with 272 (fbh1-d) and 266 (E-rad22), followed by sporulation and dissection of the tetrads to obtain spore-clones. They were tested phenotypically (resistance to geneticin meant deletion of fbh1) and genotypically (PCR amplification with a pair of primers, one of them being identical with a sequence in *nmt1* meant overexpression of *rad51*).

TABLE I

***Schizosaccharomyces pombe* strains used in this study**

Strain Nr (in our collection)	Relevant genotype and current name	Complete genotype	Source
AMC 504	rad51+ rad22+ fbh1+ (WT)	*ade6-704 ura4-d18 leu1-32 h-*	A.M. Carr
272	fbh1-d	*fbh1::kanr ade6-704 ura4-d18 leu1-3 2 h+*	A.M.Carr
AMC60	rad51-d	*rad51::kanr ade6-704 ura4-d18 leu1-32 h-*	A.M.Carr
251	nmt1-rad51	*nmt1-rad51 ade6-704 ura4-d18 leu1-32 h-*	This work
339	nmt-rad51 fbh1-d	*nmt1-rad51 fbh1::kanr ade6-704 ura4-d18 leu1-32*	This work
266	E-rad22	*E-rad22 ade6-704 ura4-d18 leu1-32 h-*	This work
317	E-rad22	*E-rad22 ade6-704 ura4-d18 leu1-32 h+*	This work
318	E-rad22 nmt1-rad51	*E-rad22 nmt1-rad51 ade6-704 ura4-d18 leu1-32 h+*	This work
319	E-rad22 nmt1-rad51	*E-rad22 nmt1-rad51 ade6-704 ura4-d18 leu1-32 h-*	This work

Culture medium and chemicals: The standard yeast extract agar (YEA) medium was used in all tests (yeast extract 0.5%, glucose 1%) to which camptothecin (Cpt) (Sigma-Aldrich USA) or hydroxyurea (HU, Sigma-Aldrich USA) were added where indicated and in the concentrations shown in figs 1 and 2. The kanamycin-like antibiotic G418 (geneticin Sigma-Aldrich USA) was added at the concentration of 400 mg/l to identify *kanr* (gene which replaces *fbh1* or *rad51*).

Testing of genotoxin sensitivity/resistance: The semi-quantitative "spot test"

was used (13).

RESULTS

Overexpression of *rad51* sensitizes cells to hydroxyurea and camptothecin. Insertion of a variant of the *nmt1* promoter is a frequently used method for overexpressing genes in *Schizosaccharomyces pombe* (14, 16).We first tested the strain with *rad51* placed under the control of the hyperactive promoter *nmt1* for its phenotype. The viability of untreated cells, estimated by the percentage of dead (magdala red dye accumulating) cells in YEA was slightly lower than of the wild-type (WT) cells (not shown).This was not so significant as to be apparent on spot tests (fig. 1, no genotoxin) as reported previously (12). This is probably due to the fact that in our strain there was a single gene under the *nmt1* control (as *nmt1* was inserted upstream of the single chromosomal gene) while Kim et al (12) constructed overexpressing strains using many copies of a plasmid carrying *rad51/rhp51* under the control of *nmt1* promoter. The sensitivity of our strain overexpressing *rad51* to hydroxyurea and camptothecin was higher compared to WT and even higher than sensitivity of the strain deleted of *rad51* (fig. 1 A).

This is not different from other types of wild-type cells, including human cells and even some cancer cells (3) which are also more sensitive when Rad51 is overexpressed. Some types of cancer cells however, especially the ones carrying mutations in BRCA2 (10) are less sensitive to DNA-damaging chemotherapeutics when Rad51 is overexpressed. We tried to reproduce the situation in *S. pombe* by combining the partial loss-of-function of Rad22 (the functional equivalent of BRCA2) (17) with Rad51 overexpression.

The double mutant E-rad22 nmt1-rad51 was not less sensitive than the single-mutant E-rad22. Rad51overexpression did not compensate for the Rad22 deficiency; in that regard the situation described in cancer cells could not be reproduced in *Schizosaccharomyces pombe*. (fig. 1B)

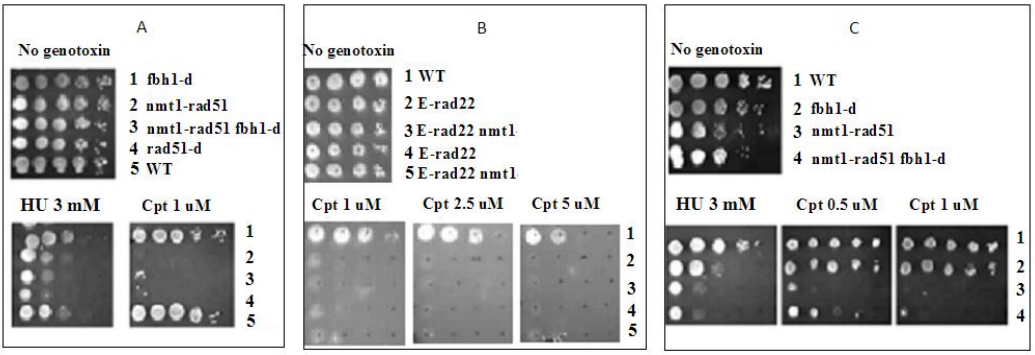

Fig. 1, A, B, C. Viability of *S. pombe* strains with and without mutations at *rad51*, *rad22*, or *fbh1* loci.

Cells were grown to stationary phase and then serially diluted to have 10^5, 10^4, 10^3, 10^2 or 10 cells/5 µl and then 5 µl of each dilution, in the decreasing order from

left to right, was dropped in a row on plates containing the solid medium YEA with or without the genotoxins in concentrations as mentioned . The plates were photographed after 5 days of incubation at 30°C. Panel A: Strains overexpressing Rad51 are hypersensitive to hydroxyurea and camptothecin. Panel B: The Rad22/Rad52 loss-of-function cannot be compensated by overexpression of Rad51. The strains 2 and 4 (317 and 266) were identically mutated at rad22 but they were derived independently and had slightly different genetic backgrounds. The same observation is available for strains 3 and 5 (double mutant's numbers 318 and 319). (tab. I) Panel C: Deletion of the gene *fbh1* alleviates the hypersensitivity to camptothecin of the strain overexpressing Rad51.

Deletion of *Fbh1* gene alleviates the hypersensitivity to camptothecin of strains overexpressing Rad51.

One question that could be raised in respect to the phenotype of Rad51 overexpression is whether it is due to the well known function of the protein in forming the nucleofilament necessary for recombination repair (1) or to a different unknown function. To answer this question we combined overexpression of Rad51 with the lack of a helicase/traslocase function that opposes the nucleofilament formation. Of the several helicases involved we chose Fbh1 which has a homologue in human cells (18, 19). We thus combined *nmt1-rad51*construct with the deletion of *fbh1* gene in the same strain.

We expected that if the excessive formation of nucleofilament had been responsible for genotoxin sensitivity it would have worsened the effect of overexpression (for example to result in less viability even in the cells untreated with genotoxins). This expectance was not confirmed. There was little difference (if any) between the viability of single mutant nmt1-rad51 and double mutant nmt1-rad51 fbh1-d in the media without genotoxin or with hydroxyurea. However, in the media containing camptothecin a clear loss of sensitivity could be perceived in the case of the double mutant nmt-rad51 fbh1-d. In other words, some resistance to camptothecin was visible on the background of Fbh1 loss of function. (fig. 1C)

DISCUSSION

We showed that overexpression of Rad51 sensitized *S. pombe* cells to the action of DNA- damaging drugs hydroxyurea and camptothecin. These two drugs damage DNA by different mechanisms but eventually result in double-strand breaks which are lethal if unrepaired. Their repairing depends on Rad51. Therefore, the absence of Rad51 (as in the case of the strain with the deletion of the gene rad51 (fig.1 A) sensitizes cells to HU and Cpt. We showed in this study that also an excess of Rad51 sensitizes cells to hydroxyurea and camptothecin (Panels A and C).

Is the deleterious effect of Rad51 overexpression due to an excessive formation of nucleofilaments ? The double-strand repair by recombination needs Rad51-dependent nucleofilament assembly in one step of the process and its helicase-dependent disassemble in another step. An excess of Rad51 may distort this balance and lead to an inefficient repair. In this case, the lack of helicase function will augment the unbalance and make the process even less efficient. Therefore we eliminated one of helicase functions by deleting the gene *fbh1* encoding the helicase Fbh1 and found that

the deletion did not add up at the sensitivity to genotoxins or worsen the viability of the cells carrying the double mutation *nmt1-rad51 fbh1-d* (figs. 1 A and C), as expected, were the recombination defects the main cause of the sensitivity. We presume that other functions of Rad51 were in cause. A previous report has shown that Rad51 is involved in cell cycling and mitosis (12), possibly by a different mechanism than that of nucleofilament formation.

Deletion of the helicase/translocase-encoded *fbh1* on the background of Rad51 overexpression had an unexpected effect: the diminution of sensitivity to camptothecin. (fig. 1 C). This is a reminiscent of the resistance of cancer cells overexpressing Rad51 to DNA-damaging chemotherapy. In our view, this finding may be interpreted in two alternative ways: 1. Hypersensitivity to camptothecin of the strain overexpressing Rad51 is not due to its effect over the nucleofilament formation and efficiency of recombination but to a different mechanism. Recombination is not affected as long as Fbh1 helicase/ translocase exists. When the helicase function does not exist, repair by recombination is more efficient and this leads to a relative resistance to camptothecin - relative because the main mechanism which is the cause of sensitivity is not suppressed - (the physiologically-based hypothesis). 2. The double genomic modification which confers the Rad51 overexpression on the background of the Fbh1 absence leads to a genomic instability and to a new mutation conferring resistance to camptothecin. The resistant cells select in the medium containing camptothecin (the genomic instability hypothesis). We cannot as yet distinguish between these two hypotheses, but the second one (genetic instability hypothesis) can be checked by genetic methods: selection of the clone presenting stably a less sensitivity to camptothecin, loss and regain of this capacity in crosses and, eventually, identification the mutated gene. That we are going to carry out further.

Relevance of our findings to the problem of cancer cells overexpressing Rad51 and their resistance to chemotherapeutics: We started this study as an attempt to reproduce in fission yeast and explain the resistance to DNA-damaging agents of cells overexpressing Rad51. Our attempt was justified by the fact that the action of DNA-damaging chemotherapeutics, the mechanisms of DNA repair and the proteins involved are similar in human cells and in yeasts. We did not find that fission yeast overexpressing Rad51 was resistant to any of two DNA-damaging agents hydroxyurea and camptothecin either Rad51 overexpression singly or accompanied by the functional equivalent of BRCA2 (Rad52/Rad22) loss-of-function. This is different from cancer cells in which association between Rad51 overexpression and BRCA2 loss-of-function is thought to cause resistance to DNA-damaging agents. However, one of our findings in this work on *S. pombe* points to recombination being involved in resistance. This is the alleviation of sensitivity to camptothecin of fission yeast cells deleted for the gene coding for helicase/translocase Fbh1. Fbh1 protein is also present in mammalian cells (19).

CONCLUSIONS

This work attempted to reproduce in fission yeast the Rad51 overexpression and resistance to DNA-damaging chemotherapeutics described in cancer cells. We found that overexpression of Rad51 in fission yeast sensitized cells to DNA damaging

agents, but sensitization was alleviated by the loss-of-function of the helicase/translocase Fbh1. This draws attention to a protein also present in human (and therefore in cancer) cells that may be involved in resistance either by its role in recombination or as a genetic stabilizer.

ACKNOWLEDGEMENTS. This work has benefitted from the grant 17075/30.09.2010 "Study of physical and functional interactions of Sp-Rad52 protein, involved in DNA double strand repair, using a genetically modified protein" attributed by UMF Iasi. We thank A.M. Carr, Sussex University Brighton UK for yeast strains and plasmids. We used the laboratory facilities of the Platform of Molecular Medicine, University of Medicine and Pharmacy "Grigore T. Popa" Iasi.

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NEWS

MULTIFOCAL GENITAL HERPES SIMPLEX VIRUS TYPE 2 INFECTION

Genital HSV reactivation is thought to be anatomically and temporally localized, coincident with limited ganglionic infection. Subclinical shedding episodes are the most common form of HSV-2 reactivation, with host clearance mechanisms leading to rapid containment.To precisely define patterns of anatomic reactivation, Johnston and co. divided the genital tract into a 22-region grid and obtained daily swabs for 20 days from each region in 28 immunocompetent, HSV-2 seropositive persons. HSV was detected via PCR and sites of asymptomatic HSV shedding were biopsied within 24 hours. They quantified CD4+ and CD8+ T cells by immunofluorescence, and HSV specific CD4+ T cells were identified by intracellular cytokine cytometry. HSV was detected in 868 of 11,603 genital swabs at a median of 12 sites per person. Bilateral HSV detection occurred on 83 days with shedding, and the median quantity of virus detected/day was associated with the number of sites positive ($p<0.001$). In biopsies of asymptomatic shedding sites, we found increased numbers of CD8+ T cells compared to control tissue (27 vs. 13 cells/mm^2, $p=0.03$) and identified HSV specific CD4+ T cells. HSV reactivations emanate from widely separated anatomic regions of the genital tract and are associated with a localized cellular infiltrate that was demonstrated to be HSV-specific in 3 cases. The authors concluded that HSV-2 reactivation can be detected at multiple, bilateral sites in the genital tract, suggesting that HSV establishes latency throughout the sacral ganglia. In addition, genital biopsies from sites of asymptomatic HSV shedding have increased numbers of CD8+ T cells compared to control tissue, and HSV-specific CD4+ T cells are found at sites of asymptomatic shedding. These findings suggest that that widespread asymptomatic genital HSV-2 shedding is associated with a targeted host immune response and contributes to chronic inflammation throughout the genital tract. (Johnston C, Zhu J, Jing L, Laing KJ et al. Virologic and immunologic evidence of multifocal genital herpes simplex virus type 2 infection. *J Virol*. 2014 Feb 19. [Epub ahead of print] PMID: 24554666)

Simona Tucaliuc

ASPECTS REGARDING THE HYGIENIC-SANITARY CONDITIONS AT THE LEVEL OF CERTAIN DENTAL MEDICINE CABINETS IN IASI COUNTY

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ASPECTS REGARDING THE HYGIENIC-SANITARY CONDITIONS AT THE LEVEL OF CERTAIN DENTAL MEDICINE CABINETS IN IASI COUNTY (Abstract) **Aim**: This baseline study aims to find out the evaluation of hygienic-sanitary conditions at the level of dental medicine cabinets through the verification of certain hygienic aspects. **Material and Methods:** The study conducted consists in monitoring the hygienic/sanitary conditions at the level of 68 dental medicine cabinets (40 private cabinets and 28 school/university dental cabinets in Iasi county), using sheets for the assessment of the hygienic/sanitary conditions adapted from the control sheets of existing dental medicine cabinets at the level of DSP (Public Health Department) Iasi. **Results and discussion:** The sheets for the assessment of the hygienic/sanitary conditions were evaluated by a specialized team and the results were illustrated in the specific charts. At the level of all the dental cabinets the study revealed non-conformities regarding the means to carry out cleaning, disinfection operations, including the management of perilous waste, the control of medical personnel. **Conclusions:** An optimization of the hygienic-sanitary conditions at the level of dental medicine cabinets is still necessary, through participation to the activity of personnel training**,** who is directly involved in dental medical assistance. **Keywords**: HYGIENIC-SANITARY CONDITIONS, DENTAL MEDICINE CABINETS, ASSESSMENT

The evaluation of hygienic-sanitary conditions at the level of dental medicine cabinets through the verification of certain hygienic aspects, as well as of the environment's parameters, with the identification of deficiencies and the implementation of some measures to fix them is an objective of priority in view of ensuring a quality dental assistance by means of removing bacterial, viral and mycotic infections as well as by assessing the epidemiologic risk (1, 2, 3).

MATERIAL AND METHODS

The study conducted consists in monitoring the hygienic/sanitary conditions at the level of 68 dental medicine cabinets (40 private cabinets, 20 from the urban environment and 10 from the rural environment) and 28 school/university dental cabinets in Iasi county, using sheets for the assessment of the hygienic/sanitary conditions adapted from the control sheets of existing dental medicine cabinets at the level of DSP (Public Health Department) Iasi (4, 5, 6, 7).

The following aspects were monitored:

- Functional structure of cabinets;
- Their location;

- Access;
- Existence of functional circuits and compliance with them;
- Washable surfaces;
- Supply with drinkable water;
- Existence of sinks for cleaning instruments and for the hygiene of hands;
- Connection to the sewage system;
- Natural and artificial lighting;
- Status of sanitary groups;
- Thermal microclimate;
- Manner of sanitary materials' sterilization;
- Compliance with norms regarding the handling operations of used instruments;
- Existence of cleaning materials;
- Existence of disinfectant and antiseptic substances approved by the MS (Ministry of Health);
- The way decontaminants are used and their existence in stock;
- The situation of medical and auxiliary personnel;
- The management of dangerous waste according to Order 219/2002;
- Cabinets' facilities.

RESULTS AND DISCUSSION

Out of the 68 dental cabinets under investigation, a number of 40 are private, the rest being school/university dental cabinets (fig. 1).

Out of the 40 cabinets of private dental medicine, 30 (75%) are from the urban environment, while 10 (25%) from the rural one (fig. 2).

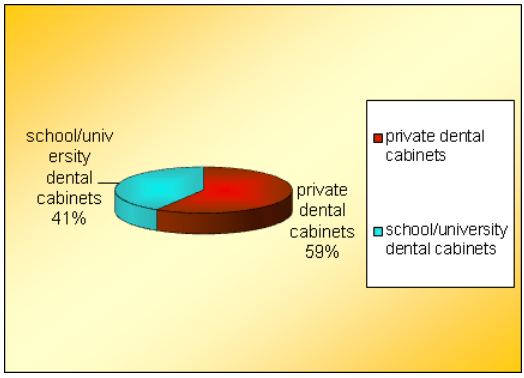

Fig. 1. Repartition or distribution of dental cabinets – depending on the form of organization.

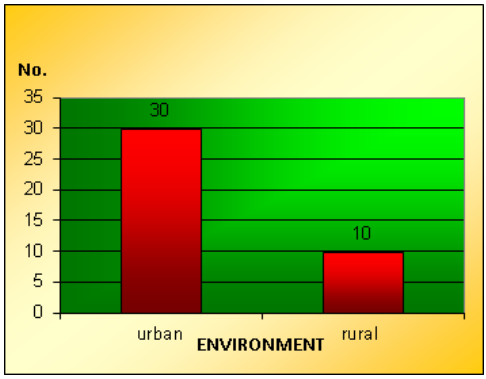

Fig. 2. Repartition or distribution of dental cabinets – depending on the environment

The number of rooms varies between 6 and 8 rooms, including the dental cabinet as such, an office, a sterilization room, dressing room, waiting room, temporary storage room for dangerous waste, 1-2 washrooms. The surface of the dental cabinet itself varies from 12 to 22 square meters.

At a number of 49 cabinets there is no sterilization room, the sterilization being conducted at the level of the cabinet itself.

Out of the 68 dental cabinets**,** 19 operated at the ground floor of blocks of flats, 5 in houses, 16 with commercial venues whose construction was destined to medical activities and the 28 school/university den-

tal cabinets operated inside the academic buildings (fig. 3).

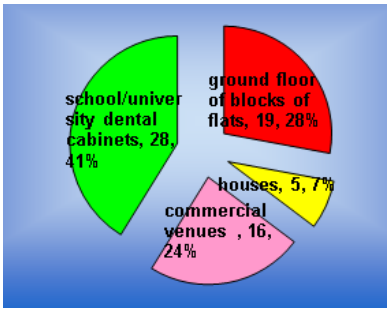

Fig. 3. Repartition or distribution of dental cabinets – depending on the location

Access to the dental practice was identified as follows:

- Separated from that of inhabitants at a number of 60 cabinets (28 school/university cabinets and 32 private dental cabinets);
- Common with that of the inhabitants at 8 private dental cabinets (blocks of flats, living houses) (fig. 4).

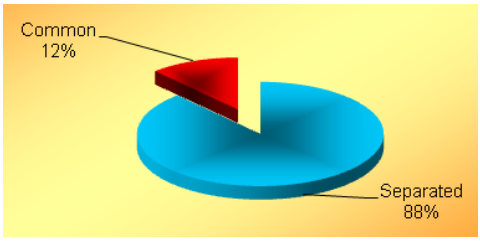

Fig. 4. Repartition or distribution of dental cabinets – depending on the access

The dental cabinets under investigation had proper functional circuits, but at a number of 10 private dental cabinets these circuits were not observed, the functional circuits being modified due to the installation of new equipment in those particular cabinets.

All cabinets under investigation had washable surfaces (floors), with differences related only to the materials used:

Out of the 28 school/university cabinets under investigation, 21 (75%) had mosaic floor and 7 (25%) floor tiles.

At the private dental cabinets under investigation a number of 13 cabinets had mosaic floor, 20 floor tiles and 7 heavy duty traffic linoleum (*tarkett*) and epoxy resin.

In rural environment there are mostly mosaic surfaces (dental cabinets in old buildings) – 7 cabinets, only 3 cabinets having tile floors (fig. 5).

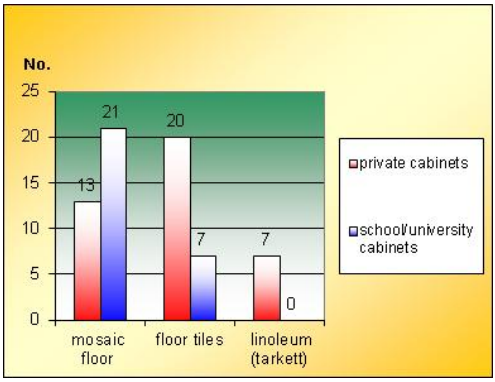

Fig. 5. Repartition or distribution of dental cabinets – depending on the surface/form of organization

The 58 dental cabinets in the urban environment (28 school/university and 30 private) are connected to the central distribution network of **potable water**, cold water being delivered on a permanent basis.

In the rural environment, out of the 10 cabinets under investigation, 5 were connected to the central water network while for the other 5 cabinets potable water supply was ensured through individual installations.

A cabinet in the rural environment connected to the centralized network had no

current water at the moment of the investigation due to some construction works, the cabinet having no supplies of potable water at the moment of the control (fig. 6).

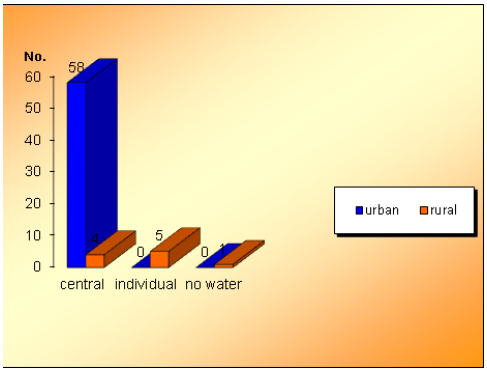

Fig. 6. Repartition of dental cabinets – depending on the potable water supply

All dental cabinets investigated has a **supply of hot water,** its existence being an essential condition for compliance with the hygienic-sanitary conditions at the level of the cabinets in view of a good performance of their activity.

Thus, out of the 40 private cabinets, 19 cabinets were connected to the city's centralized network of hot water and 21 had individual installations (thermal units and electric boilers).

Out of the 28 school/university dental cabinets, 15 were connected to the centralized network of hot water while for the rest hot water was supplied through individual installations (thermal units, electric boilers, instant boilers) (fig. 7).

In the rural environment, the 10 cabinets had mostly own installations (electric boilers, thermal units).

In all 68 dental cabinets under investigation there are sinks with separate destination: for handling the instruments and for the hygiene of the personnel's hands.

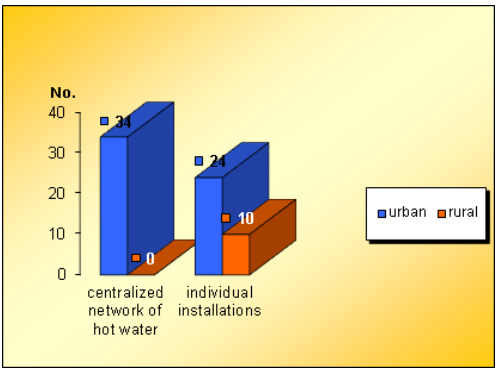

Fig. 7. Repartition of dental cabinets – depending on the means of producing hot water

There is a connection to the city's sewage system in view of evacuating **residual waste** for all cabinets (school/university and private cabinets) in the urban environment. The evacuation of residual waste for the 10 cabinets in the rural environment is conducted by means of septic tanks.

The existence of **washrooms for the medical staff separate from the patients' occurs** mainly in the case of dental cabinets destined by construction to medical activities and in the case of school/ university dental cabinets, where there are several sanitary groups.

In case of 18 private cabinets in the urban environment there is no possibility to build a second sanitary unit as the cabinets have been transformed from apartments to medical cabinets.

In the rural environment, 5 private cabinets have two sanitary groups, namely for the medical staff and for the patients.

A **natural and artificial** lighting is ensured in all dental cabinets in optimum conditions.

The thermal microclimate at the level of the 68 cabinets is ensured as follows: 19 dental cabinets were connected to the city's

heating network, 21 had individual installations (thermal units, stoves in the rural environment), while out of the 28 school/university dental cabinets 15 were connected to the centralized heating network and the rest of 13 had individual installations (thermal units) (fig. 8).

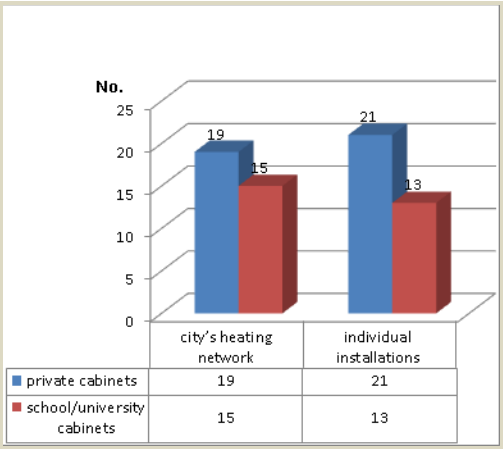

Fig. 8 Repartition of dental cabinets – depending on the means of accomplishing the thermal climate

A number of 42 dental cabinets investigated (35 of the private cabinets and 5 of the school/university cabinets, as well as 2 out of the rural environment) were endowed with **air-conditioning systems** in view of ensuring thermal comfort for the hot season.

With regard to the **sterilization of sanitary materials,** from the 68 cabinets investigated, for a number of 19 cabinets sterilization was carried out in a specially designed space – the sterilization room, while for the rest sterilization was carried out at the level of the cabinet itself. In all cases, the sterile material was not mixed up with the non-sterile one, there being no contamination danger.

All cabinets had hot air sterilizing units (*Poupinel* type), but only 16 had sterilizing units with vapors under pressure (autoclave). In all cases, the equipment used in view of sterilizing instruments and soft materials were authorized and approved by the regulating agencies.

For a number of 30 dental cabinets under investigation, there was no **technical control** conducted for the sterilizing units by specialized companies.

With regard to the correct **tallying of the sterilization procedures** as well as the **use of self-control tests**, for a number of 10 dental cabinets there were found deficiencies concerning: the proper filing of procedures by failure to cover all boxes of the Sterilization Record Book, knowledge of the sterilization technique, proper marking of self-control test kits, insufficient supplies as well as invalid expiry date for the tests – a deficiency found at one dental cabinet.

At a number of 5 cabinets, there were found deficiencies regarding the **storage and labeling** of the sterilized kits.

Handling instruments in view of sterilization is carried out properly at the 40 private cabinets, except for the 28 school/ university cabinets, which use the same solution (detergent/disinfectant) for the two stages of handling the instruments, there being no disinfectant for stage 2.

With regard to the **cleaning and disinfection materials** used at the level of the cabinets, there are sufficient quantities in stock for a number of 50 cabinets, while for the rest of the cabinets (18 school/ university cabinets) there was an absence of a proper supply of detergent-disinfectant, as well as of cleaning materials. At 8 dental cabinets (private and school/university) there was an improper storage of cleaning and disinfection materials.

The use of **decontaminants** is conducted with the observance of the instructions of use, at the concentrations at times specified by the producer at a number of 55 dental cabinets out of the 68 cabinets investigated, while at a number of 10 school/university cabinets and 3 private cabinets the decontaminants were used improperly.

The number of doctors who perform their activity at the level of dental cabinets varies from 1 to 6, depending on the dental units inside the cabinets.

A peculiar situation is the present of **auxiliary sanitary personnel (nurses)**; out of the 40 private dental cabinets, 29 were operating without a nurse and at the level school dental cabinets 5 were operating without nurses.

With regard to the personnel's **medical exams,** we found out that the provisions of the Emergency Ordinance 355/2007 regarding the surveillance of the health condition of the personnel were not complied with entirely, out of the 68 dental cabinets 10 failed to show medical exams according to the technical norms of Ministry of Health (8).

As a rule, the **protection equipment** is used in all cabinets but most of the times it is incomplete (absence of goggles and mask). The single-use gloves are used by doctors in 90% of the cases in the cabinets under investigation. The medical personnel were found to be vaccinated for B hepatitis.

Another problem encountered at the level of school/university cabinets is the **high number of pupils/students** per doctor, dental assistance being granted by a single doctor to a large number of students.

A special issue which arises in dental practice is related to the **management of solid waste,** in agreement with the Order of the Minister of Health no. 219/2002, especially of perilous waste resulted from the medical practice (9,10).

At the level of the 28 school/university dental cabinets and at a number of 30 private cabinets, there are no **temporary storage facilities for perilous waste** functionally separate from the rest of the construction, in the rest of the cases there being spaces inside constructions.

Primary recollection of waste with septic potential is carried out separately from sewage waste in all cases, but at a number of 10 private dental cabinets and at a number of 11 school/university cabinets the cutting/piercing waste was collected in carton boxes indiscriminately with septic waste.

The recipients - the bags used – are identified differently (yellow with the icon "Biologic Hazard" for perilous waste and black for domestic garbage). At a number of 10 cabinets it was assessed that the bins were not washed and disinfected every time they were emptied.

All private dental cabinets investigates **have entered contracts with a specialized authorized company for the transportation and elimination** of hazardous waste. The contract wasn't renewed in the case of 5 school/university dental cabinets.

At a number of 10 school/university dental cabinets, as well as at the level of the dental cabinets in the rural environment the **schedule for the collection of perilous waste** is not observed, the collection being carried out every two-three days or even more, in breach of the provisions of the Order of the Minister of Health no. 219/2002, due to the fact that contracts for school/university dental cabinets were not renewed and, for the rural environment, the motivation of companies being that, given

the small operational volume of medical waste the collection is not economically justified.

With regard to the cabinet facilities, it was assessed that there is proper **furniture, instruments and medical equipment** at the level of the private dental cabinets while for the 28 school/university dental cabinets a number of 5 had improper worn-out furniture, instruments and equipment.

CONCLUSIONS

At the level of school/university dental cabinets the study assessed deficiencies regarding:

- The supply with cleaning and disinfectant substances, as well as in their proper use;
- The use of the same solution (detergent- disinfectant) for the two stages of instrument handling, stage two disinfectant being absent;
- Management of perilous waste (improper collection, the waste being stored at the level of the cabinet for long intervals, sometimes for weeks, due to insufficient funds which prevent the conclusion of a contract with a specialized waste management company;
- The absence of temporary storage locations of perilous waste;
- Endowment with proper furniture, instruments and medical equipment;
- Absence of auxiliary sanitary personnel there being cabinets functioning without a medical nurse;
- The existence of a large number of patients (pupils/students) for a single doctor;
- The personnel's periodic medical exams and the proper use of the protection equipment.

At the level of private dental cabinets the study revealed nonconformities regarding:

- Compliance with functional circuits due to the installation of new equipment;
- Non-familiarization with the proper way to handle instruments in view of sterilization;
- Deficiencies regarding the sterilization process (non-familiarization with the sterilization technique, proper recording of sterilization, non-marking of self-control kits, failure to conduct a technical control of the sterilization equipment);
- Deficiencies regarding the storage and collection of perilous waste resulted from the medical activity (failure to observe the schedule of waste collection by the authorized companies, especially in the rural environment);
- The study assessed no deficiencies regarding the supply with cleaning and disinfectant substances as well as their proper use.

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NEWS

GINGIVAL EVALUATION IN CHILDREN WITH CONGENITAL CARDIOVASCULAR DISEASES

Children with congenital cardiovascular diseases who suffer from dental diseases present an increased risk of infective endocarditis. Oral inflammatory diseases may also increase the severity of their cardiovascular condition. The purpose of a study realized by a group of american researchers was to evaluate the gingival status of children with congenital cardiovascular diseases in comparison to healthy children. In the study were included 50 children, aged between 7 and 13 year old. The test group comprised 25 children with congenital cardiovascular diseases subdivided into 3 groups: *the first group*- unrepaired ventricular septal defect; *the second group* - aortic valve stenosis; *the third group* - coarctation of the aorta. The control group consisted of 25 healthy age- and gender-matched children. Plaque, calculus, gingivitis and recession were measured on 12 teeth, six sites per tooth. The results showed that children with congenital cardiovascular diseases had significantly more plaque (P<.001), calculus (P<.001), gingivitis (P<.001) and recession (P>.02) than control group. Among the congenital cardiovascular diseases groups, no statistically significant differences were found for plaque, gingivitis or recession. The conclusions of the study showed that children with congenital cardiovascular diseases had a higher prevalence of periodontal disease, evidenced by gingivitis, plaque, calculus, and recession. These children should be evaluated periodontally and their oral health monitored on a 3-month basis to prevent disease development, benefit cardiovascular condition, prevent endocarditis and to improve quality and longevity of life. (Nosrati E, Eckert GJ, Kowolik MJ et al.Gingival evaluation of the pediatric cardiac patient. *Pediatr Dent.* 2013; 35 (5): 456-462)

Irina Grădinaru

METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRIAGE IN INFANTS WITH PROTEIN-ENERGY MALNUTRITION

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METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRIAGE IN INFANTS WITH PROTEIN-ENERGY MALNUTRITION (Abstract) **Aim:** The aim of the present study was to investigate the frequency of methicillin-resistant *S. aureus* (MRSA) colonization in infants with protein-energy malnutrition (PEM) and to characterize the antibiotic susceptibility patterns in isolated strains. **Material and methods:** The study included 123 infants with PEM, admitted to the Paediatric Rehabilitation Department of „St. Mary" Emergency Clinical Children's Hospital Iaşi, during October 1st 2010 and August 30th 2011. Nasal, pharyngeal and conjunctival swabs were collected for detection of colonization upon admission and at discharge. **Results:** The study revealed a high rate of MRSA carriage among infants with PEM (62.60%), most of the isolated strains being multidrug-resistant (86.13%), with additional resistance to aminoglycosides, macrolides and inducible resistance to clindamycin. 88% of colonized infants were nasal carriers. **Conclusions:** Identification of MRSA carriers permits the implementation of measures that decrease the risk for subsequent infection. **Keywords**: *STAPHYLOCOCCUS AUREUS*, MRSA, PEM.

Staphylococcus aureus can cause a wide range of diseases, from local to deep-seated infections, in all age groups, in both hospitals and community worldwide (1). The emergence and spread of multi-drug resistant (MDR) methicillin-resistant *S. aureus* (MRSA) strains have raised the problem of finding efficient treatment of staphylococcal infections. *S. aureus* is part of the normal flora in a significant fraction of the healthy population. However, persistent carriers are known to have a higher risk of developing *S. aureus* infections (2). Nasal colonization with MRSA strains is increased in settings where people are in close contact and neonates and infants are particularly more susceptible to colonization and infection with MRSA, one of the most versatile of childhood pathogens (1, 3, 4). This risk is increased in infants with protein-energy malnutrition (PEM), a potentially fatal nutritional disorder, encountered in children world-wide and often associated with a wide variety of infections (5).

The objectives of this study were to investigate the frequency of MRSA colonization in infants with PEM, to characterize the antibiotic susceptibility patterns in

isolated strains, as well as to evaluate the performance of surveillance through cultures at various body sites for *S. aureus* colonization.

MATERIAL AND METHODS

The study included 123 infants with PEM, aged between 5 and 48 weeks, admitted to the Paediatric Rehabilitation Department of „St. Mary" Emergency Clinical Children's Hospital Iaşi, during October 1st 2010 and August 30th 2011. Of the infants included in the study, 107 were born in maternity (87%), 46 required resuscitation manoeuvres (37, 4%), and 53 received complex treatment in Neonatal Intensive Care Unit (43,1%). Nasal, pharyngeal and conjunctival swabs were collected upon admission and at discharge. The samples were subsequently processed at "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Microbiology Department.

The complete antimicrobial susceptibility patterns were evaluated using disk diffusion method, as recommended by Clinical and Laboratory Standards Institute (CLSI) guidelines 2010. We tested the sensitivity to the following antibiotics: penicillin (P), oxacillin (OX), cephoxitin (FOX), clindamycin (CM), erythromycin (E), tetracycline (TE), gentamicin (G), tobramycin (T), kanamycin (K), rifampicin (RIF), trimethoprim / sulfamethoxazole (SXT), fluoroquinolones (FQ), linezolid (LZD) and teicoplanin (TEC). Inducible resistance to CM (iCM) was assessed by double-disk (E and CM) diffusion method (D-zone test). Minimum inhibitory concentrations (MICs) for vancomycin (VA) and OX were determined using E-test method (*bioMérieux*, France). Multi-drug resistance among MRSA was defined as concurrent resistance to three or more non-beta-lactam antimicrobial agents.

RESULTS

A total of 114 *S. aureus* strains were isolated from 84 patients (68.29% of the total number of infants) upon admission (52 strains) and at discharge (62 strains), as follows: in 30 infants *S. aureus* was found both upon admission and at discharge, 22 infants carried *S. aureus* only upon admission and in 32 infants *S. aureus* was found only at discharge. In 42 patients *S. aureus* was found only in nasal swabs, in 32 cases nasal and extra nasal carriage was detected, while 10 infants were colonized only extranasally.

Of the total number of *S. aureus* strains, 101 were MRSA (88.59%), isolated upon admission (43 strains), as well as at discharge (58 strains) from 77 infants (62.6% of the total number of infants and 91.66% of colonized infants). Most MRSA strains were isolated from nasal swabs (66.33%).

The sensitivity of 30 µg cephoxitin disk testing was 99% when compared to MIC detection. MICs for OX varied between 0.125-256 µg/ml, with a heteroresistance rate of 14.85 % of the total number of strains. A high proportion of strains (42.98%) showed OX MICs of > 256 µg/mL. MICs for VA were in the susceptible range, varrying from 0.125 to 2µg/mL. MIC_{50} and MIC_{90} values were as follows: for OX – 64 µg/Ml, and > 256 µg/mL respectively, and for VA – 1 µg/mL, and 1.5µg/mL respectively.

Of 101 MRSA strains, 87 were MDR (86.13%). MRSA isolates had additional resistance to aminoglycosides (fenotype KTG – 60 strains and fenotype K – 37 strains), macrolides (93 strains), tetracyclines (82 strains) and iCM (87 strains). Twelve different antibiotic resistance patterns were observed among MRSA isolates,

the most frequent profile being: resistant to KTG, E, iCM, TE and intermediate to DO (32 strains), found upon admission (15/32) as well at discharge (17/32). Among the resistance patterns studied, we noticed the predominance at admission of the profile resistant to K, E, iCM, TE, intermediate to DO (10/17), and the predominance at discharge of isolates belonging to the profile resistant to KTG, E, iCM, TE (11/15)

Methicillin susceptible *S. aureus* (MSSA) showed increased susceptibility to antibiotics compared to MRSA strains, the majority being resistant only to P (9/13), while two of them were also resistant to aminoglycosides and other two were resistant to tetracyclines. Most MSSA strains were detected upon admission (9/13).

All strains were sensitive to LZD, VA, TEC, SXT, FQ, and RIF. There was no case of associated or subsequent staphylococcal infection in colonized infants.

DISCUSSION

In our country, the number of infants with PEM remains high due to certain specific situations: tendency to stop breastfeeding precociously, gradually intensified during the last decades; inability to ensure a proper artificial nutrition during the first months of life mainly due to lower revenue; a great number of children from disorganized families or abandoned at birth (6). Their nutritional recovery is a long process which requires continuous medical surveillance in care units and, on the other hand, long-term hospitalization predisposes to infection.

S. aureus is an important nosocomial pathogen (7). Colonization with *S. aureus* has been described as a risk factor for infection (8) and seems to be associated with an increased risk of atopic dermatitis in infants, although the latter is still disputed. A study by Lebon *et al.* showed that *S. aureus* colonization at 6 months and frequent colonization in the first year of life are associated with moderate to severe atopic dermatitis, while in a study by Skov *et al.*, no such relation was found (2, 9).

Colonization with MRSA strains poses a greater risk of infection than MSSA colonization and usually precedes invasive infection (7, 8). A study by Davis *et al.* shows that 19% of patients colonized with MRSA at admission and 25% of those colonized during hospitalization developed infection, compared with 1.5% of patients colonized with MSSA and 2.0% of patients uncolonized. MRSA carriage at admission was found to increase the risk of infection, compared with MSSA carriage or no staphylococcal colonization at admission (7). However, according to Fritz *et al.*, MRSA nasal carriage is mostly self-limited, compared to MSSA carriage which seems to be more persistent (10). The anterior nares are the most frequent carriage site (2). It is estimated that 20–40% of healthy individuals are nasal carriers (1). We have evaluated 3 sites for *S. aureus* colonization: nares, oropharynx and conjunctiva. We found that nasal cultures were able to detect carriage in 88% of colonized infants, the rest of the patients being colonized only extranasally. A study by Cursino *et al.* evaluated 4 sites for colonization in newborns from Brazil and concluded that multiple culture sites are needed, a combination of nares and umbilical samples detecting 86% of MSSA and 91% of MRSA colonization cases (11).

The rate of staphylococcal colonization among normal weight children varies greatly with age, being 40 - 50% in newborns

and infants during the first 8 weeks and falls to 21% by 6 months (11,12, 13) According to Chatzakis *et al.*, the rate of infant carriage in Greece was lower, 17.7% respectively (14). In a study by Sedighi *et al.*, in Iran, 22.5% of children from day-care centres were carriers, but the rate of MRSA colonization was very low (4.1%) (3). In Romania, a study by Constantiniu *et al.* revealed a rate of *S. aureus* colonization among children in community of 7.18%, of which only 5.4 % were MRSA (15). Among our patients, the rate of colonization with *S. aureus* was very high (68.29%), of which the majority were colonized with MRSA (91.66%).

Persistent colonization poses a higher risk of staphylococcal infections. Children are thought to have higher persistent carriage rates than adults (13), although Lebon *et al*. dispute that in infant population those carriers are rare (2). Children with persistent staphylococcal colonization often carry identical strains (10). Our study revealed that 8.33% of colonized infants carried *S. aureus* strains with identical antibiotic resistance patterns upon admission and at discharge, while 27.38% had strains with different resistance profiles which suggest that, in some cases, the source of these strains was the care unit. On the other hand, no case of subsequent infection occurred among infants colonized at admission to the hospital or in those who developed colonization during hospitalization. We note that persistent carriage needs to be evaluated periodically during hospitalization and even after discharge, since, in many cases, transition to intermittent or noncarriage state is possible. The isolation of *S. aureus* strains in a high percentage of infants after admission to hospital could be explained by the high rate of pathological antecedents together with the immune deficit due to malnutrition, but it could also be caused by close contact during hospitalization or transmission from colonized mothers. In a study by Peacock *et al*. (UK) the source for infant colonization was usually represented by the mother (12). In our study, staphylococcal colonization in mothers was not investigated.

We appreciate that the increase in MRSA colonization was facilitated by the long hospitalization duration which was needed to improve weight deficit and the acquired immunodeficiency by low nutrient intake.

Infants that were healthy carriers did not receive topical mupirocin ointment because it was not available for purchase.

We emphasize that the sanitary rules have been respected, and we tried to ensure optimal conditions for nursing, in order to limit the involvement of environmental factors in the dissemination of S. *aureus*. However, we have not managed to maintain, throughout the duration of the study, an effective ratio of staff to children, because of an acute shortage of medical staff.

CONCLUSIONS

We conclude that colonization by *S. aureus* and MRSA strains is common in infants with PEM hospitalized in Paediatric Rehabilitation Department (68.29% and 62.6%, respectively), much higher compared with studies from other countries, with the major remark that citated studies were about children with normal weight; no subsequent staphylococcal infections were reported. Antimicrobial susceptibility testing revealed a worryingly high rate of multidrug-resistance among MRSA strains

isolated from colonized infants (86.13%).

Given the age group studied and the antibiotic sensitivity spectrum of MRSA strains isolated, we consider linezolid and vancomycin as therapeutic options in the case of colonized infants that develop subsequent staphylococcal infection; cotrimoxazole can be used in infants older than 2 months, and fluoroquinolones are not recommended because of their adverse effects.

This study highlights the need to improve primary care to ensure an adequate nutritional status of infants, in order to disrupt the vicious circle malnutrition - infection - malnutrition.

Detecting MRSA colonization at admission allows us to identify a high-risk population and facilitates the implementation of strategies for decolonization and reduction of MRSA transmission and infections.

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NEWS

CARDIOVASCULAR EFFECTS OF INTENSIVE LIFESTYLE MANAGEMENT IN PATIENTS WITH TYPE 2 DIABETES

Weight loss and healthy eating are recommended for overweight and obese patients with type 2 diabetes due to the positive effects verified in the short term but long-term cardiovascular effects have not been demonstrated. In 16 U.S.A. centers were randomized 5145 patients with type 2 diabetes, overweight and obese, who received a hypo caloric diet and exercise training (intervention group) versus medical treatment of diabetes (control group). The main objective was to quantify the deaths from cardiovascular causes, cerebral, myocardial infarction, and hospitalization for angina, for a follow-up of 13.5 years. The trial was stopped early, after a period of 9.6 years, as it was reported that, although it was a significant decrease in weight (8.6% *vs.* 0.7% at one year, 6% vs. 3.5% at the final), the difference in cardiovascular morbidity and mortality was only 403 patients in the intervention group compared to 418 in the control group (p = 0,51). The remaining benefits were significant lifestyle changes: decreased glycosylated hemoglobin, partial remission of diabetes, reduction of urinary incontinence, sleep apnea, depression and quality of life. (The Look AHEAD Research Group, Cardiovascular effects of intensive lifestyle intervention in type 2 diabetes, *N Engl J Med* 2013; 369: 145-154)

Corina Dima-Cozma

NATURAL CONCEPTION FOLLOWING TOTAL FERTILIZATION FAILURE WITH INTRACYTOPLASMIC SPERM INJECTION IN A COUPLE WITH UNEXPLAINED INFERTILITY: A CASE REPORT

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NATURAL CONCEPTION FOLLOWING TOTAL FERTILIZATION FAILURE WITH INTRACYTOPLASMIC SPERM INJECTION IN A COUPLE WITH UNEXPLAINED INFERTILITY: A CASE REPORT (Abstract): With the introduction of intracytoplasmic sperm injection (ICSI), couples with severe male factor infertility have been able to achieve fertilization and clinical pregnancy rates comparable to other in vitro fertilization patients. However, despite the utilization of microsurgical sperm injection techniques, failure of fertilization still occurs in a few patients. How such fertilization failure after ICSI might impact later ICSI treatments is less understood. We report a case of total fertilization failure after ICSI using sperm from a normozoospermic husband of a patient with unexplained infertility. Six months after the cancelled cycle, the couple conceived naturally. Unfortunately, it was an ectopic pregnancy, which required laparoscopy and surgical removal of the right fallopian tube. This case shows that a failed ICSI cycle, therefore, does not imply a hopeless prognosis for future ICSI treatment. Moreover, in cases with unexplained ICSI failure, natural conception can subsequently occur. The aim of this study is two-fold: to discuss a rare case of a spontaneous pregnancy after total fertilization failure with ICSI and to develop counseling material for patients and doctors who are faced with such a rare situation. To our knowledge, this is the first report of a case of spontaneous pregnancy after total fertilization failure with ICSI. **Keywords:** TOTAL FERTILIZATION FAILURE, INTRACYTOPLASMIC SPERM INJECTION, UNEXPLAINED INFERTILITY.

Intracytoplasmic sperm injection (ICSI) is a method of micromanipulation used to deposit a spermatozoon directly into the oocyte cytoplasm. When all other forms of assisted fertilization are unsuccessful, ICSI is the method of choice to overcome male infertility. ICSI has enabled fertilization of oocytes from patients whose partners have extremely low numbers of viable sperm and a very low probability of achieving fertilization after conventional spermoocyte incubation in vitro. ICSI is possible with spermatozoa obtained from ejaculate, percutaneous epididymal sperm aspiration (PESA), microsurgical epididymal sperm extraction (MESE), or testicular sperm extraction. In addition, indications for ICSI include unexplained infertility and previous conventional in vitro fertilization (IVF) failure. Although the fertilization rates

after ICSI are relatively high (1), some injected oocytes fail to fertilize despite the presence of a spermatozoon in the cytoplasm and the absence of immaturity or degenerative alterations. For all ages and with all different sperm types, the fertilization rate after ICSI is reported to be approximately 70–80% (2). This fertilization failure after ICSI may occur because of the following reasons. First, the injected oocyte may fail to initiate the biochemical processes necessary for oocyte activation (3). Second, the biochemical processes are initiated, but they may not occur normally, thus leading to incomplete activation. Third, the spermatozoon may remain poorly accessible to oocyte factors required for chromatin decondensation and formation of the male pronucleus (4). Finally, the procedure used for ICSI may induce subtle structural changes in the organelles of the oocytes, which are undetectable by light microscopy. Although failed fertilization in some of the injected metaphase II (MII) mature oocytes is quite common, total fertilization failure (TFF) after ICSI is distressing for the infertile couple as well as for the fertility professionals.

TFF is a rare event in cases with normal oocytes and spermatozoa (5), and few patients may experience repeated TFF despite normal sperm parameters and a good ovarian response (6). In such cases, the primary reason for failed fertilization after ICSI is the lack of oocyte activation (7). Oocyte activation involves a complex series of events that result in the release of cortical granules, activation of membrane ATPase, meiotic resumption, and the formation of the male and female pronuclei with the extrusion of the second polar body. The oocyte is activated when the spermatozoon enters by either natural fertilization or ICSI. When a spermatozoon comes in contact with the oolemma and penetrates the ooplasm, intracellular calcium oscillation occurs, and this increase in calcium concentration induces oocyte activation and the initiation of zygote development (8). Several authors have proposed different techniques of artificial oocyte activation. Some assisted activation treatments, such as puromycin (9), promote an increase in the intracellular free calcium concentration by releasing calcium from cytoplasmic stores; other activation treatments, such as electrical stimulus (10), promote the influx of calcium from the extracellular medium. Positive results have been obtained by using a combination of calcium ionophore and puromycin (11). Similar to other new assisted reproductive procedures, the impact of either electrical or chemical oocyte activation on the health of the embryo must be evaluated by conducting larger studies, before they can be considered for routine clinical purposes.

For the typical patient who fails to achieve fertilization with ICSI, models proposed to explain why fertilization failed may be less helpful than the counseling requested regarding future treatment. Unfortunately, the efficacy of subsequent infertility treatments following TFF after ICSI remains largely unknown. Therefore, one aim of this study was to develop information that would be useful in counseling patients who experienced TFF with ICSI.

CASE REPORT

A 39-year-old woman and her 45-year-old husband were referred to our clinic for unexplained infertility; they had been married and trying to conceive for more than 2 years. Her physical and gynecological ex-

aminations revealed normal results, including hysterosalpingography and routine blood tests. Her menses were regular. However, her anti-mullerian hormone serum concentration (0.82 ng/ml) and antral follicle count (6) were indicative of low ovarian reserve. Other blood hormone concentrations were within the normal ranges (day 3 follicle-stimulating hormone (FSH) 7.01; thyroid-stimulating hormone 1.1 mUI/ml; prolactin 22.8 ng/ml). The mean sperm parameters were as follows: volume 2.7 ml; count 50.53×10^6/ml (1 specimen had relatively low numbers); motility (A + B) 42.0%, and normal morphology 7.0%.

First, we advised them regarding the optimal timing for intercourse, and, after 3 months, we administered 4 cycles of stimulated intrauterine insemination (IUI), which were unsuccessful. Subsequently, after obtaining informed consent, we offered IVF to the couple. In May 2012, the couple underwent their first IVF cycle. A long GnRH agonist protocol was applied at a daily low dose of 0.05 mg (Gonapeptyl®; Ferring, Switzerland) starting from day 21 of the previous cycle. Ovarian stimulation was achieved using a combination of recombinant FSH and recombinant luteinizing hormone (rLH), to a total dose of 300 IU FSH and 75 IU LH per day (Gonal F® and Pergoveris®; Serono, Switzerland). An injection of 250 mcg (6500 IU) of recombinant human chorionic gonadotropin (hCG, Ovitrelle®; Serono, Switzerland) was administered when the leading follicle reached a mean diameter of 20 mm. Transvaginal ultrasound-guided needle aspiration of oocytes was performed 36 h after the hCG injection. All oocyte-handling procedures were conducted on warm stages (37^oC) using conventional methods. We retrieved 10 oocytes: 8 were mature (MII) and 2 were immature. We performed ICSI for all 8 MII oocytes after a 4-h incubation period. The injected oocytes were cultured for several hours in Cook MINC incubators, which provide a special low-oxygen environment for the embryos; the incubators are supplied by a triple gas mixture containing 5% O_2, 6% CO_2, and 89% N_2, as opposed to the standard air supply, which contains 5% or 6% CO_2 and 20% O_2. Fertilization was assessed 18 h after ICSI by 2 embryologists: none of the 8 injected oocytes exhibited pronuclei, and all were judged unfertilized after a final assessment conducted 6 h later (Fig. 1).

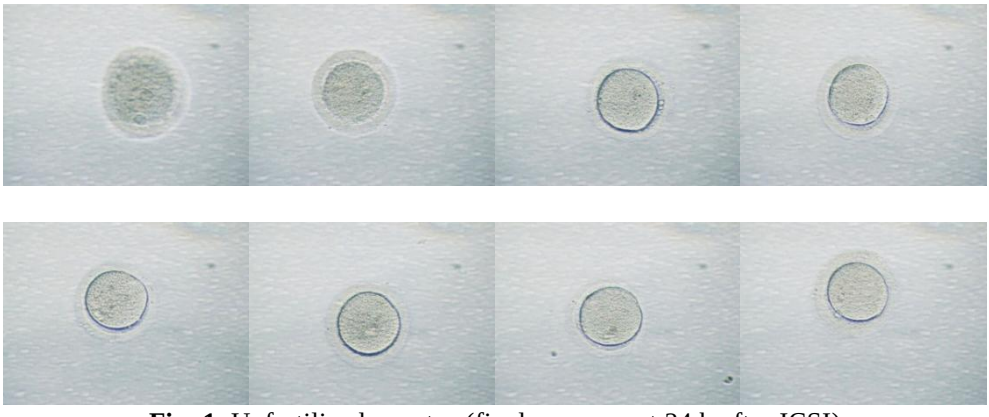

Fig. 1. Unfertilized oocytes (final assessment 24 h after ICSI)

All possible explanations responsible for TFF were analyzed, and all external factors, such as contamination of culture media and improper gamete micromanipulation, were excluded. The lack of fertilization was attributable to the sperm's inability to activate the oocytes, or to the inability of the oocytes to decondense the spermatozoa. However, it was impossible to determine whether the oocytes or spermatozoa were defective as the couple did not agree to an experimental protocol to overcome their complete fertilization block of unknown causes (testing the ability of the husband's sperm to activate mouse oocytes and/or treatment of the patient's oocytes with calcium ionophore in a subsequent ICSI cycle were proposed). After 6 months, the patient asked for a consultation for 6-weeks amenorrhea and a positive pregnancy test. The transvaginal ultrasound examination revealed an ectopic gestational sac, and the patient underwent operative laparoscopy. Surgical findings were as follows: small amount of blood in the peritoneal cavity, bloated right fallopian tube, normal uterus and ovaries, and normal left fallopian tube. After the surgical removal of the right fallopian tube, the patient's recovery was uneventful. A subsequent histological examination of the removed block confirmed the diagnosis of a tubal pregnancy.

DISCUSSION

In this contribution, we describe the case of an infertile couple with unexplained TFF with ICSI. As all 8 oocytes were mature, with no morphological anomalies, and for the ICSI procedure only motile spermatozoa were used, concerns regarding possible technical errors were raised. The risk of oocyte damage by the ICSI procedure is low in humans and may be related to both the skill of the person performing the injection procedure and to the quality and quantity of the gametes used during the procedure. The embryologist conducting the ICSI procedure is a significant predictor of fertilization, whereas laboratory conditions such as the type and model of incubators and culture of oocytes (individually versus grouped) do not affect the fertilization rates (12).

Although ICSI is now considered a routine procedure, it remains a very demanding technique to master, partly due to its inherent technical difficulty and partly due to the heterogeneity of the cases. It is generally agreed that the ICSI procedure is subject to a learning curve. One common technical failure may result in the spermatozoon not being deposited within the cytoplasm of the oocyte. In this situation, the oocyte membrane may not have been broken while attempting to aspirate the ooplasm into the ICSI needle. Thus, the spermatozoon is then deposited next to the oocyte membrane, so that when the oolemma returns to its original position, the spermatozoon is pushed out into the perivitelline space (Fig. 2A), or is trapped inside a sac formed by the membrane (Fig. 2B). The spermatozoon may also adhere to the tip of the injection needle or remain within the injection needle and be inadvertently pulled out upon withdrawal of the needle from the cytoplasm. The degeneration of oocytes after ICSI is often a result of a faulty ICSI technique, e.g., use of an injection pipette that is too large or not sharp enough. Aspiration of the ooplasm is always performed to ensure that the oocyte membrane is broken during injection. However, if excessive ooplasm is aspirated, the oocyte frequently degenerates (Fig. 2C).

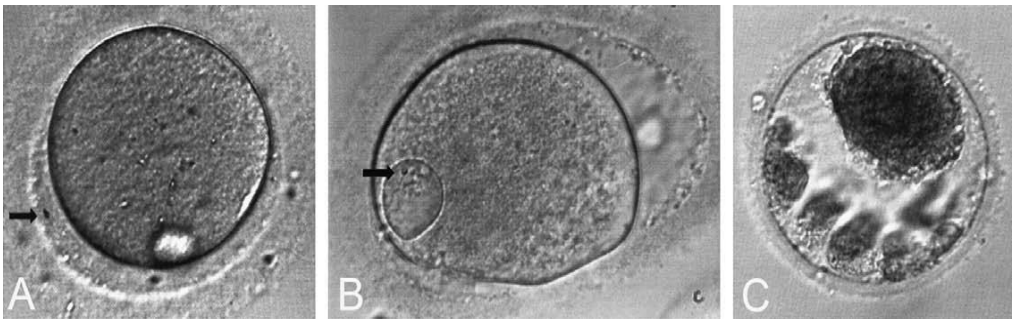

Fig. 2. Damaged oocytes during ICSI (A-spermatozoa in perivitelline space; B-spermatozoa trapped inside a membrane sac; C-degenerated oocyte after excessive cytoplasm aspiration)

None of the previously described aspects were found in this case. When fertilization failure occurs in most or all of the injected oocytes, despite the procedure being performed by experienced practitioners using normal spermatozoa, TFF is attributable to oocyte dysfunction, oocyte activation failure, or inability of the spermatozoon to be decondensed and processed by the oocyte.

Despite frequent use and success of IVF treatment for infertile couples, cases of TFF continue to be encountered. Their consequences are devastating to the patients, with lost resources, incurred costs, and much distress. Understanding the etiology of FF is, therefore, of critical importance to assist patient counseling and optimize treatment.

The incidence of TFF after conventional IVF using sperm of normal quality has been reported to range from 5% (13) to as high as 15–20% (14). While ICSI has overcome many fertilization problems, it does not completely eliminate TFF. In a randomized clinical trial comparing outcomes after ICSI or IVF for cases of non-male factor infertility, Bhattacharya et al. (2001) documented a TFF rate of 2% versus 5% for ICSI and IVF, respectively (15). Indeed, several large studies using ICSI for a variety of infertility diagnoses reported TFF at rates ranging from 1.3% in 1779 cycles(16) to 3% in 2732 cycles (17). The possible etiologies underlying TFF are complex and may relate to the cycle-specific parameters, yield and quality of oocytes, availability of motile sperm, and/or to severity of sperm defects (18, 19, 20). Sperm abnormalities may result in abnormal sperm decondensation and/or aberrant pronuclear development, migration, apposition, and first mitosis (21). Some ICSI oocytes fail to activate, which, under some circumstances, has been successfully overcome by manipulating intracellular calcium levels in oocytes by using calcium ionophore (22) or electric pulses (23). Several early studies concerning gamete ultrastructure (24) and staining of DNA or whole chromosomes (25) led to the identification of abnormal chromatin patterns and/or chromosome numbers, of either paternal or maternal origin, in non-fertilized oocytes. Recent studies of microtubules also revealed abnormal spindle and interphase microtubules, indicating that defects in cytoplasmic components of oocytes may be the main cause of failed fertilization (26).

This case was extremely challenging

from a counseling perspective. For couples experiencing TFF after ICSI, little data exists for patient counseling, especially with respect to the efficacy of continued therapy and the future hope of a successful live birth. To date, only 2 studies have analyzed the outcome of a subsequent conventional ICSI cycle after a previous TFF. In 1 study (published in 2003), TFF reoccurred only once in 23 repeated ICSI cycles; the mean cycle fertilization rate in that population was 69.2%, and the clinical pregnancy rate per transfer was 45.4% (27). In the other study (published in 1995), TFF reoccurred in 5 of the 27 repeated cycles (17). The differences between fertilization rates remain unexplained, although fluctuations in gamete quality may play a contributory role. A history of failed fertilization may be related to a gamete abnormality that may be modified or corrected during the next cycle. Pretreatment endocrine assays and semen analyses have proven to be of little value in predicting failed fertilization. Very subtle improvements in semen parameters and/or oocyte yield/quality may result in fertilization during a subsequent ICSI attempt. Otherwise, the options of donor sperm insemination, donated oocytes or embryos, adoption, and remaining childless should be discussed with the couple (28).

CONCLUSIONS

Despite the important advances in assisted reproductive technologies, successful fertilization for every patient is not guaranteed, even with ICSI. In such cases, new laboratory techniques can aid a subsequent ICSI cycle. However, from a practical point of view, especially in patients with normal sperm parameters, conventional ICSI can be considered a good alternative. It is important to note that no unified theory exists to explain the phenomenon of TFF, since it might be the result of multiple, and, as our case shows, sometimes, temporary anomalies. Therefore, for couples with unexplained infertility (normal sperm parameters and optimal ovarian response) and TFF after ICSI, a subsequent treatment cycle with conventional IVF or split ICSI/IVF may be recommended.

ACKNOWLEDGEMENTS:

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NEWS

SCHIZOPHRENIA AND VIRUS-GENES INTERACTION

Schizophrenia is a severe mental disorder, which affects approximately 1% of the global population. The disease is caused by genetic and environmental factors as well as gene–environment interactions. An international study conducted by Borglum and colleges show that interaction between genes and cytomegalovirus (CMV) influences the risk of developing schizophrenia. Women that have been infected by the virus will have an increased risk of giving birth to a child who later develops schizophrenia, if the child has the gene variant associated with this mental disorder. By using single nucleotide polymorphisms (SNP), the researchers have showed a strong association between schizophrenia and a number of loci such as: SNP rs4757144, located in an intron of the circadian rhythm-associated gene *ARNTL* on 11p15, SNP rs8057927 in the intron of gene *CDH13* on 16q23 (both genes previously linked to schizophrenia or other psychiatric disorders) and, for first time, one region at chromosome 10p11 overlapping the gene *ZEB1*. This study also identified a significant interaction between maternal CMV infection and *CTNNA3* of the progeny, a gene that has not previously been associated with schizophrenia. The results emphasize the importance of environmental factors in disease development. These results may suggest also that a vaccine against CMV may help to prevent schizophrenia (Borglum AD, Demontis D, Grove J, Pallesen J *et all*. Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci. *Mol Psychiatry*. 2013 Jan 29).

Cătălina Luncă

LOCAL PERIPROSTATIC ANESTHESIA BETWEEN OPTION AND NECESSITY IN TRANSRECTAL ULTRASOUND - GUIDED PROSTATE BIOPSY

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LOCAL PERIPROSTATIC ANESTHESIA BETWEEN OPTION AND NECESSITY IN TRANSRECTAL ULTRASOUND-GUIDED PROSTATE BIOPSY (Abstract) According to the European Association of Urology guidelines, local periprostatic anesthesia during ultrasound guided biopsy is "state of the art" without specifying the exact benefits and character of choice vs. necessity of this maneuver. **Aim:** To determine the benefits of using periprostatic anesthesia as standard method of analgesia in patients undergoing transrectal ultrasound guided prostate biopsy. **Material and methods:** We conducted a prospective randomized study involving 100 biopsy patients. The patients were randomized in two groups, 50 patients benefiting from local periprostatic anesthesia with 10 ml of lidocaine and the remaining 50 without local anesthesia. In our clinic we use the 12-core prostate biopsy procedure using 18G/20 cm caliber needles. To assess perceived pain intensity during the procedure, immediately after biopsy we applied to patients a VAS questionnaire (Visual Analogue Scale) as a simple method of quantitative evaluation of a symptom the perception of which varies greatly between individuals. **Results:** A reduction in perceived pain by 45.06% (30.47 vs. 16.74) was recorded in the group receiving local periprostatic anesthesia. It is also worth mentioning that the patients receiving anesthesia said that anesthesia punctures were the most painful (the remaining punctures being much less painful), while patients without anesthesia reported pain intensity levels more or less equal in all 12 performed punctures. **Conclusions:** Local anesthesia is a necessity in ultrasound guided prostate biopsies as it significantly reduces pain intensity in patients undergoing this diagnostic procedure. **Keywords:** PROSTATE BIOPSY, LOCAL ANESTHESIA, PAIN.

The European Association of Urology guidelines state that local periprostatic anesthesia during the ultrasound guided biopsy is a "state of the art" method without specifying its exact benefits and choice vs. necessity of this maneuver (1). Consequently, we decided to conduct a comparative study in patients who already underwent an ultrasound guided transrectal prostate biopsy in an effort to demonstrate whether local periprostatic anesthesia with lidocaine 1% (2) has advantages or not

over no anesthesia in relation with perceived pain during core biopsy.

MATERIAL AND METHODS

In the interval May 21, 2010- October 29, 2012, at the Urology Clinic of the „C.I. Parhon" Hospital in Iaşi, 300 ultrasound guided transrectal prostatic biopsies were performed. The study was conducted on 100 patients who underwent biopsy in the interval September 23, 2010- August 25, 2011 with the purpose of determining whether local periprostatic anesthesia is a state of the art method or a necessity in ultrasound guided transrectal prostatic biopsy.

In our clinic we use the 12-core prostate biopsy procedure (fig.1) using 18G/20cmcaliber needles (fig.2) (3,4,5). For guiding we use a BK Pro Focus UltraView ultrasound equipped with an endorectal transducer allowing the simultaneous visualization on the screen of the transverse and longitudinal planes (fig. 3). This type of visualization facilitates the precise identification of all prostate regions and especially the accurate location of puncture trajectory in different zones of the prostate, thus simplifying the punctures and allowing a high standardization of the biopsy scheme.

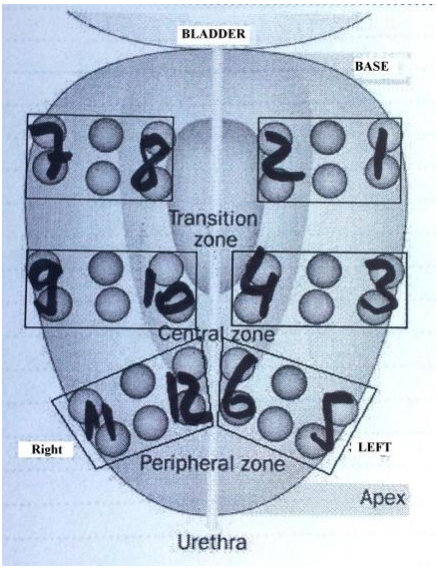

Fig. 1. 12-core biopsy scheme

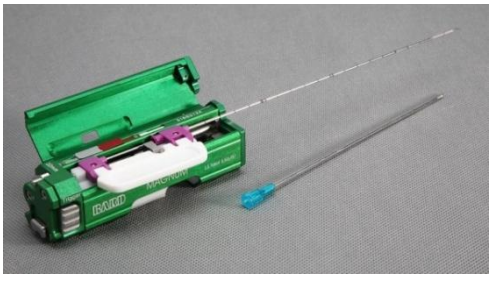

Fig. 2. Biopsy gun with a fixed needle

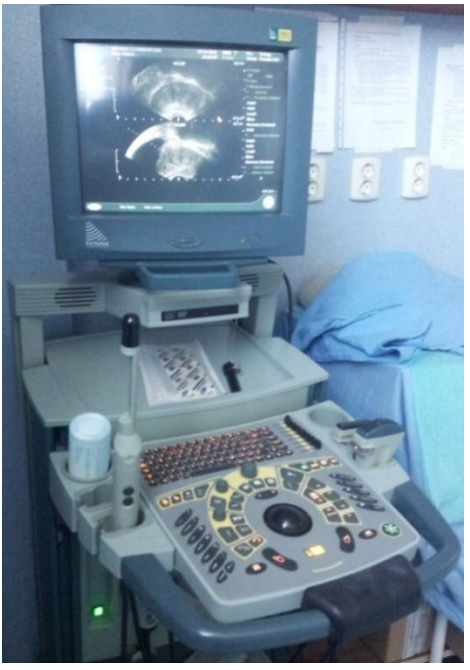

Fig. 3. BK Pro Focus UltraView ultrasound

Also, minimal analgesia with 1 vial of Ketorol and 1 vial of Algocalmin administered 30 minutes before the puncture is provided in all patients.

The study was conducted on a randomized group of 100 patients: all of them were

given the above mentioned minimal analgesia and 50 were also given a periprostatic 10 ml injection of lidocaine (5 ml per side) (6, 7).To assess perceived pain intensity during the procedure, just after biopsy the patients were asked to fill-out a Visual Analogue Scale questionnaire (VAS) as a simple method of quantitatively evaluating pain perception which varies greatly between individuals (fig. 4).

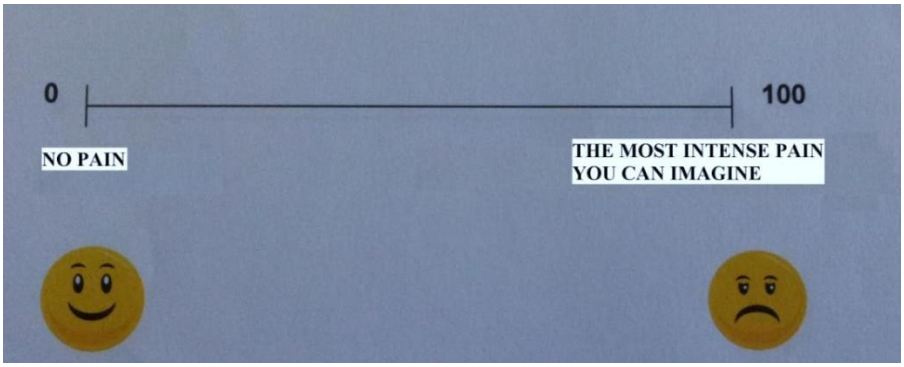

Fig. 4. Visual Analogue Scale (VAS)

The advantage of using VAS is that pain is measured on a 0-100 scale, thus being more sensitive to small variations. Furthermore, the method can be easily explained to patients, who have no problem in understanding the relatively simple manner of rating pain. The scale gives a quantifiable number reflecting the pain perceived by the patient (8).The questionnaire was applied immediately after the completion of the procedure in order to obtain a more accurate evaluation of pain intensity. The patients were asked to rate perceived pain intensity over the whole procedure. This prevented us from strictly assessing the pain felt during biopsy punctures, but added validity to the evaluation of the same type of pain in all patients by the fact that the comparison of the obtained scores is possible and the obtained results reliable. These obstacles arise every time in studies evaluating the perceived level of pain and comparing it between different groups of persons. The difficulty stems from the major differences in pain perception between persons and also from the way pain is defined in relation with a person's social status, physio-pathological history and even professional training (9).

The obtained ratings were added up, an arithmetic average was calculated for each group, and then comparisons were made (tab. I).

TABLE I

Results of the study groups

Groups of patients	**Periprostatic anesthesia**	
	With	**Without**
Number of patients	50	50
Average VAS value	16.74	30.47
Extreme VAS value	0 – 39	2 – 82

In the group with local periprostatic anesthesia a decrease in the perceived pain level by 45.06% (30.47 *vs.* 16.74) – value p 0.00064, was noticed. Furthermore, the patients who received periprostatic anesthesia reported anesthesia punctures as being

more painful (the remaining punctures being less painful), while the patients on no periprostatic anesthesia reported a more or less equal level of pain intensity in all 12 punctures performed. Results distribution shows that in the group with periprostatic anesthesia the highest values of perceived pain intensity were much lower than in the group with no periprostatic anesthesia (82 *vs.* 39) and that there were less VAS scores below 20 in the group with no periprostatic anesthesia (18 patients), while the periprostatic anesthesia group frequently presented VAS scores below 20 (32 patients) (fig. 5).

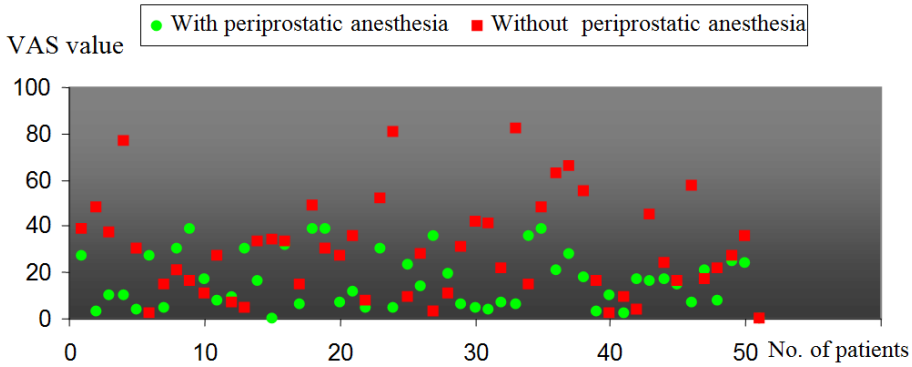

Fig. 5. Distribution of VAS obtained values

CONCLUSIONS

The obtained results are in favor of periprostatic anesthesia regardless of the analysis method. Nevertheless, we need to mention that the only reliable method for statistically analyzing the data obtained by VAS in view of qualitative comparison of groups of persons is represented by the arithmetic average of all obtained ratings. VAS does not provide accurate information when patients are compared individually due to the high degree of subjective rating and difficulty in measuring pain. However, comparing the average scores of the two groups we feel entitled to state that local periprostatic anesthesia is a necessity in ultrasound guided prostate biopsy, significantly reducing perceived pain in the patients undergoing this diagnostic procedure. Under these conditions, as a result of this study we have adopted local periprostatic anesthesia with lidocaine as a mandatory standard method in transrectal ultrasound guided prostate biopsy protocol.

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NEWS

TIME TO OFFER A POLYPILL TO PEOPLE AGED 50 OR OVER?

In David S. Wald and his coauthors study, the clinical and academic aims are to bridge the interventional and preventive approaches to cardiovascular disease. He coordinates the Polypill Prevention Programme, a novel service that adopts the polypill approach in coronary heart disease and stroke prevention. The concept of a "polypill" as a tablet or capsule consisting of a combination of drugs to simultaneously reduce several cardiovascular risk factors first captured worldwide attention in 2003. Suggested components for this initial polypill were aspirin, a statin, 3 blood pressure-lowering drugs at half the standard dose (eg, a thiazide, an angiotensin-converting enzyme [ACE] inhibitor, and a calcium-channel blocker), and folic acid. Since that time, several polypill formulations have been manufactured and tested in clinical trials for primary and secondary prevention of cardiovascular disease. Some are already commercially available in India. The clinical trials demonstrated the feasibility and safety of a polypill in lowering blood pressure and low-density lipoprotein (LDL) cholesterol levels in people with 1 or more cardiovascular disease risk factors, although these effects were generally less than predicted from early research. The use of a polypill in the primary prevention of cardiovascular disease has been a focus of research at the Wolfson Institute, where the inventors of the polypill work. A Polypill Prevention Programme was established in 2006 to provide a service applying the polypill approach to the prevention of first heart attacks and stroke; separate component drugs were used at the correct doses, because a single polypill was not yet available. Using a randomized, double-blind, placebo-controlled, crossover design, they were able to estimate risk factor reductions with the polypill more accurately than in previous polypill trials, which used a parallel-group design. The polypill consisted of amlodipine 2.5 mg, losartan 25 mg, hydrochlorothiazide 12.5 mg, and simvastatin 40 mg, and was manufactured for the study by Cipla (Mumbai, India). For the first time in a study of the polypill, the only criteria for participation were age (≥50 years) and no history of cardiovascular disease. Using the observed reductions in blood pressure and LDL cholesterol and evidence of the quantitative relationship between risk factors and risk, Dr. Wald and his coauthors calculated that this polypill would result in reductions of 72% for ischemic heart disease events and 64% for strokes. These results were greater than those in earlier trials.No serious adverse events were reported during the study (Wald DS, Morris JK, Wald NJ. Randomized polypill crossover trial in people aged 50 and over. *PLoS One*. 2012;7: e41297).

Doina Butcovan

MANAGEMENT OF BORDERLINE OVARIAN TUMORS

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MANAGEMENT OF BORDERLINE OVARIAN TUMORS (Abstract): Borderline ovarian tumors are an intermediate stage between benign cystadenomas and adenocarcinomas. **Aim:** The paper evaluates the management of borderline ovarian tumors (BOTs) in the patients admitted and treated in our clinic in the interval January 2003 – June 2011. **Material and methods:** The observation sheets and pathology results of 264 patients with malignant ovarian tumors were analyzed. **Results:** Of the 264 malignant ovarian tumors 74 (28.03%) were low malignant potential. Patients with BOT were aged 18-72 years (mean 46 ± 6.2 years) and those with invasive tumors 14-83 years (mean 53 ± 9.8 years). 92.18% were in stage I. Sixty tumors were graded G1. 53.52 % of the tumors were over 10 cm (maximum 30 cm) in size. The histological types were: serous – 35 cases, mucinous – 19 cases, mixed (serous and mucinous) – 8 cases, and endometrioid - 2 cases. Fifteen patients presented intraepithelial carcinoma and 11 noninvasive implants into the peritoneal cavity. Five women had recurrences. **Conclusions:** Ovarian borderline tumors with histological characteristics of carcinoma, but with good behavior are now with better defined histological features. The biggest challenge in the management of women with these tumors is to identify the subset that will behave in a malignant fashion and to develop effective treatment for them. **Keywords:** BORDERLINE OVARIAN TUMOR, TREATMENT, TUMOR PROGRESSION

BOTs behavior is enigmatic, pathogenesis unclear and clinical management controversial, especially for serous borderline tumors. From oncologic point of view there are numerous debates on the surgical treatment of these patients, taking into account fertility sparing and outcomes. So the question was raised if BOT patients are not in general overtreated, and on the other hand how can the patients at high risk be distinguished?

MATERIAL AND METHODS

In the interval January 2003 – June 2011, 264 women with ovarian cancer were treated surgically in our clinic. During this interval we reviewed the medical records and pathological findings of the patients with BOTs. Histological criteria of inclusion in this category of tumors were: proliferative activity of epithelial cells (epithelial multi-layering of more than 3 layers, loss of cell polarity), no more than 4 mitoses/10 HPF (high power field), moderate to marked nuclear atypia (nuclear pleomorphism), increased nuclear/cytoplasmic ratio, slight-to complex branching of epithelial papillae, detached cellular clusters in the glands, and absence of frank stromal invasion (the most important criterion of

diagnosis). The patients were followed-up until June 2012. We tried to establish all parameters which must be taken on consideration for a good management decision.

RESULTS

74 (28.02%) women had BOTs. The mean age of the BOT patients was 46±6.2 years (range 18-72 years) as compared to 53±9.8 (range, 14-83 years) in the women with invasive ovarian carcinoma.

The average size of the tumors was 10 cm, in 53.52 % of the patients being over 10 cm (maximum size 30 cm). The largest were more frequently mucinous BOTs. As to pathological type, 35 (54.68 %) were serous tumors (fig. 1), 19 (29.68 %) mucinous tumors (fig. 2, 3), 8 (12.5 %) mixed (serous and mucinous), and 2 (3.14 %) endometrioid (fig. 4). Serous BOTs were bilateral in 4 cases.

A particular variant of BOT is serous carcinoma with micropapillary architecture, a highly aggressive variant with a bad course as compared with BOTs in general, but with a better prognosis than invasive ovarian cancer. Seven of our patients had this variant. Micropapillary pattern is usually associated with the risk of invasive implants. To complicate the pathological notion of BOT, in the last few years, the presence of one or more discrete foci of stromal microinvasion (less than 3 mm in diameter and smaller than 10 mm²) was included in the category of BOTs as intraepithelial carcinoma. Of our study patients 14 presented intraepithelial carcinoma (fig. 5 a, b, c, d).

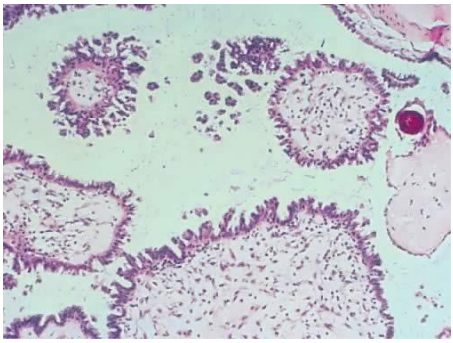


Fig. 1. Serous borderline ovarian tumor

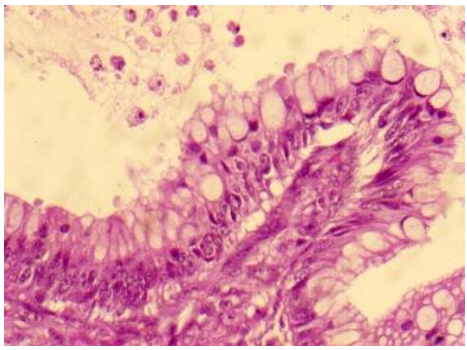


Fig. 2. Intestinal-type mucinous BOT

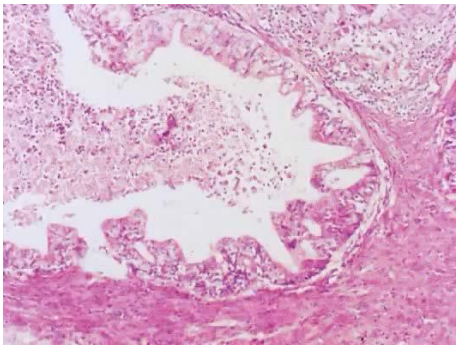


Fig. 3. Endocervical-type mucinous BOT

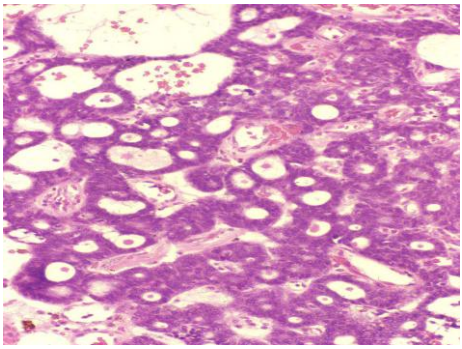
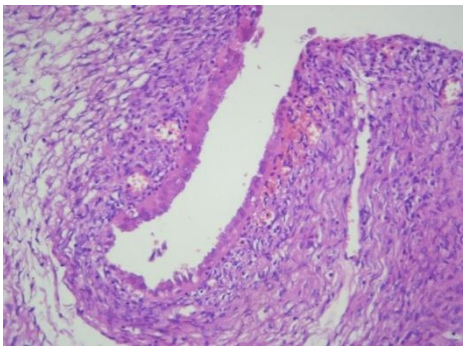
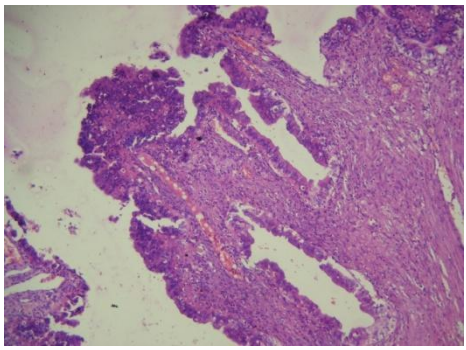


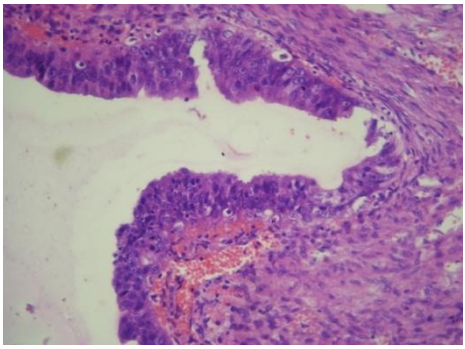
Fig. 4. Endometrioid BOT



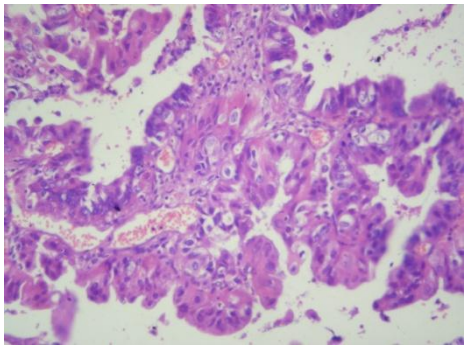
a. Serous cyst



b. Transition from cystadenoma to BOT



c. BOTs zone



d. Intraepithelial microinvasion

Fig. 5. Transition from cystadenoma to BOT and intraepithelial microinvasion

Peritoneal dissemination of BOT is called implants and not metastasis, because even the women with extraovarian disease have a good course. These implants are an important component of staging, and are in fact the most important prognostic indicator. They are classified as "invasive" and "noninvasive" based on their microscopic appearance, and the noninvasive ones are subdivided into epithelial and desmoplastic. Invasive implants are characterized by cellular atypical, a haphazard and disorderly infiltration of normal tissues, histological resembling a low-grade serous adenocarcinoma. In contrast with the well-defined limits of desmoplastic implants, the invasive implants exhibit irregular borders. Eleven (17.18%) of our study patients had noninvasive peritoneal implants from serous BOT.

Mucinous BOTs are in general unilateral. There are two types: intestinal-type (more frequent) and endocervical-like (with risk for recurrence and death). When present on both ovaries and as advanced stages they may be primary from the ovaries, but more often metastatic from the digestive tract. Immunohistochemistry (IHC) using cytokeratines is of benefit in the differential diagnosis of primary ovarian carcinoma from metastatic intestinal adenocarcinoma. *Pseudomixoma peritonei* is almost always the result of intraperiotoneal dissemination of a non-ovarian mucinous neoplasm, especially from appendicle tumors. In our study there were 2 cases with *pseudomixoma*

peritonei.

Only 4 women were in stage II, the others being in stage I – 93.55 % (fig. 6, 7, 8 a,b). Fifty-one tumors were G1.

Primary treatment for BOTs is surgery. Total abdominal hysterectomy with bilateral salpingo-ooforectomy, peritoneal cytology, multiple peritoneal biopsies is the golden standard in women in peri- and postmenopause, those who completed childbearing or do not want to preserve the fertility. Appendicectomy is a particular procedure, necessary in cases with mucinous tumors. Because BOTs are frequent during the reproductive period, the diagnosis is made at early stage and women have an excellent prognosis. For that reason the treatment of choice preserves fertility (that means keeping the uterus and part of one ovary at least). Even though the risk of recurrence is substantial after conservative treatment of BOTs, the survival rate is not altered by this approach. The decision of cystectomy instead of adnexectomy depends on such factors as woman's age, desire of childbearing, surgeon and patient expectation before treatment, but clear guidelines do not exist in practice. Usually it is performed a unilateral adnexectomy or associated with cystectomy for the other ovary when BOTs are bilateral. Biopsy of the controlateral normal ovary must be avoided, for protection from adhesions, which could decrease the fertility. None of our patients was treated by laparoscopic surgery. In general we did not perform pelvic and para-aortic lymphadenectomy in patients with BOTs.

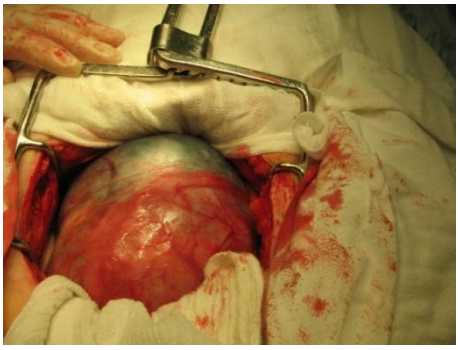


Fig. 6. DE 60 years – stage IA

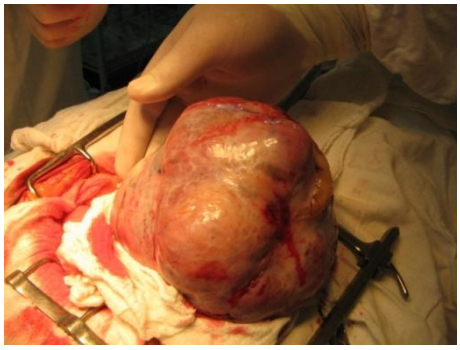


Fig. 7. AT 62 years – stage IA

a.

b.

Fig. 8. CM 30 years– Stage IC (a - during surgery, b - micropapillary aspect)

In the study group there were 5 cases with serous BOT recurrence at 5, 3, 2 and 1 year, respectively from primary surgery. Of the cases with recurrence after 1 year, one had BOT of the other ovary even though she received chemotherapy after surgery, and in the second BOT recurred as invasive ovarian cancer. The problem in these cases is that it could have been some foci of invasive ovarian cancer undetected at the pathological exam after primary surgery (the women received adjuvant chemo one month after diagnosis). The patient with recurrence after 2 years also presented invasive carcinoma and the initial stage was IA without adjuvant treatment.

DISCUSSION

BOTs account for 10-15% of malignant ovarian tumors (1), but in our study they represented 28.03%. Patients, with a mean age of 38 years, are younger than those with invasive carcinoma (2). In our patients mean age was 46 years, but significantly less than of invasive cancer patients. BOTs are epithelial tumors: 50% serous, 46% mucinous, and almost 4% mixed endometrioid, clear cell, or Brenner tumors. In our study series 54.68% were serous BOTs. Bilateral tumors were described in the literature in 25-50% of the cases (3). 90% are typical serous BOTs and 10% micropapillary BOTs progressing to low-grade serous ovarian carcinoma. Of our patients 9.47% had a micropapillary pattern.

20-46% of serous BOTs present peritoneal implants – only 17.18% in our cases. Peritoneal implants may occur in 66% of the patients with exophitic lesions on ovarian surface as compared to 5% of those with endophitic lesions. In 2000, Seidman proposed that since serous BOT with invasive implants have a clinical course similar to a low grade invasive carcinoma with a 34% mortality rate, it is more reasonable to classify these tumors as carcinomas (4). In agreement with FIGO and WHO staging the diagnosis of BOT is made on histological type and not on extraovarian spread. Patients with serous tumors and noninvasive implants have a low rate of recurrence and a survival rate of 100% (5). Unlike noninvasive implants, cases with invasive implants have a rate of recurrence of 45% instead of 11% for a follow-up period of 15 years (6). Micropapillary serous carcinoma (MPSC) has a powerful association with invasive implants and a survival rate at 10 years of only 60% (7). Recurrences occur in 77% of women with invasive carcinoma (2 cases in our study patients), with a high mortality rate until 74% (8). Sometimes pathologists acknowledge the distinction between invasive and noninvasive implants at time can be very difficult. Molecular genetic studies including mutational analysis and allelic imbalance will determine whether different kinds of implants have distinct molecular genetics profiles and whether these profiles can predict outcome. However, till now studies on noninvasive implants alone have been conducted. Allelic imbalance and the KRAS mutation status are similar in BOT and synchronous peritoneal implant in the same patient.

Mucinous BOTs are in 90% of cases unilateral and 85% of them are intestinal-type and 15% endocervical-like (9). Usually mucinous BOTs restricted to ovaries.

The correct diagnosis of BOT through frozen sections gives the surgeon the option to do a conservative treatment. This exam differentiates borderline from invasive ovarian tumors having a sensitivity of 67-97 % and a specificity of 97-100 % (10). Another study indicates a sensitivity of 71.1 %, PPV (positive predictive value) 84.3 %, over-diagnosis 6.6 %, and under-

diagnosis 30.6 % (11). Mucinous tumors and tumors over 10 cm in size are predictors of BOT under-diagnosis. For tumors under 10 cm a minimum of one section/cm of maximum diameter (without cystic areas with smooth wall) and two sections/cm for tumors over 10 cm are recommended.

If cystectomy is performed, the rate of recurrence is higher than in the cases with adnexectomy, 12-58 % vs. 0-20 %. On the other hand, bilateral cystectomy significantly increased the cumulative pregnancy rate without increasing significantly the cumulative recurrence rate (12). But in these women longer follow-up is needed because of the likelihood of late recurrence.

Pelvic and para-aortic lymph node involvement in women with serous BOTs seems to be present in 7-23 % of the cases with lymph node biopsy at the time of surgery (13). Lymphadenectomy is not necessary for disease staging, and when done it usually does not significantly improve the course. Recent studies show that lymphadenectomy can be avoided even in advanced BOTs stages because it does not influence recurrence and survival rate (14, 15). So, in BOTs lymphadenectomy is limited to research.

Fertility outcome in women with conservative treatment for BOTs is good and spontaneous fertility rate varies between 32 % and 65 % (16). But these patients frequently need ovarian stimulation for having a pregnancy. The next question is if ovarian stimulation is an option in these women given the debates regarding the link between ovarian stimulation and the risk for ovarian cancer. A multicentre retrospective study on 30 patients with a history of BOTs that underwent infertility treatment found recurrences in only 4 of them (16 %) 93 months after the treatment for BOT and 43 months after ovarian stimulation. The conclusion is that in women with early stage BOTs ovarian stimulation is safe (17). The need for completion surgery as a usual practice is disputed and depends of some factors (histological subtype, stage, type of conservative treatment and patient preference). But the number of patients which choose definitive treatment soon after finishing family planning is high because of psychological stress.

The 5-year survival rate in patients with stage I BOTs is 95-97 %, but at 10 years only 70-95 % because of late recurrences. Chemotherapy regimes are similar with those for invasive epithelial ovarian cancer. Clinical trials are needed for getting a clear opinion on the role of chemotherapy in BOTs.

Tumor DNA ploidy could be used for the identification of patients which will develop an aggressive disease (with tumor aneuploidy). Adjuvant therapy is not indicated for stage I diploid tumors (18). Also the presence of BRAF (codon 599) and KRAS (codon 12 and 13) in serous tumors and KRAS mutations in mucinous tumors is associated with tumor proliferations (19). These mutations are involved in early tumorigenesis, but the mutation is not sufficient for malignant transformation. KRAS and BRAF mutations are rare in high-grade serous carcinomas, different from p53 mutation which is present in more than 50% of high-grade carcinomas, and is rare in BOTs and low-grade carcinomas.

CONCLUSIONS

The mean age of the BOTs patient was 46±6.2 years, less than those with invasive ovarian carcinoma.

The presence of extraovarian implants - 11 of our study patients and the micropapillary type -7 from our cases are a challenge for gynecologists.

Given patients' age and the need of fertility

sparing, their intraoperative assessment by peritoneal washing, ooforectomy for the affected ovary, infracolic omentectomy and multiple peritoneal biopsies is very important.

The opportunity of adjuvant CHT is a matter of dispute, especially for patients with invasive implants associated with micropapillar architecture.

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CORRELATION BETWEEN ADIPONECTIN, LEPTIN, INSULIN GROWTH FACTOR-1 AND BONE MINERAL DENSITY IN PRE AND POSTMENOPAUSAL WOMEN

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School of Medicine
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CORRELATION BETWEEN ADIPONECTIN, LEPTIN, INSULIN GROWTH FACTOR- 1 AND BONE MINERAL DENSITY IN PRE AND POSTMENOPAUSAL WOMEN (Abstract): Body weight positively correlates with bone mass, gravity being a stimulating bone stress. Adipose tissues could influence bone metabolism especially in postmenopause. Also, muscle mass positively correlates with bone mass and is dependent on physical activity, but also on some hormones with direct effect on bone, such as insulin growth factor-1(IGF-1). **Aim**: Our study investigated the relative influence on lumbar bone mass of pre and postmenopausal women of body weight, leptin, adiponectin and IGF-1. **Material and methods**: Cross-sectional study including six groups of 8 to 15 pre and postmenopausal healthy volunteers with different weight; body mass index (BMI): normal and underweight (BMI<25 kg/m^2), overweight (BMI 25-30 kg/m^2), and obese (BMI>30 kg/m^2). Lumbar bone mineral density (BMD) and body composition (BC) were evaluated by dual X ray absorptiometry (DXA, Hologic), while serum leptin, adiponectin and IGF-1 were measured by ELISA. **Results**: Body weight, fat and lean mass positively correlated with BMD, irrespective of age ($p<0.05$, ANOVA). Leptin was an independent predictor for bone mass only in postmenopausal women ($p<0.05$). Adiponectin negatively correlated ($R2=0.1975$) and IGF-1 positively correlated with body mass ($p<0.05$). **Conclusions**: Increased body weight prevents bone loss after menopause. Leptin and IGF-1 have adjacent positive roles in preserving bone mass during postmenopause. **Key words**: BODY COMPOSITION, LEPTIN, ADIPONECTIN, IGF-1, MENOPAUSE.

In recent years, many studies have been published showing the link between bone mass, body composition, and hormonal parameters. Their conclusions are: body weight has an important predictive effect on bone mass; body weight influences bone mineral density through both fat mass and lean mass; fat mass acts through several mechanisms, once through the pressure it exerts on the skeleton, then by its association with bone active hormones secretion, estrogen and leptin secretion by adipocytes (1), muscle mass is dependent not only on physical activity but also on hormones, such as IGF-1 and testosterone, that have a direct effect on bone (2).

MATERIAL AND METHODS

Cross-sectional study on 6 groups of about 8 women each. Age dstribution: 31

premenopausal women and 37 postmenopausal women (at least two years of menopause). BMI: normal and underweight (BMI<25 kg/m^2), overweight (BMI 25-30 kg/m^2) and obese (BMI>30-40 kg/m^2). Age-group and BMI distribution: premenopausal women 16 normal weight and underweight, 9 overweight and 6 obese; postmenopausal women: 8 normal weight and underweight, 11 overweight and 17 obese. All women volunteered to participate in this study, and were recuited into the study from patients, students and staff of Iasi County „St. Spiridon" Emergency Hospital. A signed informed consent was obtained from all women.

Exclusion criteria were: diabetes mellitus, osteoporosis under treatment, hormone replacement therapy, severe liver or kidney disease, hyperthyroidism, primary hyperparathyroidism, hypogonadism, growth hormone deficiency, Cushing's syndrome, corticotherapy for over a year, anorexia nervosa, malabsorption syndrome, genetic syndromes. A fasting blood sample was collected, centrifuged, and stored in a refrigerator (-30° C) for estrone and estradiol determinations. Then, all volunteers' height and weight were measured, and subjected to osteodensitometry by dual X-ray absorptiometry (DXA). A certified technician measured bone mineral density (BMD) of the lumbar spine on Hologic DXA. Total fat and lean masses were expressed in grams. Lumbar BMD was expressed in g/cm^2. Leptin (ng/ml), adiponectin (mg/l), IGF-1(μg/l) were determined using commercial ELISA kits (Diagnostic Automation Inc., Calabasas, CA). The results were statistically processed using NCSS 2007 software, simple Pearson correlation, student t test, the correlation being considered significant when $p<0.05$. Multivariate regression (ANOVA) was used to exclude systematic biases.

RESULTS

Lumbar BMD was significantly lower in all postmenopausal women regardless of body weight compared with premenopausal women ($p<0.05$). We found that lumbar mineral density in obese postmenopausal women was similar to that in obese premenopausal women, while normal weight or underweight postmenopausal women had a significantly lower BMD than premenopausal women ($p<0.05$) (fig. 1).

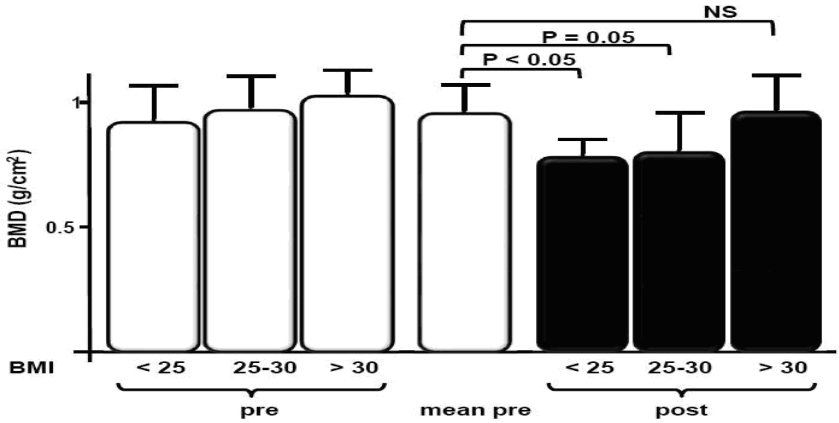

Fig. 1. Mean lumbar bone mineral density of premenopausal (left) and postmenopausal (right) women

BMD in lean postmenopausal women was significantly lower than in premenopausal women ($p<0.05$). BMD in obese postmenopausal women was comparable to that of premenopausal women.

Body weight, expressed as BMI (kg/m^2), positively correlated with lumbar BMD, Z score for all women (fig. 2).

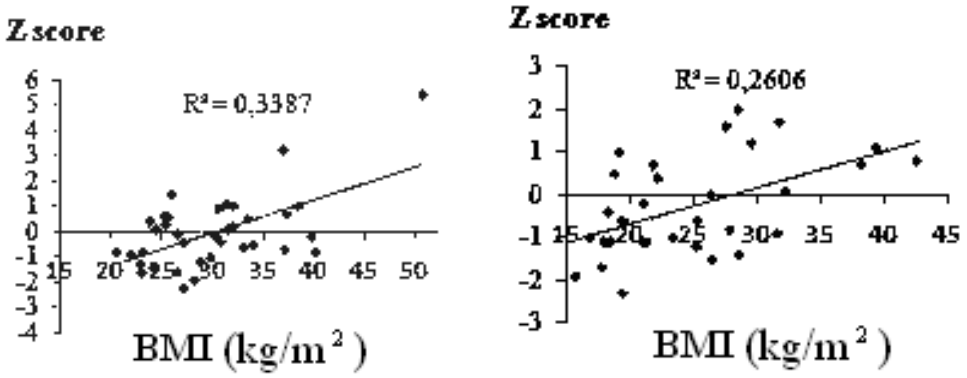

Fig. 2. Relation between lumbar Z score and BMI in premenopauzal women (right) and postmenoausal women (left)

Leptin positively correlated with fat mass, while between leptin and Z score there was a significant correlation in all study groups, suggesting that leptin itself may have direct effect on bone ($p<0.05$, fig. 3).

IGF-1 correlated positively with bone mineral density in premenopausal women ($r^2=0.1854$, $p<0.05$), while adiponectin correlated negatively ($r^2=0.1975$) (fig. 4, 5).

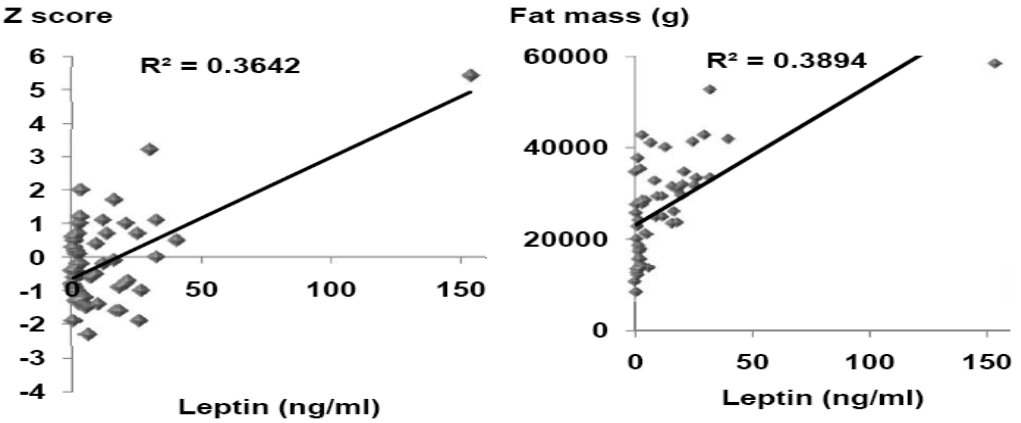

Fig. 3. Correlation between leptin and lumbar Z score (right), and between leptin and lean mass (left)

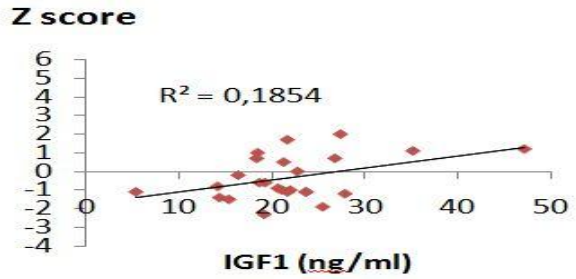

Fig. 4. Positive correlation between IGF1 and lumbar Z score

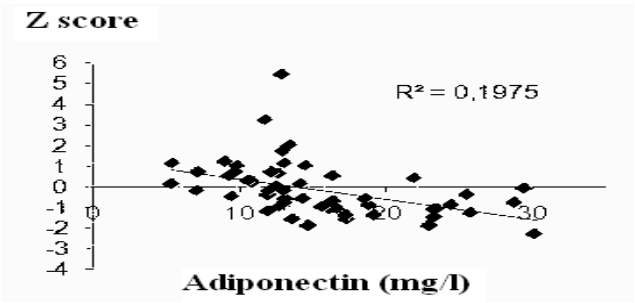

Fig. 5. Significant negative correlation between adiponectin and lumbar Z score

DISCUSSION

One factor correlated positively to bone mineral density is body weight. This can be explained by the mass effect, gravitational stress stimulating bone formation (3). Not completely elucidated is the relative importance of various body compartments, which may influence bone acquisition by mechanisms other than mechanical stress. Body weight is the sum of lean mass to fat mass. Increased muscle mass, combined with physical exercise, may contribute to a higher bone mass at young ages (4). Muscle mass is dependent not only on physical activity but also on hormones that have a direct effect on bone, such as IGF1 and testosterone (5). We demonstrated that IGF1 level positively correlates with bone mass (fig. 4), thus partially explaining the correlation between lean mass (source of IGF1 synthesis) of and bone mass. Low IGF1level is considered by other authors to be predictive of low bone mass and osteoporosis (6).

Adipocyte secretes specific peptide hormones, called adipocitokine (7). Leptin is considered to be a mediator between adipocytes and bone metabolism. Osteoblasts have leptin receptors, which stimulate bone formation through a direct mechanism, but inhibit the recruitment of osteoclast precursors, reducing osteolysis (8). However, by central effect, leptin stimulates the sympathetic nervous system leading to bone demineralization. The literature on the role of leptin on bone are conflicting, some authors describing a positive predictive role, while others suggest either the loss of leptin influence on bone when normalized in adipose tissue, or a negative predictive role in some populations (9, 10). In vitro and on animal models, adiponectin

seems to have direct effects mediated by receptors located on osteoblast, determining an increased bone turnover with predominance of osteoclast activity, possibly associated to bone mass loss (11). The effects observed in vitro and on animal models have been confirmed by some studies (12). In agreement with these studies, we found a negative correlation of adiponectin with bone mass.

CONCLUSIONS

We have demonstrated that the various body compartments influence bone mass acquisition in different ways, mass effect-independent mechanisms included. Thus, lean tissue mass has a more important predictive role on bone mass than fat tissue mass or total body weight. This effect could be explained by a hormonal profile favoring muscle development and collagen matrix, such as higher levels of IGF1.

Adipokine secretion profile has a buffer effect, adiponectin being a negative predictor of bone mass acquisition.

Maintaining aceptable bone mass in postmenopausal women is essentially influenced by premenopausal bone mass acquisition, which depends on weight, lean mass, estrogen impregnation, and IGF1 levels as positive factors, but also on adiponectin as possible adverse prognostic factor.

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ATITUDINE TERAPEUTICĂ ÎN CAZ DE REACȚIE ALERGICĂ LA MEDICAȚIA ANTITUBERCULOASĂ. PREZENTARE DE CAZ

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THERAPEUTIC ATTITUDE IN CASE OF ALLERGIC REACTION TO ANTITUBERCULOSIS MEDICATION – A CASE REPORT (Abstract). Medication used to treat tuberculosis is responsible for numerous side effects that have a major impact on patient's health, with a negative influence on the quality of life. Patients of all ages must be trained in order to recognize all the adverse effects and the undesirable reactions produced by antituberculosis medication. We report the case of a 24-year-old woman with generalized psoriasis and psoriatic arthropathy who was treated against tuberculosis. After 7 days of treatment with isoniazid, rifampicin, ethambutol and pyrazinamid she had severe allergic reaction with generalized pruritus and rash which were difficult to control with antiallergic treatment. Conclusions: Particular to this case is the absence in patient's history of the allergic reactions to medication. The re-establishment of treatment of tuberculosis was done by clinical trials and the patient had responded to only three drugs: isoniazid, rifampicin and ofloxacin, so the patient received an individual treatment due to adverse reactions to tuberculosis medications.
Key words: ALLERGIC REACTIONS, ANTITUBERCULOSIS MEDICATION, BACTERIOLOGICAL TEST

Tuberculoza rămâne o problemă de sănătate publică în România, dar şi pe plan mondial. Tuberculoza este o maladie infecțioasă, socială, asociată cu sărăcia şi malnutriția. Se estimează că în România sunt aproximativ 30.000 de bolnavi în toată țara (1).

Pacienții diagnosticați cu tuberculoză pulmonară sau extrapulmonară beneficiază de medicație antituberculoasă gratuită prin Programul Național de Control al Tuberculozei (PNCT). Tratamentul şi monitorizarea rezultatelor terapeutice sunt disponibile în studiile publicate şi documentația OMS (2, 3, 4, 5, 6).

Inițierea terapiei antituberculoase se face în spital sub directa observare a medicului pneumolog. Înainte de începerea oricărui tratament sau de reluarea unui tratament este necesar un examen clinic complet şi dozarea enzimelor hepatice, bilirubinei, creatininei, ureei, efectuarea unei hemograme. La pacienții cu vârstă peste 50 ani este recomandabil să se facă şi un examen oftalmologic (dacă se intenționează folosirea Etambutolului) precum şi o audiogramă (în cazul folosirii aminoglicozidelor sau a antibioticelor peptidice).

Pacienții de toate vârstele trebuie instruiți pentru a putea recunoaşte şi semnala efectele adverse şi reacțiile nedorite produse

de medicamentele anti-tuberculoase. Dacă apar simptome ce evocă toxicitatea medicației antituberculoase vor fi recomandate examene de laborator pentru a le identifica, iar conduita terapeutică va fi modificată corespunzător (7). Indiferent de schemele de tratament abordate de medicul pneumolog, medicația antituberculoasă este responsabilă de numeroase reacții adverse (8, 9).

PREZENTAREA CAZULUI

O pacientă de 24 ani, din mediu rural s-a internat în Clinica de Pneumologie Iași în aprilie 2011 cu diagnosticul de hemoptizie, observație tuberculoză pulmonară dreaptă, caz nou. De aproximativ o lună și jumătate pacienta prezenta inapetență, episoade infecțioase respiratorii recurente.

Din antecedentele personale patologice s-a remarcat diagnosticarea în decembrie 2010 cu psoriazis vulgar generalizat și artropatie psoriazică pentru care a urmat tratament cu Leflunomid 100 mg/zi apoi 20 mg/zi, ulterior cu Metotrexat 7,5 mg/săptămână și topic cu un derivat cortizonic, Hidrocortizon unguent cu o evoluție parțial favorabilă. Anterior internării în Clinica de Pneumologie pacienta a fost internată în Clinica de Dermatologie unde în urma unei radiografii pulmonare s-a decelat o opacitate în câmpul pulmonar mediu drept.

Examenul clinic a relevat la nivelul tegumentelor placarde eritematoase cu scuame toraco-abdominale, stare generală relativ bună, afebrilă cu absența sindromului inflamator, TA 100/60 mm Hg. Datele de laborator au arătat valori în limite normale ale funcției hepatice, renale, hematopoietice.

Radiografia pulmonară pune în evidență prezența unei opacități infiltrative cu hipertransparență centrală în câmpul pulmonar mediu drept, nodul pulmonar LID.

La examenul bacteriologic repetat de spută BK a fost negativ. S-a efectuat examen fibrobronhoscopic: fără modificări la examinarea arborelui bronșic bilateral, s-a prelevat aspirat bronșic pentru BK. La examenul bacteriologic de aspirat bronșic BK a fost negativ. Spirometrie în limite normale. De asemenea se face testarea la Quantiferon TB GOLD, iar rezultatul a fost pozitiv.

Datele obținute clinic și paraclinic au pus în discuție inițierea tratamentului antituberculos categoria I de tratament: Izoniazidă (HIN), Rifampicină (RIF), Etambutol (EMB) și Pirazinamidă (PZM) . Tratamentul este tolerat bine, pacienta prezintă stare generală bună fără reacții alergice la medicația antituberculoasă. După 7 zile de tratament apare o reacție alergică severă cu prurit și eritem generalizat, se decide întreruperea tratamentului antituberculos. Se reîncepe testarea separată a tuberculostaticelor, dar pacienta prezintă reacție adversă severă cutanată la: HIN, RIF, PZM fără a se mai încerca testarea și la EMB. Reacția adversă cedează cu dificultate la medicația antialergică. Medicul curant a completat fișa pentru raportarea spontană a reacțiilor adverse la medicamente în cazul de față la medicația antituberculoasă și a fost trimisă la Agenția Națională a Medicamentului. După o perioadă de aproximativ 2 luni timp în care pacienta este urmărită clinic, paraclinic și radiologic pacienta primește tratament pentru psoriazis cu o evoluție foarte bună a acestuia se decide intervenția chirurgicală cu excizia formațiunii în vederea unui diagnostic de certitudine. După întreruperea oricărei terapii pacienta se internează în Clinica de Chirurgie toracică cu diagnosticul de tuberculom lob inferior drept și se va interveni chirurgical pentru lobectomie inferioară dreaptă, drenaj pleural. Fragmentul pulmonar se trimite la laboratorul de bacteriologie și se confirmă BK pozitiv în piesa operatorie.

Se decide reînceperea tratamentului antituberculos cu Ofloxacină 300 mg/zi, Rifampicină 150 mg/zi, tolerează, a doua zi se

creşte doza la 300 mg/zi, tolerează, a treia zi se creşte doza la 450 mg/zi. În ziua a patra se introduce Etambutol 400 mg/zi, după 3 ore pacienta acuză prurit la nivelul feței dorsale a mâinii drepte şi la nivel toraco-abdominal, eritem diseminat pe abdomen şi torace prezența de micropapule la nivelul membrelor superioare. Se administrează medicație antialergică şi se opreşte administrarea Etambutolului. După două zile se introduce Izoniazidă 100 mg/zi, pacienta tolerează medicația, se creşte treptat doza la 250 mg/zi. Starea generală a pacientei este bună suportă bine tratamentul individualizat, pacienta se externează pentru continuarea terapiei antituberculoase la domiciliu.

DISCUȚII

Raportat la numărul total de pacienți internați şi tratați cu medicație antituberculoasă în Spitalul Clinic de Pneumologie Iaşi în perioada 2007-2011 incidența reacțiilor adverse este următoarea pe ani (1,46% / 2007, 2,97% / 2008, 2,17% / 2009, 3% / 2010 şi 3% / 2011). Numărul de pacienți tratați cu medicație antituberculoasă a scăzut semnificativ în anul 2010 şi 2011, în schimb incidența reacțiilor adverse este sensibil mai mare în aceşti ani. Cele mai multe reacții adverse s-au semnalat la un singur medicament Pirazinamida 500 mg (75,6%), în timp ce (24,4%) reprezintă reacțiile adverse semnalate la mai multe medicamente din schemele de tratament. În ce priveşte repartiția pe sexe a fost mai crescută la sexul masculin (88,8%) comparativ cu cel feminin, grupa de vârstă cea mai afectată fiind 45-54 ani, preponderent din mediul rural.

CONCLUZII

Particularitatea cazului prezentat constă în faptul că pacienta nu a mai avut episoade de reacții alergice la medicamente. Datorită reacțiilor adverse severe la Pirazinamidă şi Etambutol va beneficia de tratament individualizat format din: Izoniazidă 250 mg/zi, Rifampicină 450 mg/zi şi Ofloxacin 300 mg/zi.

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INCIDENTAL DISCOVERY OF HUMAN TRICHINELLOSIS ASSOCIATED WITH ORAL SQUAMOUS CELL CARCINOMA. CASE REPORT

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INCIDENTAL DISCOVERY OF HUMAN TRICHINELLOSIS ASSOCIATED WITH ORAL SQUAMOUS CELL CARCINOMA. CASE REPORT (Abstract): Trichinellosis is a parasitic infestation that is caused by roundworms of the Trichinella type and which affects the skeletal muscles. In most parts of the world there have been reports of trichinellosis in humans, with a lower incidence in countries in which pork is not traditionally eaten, such as India. This article reports a rare case of an incidental finding during a reconstructive surgery procedure on a 67-year-old man suffering from squamous cell carcinoma of the floor of the mouth. The intervention helped prevent long-term morbidity caused by trichinellosis in our patient. The case was successfully resolved by our reconstructive surgery followed by antiparasitic medication. We also discuss the pathogenesis and carcinogenic potential of trichinellosis, suggesting the critical importance of timely diagnosis and treatment of this infestation. **Keywords**: ORAL CANCER, PARASITOSIS, MIOCUTANEOUS FLAP, TRICHINELLA SPIRALIS, TRICHINELLOSIS.

Trichinellosis is caused by helminth (roundworms) of the species *Trichinella*. It is spread by the consumption of undercooked or raw meat products, infested by encysted larvae (1). Most often the meat is pork or wild boar but can also come from other species such as bears or dogs, depending on culinary customs (2). During the initial phase of the infestation the larvae are released from their cysts and begin to invade the wall of the small intestine, where they develop into adults and can cause digestive symptomatology such as abdominal pain, vomiting, diarrhea, or even unspecific systemic signs such as rashes, fatigue, fever, arthralgia, myalgia (3). After about one week, the females release new larvae that begin to migrate using the capillaries towards various organs such as the lymph nodes, myocardium, retina, however only larvae that reach the skeletal muscles survive and begin to affect these cells,

causing fever, muscle pains and rash (4).

The presenting complaints most commonly include myalgia, fever, rash and fatigue (5). Some cases may be completely asymptomatic, however complications have been described, such as myocarditis, central nervous system damage or pulmonary damage. In rare cases there is neurologic damage such as respiratory paralysis, ataxia, or even cerebral venous sinus thrombosis from contamination of the central nervous system (6). Pyomyositis is a rare complication by primary infection of the skeletal muscle, usually caused by Staphylococcus aureus, and is endemic in tropical areas (7). In most cases, the diagnosis is based on symptoms and confirmed by specific antibodies in the blood or by tissue biopsy.

Once diagnosed, the infection can be treated with mebendazole, albendazole or other antiparasitic medication, rapid treatment neutralizing the parasites and preventing further deterioration of the symptoms (8). Early administration of anthelmintic drugs decreases the likelihood of larval encystation, especially if administered within the first days after contact (9). However, most cases are diagnosed much later, when the symptomatology is significant (10). If left untreated, the infected humans represent a dead-end host, and the parasite remains in a cystic stage for the rest of the host's life.

CASE REPORT

A 67-year-old man was referred to the Oral and Maxillofacial Surgery Clinic of the „Sf. Spiridon" Emergency Hospital, Iasi, Romania with the suspicion of squamous cell carcinoma of the floor of the mouth. The patient had a medical history of hypertension, atrial fibrillation, varicose veins in the lower extremities and total bimaxillary edentation. His treatment included Acenocumarol, Digoxin, Carvedilol, Spironolactone, and Perindopril-Amlodipine. He resided in a rural area of Romania and was retired. The patient was a smoker for about 50 years (roughly 10 cigarettes per day) and an occasional alcohol consumer. Occasionally, he enjoyed eating undercooked pork or boar. The patient declared that he initially noticed the oral tumor in early 2017 and that at first it was not painful, becoming more and more uncomfortable as the tumor started to grow.

Extraoral physical examination identified only a right submandibular lymphadenopathy. Intraoral examination revealed normally colored vestibule and tongue, with a mid-sized palate and no invasion of the superior and inferior vestibule. We identified a neoplastic mass of the floor of the mouth extending to the anterior third of the tongue, ulcerated, hardened and painful both spontaneously and upon contact. Tongue with normal tonus and reduced mobility due to the fixation to the floor of the mouth in the anterior region.

Orthopantomography revealed an area of radio transparency circumscribing 33, poorly delimitated. Thoracic X ray revealed signs of aortic atherosclerosis and otherwise normal pulmonary areas.

Cardiology exam revealed signs of digitalis toxicity on EKG, subsequently Digoxin was stopped. Acenocumarol was stopped and replaced with Clexane twice a day. Echocardiography revealed mitral annular calcification and thickening of the tricuspid and aortic valves, mild bilateral atrial dilation and mild aortic atherosclerosis, with an ejection fraction of 55%.

The hematological exam was largely normal, except for an increased MCV of 101.3/fL and slight elevation of monocyte percentage to 13.3%.

During hospital stay the patient presented an allergic reaction characterized by acute

urticaria of probable food cause and corticoid and antihistaminic treatment was initiated.

A decision was taken to perform a bilateral radical neck dissection followed by the excision of the tumor with adequate safety margins, followed by immediate reconstruction with pedicelled pectoralis major myocutaneous flap, a versatile flap with an excellent reach to face oral cavity and neck region (11). Upon inspection of the muscular fibers we noticed a peculiar aspect of skeletal muscle fibers in the thoracic, head and neck areas, suggestive of cystic, parasitic lesions, (fig.1) prompting the decision to send a small fragment of the pectoralis minor for extemporaneous anatomopathological examination (fig.2). The results showed skeletal muscle fibers containing cystic elements, outlined by a connective tissue and containing spiraled parasitic organs belonging to a possible nematode, highly suggestive of *Trichinella spiralis* infestation. After discussion with the pathologist we decided to send another muscle biopsy sample from the platysma muscle to obtain hematoxylin and eosin-stained (H&E) sections from the paraffin wax-embedded sample to better identify the parasitic nematode. The sections were examined by a clinical biologist from the Iasi Infectious Hospital confirming the cystic stage of a *Trichinella spiralis* infestation.

Hematoxylin and eosin-stained (H&E) sections from paraffin wax-embedded muscle revealed skeletal muscle fibers containing cystic elements, outlined by connective tissue and containing spiraled parasitic organs identified as *Trichinella spiralis* (fig.2-3). Masson's trichrome stain on paraffin-embedded sections showed the cystic stage of a *Trichinella spiralis* nematode surrounded by muscle fibers and connective tissue highlighted in a bright blue color (fig.4-5). The patient was discharged after receiving a full treatment with Mebendazole 400 mg three times a day for 10 days, supportive drugs and is being followed up regularly without complaints (12).

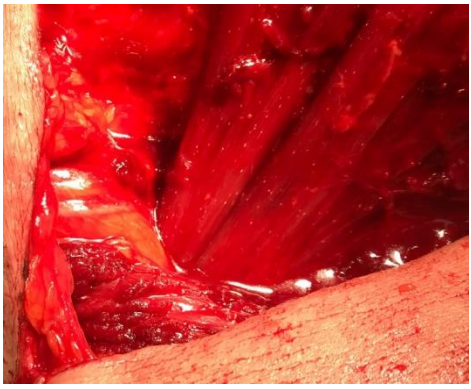

Fig. 1. Intraoperative photograph showing a portion of pectoralis minor covered by what looks like small cystic lesions, compatible with a parasitic infestation

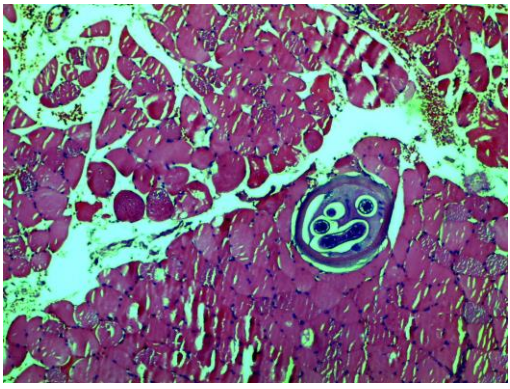

Fig. 2. Hematoxylin and eosin-stained (H&E) sections from paraffin wax-embedded muscle, magnification X10 - skeletal muscle fibers containing cystic elements outlined by connective tissue and containing spiraled parasitic organs identified as *Trichinella spiralis*

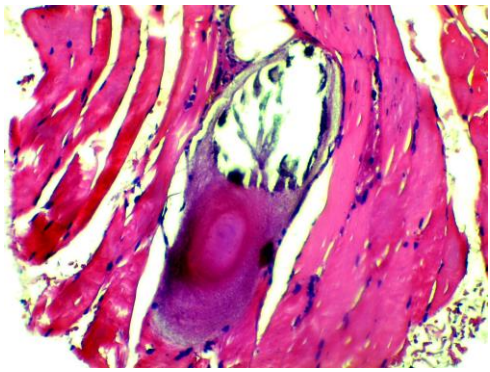

Fig. 3. Hematoxylin and eosin-stained (H&E) sections on paraffin-embedded sections of pectoralis minor muscle, magnification X20

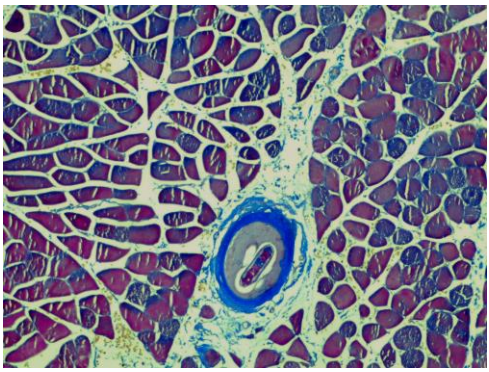

Fig.4. Masson's trichrome stain on paraffin-embedded sections, magnification X10 – the connective tissue is highlighted in a bright blue color

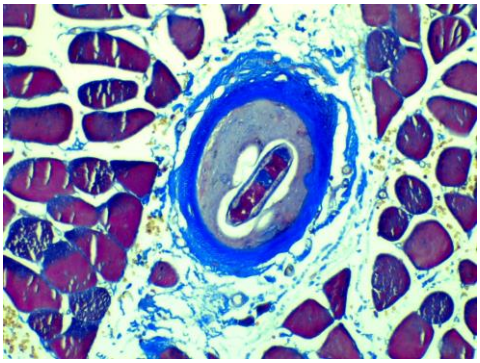

Fig.5. Masson's trichrome stain on paraffin-embedded sections, magnification X20 – cystic stage of a *Trichinella spiralis* nematode surrounded by connective tissue and muscle fibers.

DISCUSSION

Trichinella infestation has been reported in association with carcinomas of oral cavity and breast (13). The nematodes seem to target the oxygenated skeletal muscles (14). In typical cases the larvae can be seen encased in a cyst wall composed of the host tissue, with lymphocytic and eosinophilic infiltrate, producing a characteristic appearance in biopsy samples that provides a diagnostic (15).

Bruschi (16) showed the cytotoxic role of eosinophils *in vitro*, in human antibody dependent cellular cytotoxic reactions that can be attributed to interleukin-5 produced by the Th2 subset of CD4+ T cells. The coexistence of trichinosis infestation and tumor tissues has been previously observed in other reports such as that by Jozo Kristek (17). It remains to be proven if trichinosis is a carcinogen involved in the development of malignancies of the oral cavity, constant irritation by the parasitic cysts may be responsible for the upregulation of VEGF and other modifications of external kinases (18).

Ever since the discovery of the antitumor effect of *Trichinella spiralis* in the 1970s many research teams have proved with relative success that *Trichinella spiralis* possesses antitumor activities. Unfortunately, the research has proved to be inconsistent with little advancement in the field over the past years. Consequently, the underlying mechanisms explaining the inhibitory effects remain unclear, and there have been no clinical trials that provide adequate evidence in support of a signifi-

cant effect of *Trichinella spiralis* over the prevention or treatment of cancers (19). Meanwhile, some other researches also suggested that *Trichinella spiralis* may cause or contribute to coinfection with a tumor (20).

The presented case represents an incidental discovery of a *Trichinella spiralis* infestation and as such it cannot be confirmed or ruled out as a cause of the squamous cell carcinoma that prompted the need for surgery, but recent research shows that coinfection is a possible cause for the development of cancers (18-22). The presence of the cystic parasites in the muscular fibers represents a constant local inflammation source that can be responsible for the appearance of a neoplastic process. The cystic nematode remains surrounded by connective tissue causing little disturbance to the systemic biochemistry and hematologic tests, in many cases the infestation can remain undetected for years. The incidental discovery of the parasitic infestation represents a unique opportunity because it allowed us to perform an adequate examination and provide treatment.

CONCLUSIONS

We report a case of asymptomatic *Trichinella spiralis* infestation, diagnosed incidentally while the patient underwent surgery for oral squamous cell carcinoma. To our knowledge this is the first case reported in our geographical area. Our findings come to support and lend credibility to the claims of carcinogenic potential, considering the presence of this parasite in other case reports associated with cancers in general and specifically with oral cancers. We believe further research is warranted to verify this claim with a larger cohort. The fortuitous discovery of the parasitic infestation represents a rare opportunity to perform adequate pathological examinations to document the cystic stage of infestation in humans, rarely seen today due to the invasive nature of muscle biopsies and the possibility to identify the parasite using hematological tests.

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NEWS

LETHAL HYPOTHERMIA-THE IMPORTANCE OF POSTMORTEM CONCENTRATION OF ENDOCAN

Endocan is an endothelial cell-associated proteoglycan expressed and secreted by vascular endothelial cells of various organs, preferentially by the lung in response of pro-inflammatory cytokines. This molecule is known to be a good biomarker for multi-organ failure and endothelial dysfunction especially in cases of sepsis, severe sepsis and septic shock. In a study, endocan values were determined in three biological samples (femoral serum, vitreous humor and urine) and three different group cases (hypothermia, sepsis, non-hypothermia/ non-sepsis cases), with a total amount of 76 autopsy cases prospectively selected by several inclusion criteria. The results of the study indicate that generalized inflammation and endothelial activation with increased endocan concentration in autopsy collected biological samples it is not a characteristic of hypothermia fatalities following cold exposure. The limitations of the study are linked by the small number of autopsy cases, precised selection criteria and, being a prospective study, much more cases would be needed to confirm the findings (E. Descloux *et al.* Endocan concentrations in postmortem serum, vitreous humor and urine in victims of lethal hypothermia. *Journal of Forensic and Legal Medicine* 2017; 50: 39-43).

Andreea-Alexandra Velnic

COLON CAPSULE ENDOSCOPY COMPARED TO COLONOSCOPY FOR COLORECTAL NEOPLASMS DIAGNOSIS: AN INITIAL EXPERIENCE AND A BRIEF REVIEW OF THE LITERATURE

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COLON CAPSULE ENDOSCOPY COMPARED TO COLONOSCOPY FOR COLORECTAL NEOPLASMS DIAGNOSIS: AN INITIAL EXPERIENCE AND A BRIEF REVIEW OF THE LITERATURE (Abstract): Conventional colonoscopy is regarded as the gold standard procedure for the diagnosis of colorectal neoplasms despite its several limitations including invasiveness, discomfort and potential complications. Colon capsule endoscopy (CCE) could be an attractive alternative method to the invasive colonoscopy for the diagnosis of colorectal cancer and polyps. Publications regarding the use of CCE for detecting colorectal cancer and polyps, as well as our experience, were reviewed. The new second-generation CCE (PillCam COLON 2) has improved accuracy compared with the first-generation system for detecting colorectal neoplasms. **Key words**: COLON CAPSULE ENDOSCOPY, COLONOSCOPY, COLORECTAL POLYPS.

Colorectal cancer (CRC) is a leading cause of morbidity and mortality worldwide. Approximately 90% of CRC develop from benign adenomatous polyps, and current guidelines recommend screening colonoscopy with subsequent polypectomy to prevent progression into cancer (1). With such screening program in the United States, a decrease in incidence and mortality of CRC was reported (2, 3). However, colonoscopy has several limitations including invasiveness, discomfort, embarrassment, high cost, sedation, and potential complications. Therefore, many patients are reluctant to undergo this procedure, and generally, participation in colonoscopy screening is unacceptable low (4).

Colon capsule endoscopy (CCE) is a new, safe non-invasive and patient-friendly procedure for visualizing the colon with no need of sedation, intubation, air insufflation or radiation; this new technology might become an alternative method to the invasive conventional colonoscopy in CCR screening and polyp detection.

In this paper we aim to present our personal experience with CCE, to summarize the recent impressive technical achievements, and to review the most recent studies addressed to this subject.

COLON CAPSULE ENDOSCOPY: DEVICE DESCRIPTION

The PillCam Colon capsule 1 (PCC1)

endoscope (Given Imaging Ltd., Yoqneam, Israel) measures 11 x 31 mm and has two cameras that enables the device to acquire video images from both ends with a frame rate of 4 images per second, and a total operation duration of about 10 hours (5). After an initial few minutes of image transmission, the capsule enters a delay mode of approximately two hours, after which (when capsule is probably in terminal ileum) it "wakes up" and restarts the transmission of video images. The recorded data are downloaded into the Given Imaging RAPID workstation for review of the colon video.

The PillCam Colon 2 (PCC2) (Fig. 1) – the second generation capsule endoscopy system – measures 11.6 x 31.5 mm, and the angle of view has been increased to 172 degrees for each camera (from 154° in PCC1), offering a panoramic view. The new Data Recorder 3 is a "thinking" recorder which controls the capsule image rate in real time (4 images per second when the capsule is stationary and 35 images per second when it is moving) as opposite to the fixed rate of 4 images per second of PCC1; this technical improvement conserves battery energy (5). The Data Recorder 3 also recognizes location of the capsule (stomach, small intestine) and how long it has been there, thus assisting medical staff and patient through the procedure (administration of a prokinetic or laxative). The new RAPID software includes a graphic interface tool for polyp size estimation in millimeters.

Fig. 1. PillCam Colon 2 and Data Recorder 3

COLON PREPARATION

The bowel preparation procedure is similar to that used for conventional colonoscopy. However, it is more rigourous since no suction of liquid remnants is possible during capsule endoscopy, unlike during colonoscopy. Briefly, it consists of clear liquid diet on the day before capsule ingestion and 3 liters of polyethyleneglycol (PEG) ingested in the evening before procedure; another liter of PEG is ingested in the next morning, then the capsule is swallowed with a glass of water (6). One or two "boosters" (sodium phosphate) are administered after 1-2 hours if the capsule has entered the small bowel, followed by a

laxative (bisacodyl suppository) if the capsule has not been excreted (6, 7). The reason for the "vigorous" bowel preparation is to maintain the colon cleanliness and to promote the capsule progression through the gastrointestinal tract.

CONTRAINDICATIONS

CCE should not be performed in patients with dysphagia, known or suspected bowel obstruction, surgical anastomosis, diverticulosis, cardiac pacemaker or other implanted electromedical devices, pregnant female patients, known allergy to PEG, sodium phosphate or bisacodyl.

COMPARISON OF COLON CAPSULE ENDOSCOPY WITH CONVENTIONAL COLONOSCOPY

Several studies have been performed with the first-generation colon capsule endoscopy (PCC1) which showed promising accuracy compared with conventional colonoscopy for detection of colorectal significant lesions (8-13).

We have analyzed the first results with PCC1 at our institution in patients with or without personal or family history of CRC or polyps and compared them with those obtained at colonoscopy. Patients included were 17 men and 11 women, aged 26 to 74 years (mean age: 56 $\pm$ 12 years) who underwent both capsule and colonoscopy examinations at the Iasi Institute of Gastroenterology and Hepatology between May 2008 – December 2010. Colon preparation with PEG was similar for capsule endoscopy and colonoscopy. All CCE recordings were read by the same physician, and all colonoscopies were performed by two experienced endoscopists. For statistical analysis, colonoscopy was considered the gold standard, and per-patient comparisons were made as in other studies. No CRC were detected by CCE and colonoscopy, but colorectal polyps were found in 9 (32.1%) patients by colonoscopy and in 7 (25%) patients by CCE, the agreement between these two examinations being significant (p =0.19) (Fig. 2).

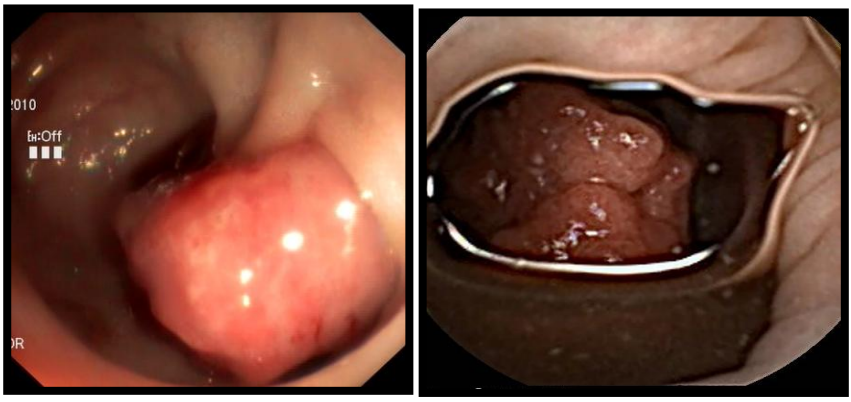

Fig. 2 a, b. Colonic polyp seen at colonoscopy (a) and colon capsule endoscopy (b)

The sensitivity and specificity of CCE for the detection of colorectal polyps (any size) were 73.5% and 81.2%, respectively. The positive predictive value of CCE was 69.8% and the negative predictive value was 83.1%. The tolerance of CCE procedure was excellent, and no complications were reported.

Colon capsule endoscopy was compared to conventional colonoscopy for the detection of colorectal polyps and cancer in several studies (7-17). Van Gossum et al (14) in a prospective, multi-center study found that sensitivity of CCE for detecting colonic polyps, advanced adenomas, and colorectal cancer was relatively low in comparison with colonoscopy. A low sensitivity of CCE for detection of relevant colorectal polyps was also reported in another recent published study (15). Sacher-Huvelin et al (16) in a prospective, multi-center trial to compare CCE (first generation of colon capsule) and colonoscopy in asymptomatic subjects enrolled in screening or surveillance programmes for the detection of colorectal neoplasia, have found that CCE cannot replace colonoscopy as a first-line investigation for screening and surveillance of patients at risk of cancer. On the other hand, several studies have reported that CCE was effective in detecting colorectal polyps. Thus, Devière at al (13) in a multi-center European study of detecting colon cancers and polyps using CCE (PillCam Colon 1) compared to conventional colonoscopy found encouraging accuracy of CCE for detecting polyps and suggested that this new method for visualizing the colon may be an alternative for patients who cannot or are unwilling to undergo colonoscopy. Gay et al (10) found CCE to be an effective method in detecting clinically relevant colonic findings in patients with an indication of conventional colonoscopy. Two recent meta-analyses (11, 12) reported CCE to be a reasonable method for screening asymptomatic subjects for colorectal polyps.

The most, if not all above mentioned studies were performed with the first generation of CCE. Recently, Eliakim et al (17) reported an improved accuracy of the second-generation CCE (PCC2) compared with the first-generation system (PCC1) in detecting colorectal polyps. Thus, the sensitivity to diagnose patients with colorectal polyps increased from 60% in the previous study with PCC1 (9) to 90% and the only reason for greater diagnostic yield was the technological improvements in the PCC2 system. Hassan et al (18) have evaluated the cost-effectiveness of CCE based on a Markov mathematical model and found the cost-effectiveness potentially superior to colonoscopy.

CONCLUSIONS

Colon capsule endoscopy is a safe, minimally invasive, very well accepted by patients, easy to perform procedure which with new technological improvement could become an option for the screening of colorectal polyps and cancer.

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ROLUL EXAMENULUI ECOGRAFIC ÎN CAZUL TUMORILOR OCULOORBITARE

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THE ROLE OF ULTRASONOGRAPHY EXAM IN ORBITAL-OCULAR TUMORS (Abstract): Ophthalmology is one of the specialties that have particularly benefited from the contribution of ultrasonography exam as a method of investigation. Ultrasonography is very much essential for diagnostic to complement other clinical and laboratory investigations, providing images in real time. The basic principle of diagnostic ultrasound is to study and to interpret the changes they undergo when crossing ultrasonic waves diverse biological properties different sound, and such injuries can be traced in the dynamics or can be documented on photographic paper and thus can diagnose correct certain eye diseases. The indications for performing ultrasound consist in: measurement of distances and volumes, examine difficult or inaccessible case of opaque media; ophthalmoscopic view of a mass lesion, examine the orbit or optic nerve. The advantages of ultrasound for orbital-ocular tumors are represented by the fact that ultrasound is a noninvasive method, safe, well tolerated, less expensive that the advantage of determining the position and distance from structures ocular tumor. High frequency ultrasound provides excellent resolution of 0-1 to 0.01 mm, and serial scans allow tracking progress and measuring lesion diameters tumor while allowing monitoring and evaluation of stereotactic radiation treatments applied to small tumors. In conclusion ultrasound allows not only early diagnosis of eye tumors, but accurate assessment of the proposed therapy and of the evolution of detected mass lesions or tumors. **Key words**: ULTRASONOGRAPHY EXAM, ORBITAL EXAM, OCULAR TUMORS, OUTCOME.

Ultrasunetul este un fenomen de undă şi se încadrează în categoria oscilațiilor şi undelor mecanice, constituind o parte a acusticii. Ultrasonografia este o metodă de investigație paraclinică (imagistică) bazată pe principiul emiterii undelor sonice la țesuturi şi al recepționării şi interpretării undelor reflectate. Oftalmologia este una dintre specialitațile care au beneficiat în mod deosebit de aportul ecografiei ca modalitate de investigație, examenul ecografic fiind de foarte multe indispensabil în scop diagnostic pentru complementarea celorlalte investigații clinice şi paraclinice, furnizând imagini în timp real.

Indicațiile efectuării examenului ecografic în oftalmologie sunt (1): **1.** măsurarea unor distanțe sau volume (biometrie,

grosime corneeană); **2.** examinarea dificilă sau inaccesibilă în caz de medii opace (cataractă, leucom corneean); **3.** vizualizarea oftalmoscopică a unei leziuni de masă retinocoroidiene; **4.** depistarea eventualelor leziuni posttraumatice şi urmărirea evoluției acestora în timp (hifemă, detaşarea corpului ciliar, recesia unghiului irido-corneean, decolarea de retină); **5.** examinarea orbitei sau a nervului optic; **6.** documentarea evoluției tumorilor oculare în timpul tratamentelor de iradiere (2).

Modalități de producere a ultrasunetelor cu aplicabilitate în oftalmologie

În oftalmologie sunt folosite unde cu frecvență cuprinse între 10-50mHhz. Ultrasunetele sunt generate şi emise de un transductor piezoelectric cu un cristal de cuarț situat în interiorul sondei exploratoare; emisia este discontinuă, astfel încât transductorul este şi emițător şi receptor al ecourilor ultrasonice. Ultrasunetele reflectate sunt recaptate de sondă şi sunt transformate în semnal electric care este amplificat şi vizualizat pe monitorul ecografului. Leziunile pot fi urmărite în dinamică sau documentate pe hârtie fotografică (3). Se folosesc: **1.** modul A scan biometry – pentru măsurători axiale ale globului ocular; **2.** UBM – ultrabiomicroscopie sau VHFU (very high frequency ultrasound – ultrasunete de înaltă frecvență) pentru examinarea polului anterior; **3.** modul B-scan cu/fără A scan vector, cu/fără display tridimesional; **4.** modul B-scan şi A-scan standardizat; **5.** modul 3D tridimensional (modul C) – tehnică prin care se obține reconstrucția statică şi procesarea tridimensională a imaginii după stocarea multiplelor secțiuni obținute prin modul B; **6.** modul Doppler color pentru vizualizarea neoformațiilor vasculare şi a şunturilor arterio-venoase; **7.** Doppler în undă continuă; **8.** Doppler pulsatil (4).

Ecografia modul A (amplitudine) – este emis un fascicul subțire de unde paralele ce traversează ochiul şi scanează un ax mic de țesut. Se analizează: **1.** distanța de la sondă la structura examinată (biometrie); **2.** amplitudinea ecoului (înălțimea spiculilor) care depinde de natura interfeței reflectante (ecografie cantitativă). Ecograma A normală (fig. 1) cu sonda aplicată pe cornee prezintă: **1.** ecou înalt dat de cornee; **2.** linie izoelectrică corespunzătoare UA din CA; **3.** două ecouri înalte (egale) date de capsula anterioară şi posterioară a cristalinului; **4.** linie izoelectrică lungă corespunzătoare vitrosului; **5.** ecou înalt generat de peretele posterior al globului ocular; **6.** ecouri descrescătoare corespunzătoare țesutului retroocular.

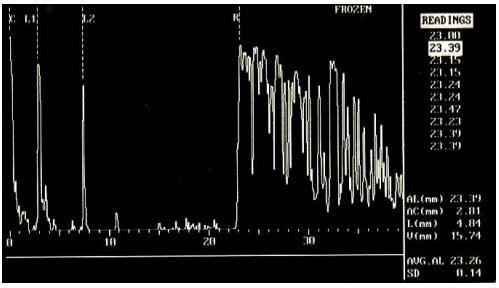

Fig. 1. Ecogramă modul A normală (axial orizontal)

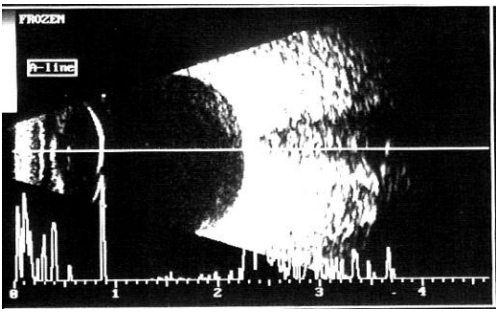

Fig. 2. Ecogramă modul B normală (axial orizontal)

Ecografia modul B – este emis un fascicul de unde oscilant ce traversează ochiul şi analizează o felie de țesut. Ecograma B

normală (fig. 2) prezintă: **1.** zonă ecogenă inițială dată de cornee; **2.** zonă ecotransparentă corespunzătoare umorului apos; **3.** două ecouri ecogene, convex-concav, subțiri date de cele două capsule cristaliniene, între care se observă o zonă ecotransparență (cristalinul); **4.** zonă ecotransparentă (vitrosul); **5.** ecou hiperecogen concav generat de peretele scleral posterior; **6.** ecouri corespunzătoare grăsimii retrooculare (5).

Examinarea globului ocular – Globul ocular se examinează de la nivelul polului posterior spre periferie, evaluând fiecare cadran prin scanare atentă cu sonda de la limbul sclerocorneean spre fornixul conjunctival. Pacientul stă întins sau în şezut cu capul sprijinit pe tetiera scaunului şi cu privirea orientată spre tavan, se aplică anestezic corneean topic; copiii pot fi examinați cu anestezie generală sau sub sedare uşoară. Capătul sondei (oval sau rotund) se aplică în contact cu corneea (după anestezie) prin intermediul unei fine pelicule de gel si se scanează în toate cadranele urmărind integritatea structurilor oculare.

TABELUL I

Metodologia şi folosirea ecografiei oculare (după Atta HR 1996) (6)

Informații	Modul ecografic
Leziuni detectate	
Examinare topografică	
Forma	B-scan
Localizare	B-scan
Extindere	B-scan
Examinare cantitativă	
Reflectivitate (măsurarea amplitudinii ecourilor)	A şi B-scan
Structură internă (regularitatea amplitudinii ecourilor şi a intervalului acestora de succesiune)	A-scan
Atenuare sonică (datorată absorbției ultrasunetelor la trecerea prin țesuturi)	A-scan
Examinare kinetică	
Mobilitate	B-scan
Mobilitate după mişcarea globului ocular	A şi B-scan
Vascularizație	A-scan

Se fac *6 scanări obligatorii*: **1.** Axial – cristalinul şi capul nervului optic sunt plasate în centrul ecogramei: **a.** orizontal cu proba perpendiculară pe axul central al corneei, markerul de pe lateralul sondei nazal (ora 3 pentru OD şi ora 9 la OS) – pentru scanarea polului posterior; **b.** axial vertical – marker la ora 12; **2.** Transversal 12-6 (sonda la ora 12 scanează regiunea orei 6); **3.** Transversal 6-12 (sonda la ora 6 scanează regiunea orei 12); **4.** Transversal 9-3 (sonda la ora 9 scanează regiunea orei 3); **5.** Transversal 3-9 (sonda la ora 3 scanează regiunea orei 9); **6.** Longitudinal 3-9 pentru OD şi 9-3 pentru OS pentru evaluarea regiunii maculare. Există 4 grupe mari de aspecte topografice anormale: opacitățile membranoase, leziunile de masă tumorală, opacități vitreene (unice/multiple), anomalii de contur ale globului ocular. *Examinarea cinetică* (în dinamică) a structurilor normale şi anormale este foarte importantă şi evidențiază: **1.** mobilitatea țesuturilor analizate simultan cu mişcarea globului ocular; **2.** vascularizația intratumorală decelabilă prin modul A – vibrații fine şi rapide verticale în interiorul ecoului tumoral (7). *Examinarea orbitei* – este o investigație utilă în patologia orbitei care permite localizarea topografică a leziunilor orbitare anterioare. În cazul leziunilor orbitare, CT şi RMN sunt superioare ca metode de diagnostic ecografiei. Ecografia de orbită este indicată în: **1.** exoftalmie unilaterală şi deplasarea globului ocular; **2.** tulburări de motilitate oculară şi durere oculoorbitară; **3.** congestia oculară unilaterală şi creşterea PIO (fistula carotido-cavernoasă traumatică); **4.** edemul sau atrofia nervului optic asociat cu ocluzia venoasă; **5.** pliuri coroidiene (tumori intraconale, oftalmopatia tiroidiană severă); **6.** traumatismele orbitei (8). Examinarea ecografică a orbitei se începe tot cu modul B pentru stabilirea

formei şi topografiei leziunii. Urmează modul A pentru evaluarea reflectivităţii şi corelarea directă cu structura histologică. Se impune examinare bilaterală şi compararea imaginilor ecografice.

Calea transoculară se realizează prin plasarea transductorului direct pe globul ocular, în incidenţa transoculară axial orizontal cu planul de secţiune al nervului optic. Unghiul anterior al triunghiului dat de nervul optic este în general între 40-70° (dacă se măreşte poate indica lărgirea patologică a nervului optic sau a tecilor nervului optic). Dacă scanările se fac superior sau inferior de nervul optic, triunghiul hipoecogen determinat de nervul optic este absent, iar modelul retrobulbar apare ca o zonă uniformă albă. *Calea paraoculară* se realizează prin plasarea transductorului pe pleoapele închise, în spaţiul dintre glob şi marginea orbitei; se evidenţiază leziunile plasate anterior (tumori de glandă lacrimală, mucocel, chist dermoid). *Examenul ecografic în cazul tumorilor intraoculare (9)* – ecografia poate analiza tumori cu mărimi de peste 0,5mm, însă precizarea diagnosticului final se face numai prin corelarea datelor examenului clinic şi ecografic cu alte investigaţii, iar certitudinea aparţine examenului anatomopatologic.

Tumori coroidiene – *Melanomul malign de coroidă* (MMC) – tumoră melanocitară malignă, una dintre cele mai frecvente tumori maligne primare ale ochiului – 75%. Criterii de diagnostic ecografic în cazul MMC: *Modul A* – **1**. ecou iniţial intens reflectiv urmat de un grup de ecouri cu reflectivitate medie şi mică (reflectivitate internă 5-60% din ecoul scleral), bine individualizate, cu structură omogenă; **2.** vitrosul pare scurtat, coloană de ecouri descrescătoare, ecourile nu ajung la linia izoelectrică; **3.** oscilaţii spontane, rapide ale ecourilor verticale vascularizaţie intratumorală – semn specific în MMC; **4.** ecografia permite măsurarea biometrică a tumorii. *Modul B* – **1.** zonă ecogenă compactă, densă, relativ omogenă, bine conturată, cu aspect în dom (membrana Bruch este intactă) sau de ciupercă (tumora a perforat membrana Bruch) care proemină în aria de ultrasonotransparenţă a vitrosului; **2.** zonă cu reflectivitate scăzută la baza tumorii – excavaţia coroidiană care deprimă conturul peretelui scleral; **3.** umbra acustică – umbră orbitară din cauza absorbţiei mari a ultrasunetelor de către tumoră; **4.** unghiul kappa format de orizontala ce trece prin primul ecou intratumoral; **5.** tendinţă de creştere a leziunii la examinări repetate; **6.** DR secundară (fig.3). *Nev coroidian tumoră benignă melanocitară a coroidei* **(**fig. 4**) –** modul A: reflectivitate crescută 90-100%, spike cu platou. Modul B – mică elevaţie plată sau în dom care nu creşte la examenele ulterioare sau creşte foarte lent.

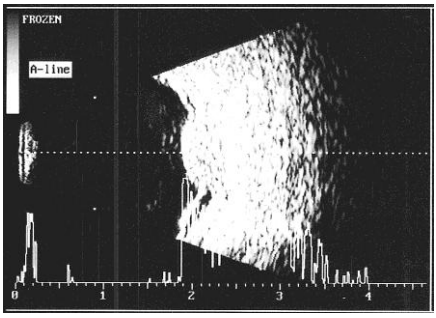

Fig. 3. MMC cu DR exsudativă secundară

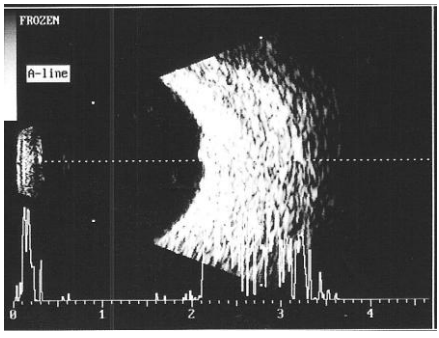

Fig. 4. Nev coroidian

Hemangiomul coroidian este un hamartom vascular congenital benign al coroidei. Modul A – structură internă regulată cu reflectivitate crescută şi atenuare sonică slabă. Ecografia cinetică demonstrează caracterul non vascular al afecţiunii. Modul B – proeminenţă ecogenă în dom sau îngroşare difuză a stratului retinocoroidian, localizată la nivelul polului posterior. Nu prezintă excavaţie coroidiană sau fenomenul de atenuare sonică la nivelul grăsimii retrooculare. *Tumori metastatice coroidiene* – modul B zonă ecogenă de tip infiltraţie cu una sau două proeminenţe şi cu bază tumorală întinsă, mai extinsă decât înălţimea tumorii; zona de predilecţie este la nivelul polului posterior. Modul A – ecouri interne neregulate cu amplitudine între 40-80%, înălţimea spikeului *tumorale creşte către scleră* (la MMC scade către scleră) (10). Atenuare sonică slabă.

Tumori retiniene – *retinoblastomul (RB) este un neoplasm primar malign al retinei, cea mai frecventă tumoră intraoculară la copii.* Pe ecografia în modul A şi B imaginea unui retinoblastom compact este similară unei tumori coroidiene, cu diferenţa că retinoblastoamele apar cu precădere retroecuatorial. Forma chistică este nesistematizată, pretând la confuzii cu hemoragia vitreană organizată şi fibroplazia retrolentală. *Foarte importantă este prezenţa calcificărilor intratumorale în cazul retinoblastomului care sunt hiperreflective pe ecograma B, foarte absorbante, lăsând un „con de umbră" posterior* (11). *Forma solidă* – o formaţiune tumorală intraoculară, hiperecogenă în aria de ultrasonotransparenţă a vitrosului. Prezenţa zonelor cu reflectivitate înaltă intratumorală reprezintă unul din criteriile importante de diagnostic (90-95% din cazuri). *Forma chistică* – o masă hiperecogenă cu structură heterogenă cu tendinţa de a ocupa întreg vitrosul cu zone intens reflective determinate de calcificări, axul anteroposterior al globului ocular este normal. În cazul RB fără calcificări se observă masă tumorală neregulată cu reflectivitate difuză, vascularizată. **Leziuni tumorale orbitare –** există 4 modele ecografice de leziuni de masă: chistice, solide, angiomatoase şi infiltrative. Conturul poate fi rotund, bine definit şi să comprime structurile adiacente sau conturul neregulat poate fi sugestiv pentru tumori angiomatoase sau infiltrative. Ecourile interne – ecourile de amplitudine mică în interiorul tumorii indică tumori solide homogene. Tumorile orbitei sunt mai puţin ecogene comparativ cu grăsimea orbitei prezentându-se sub forma unor lacune, limitele putând fi bine conturate sau estompate. Structuri tumorale orbitare: **1.** structuri heterogene foarte ecogene (hemangiomul cavernos capilar, hematomul orbitar necolectat); **2.** structuri dure – moderat ecogene, omogene (tumori primitive, metastaze); **3.** structuri lichide – neecogene, omogene (mucocelul, chistul dermoid). **Tumorile infiltrative** (limfom, carcinom metastatic, sarcom) sunt cel mai dificil de diagnosticat cu ajutorul ultrasunetelor deoarece modificările inflamatorii ale orbitei pot produce aspect similar mai ales dacă edemul inflamator inplică nervul optic şi spaţiul sub-Tenon; acelaşi aspect poate fi greu de diferenţiat şi în caz de cicatrici ale ţesuturilor orbitei după chirurgie anterioară la nivel orbitar. **Tumori chistice – 1.** *mucocel* – rotund, bine conturat, bine demarcat de ţesuturile din jur, modul B – cavitate ultrasonotransparentă, modul A intern ecou liniar; fără absorbţie a ultrasunetelor la nivelul leziunii, ceea ce permite vizualizarea peretelui posterior; **2.** *chist dermoid*; **3.** *hemangiom cavernos* – rotund, bine delimitat (în special marginea anterioară), absorbţie joasă-moderată, ecouri interne de joasă

amplitudine; **4.** *hemangiopericitom*; **5.** *limfangiom chistic*. **Tumori solide –** *tumorile nervului optic* – **1.** rotunde, margine anterioară bine definită, marginea şi extensia posterioară sunt greu de apreciat din cauza absorbției crescute a ultrasunetelor la nivelul leziunii; pe A scan homogenitate acustică cu ecouri de amplitudine mică; **2.** produc lărgirea umbrei sonotransparente determinate de nervul optic, uneori indentarea nervului optic în porțiunea anterioară. **Tumori angiomatoase – 1.** margini neregulate, bună transmitere prin leziunea de masă; **2.** limfangiom difuz – localizarea, mărimea şi granițele se demonstrează cu ajutorul modulului B; transmiterea ultrasunetelor la nivelul leziunii este bună şi nu apare atenuare sonică; prezintă pulsații pe A scan. **Tumori infiltrative – 1.** *limfoame* – contur neregulat, lobulat, demarcate față de țesuturile din jur, conținut solid cu mare capacitate de absorbție a ultrasunetelor, nu se poate obiectiva extensia posterioară a tumorii, homogenitate acustică, ecouri medii ca amplitudine pe A scan; **2.** *carcinom metastatic* – spații infiltrative sonotransparente infiltrative în grăsimea orbitară; **3.** *histiocitom fibros*; **4.** *rabdomiosarcom*. **Pseudotumoră inflamatorie** – pe B scan se observă leziune de masă tumorală în interiorul orbitei şi edem inflamator al structurilor orbitare normale. Caracteristicile acustice sunt similare tumorilor infiltrative: contur neregulat, absorbție puternică a ultrasunetelor.

Avantajele ecografiei în cazul leziunilor tumorale oculoorbitare – ultrasonografia este o metodă neinvazivă, sigură, bine tolerată, puțin costisitoare care oferă avantajul determinării poziției şi distanței tumorii față de structurile oculare. Frecvența mare a ultrasunetelor oferă o rezoluție excelentă de 0-1 la 0,01 mm, iar ecografiile seriate permit urmărirea evoluției leziunii în timp şi măsurarea diametrelor tumorii permițând astfel monitorizarea şi evaluarea tratamentelor de iradiere stereotactică aplicate în cazul tumorilor de mici dimensiuni.

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SINDROMUL METABOLIC LA PACIENTUL INFECTAT HIV CU RĂSPUNS FAVORABIL LA TRATAMENTUL ANTIRETROVIRAL

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METABOLIC SYNDROME IN HIV INFECTED PATIRENTS WITH FAVORABLE RESPONSE AT ANTIRETROVIRAL THERAPY (Abstract): **Aim:** The assessment of the metabolic syndrome prevalence, as defined by the American Heart Association / The US National Cholesterol Education Program Adult Treatment Panel, (AHA / NCEP III updated), has been made in 68 polyexperimented HIV infected patients that received antiretroviral therapy over 24 months and who have a favorable viroimunologic response (undetectable plasmatic viral load). **Materials and methods:** this cross-sectional analytical study has been carried out on a cohort of 68 patients clinically and paraclinically monitored at the HIV /AIDS Department. **Results:** The response to antiretroviral therapy clinically and paraclinically evaluated after two years from the initiation of the novel HAART scheme has been favourable in all the 68 patients. Only 4 (0.58%) of the patients fulfill the criteria of the metabolic sindrome definition. The statistical analysis does not indicate an increase in the risk of the metabolic syndrome development in these polyexperimented patients, where the clinical symptoms connected with the lipodistrophic syndrome with lipoatrophy are preponderent, associated with different types of antiretroviral drugs. **Conclusions:** The metabolic syndrome criteria are not applicable in the case of HIV infected patients (infected in the first year of life), polyexperimented at HAART therapy with favourable viroimmunologic response. **Key words:** HIV PATIENTS, METABOLIC SYNDROME, HAART

Sindromul metabolic pare a avea o prevalenţă mai mare la pacientul infectat cu HIV faţă de cel neinfectat, supoziţie infirmată printr-o multitudine de studii clinice (1). În populaţia generală prevalenţa sindromului metabolic variază între 22-24% crescând odată cu vârsta (1). Prevalenţa creşte însă la pacientul infectat cu HIV cu tratament antiretroviral

Introducerea terapiei Highly Active Anti-Retroviral Therapy (HAART) a redus semnificativ progresia bolii spre stadiul de boală SIDA, morbiditatea şi mortalitatea asociată cu HIV (2,3). Pe termen lung medicaţia antiretrovirală induce modificări ale metabolismului glucidic, lipidic şi modificări morfologice (acumularea de grăsimi, lipoatrofia) (4). Studii recente de cohortă sugerează o creştere a sindromului metabolic şi a riscului cardiovascular (RCV) la pacienţii cu HAART şi viremie nedetectabilă (5). Pe termen lung nu au fost clar definiţi: rolul HIV, al terapiei HAART şi al

factorilor genetici în generarea modificărilor metabolice ce însoţesc sindromul metabolic, factor de risc cardivascular (1,5). Terapia HAART induce modificări ale metabolismului lipidic: hipercolesterolemia (30%-95%), hipertrigliceridemia (50%-90%), sindromul lipodistrofic (20%-83%), insulinrezistența (25%-62%), diabetul zaharat (5%-10%) şi hipertensiunea (20%-74%) (6,7). Fiecare clasă de medicamente ARV influenţează diferit metabolismul lipidic: inhibitorii de protează sunt asociaţi cu creşterea TGL la peste 25% din pacienţii la un an de la iniţierea tratamentului (8-10), inhibitorii nucleozidici de revers transcriptază sunt asociaţi cu creşterea colesterolului total al LDL-C şi al trigliceridelor iar inhibitorii nonnucleozidici de reverstranscriptază cu creşterea HDL-C, dar pe termen lung cresc LDL-C şi trigliceridele.

MATERIAL ŞI METODĂ

A fost efectuat un studiu transversal pe 68 de pacienţi diagnosticaţi cu infecţie HIV între 1989-1990. La momentul iniţierii noii scheme HAART la subiecţii în eşec terapeutic (viremie plasmatică >1000 copii/ml, scădere accelerată a LCD4, fără infecţii oportuniste manifeste clinic), pacienţii fiind la minim 2 scheme HAART în antecedente. A fost efectuat examenul clinic obiectiv pe aparate şi sisteme initial, apoi din 6 în 6 luni. Examenul clinic a semnalat scăderea ţesutului celular subcutanat la nivelul extremităţilor, la nivel facial, temporal, nazolabial şi genian, semne caracteristice sindromului lipoatrofic. Au fost măsurate circumferinţele braţului, coapsei, toracelui, taliei şi şoldului folosind panglica metrică, talia cu taliometrul şi greutatea cu cântarul şi calculul indicelui de masă corporală (IMC Dubois). În conformitate cu protocolul şi cu consimţământul subiecţilor, probele biologice au fost recoltate dimineaţa „*à jeun*", după cel puţin 12 ore de post. Din lotul studiat 19% au fost la a 2-a schemă, 52% la a 3-a schemă, 27% la a 4-a şi 2% la a 5-a schema HAART. Testele de rezistenţă genotipică (TRG) au fost efectuate la 10 pacienţi, noua schemă HAART fiind în concordanţă cu algoritmul de interpretare a TRG. Au fost analizate datele demografice (vârstă, sex, mediu de provenienţă), nivelul iniţial, dinamica LCD4 şi a viremiei plasmatice (tab. I, II). Evaluarea sindromului metabolic a fost făcută pe baza definiţiei AHA /NCEP III actualizată luându-se în calcul cel puţin trei din următoarele criterii: obezitate abdominală, hipertrigliceridemie, scăderea HDL-C, hipertensiunea arterială, creşterea glicemiei „*à jeun*".

Au fost urmăriţi:

- circumferinţa taliei: barbati ≥102 cm; femei ≥88 cm;
- trigliceride ≥150 mg/dl;
- scăderea HDL colesterolului (HDL-C) la bărbaţi ≤40mg/dl, la femei ≤50mg/dl;
- creşterea tensiunii arteriale ≥130/85 mm Hg sau utilizarea de medicamente antihipertensive;
- glicemia „*à jeun*" ≥110 mg/dl sau utilizarea de medicamente antidiabetice.

A fost evaluată şi Proteina C reactivă ca marker predictiv al riscului coronarian.

Eşantionul studiat fiind mic, analiza statistică s-a bazat pe indicatori statistici descriptivi (valori medii, intervale de încredere 95%) şi teste comparative (*t Student*, odds ratio) folosind programe tip *SPSS*, Microsoft Excel + Analyse-it, $p<0.05$ a fost considerat semnificativ statistic.

REZULTATE ŞI DISCUŢII

Din cei 68 de pacienti înrolaţi, 63% (43) se aflau în stadiul de boală SIDA si 47% (25) în stadiul de infecţie HIV.

La doi ani de la iniţierea ultimei scheme HAART, toţi pacienţii au menţinut un răspuns viroimunologic favorabil (tab. III, IV).

TABELUL I

Distribuţia pacienţilor după clasificarea CDC 1993

după valorile LD4 (cel/ml)		categorii clinice		
		A	B	C
1	18	2	13	12
2	14		10	18
3	10		5	8

TABELUL II

Caracteristicile lotului studiat

Caracteristici	Valori medii
Vârsta (ani)	20,9
Masculin/feminin	38/30
IMC (kg/m2)	19,94
Talie (cm)	
Barbati	78,42
Femei	79,52
Fumat (%)	10
LCD4 (nadir) cel/ml	227
LCD4 cel/ml	563
Viremia plasmatică	
<400 cp/ml (%)	68
>400 cp/ml (%)	32
Durata tratamentului ARV	24
Inhibitori de protează (%)	88,8
Fără inhibitor de protează	11,2
Lipodistrofia (%)	
Absentă	2
Lipoatrofie	84
lipohipertrofie	10
Mixtă	4

TABELUL III

Dinamica LCD4

		Mediana = 323		
LCD4 (copii/ml)	N	≤	>	mediana
LCD4 iniţial	68	60	8	212,0
LCD4 la 2 ani	68	10	58	541,5
p	<0.0001			

TABELUL IV

Dinamica viremiei plasmatice

Groups	N	≤	>	Median
VP iniţial	68	0	68	70500,0
VP la 2 ani	68	68	0	46,0
p	<0.0001			

TABELUL V

Valorile obţinute ale parametrilor definitorii pentru sindromul metabolic

talie (cm)	
>102 bărbaţi	0
> 88 femei	0
TA	
≥135/80mmHg	4(0,58%)
HDL - Colesterolul	
bărbaţi ≤40 mg/dl	36(94%)
femei ≤50 mg/dl	28(93%)
trigliceridele ≥150mg/dl	60(88%)
glicemie ≥110 mg/dl	6(0,88%)

Pacienţii evaluaţi au avut o durată medie a terapiei HAART de 14 ani. Din cei 68 de subiecţi analizaţi 4 au risc de a dezvolta sindrom metabolic (p<0,1, nesemnificativ statistic), doi de sex masculin şi doi de sex feminin. Prevalenţa SM a fost de 0,58% (CI 95%). Nu au existat variaţii legate de vârstă, lotul studiat fiind unitar din acest punct de vedere. Sindromul lipodistrofic cu lipoatrofie a fost prezent la 55 (80%) versus 13 pacienţi (20%, p=0,0001). Hipertrigliceridemia a fost prezentă la 88% dintre subiecţi, HDL-C scăzut sub limita de protecţie, peste 90% nefiind diferenţe între sexe. Modificările glicemiei „a jeun", valorile tensiunii arteriale, sunt puţin implicate în definirea SM la acest lot (tab. V). Lipoatrofia ar trebui asociată cu SM deoarece apare ca o consecinţă a redistribuirii prin exces sau pierdere a grăsimilor şi se poate asocia cu insulinorezistenţa. Pacienţii aflaţi în multiplu eşec terapeutic au recuperat parţial deficitul în greutate, cei cu lipoatrofie mai greu şi incomplet (p=0,003), neexistând o corelaţie între modificările distribuţiei grăsimilor în corp şi valorile lipidelor sanguine. Nu a putut fi făcută o corelaţie semnificativă statistică între IMC şi sindromul lipodistrofic. Analiza multivariată arată că stavudina (odss ratio 1,87; CI 95% 1,23-3,12; 0.002) se asociază cu sindrom metabolic. Analiza statistică nu evidenţiază riscul apariţiei sindromului metabolic la aceşti pacienţi, poliexperimentaţi la care predomină manifestările clinice legate de sindromul lipodistrofic cu lipoatrofie,

asociat diverselor clase de medicamente antiretrovirale.

CONCLUZII

Criteriile definitorii ale sindromului metabolic nu sunt aplicabile pentru pacienţii cu infecţie HIV (infectaţi în primul an de viaţă) poliexperimentaţi la HAART cu răspuns viroimunologic favorabil. Multiplele scheme de terapie antiretrovirală la adultul tânăr infectat în primii ani de viaţă generează modificări ale fizionomiei şi metabolismului lipidic, nesemnificativ ale glicemiei „a jeun“ sau provocate influenţând criteriile de încadrare în definiţia de caz. Recuperarea indicilor atropometrici este lentă şi parţială în condiţiile răspunsului sustenabil la HAART. Efectuarea de studii pe cohorte mari de pacienţi din această categorie ar permite identificarea altor criterii de diagnosticare a SM. Rezultatele sunt asemănătoare şi pentru evaluarea riscului cardiovascular. Este posibil ca lotul studiat, redus numeric, să influenţeze rezultatele statistice obţinute. Noile clase de medicamente antiretrovirale par a avea, cel putin pe termen scurt, reacţii adverse reduse asupra metabolismului lipidic, glucidic şi al valorilor tensiunii arteriale.

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COMPLICAȚIILE NEFROSTOMIEI PERCUTANATE ÎN TRATAMENTUL INSUFICIENȚEI RENALE OBSTRUCTIVE

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PERCUTANEOUS NEPHROSTOMY COMPLICATIONS IN OBSTRUCTIVE RENAL FAILURE (Abstract): **Objectives**: The study aimed to identify the complications recorded at the patients with obstructive renal failure treated with percutaneous nephrostomy (PCN). **Material and method**: The retrospective study investigated the data of 244 patients admitted in our department and treated with percutaneous nephrostomy for obstructive renal failure during January 2005 – December 2007. Demographical data, investigation, indication, complications and hospital stay were recorded. **Results**: Median preoperative/discharge haematological values were hematocrit 27.4% comparing to 25.8% and haemoglobin 9.9g% comparing to 9.3g%. An anaemic syndrome was noted at admission time in 161 (65.9%) patients and at discharge time 202 (82.8%). 126 (51.6%) patients were treated with blood transfusion with an average dose of 2.47 red blood cell units per patient. In 25 (10.1%) patients the nefrostomy tube was clamped in order to control the postoperative bleeding. The median hospitalization time was 8.7 days with a median ICU stay of 3.3 days. The recorded mortality was 3.2% (8 patients) with an average time of 5.8 days between the operatory time and exitus time. **Conclusions:** The most frequent causes of obstructive renal failure necessiting percutaneous nephrostomy are malignancies. Preoperative anaemic syndrome is aggravated in the postoperative period and frequently need red blood cell administration. In experienced hands, PCN catheter placement is safe and usually successful **Key words**: RENAL FAILURE, PERCUTANEOUS NEPHROSTOMY , COMPLICATION

Insuficiența renală (IR) postrenală se întâlneşte la aproximativ 5% din cazurile de IR. Obstrucția tractului urinar determină insuficienta renală în cazul interesării bilaterale a fluxulului urinar renal cu excepția unei disfuncții renale preexistente când obstrucția poate interesa un singur rinichi (1).

Abordarea terapeutică în urgență a obstrucției tractului urinar superior include tehnicile minim invazive: cateterismul ureteral sau nefrostomia percutanată.

Nefrostomia percutanată constă în plasarea ghidată imagistic a unui cateter în sistemul colector renal (2).

Plasarea unui cateter de un calibru suficient pentru a asigura drenajul adecvat al sistemului colector sau a per-mite dilatarea traiectului şi efectuarea procedurii intervenționale planificate defineşte nefrostomia percutanată reuşită. Rata de succes a procedurii percutanate este de 98%–99%

fiind mai redusă la pacienții cu sistem colector nedilatat, litiaza renală complexă sau calculi coraliformi (3,4,5).

Prima descriere a nefrostomiei percutanate a fost făcută în 1955 (6) sub forma unei proceduri efectuate sub o ghidare imagistică limitată motiv pentru care a fost acceptată cu anumite rezerve;

Perfecționarea instrumentarului utilizat precum și a tehnicilor radiologice intervenționale au permis extinderea acestui procedeu terapeutic. În România prima intervenție percutanată renală a fost efectuată în 1985 în Clinica Urologică Târgu Mureș de Profesorul Radu Boja.

Nefrostomia chirurgicală deschi-să de decompresiune a reprezentat tratamentul standard al obstrucției maligne ureterale în etapa predecesoare impunerii chirurgiei minim invazive (7), ulterior această opțiune terapeutică a fost aproape în totalitate înlocuită de nefrostomia percutanată datorită complicațiilor cu incidență redusă (8,9).

Complicațiile asociate cu NSP sunt puține și de obicei minore. În general rata complicațiilor procedeului de nefrostomie percutanată este sub 10% (4,10), dar la pacienții cu neoplazii avansate au fost raportate frecvențe crescute ale complicațiilor (4,10,11) explicate prin statusul biologic precar și diateza hemoragică uremică.

Complicațiile majore după abordul percutanat se situează în jurul cifrei de 5% (12,13), iar complicațiile minore se întâlnesc în peste 70% din cazuri, în timp ce mortalitatea variază între 0,5-1%. Cea mai întâlnită complicație este hemoragia antrenând o pierdere parțială a funcției renale sau chiar nefrectomia și impunând frecvent transfuzii de sânge cu riscurile asociate de hepatită și infecție cu HIV.

Reducerea complicațiilor hemoragice este posibilă prin obținerea unui traict care abordează sistemului colector prin fornix sau vârful papilei unui calice posterior la care se ajunge străbătând teritoriul relativ avascular dintre diviziunile anterioare și posterioare ale liniei Brödel (7,8).

OBIECTIVE

Scopul studiului a fost de a evalua complicațiile drenajului urinar percu-tan în abordarea terapeutică a pacienților cu insuficiență renală obstructivă.

MATERIAL ŞI METODĂ

Lucrarea reprezintă un studiu retrospectiv al experienței Clinicii Urologice Iași în soluționarea prin abord percutanat renal a patologiei obstuctive urinare asociate cu insuficiența renală. Au fost incluși în studiu 244 de pacienți cu insuficiență renală obstructivă internați in Clinica de Urologie și Transplant Renal Iași la care s-a practicat nefrostomie percutanată în perioada ianuarie 2005 – decembrie 2007.

Selecția pacienților s-a efectuat pe baza diagnosticului clinico-imagistic și biologic (uree,creatinină) de insuficiență renală obstructivă.

Procedurile intervenționale s-au realizat sub control imagistic asigurat de un echipament radiologic mobil (Siremobil Compact L Siemens) și un ecograf BK MiniFocus. Pentru drenajul urinar s-au utilizat catetere de uz general (sonde cu balonaș tip Foley 20 Ch) și dedicat (truse de nefrostomie a minima 8, 12F), fire ghid 0,038". Dilatarea acută a traiectului s-a realizat cu dilatatoare fasciale de dimensiuni progresive pană la 12 F în cazul NSP a minima, și respectiv cu setul de dilatatoare Amplatz în cazul montării unor sonde autostatice tip Foley.

Parametrii analizați în prezentul studiu au fost reprezentati de: afecțiunea care a

determinat obstrucția urinară, complicațiile postoperatorii, hematocrit, hemoglobină, urocultură, tipul de cateter utilizat.

Prelucrarea statistică a datelor a fost realizată prin utilizarea programului SPSS 17.0 for Windows.

REZULTATE ŞI DISCUȚII

Lotul de pacienți a fost alcătuit din 244 de pacienți (121 femei şi 123 bărbați) diagnosticați cu insuficiență renală obstructivă). Vârsta medie a pacienților a fost de 61,14 cu limite între 21 şi 90 de ani. Mediul rural a fost preponderent (59,42% rural vs 40,78% urban).

Evaluarea patologiei care deter-mină obstrucția aparatului urinar ce impune efectuarea NSP obiectivează o pondere majoră a patologiei neoplazice, afecțiuni în care tentativele de protezare endoureterală (stent ureteral JJ) eşuează din cauza condițiilor locale sau datorită evoluției ulterioare nefavorabile în condițiile drenajului urinar intern(recuperare funcțională nesatisfăcătoare, complicații infecțioase). Cele mai frecvente neoplazii sunt cancerul de col uterin, tumorile vezicale infiltrative şi cancerul de prostată local avansat (tab. I).

TABELUL I

Etiologia obstrucției urinare ce a impus efectuare nefrostomiei percutanate pentru tratamentul insuficienței renale

Etiologia obstrucției urinare	Nr. cazuri	Frecventa
LITIAZĂ URETERALĂ	19	7,79%
HIDRONEFROZĂ CONGENITALĂ	6	3,46%
LITIAZĂ RENALĂ	2	0,82%
CISTITĂ INCRUSTATĂ	1	0,41%
FIBROZĂ RETROPERITONEALĂ	1	0,41%
MEGAURETER OBSTRUCTIV	2	0,82%
TUBERCULOZĂ URINARĂ	7	1,86%
STENOZĂ URETERALĂ MULTIPLĂ	3	1,23%
UROPIONEFROZĂ	4	1,64%
NEOPLASM COL UTERIN	77	31,54%
NEOPLASM VEZICAL	53	21,72%
NEOPLASM PROSTATIC	31	12,70%
NEOPLASM RECTAL	7	1,86%
NEOPLASM COLON	1	0,41%
NEOPLASM MAMAR	1	0,41%
NEOPLASM OVARIAN	3	1,23%
NEOPLASM SIGMOIDIAN	4	1,64%
NEOPLASM UTERIN	1	0,41%
NEOPLASM VEZICAL + NEOPLASM PROSTATIC	2	0,82%
TUMORĂ PELVINĂ NEPRECIZATĂ	13	5,32%
TUMORĂ VAGINALĂ	1	0,41%
ALTE NEOPLAZII	5	2,05%

Nefrostomia percutanată este recunoscută ca fiind o intervenție cu risc hemoragic semnificativ. Sângerări asimptomatice clinic sunt frecvent întâlnite, hematuria moderată este prezentă la aproximativ 50% dintre pacienți după poziționarea NSP (14) în timp ce sângerările retroperitoneale documentate prin tomografie computerizată se întâlnesc în 13% din abordurile percutanate renale (15). Hemoragiile ce necesită transfuzii sanguine au o incidență de 1-4 % (4,5,12,16) . La 10,12% din pacienți supuşi intervenției s-a inregistrat apariția unei hematurii importante ce a necesitat manevre de tamponadă locală (clamparea sondei de nefrostomie) şi mijloace de compensare hematologică. La 126 de pacienți s-au efectuat transfuzii cu o medie de 2,47 unități de sânge per pacient.

Leziunile vasculare (17,18) care impun embolizare sau nefrectomie sunt întâlnite în 0.1-1% din cazuri.

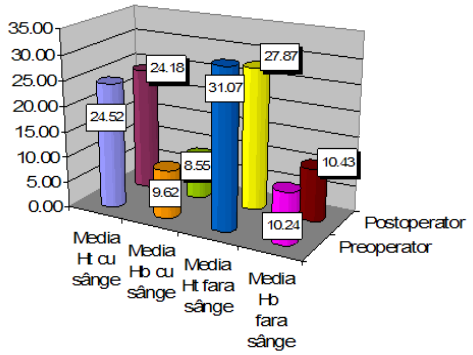

Fig.1. Bilanțul hematologic al pacienților care au primit sânge *vs.* pacienți netransfuzați

Alte complicații asociate NSP sunt mult mai rare şi include leziunile colonului (19,20). Perforația colonului citată în 0,2% (20) (retroperitoneală) a fost identificată într-un singur caz (0,4%) la 5 luni de la efectuarea abordului percutanat fiind soluționată conservator.

Nu au fost consemnate leziuni splenice (21) sau puncții ale veziculei biliare (22). Complicațiile pleurale posibile în abordul percutanat (pneumotorax, hemotorax, pleurezii – 0,1 – 0,2% (4,17)) nu au fost intâlnite în lotul studiat deoarece nu s-au impus traiecte de nefrostomie supracostale.

Şocul septic raportat în literatură cu o incidență de 1-3% (4,23,24) a fost clinic manifest în 22 de cazuri 9,82%, pacienții fiind frecvent infectați la internare.

Eşecul plasării nefrostomiei s-a înregistrat în 3,24% din cazuri impunându-se puncționarea sistemlui colector cotrolateral sau intervenția unui medic mai experimentat.

Depoziționarea precoce a sondei s-a înregistrat în 9 cazuri (3,68%).

Complicațiile tardive apar datorită disfuncțiilor cateterului (depozitionare sau ocluzie) (25) şi se întâlnesc la 40-50% din pacienți; acestea scad cu calibrul crescut al cateterului, materiale mai moi şi prezența mijloacelor autostatice.

Cel mai frecvent se constată depoziționarea cateterului sau ocluzia acestuia. Manifestările clinice ale blocajului cateterului sunt: scurgerea de urină pe lângă cateter, creşterea ureei şi creatininei, pielonefrită acută. În aceste condiții se impune înlocuirea cateterului, lucru care se poate efectua în condiții de spitalizare de zi, cu excepția cazurilor de pielonefrita acută.

Au fost înregistrate 8 decese (3,27%) survenite la un interval mediu de 5,79 zile de la momentul operator. În 50% din cazurile de deces cauza determinantă a fost sindromul de disfuncție multiplă de organ, un singur caz de deces a survenit în contextul unui hematom lombar voluminos.

CONCLUZII

Numărul complicațiilor înregistrate, mai ales a celor majore (hemoragice şi infecțioase) a fost relativ redus având în vedere că

nefrostomiile percutanate au fost practicate, în cele mai multe cazuri, la pacienți aflați în condiții clinice grave.

Ghidajul combinat: ecografic și fluoroscopic, utilizat pentru realizarea puncției percutanate a facilitat obținerea unui abord corect al sistemului pielo-caliceal și consecutiv posibilitatea efec-tuării unei dilatații acute a traiectului de nefrostomie până la un diametru ce a permis poziționarea unor sonde autostatice cu balonaș (Foley).

Utilizarea sondelor Foley asigură o întreținere facilă prin prețul redus al materialelor, risc redus de depoziționare și prin posibilitatea înlocuirii sondelor în condiții de ambulator .

Pacienții care beneficiază de această modalitate de tratament necesită transfuzii repetate în scopul redresării sindromului anemic determinat de malignitate, insuficiența renală și accentuat de hemoragiile declanșate de intervenția percutanată.

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ATYPICAL MENINGIOMAS OF CEREBELLOPONTINE ANGLE. A FIVE CASE SERIES

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ATYPICAL MENINGIOMAS OF CEREBELLOPONTINE ANGLE: A FIVE CASE SERIES (Abstract): Cerebellopontine angle (CPA) meningiomas represent a subset of posterior fossa tumors accounting approximately 10% of all brain tumors, and out of all these, meningioma is the second most common tumor of the CPA. Surgical treatment of CPA meningiomas is challenging because of their relationship with important and vital neurovascular structures of the CPA. **Aim:** Identification of clinical, neuroimaging and surgical features of atypical CPA meningiomas. **Material and methods:** Out of all the 1287 intracranial meningiomas diagnosed and operated in "Professor Dr. N. Oblu" Emergency Clinical Hospital Iasi, from 1990-2015 were identified 44 CPA meningiomas. Of these were identified 5 atypical meningiomas (AM) of CPA. The results of our five-case series were compared to those of the existing studies. **Results:** This is an observational clinical study for five cases of atypical CPA meningiomas. CPA meningiomas have distinct clinical syndromes, associated with anatomical localization. Compared to the previous studies, the number of atypical CPA meningiomas from our series is higher, with average duration between the onset of symptoms and admission longer. As well as in literature, in our case series the retro sigmoid approach was preferred and the only subtotal tumoral resection of AM were those with extension towards the ventral petrous face and facial palsy was the most common postoperative complication. **Conclusions:** Surgical treatment of atypical CPA meningiomas has become increasingly safe, but these tumors remain challenging lesions, and the resection of these tumors is associated with increased rates of complications. **Keywords:** ATYPICAL MENINGIOMA, WHO GRADE II MENINGIOMAS, CEREBELLOPONTINE ANGLE MENINGIOMAS, POSTERIOR FOSSA MENINGIOMAS.

CPA tumors represent approximately 10% of all brain tumors (1). Out of all these, meningioma is the second most common tumor of the CPA after vestibular schwannoma, counting 5-15% of all the tumors of this region (2).

CPA meningiomas arise from epithelial cells, which aggregate in clusters around the tips of arachnoid villi. In CPA, they are located within the internal auditory canal, in the region of *porus acousticus*, *clivus*, *exit foramina* of the trigeminal nerve (Meckel's cavum), superior and inferior petrosal sinuses, sigmoid sinus, jugular foramen and torcula (3, 4, 5, 6, 7).

Like other intracranial meningiomas in general, CPA meningiomas are typically slow growing, usually in a different direction and can produce symptoms when they comprise nerve structures or cranial nerves (8). Due to this slow growing, CPA meningiomas can produce pressure indentation in the cerebellum and brainstem, but these can adapt themselves and undergo considerable distortion (7), so that meningiomas can reach gigantic dimensions, which may exceed 100 mm at the time of diagnosis (9).

Meningiomas arising within the CPA can displace or surround the vessels and cranial nerves rather than invade them, but depending on the histology of meningiomas, they can strongly adhere to these structures (5, 7), due to which these tumors represent a very important surgical challenge. Despite the advanced clinical techniques, the maximal surgical resection can't be performed without determining the patient face a high morbidity and mortality risk (2), and these are attributed precisely to the way the tumor surrounds or infiltrates cranial nerves and/or brainstem (10). Thus, the meningiomas of these challenging locations have higher subtotal resection complications rates (8, 11, 12, 13) and represent a challenge even in the hands of expert neurosurgeons (14).

MATERIAL AND METHODS

There were identified 5 CPA meningiomas diagnosed and operated in "Professor Dr. N. Oblu" Emergency Clinical Hospital Iasi, Romania, from 1990 to 2015. Also, specialty literature has been revised and the key words which were identified are *atypical meningioma* and *cerebellopontine angle meningioma*, *skull base meningioma* and *posterior fossa meningioma*. The results of our five-case series were compared to those of the existing studies.

RESULTS

Out of all the 1287 intracranial meningiomas diagnosed and operated in "Professor Dr. N. Oblu" Emergency Clinical Hospital Iasi, from 1990 to 2015, there were identified 44 CPA meningiomas. These represent a percentage of 3.41% from the total of diagnosed and operated meningiomas in our hospital from 1990 to 2015. According to the WHO grade of CPA meningiomas, this consists mainly of WHO grade I meningiomas, counting 84% (37 cases), followed by WHO grade II meningiomas (AM), counting 11.36% (5 cases) and WHO grade III meningiomas, counting 4.5% (2 cases) (fig 1).

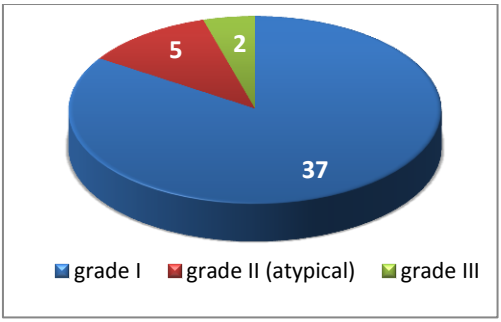

Fig. 1. CPA meningiomas distribution according to the WHO grades (1990-2015).

Case 1. Female patient aged 59 is hospitalized for headache and nausea, vertigo, gait instability, adiadochokinesia and hy-

poacusia with mild CN XII paresis for thirty months. Subsequent head-MRI demonstrated a 44 mm heterogeneously enhancing lesion rising from posterior petrous face, which occupies CPA (fig 2). The patient underwent left retro sigmoid craniotomy with gross total resection (GTR). Postoperatively, the evolution was favorable with left facial palsy and swallowing difficulty. On the sixth day of surgery, the patient began suffering from febrile syndrome and diarrhea (further confirmed *Clostridium difficile),* tachypnea, finally causing septic shock and death.

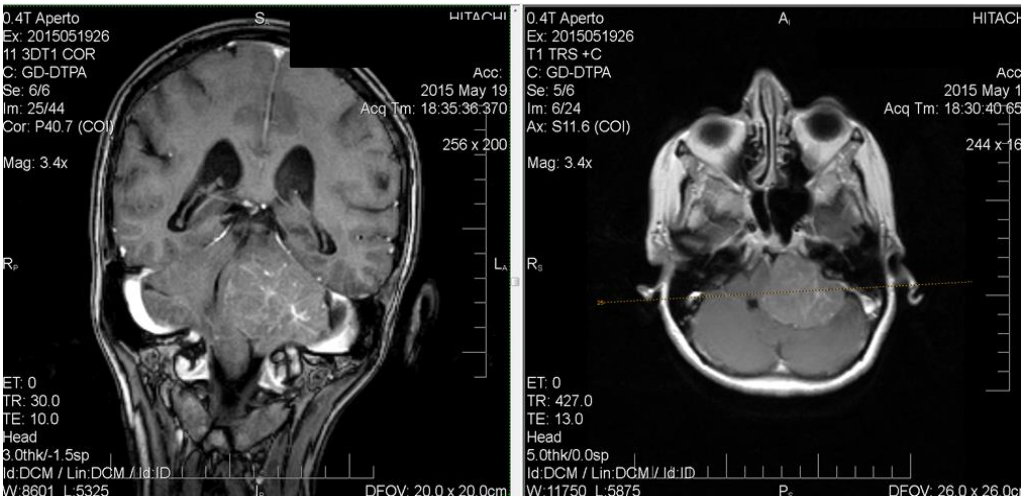

Fig. 2. Case 1. Preoperative coronal and axial T1-weighted images with contrast showing a large left CPA atypical meningioma.

Case 2. Female patient aged 48, comes to a consultation with a 2-year headache history, left cerebellar dysfunction, left hypoacusia, dysphonia, hypoesthesia in the left extremity and hemifaces, characterized by a two-week acute evolution. A head-CT revealed a 40 mm left CPA mass (fig 3. A). The performed surgery involved a left retro sigmoid craniotomy with subtotal gross resection (STR) (fig 3. B). Two days after the surgery, the patient developed hydrocephaly and underwent right frontal external ventricular drain, which was subsequently suppressed. Clinical evolution was favorable, with MRI follow-up demonstrating the increase in size of the lesion (fig 4. A). Eight years after the surgery, the patient comes again for gait instability and left-sided hemiparesis. Head-MRI scan highlighted tumor growth (fig 4. B. C), but the patient refuses surgical intervention.

Case 3. Female-patient aged 57 is hospitalized for a 12-month headache, hypoacusia and cerebellar syndrome. Head-MRI examination emphasized a left CPA meningioma, measuring 45 mm (fig 5). The patient underwent left retro-sigmoid craniotomy with STR, followed by a transient facial palsy.

Case 4. Female-patient aged 75 is admitted for intracranial hypertension syndrome, progressive spastic paraparesis, gait instability, left hypoacusia, with a nineteen-month insidious onset. Head-MRI scan revealed a meningioma of the free

margin of the tentorium developing infratentorial in the left CPA (fig 6. A). The patient underwent left sub temporal approach with GTR and partial resection of the tentorium infiltrated, followed by a favorable clinical evolution and remission of symptoms. MRI follow-up at six months (fig 6. A) and twelve months (fig 6. B) demonstrates the absence of recurrence.

Case 5. Female-patient aged 40 is hospitalized for right hypoacusia, disorder of equilibrium, vertigo, lasting for approximately 24 months, which add acute headache lasting for 7 months prior to the admission. MRI scan revealed a right sigmoid sinus and superior petrosal sinus meningioma with infratentorial extension in the right CPA (fig 7. A. B). The patient underwent left retro sigmoid craniotomy with GTR (fig 7. C). The evolution was favorable with left facial palsy which was subsequently remitted.

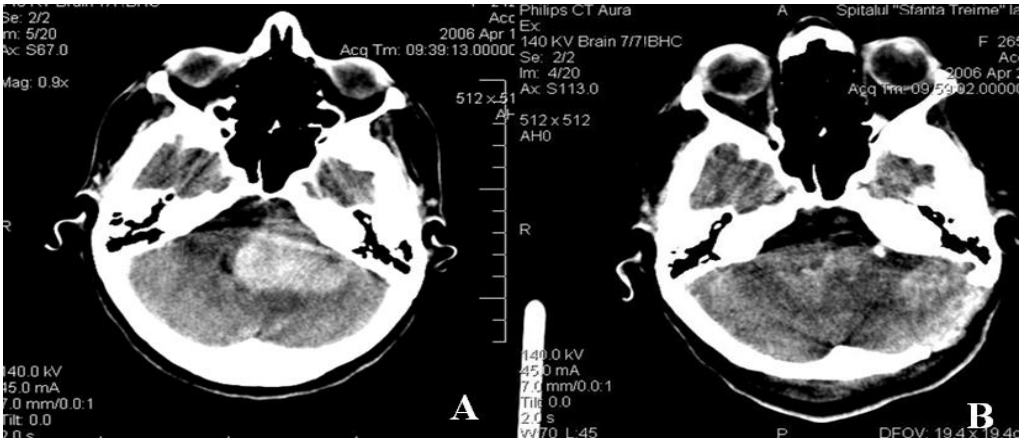

Fig. 3. Case 2. Preoperative (A) and postoperative (B) axial CT images with contrast.

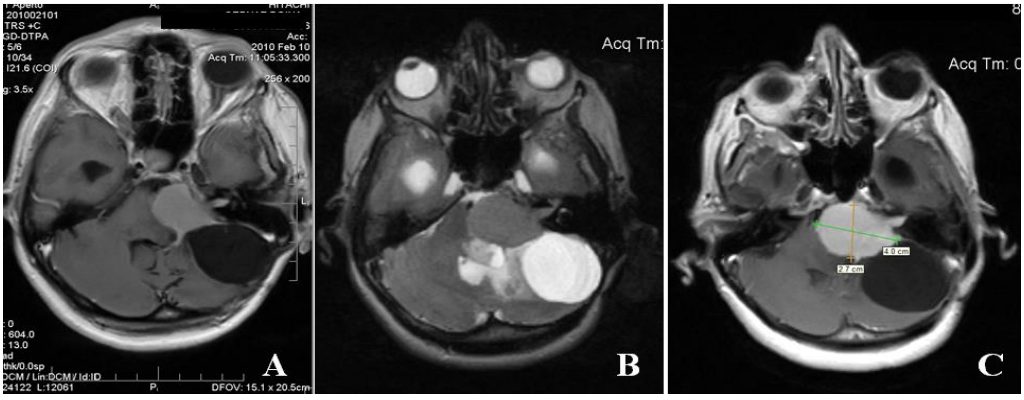

Fig. 4. Case 2. Left CPA atypical meningioma 4 years after surgery (A), 8 years after surgery (B) and 9 years after surgery (C). The increase in size by 3 mm of AM is observed 4 years after surgery (B) and stationary images between (B) and (C).

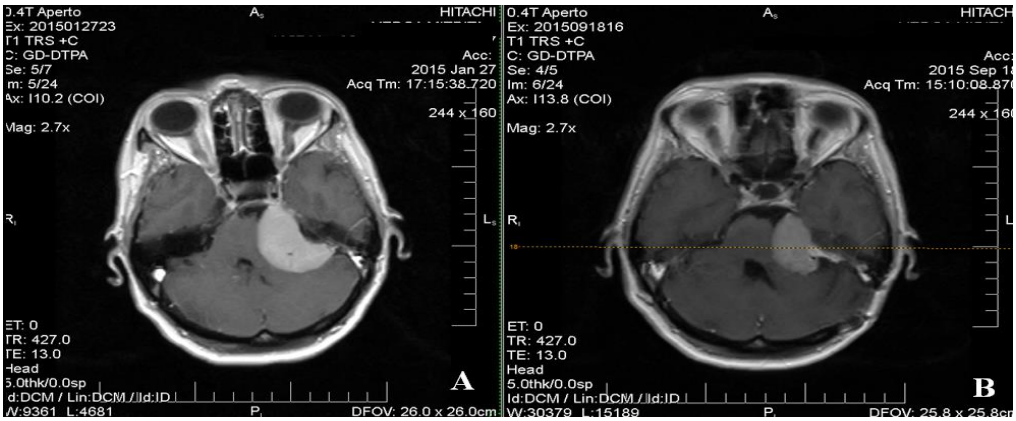

Fig. 5. Case 3. Left CPA atypical meningioma. Preoperative (A) and postoperative (B) axial T1-weighted images with contrast.

Fig. 6. Case 4. Atypical meningioma of the free margin of the tentorium with extension in the left CPA (A). MRI scan at six months (B) and twelve months (C) demonstrates the absence of tumoral recurrence.

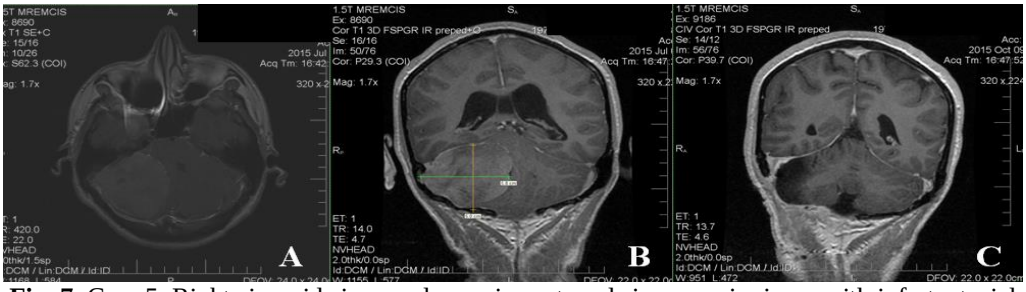

Fig. 7. Case 5. Right sigmoid sinus and superior petrosal sinus meningioma with infratentorial extension in the right CPA (A), (B). Postoperative MRI (C).

DISCUSSION

Out of the total of 44 CPA meningiomas operated from 1990 to 2015 in "Professor Dr. N. Oblu" Emergency Clinical Hospital Iasi, Romania, the AM represent only 11.36% (5 atypical CPA meningiomas) of them, compared to the 1-12.2% percentage reported in the preexistent studies (9, 15, 16, 17, 18, 19, 20, 21, 22) (tab. I). Although the number of CPA meningi-

omas, as they mentioned in literature, ranges from 28 to 193 of cases, and the follow-up time were like the ones in our series, studies could not identify more than 3 atypical CPA meningiomas for at least 20 years (9, 15). We noticed that compared to the literature, the number of atypical CPA meningiomas from our case series is higher, and this could be justified by the geographical proximity to Chernobyl and secondary irradiation to the disaster (23).

In 1917, Cushing described the *syndrome of the cerebellopontine angle* as being represented by symptom progression: 1) auditory and vestibular changes, 2) headache, 3) ataxia, 4) involvement of adjacent cranial nerves, 5) hydrocephalus, 6) dysarthria and dysphagia and 7) cerebellar and brain stem signs (24, 25). Later, Bartels and Arrington (1994) described extensively the typical course of CPA syndrome as: hearing loss, balance disturbance, altered facial sensation, facial pain, and later nystagmus, impaired coordination, facial palsy, swallowing difficulty, impaired vision and eventually long tract signs (26).

TABLE I
Number of atypical meningiomas of the CPA in the main studies (2005-2018).

Authors, Year	Follow-Up Period	CPA Meningiomas	AM	Percent (%)
2018, Magill (15)	1991-2015	51	1	2.0%
2017, He (16)	2012-2015	49	1	2.0%
2017, Lynch (9)	1986-2014	28	3	12.2%
2015, Gao (17)	2010-2013	193	2	1.0%
2013, Agarwal (18)	2005-2013	34	3	8.8%
2013, Nowak (19)	2002-2011	48	2	4.1%
2011, Baroncini (20)	1994-2007	69	3	4.0%
2009, Qu (21)	1996-2006	42	1	2.3%
2005, Roser (22)	1978-2002	74	2	2.8%

As for the early symptomatology of CPA meningiomas, this often consists of complicated symptoms and the first ones are: headache, vertigo or hearing loss and tinnitus, often neglected by patients (16). This makes the time between early symptoms and admission long, and patients to present with large intracranial meningiomas. Regarding the CPA meningiomas, in literature studies the average duration between the onset of symptoms and admission was different: 7.0 months (range 0.1-120 months) (18), 15.9 months (range 1-60 months) or 19.4 months (16), compared to our average period of 21.8 months which is higher (range 19-30 months). The average duration for our case series is longer probably because the other series (16, 18) included all WHO grades meningiomas (grade I, II and III), while our series contains only grade II (AM).

The three most commonly seen complaints in all the four cases from our study were: CN VIII nerve findings (hearing disturbance), headache and cerebellar syndrome in accordance with literature (16). Regarding cranial nerves injury in atypical CPA meningiomas, CN VIII was the most frequently affected by tumors in our series, followed by CN XII (case 1) and CN V, CN IX, CN X and CN XI (case 2), symptoms of lower cranial nerves present only in two cases of posterior face petrous meningiomas (case 1 and 2) (tab. II).

TABLE II
Symptomatology of CPA meningiomas

	Case 1	Case 2	Case 3	Case 4	Case 5
ONSET	30 months	24 months	12 months	19 months	24 months
CN V	-	hemifacial hypoesthesia	-	-	-
CN VIII (HYPOACUSIS)	+	+	+	+	+
CN IX, X, XI, XII	unilateral hypoglossal nerve palsy	dysphonia	-	-	-
INTRACRANIAL HYPERTENSION SYNDROME	+	+	+	+	+
CEREBELLAR SYNDROME	+	+	+	+	
PYRAMIDAL SYNDROME	hyperkinetic reflexes, Babinski -	-	-	progressive spastic paraparesis	-

Moreover, literature records, in the case of CPA meningiomas, the involvement of CN VIII in a high percentage 50-70% (5, 7, 25, 27, 28, 29, 30), aspect confirmed by our series of cases also, hearing disturbance being present to all the patients. In addition to this, the deficits of one or more cranial nerves (CN V to XII) were found in literature in a percentage that involves 30% of the patients in the case of CPA meningiomas (5, 7, 25, 28, 29, 30, 31). Despite this, it is not difficult to notice that regardless the large dimensions of CPA meningiomas indicated by radiographic studies, the involvement of cranial nerves except of CN VIII is very low, being present in only two cases (posterior petrous face meningiomas, case 1 and case 2).

Cerebellar signs (ataxia, adiadochokinesia) were reported in literature in a variable percentage of 30-90% in the case of CPA meningioma (7, 25, 27, 28, 30, 32), also present in all the five cases from our study.

MRI data offers important information to the neurosurgeon about the dislocation of the cranial nerves or other anatomical structures (33, 34). As in literature, meningiomas of the CPA from our series are broad-based/sessile with dural tail (35, 36), while dural insertion was discovered intraoperatively, since the evaluation of preoperatively meningioma dural insertion is difficult to obtain (37).

TABLE III
CPA meningiomas diameters in the main literature studies (2011-2018).

	Diameter (mm)	Average (mm)
2018, Magill (15)	8-62 mm	30 mm
2018 Spena (14)	22-51 mm	37 mm
2017, He (16)	40-55 mm	-
2017, Lynch (9)	20-105 mm	-
2013, Agarwal (18)	9-46 mm	23 mm
2013, Nowak (19)	25- >40 mm	-
2011, Baroncini (20)	28-33 mm	30 mm (+/-11mm)
2011, Peyre (41)	18-45 mm	25 mm

Due to the non-specific symptomology at the moment of the diagnosis, meningiomas have greater dimensions than 44 mm (case 1), 40 mm (case 2), 45 mm (case 3), 32 mm (case 4) and 58 mm (case 5), with the average of 43.8 mm, higher than the average in studies (tab. III).

CASE 1, CASE 2, CASE 3

Posterior petrous face meningiomas in the CPA represent a surgical challenge due to their proximity to the cranial nerves, brainstem and cerebellopontine angle vasculature (8, 12, 38). The posterior petrous face of the temporal bone is somewhat unique in relation with the venous sinuses, because the superior and inferior petrosal, cavernous and sigmoid form a ring which surrounds this posterior face (39). Usually, these tumors have large dimensions when identified, mostly over 30 mm (39), which makes it difficult to establish anatomical relationships. In the case of our series, the two out of three petrous face meningiomas were gigantic with many symptoms like lower cranial findings and signs of brainstem and cerebellar compression (tab 2). Also, studies from literature have shown that middle petrous face tumors (along with middle and posterior petrous locations, most frequently presents with hearing loss and vestibular symptoms (15, 40), same as in the case of our patients. In case of meningioma extension towards the ventral petrous face, because of the difficulties encountered in safely removing, the rate of GTR is lower for this tumor groups than in retro meatal tumors (41), as in two of our cases where STR was performed (case 2 and case 3). Case 2 has developed post-operative hydrocephalus which was subsequently remitted, aspect recorded in literature in the petrous face meningioma case, especially in those with posterior extension (15).

CASE 4

Meningiomas along the free tentorial margin with extension in CPA present a high surgical difficulty, because the tentorial notch is not attached of any bone structures and it encircles the peri mesencephalic cistern which contains important vascular and neural structures (42). The lateral incisura space contains anterior and posterior choroidal arteries and trochlear and trigeminal nerves (42). Meningiomas localized in the lateral incisura space are most frequently accessed by a sub temporal approach, since it offers the most direct and shortest route, even though complications in the temporal lobe retraction may arise (42). The preoperative venogram is important in preserving the vein of Labbé (43, 44, 45).

Infratentorial meningiomas have large dimensions at the moment of their diagnosis and this is because the infratentorial symptoms, like headache and vertigo, are non-specific (46). In the case of our patient, the 24-months onset symptoms consisted in headache and gait instability, late onset of hypoacusia, a month before hospital admission (tab 2). In these cases, sub temporal approach gives the possibility of total resection of tumor, both of supra and infratentorial components (fig 6. B).

CASE 5

Meningiomas with dural attachment over the transverse-sigmoid junction with CPA extension should be differentiated from the meningiomas of the cerebellar convexity, tentorium and petrous bone with marginal attachment to the venous sinuses, although a major part of the studies includes them all in these main groups (32, 39, 47, 48, 49). At this level, as well as

along the transverse sinus and periocular region, arachnoidal cap cells are more relatively abundant than in other parts of the posterior fossa (50, 51).

The localization of the meningiomas away from the cranial nerves, brainstem and fourth ventricle with cerebrospinal fluid pathway, determines the lack of neurological symptomology, or makes it poor and non-specific (52), characterized by headache, vertigo and hypoacusia, like in the case of our patient (case 5) (tab 2). In managing these types of meningiomas, the close relationship with transverse, sigmoid and petrous sinuses is the main issue, but, despite of all these, the superficial location away from the cranial nerves and brainstem involves a low surgical risk when venous sinuses are preserved (52).

Some of the authors consider that the invasion of the venous sinuses must be treated conservatively (53), while others suggest an aggressive approach, with sinus resection and its reconstruction (43, 54, 55, 56, 57). Nevertheless, aggressive management can have a potential serious risk of intraoperative venous infarction and the reconstruction of the superior petrosal sinus outflow into the transverse-sigmoid sinus junction is a difficult one (52).

The residual tumor nodules within the sinus lumen can be treated by radiosurgery, which is a useful adjuvant treatment in subtotal resections or tumor regrowth (58, 59, 60, 61).

In the past ten years, studies of skull base meningiomas and particularly a CPA meningioma focused mainly on functional outcome and life quality (62, 63, 64, 65). Therefore, in our series, facial palsy was the most common postoperative complication, and the functional reeducation may be helpful for the patient (66).

In this regard, recent studies have shown that a complete resection with dura and underlying bone, which corresponds to Simpson grades I and II, has not been positively correlated to a lower recurrence rate in relation to skull base meningiomas (67). Thus, in an era of multimodal treatment, GTR is not mandatory to obtain a good outcome and good tumor control (68). Moreover, residual tumor can be controlled by a conservative treatment, such as radiotherapy, even if atypical CPA meningiomas or higher grades of histopathology have an increased recurrence risk (16). In recent years, meningioma cell biology has progressed significantly and developed a various therapy for adjuvant interventions (69) and anti-proliferative activity agents against meningioma such as flavones and flavanols from wine (70, 71, 72).

Of the five AM of the CPA, only three were gross total resected and two subtotals resected, due to extension towards the ventral petrous face and intrinsic difficulties (tab 4). Retro sigmoid approach is perhaps one of the most commonly used surgical approach for resection of CPA meningiomas (28, 73); 4 of our patients were operated using this approach, while one of them underwent sub temporal approach (tab 4). When approaching a CPA meningioma, should be considered: its expansion to the internal auditory canal, tentorial attachment, invasion of the cavernous sinus and its expansion towards the jugular foramen (28, 73). The preservation of arachnoidal plane and incorporation of arterial branches of the CPA are also important in choosing the best surgical strategy.

As for the resectability rate, petrous meningiomas WHO I have a greater GTR rate than WHO II or gigantic meningiomas, due to the preserved arachnoid layer pro-

tecting the neurovascular structures of CPA and brainstem (74). In addition to this, progression-free survival in WHO grade II is lower compared to WHO grade I (74). Moreover, AM are known for their tendency to relapse after the surgery (75).

TABLE IV
Characteristics of surgery and postoperative complications

Patient	Age, Sex	Tumor Size	Tumor Location	Surgical Approach	Resection	New CN Deficit
1	59, F	44 mm	L	Retro sigmoid	GTR	facial palsy, swallowing difficulty
2	48, F	40 mm	L	Retro sigmoid	STR	-
3	57, F	45 mm	L	Retro sigmoid	STR	facial palsy
4	75, F	32 mm	L	Sub temporal	GTR	-
5	40, F	58 mm	R	Retro sigmoid	GTR	facial palsy

Literature studies reported that large meningiomas (>30 mm) of the CPA are associated with a rate of complications (76). In the case of our series, all the CPA meningiomas measure less than 30 mm in diameter, and the postoperative complications consisted in CN VII paralysis (case 1, case 3, case 4) and transient swallowing difficulty (case 1).

CONCLUSIONS

Cerebellopontine angle meningiomas have distinct clinical syndromes, associated with anatomical localization. Compared to the previous studies, the number of atypical CPA meningiomas from our series is higher, with average duration between the onset of symptoms and admission longer. As well as in literature, in our case series the retro sigmoid approach was preferred and the only subtotal tumoral resection of AM were those with extension towards the ventral petrous face and facial palsy was the most common postoperative complication. Surgical treatment of atypical CPA meningiomas has become increasingly safe, but these tumors remain challenging lesions, and the resection of these tumors is associated with increased rates of complications.

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STUDIU COMPLEX ASUPRA *PLACENTEI PRAEVIA*

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A COMPLEX STUDY ON *PLACENTA PRAEVIA* (Abstract): **Material and methods:** The study was carried out on 53 cases with *placenta praevia*, admitted to the "Cuza Vodă" Obstetrics and Gynecology Hospital, Iaşi, where the prevalence was 3.84% between 2006 and 2009. **Results**. The onset, increased with parity, was during the 8th month (30.19%), with hemorrhage (77.35%). An obstetrical pathological history was found in 33 cases (62.26%).
Key words: PLACENTA PRAEVIA, MATERNAL FETAL RISK, GESTATIONAL MANAGEMENT

Placenta praevia rămâne în atenţia specialiştilor prin complicaţiile patologice materne şi fetale şi prin decesele determinate de anormalitatea inserţională placentară (1, 2,3,4). Incidenţa cazurilor de *placenta praevia* este corelată, în special, cu vârsta (creşte de 3-4 ori la gravidele peste 35 de ani, comparativ cu cele între 15-24 de ani, indiferent de paritate) şi cu paritatea (creşte de 3 ori la marile multipare, comparativ cu primiparele). De asemenea, incidenţa este crescută în sarcinile gemelare, comparativ cu cele unice, din cauza întinderii mari a sediului implantaţiei (5,6).

Factorii etiopatogenici implicaţi în apariţia cazurilor de *placenta praevia* sunt evidenţiaţi prin două ipoteze, care nu se exclud reciproc şi care se referă la implantaţia joasă (*placenta praevia* primară), posibil a fi determinată de neconcordanţa între ritmul de diviziune şi progresiune tubo-uterină a blastocistului şi stadiul de pregătire pentru nidaţie a endometrului, prin insuficienţa secretorie a corpului progestativ ovarian, astfel încât blastocistul ajunge până în regiunea istmică şi se implantează la acest nivel. Implicarea miometrială este, de asemenea, în studiu, un aspect la care ne referim şi noi (7,8).

MATERIAL ŞI METODĂ

În studiu, am inclus 53 (3,84%) cazuri de *placenta praevia*, din totalul pacientelor asistate în Spitalul Clinic de Obstetrică şi Ginecologie "Cuza Vodă" Iaşi în perioada 2006-2009.

Culegerea datelor s-a realizat prin analiza foilor de observaţie, urmărind: incidenţa pe grupuri de vârstă, motivele internării, paritatea, antecedentele obstetricale patologice, istoricul sarcinii, simptomele de debut, rezultatele examenului obiectiv, programul terapeutic şi starea nou-născutului.

REZULTATE ŞI DISCUŢII

1. Relaţia dintre vârstă, paritate şi prezenţa placentei praevia. Deşi în toate grupu-

TABELUL I

Distribuţia pe grupuri de vârstă a cazurilor cu *placenta praevia*

Vârsta (ani)	Nr. cazuri	%*)
16-20	1	1,89
21-25	11	20,75
26-30	17	32,08
31-35	7	13,21
36-40	9	16,98
peste 41	8	15,09
Total	**53**	**100,00**

*) pentru valorile mai mici de 10, proporţiile sunt orientative

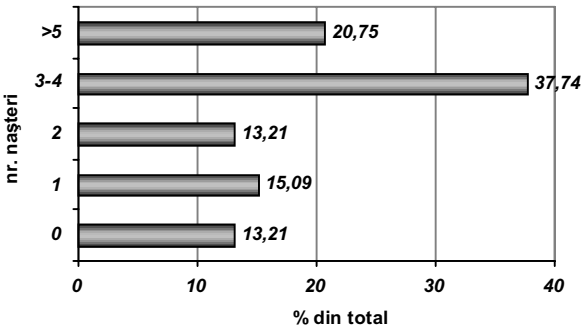

Fig. 1. Incidenţa *placentei praevia* pe grupuri de paritate

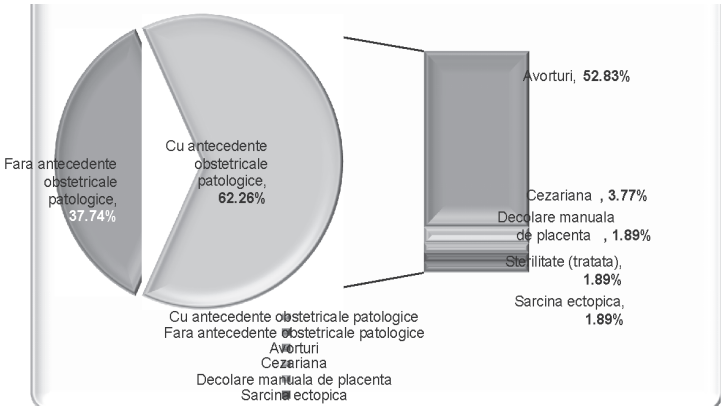

Fig. 2. Prevalenţa antecedentelor obstetricale patologice

rile de vârstă s-au înregistrat cazuri de *placenta praevia*, cele mai frecvente (52,83%) au fost semnalate în intervalul demografic 21-30 ani (tab. I).

În funcţie de paritate, cazurile au prezentat o distribuţie neomogenă, evidenţiind o incidenţă care creşte odată cu cea a parităţii : secundipare – 13,2% ; terţipare – 20,75% şi 37,7% - multipare, dar la primipare, incidenţa a fost mai scăzută (13,21%) (fig. 1).

2. Antecedentele obstetricale ale cazurilor incluse în studiu. S-au înregistrat 18 gravide cu 1-2 avorturi (33,96%) şi 10 peste 2 avorturi (18,86%) ; 2 cazuri cu cezariană, 1 caz cu două manevre de decolare manuală de placentă, 1 caz cu sarcină ectopică şi 1 cu tratament pentru sterilitate. Deci, din cele 53 gravide cu *placenta praevia*, 33 (62,26%) au prezentat un trecut obstetrical patologic (fig. 2).

3. Relaţia dintre momentul debutului afecţiunii şi evidenţierea manifestărilor clinice. În cercetările unor autori (2,3,5) s-a menţionat că manifestările clinice în cazul *placenta praevia* apar în luna a 8-a (40%) şi în ultima lună (30%), restul distribuindu-se uniform în lunile 5, 6 şi 7. În cazul nostru, debutul a fost cel mai frecvent în luna a 8-a, urmată îndeaproape de a 9-a, iar cu valori mult mai mici, au fost lunile 6 şi 7 şi cel mai puţin, luna a 5-a (fig. 3).

4. Unele caracteristici ale debutului. Noi am constatat că acesta este reprezentat de cele mai multe ori de hemoragie (77,35%) şi, în măsură mult mai mică, de ruptura prematură a membranelor (15,09%) şi imi-

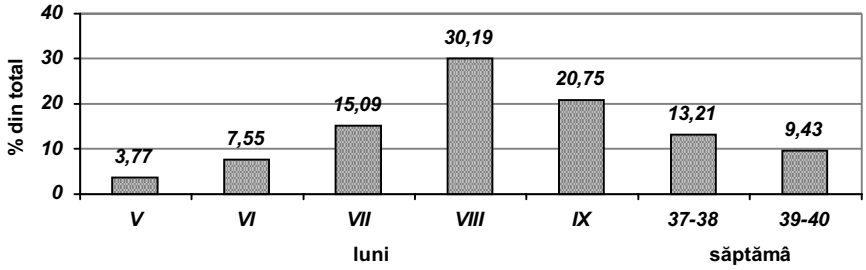

Fig. 3. Momentul debutului afecţiunii

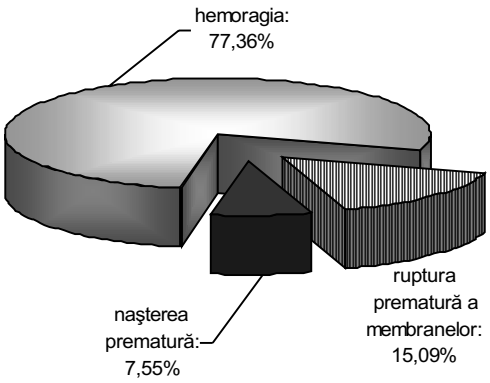

Fig. 4. Simptomatologia principală de debut

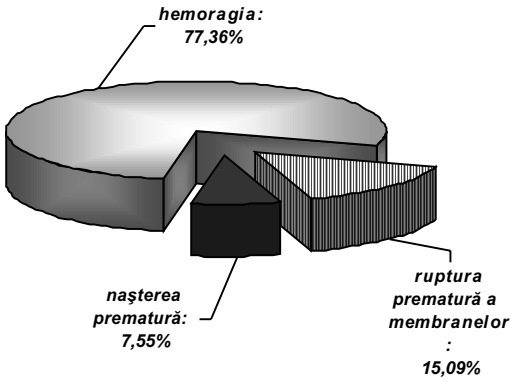

Fig. 5. Aspecte privind structura manevrelor terapeutice

nenţă de naştere prematură (7,54%).

Datele noastre concordă cu cele obţinute de alţi autori (1,2,8,9), care susţin că simptomul de debut este reprezentat, îndeosebi, de hemoragie (70%), urmată de ruptura prematură a membranelor (15%), iar în rest fiind vorba de asocieri ale celor două manifestări (fig. 4).

5. Studii histopatologice. Debutul, cel mai frecvent, a fost hemoragic, ceea ce presupune apariţia unor modificări miometriale, motiv pentru care s-au realizat investigaţii histopatologice, pentru a sublinia tipul reacţiei miometriale.

În cazurile cu absenţa parţială sau totală a *deciduei* bazale şi dezvoltarea imperfectă a stratului de fibrinoid, a determinat favorizarea contactului direct între vilozităţi şi miometru (*accreta*), urmat de invazia miometrului (*increta*) şi traversarea miometrului (*percreta*).

Studiul a evidenţiat că, la nivelul miometrului, au fost prezente toate cele trei tipuri caracteristice de joncţiuni ale fibrei musculare netede, respectiv caveolele, zonele asherens şi joncţiunile digitiforme, dar mult modificate ca repartiţie şi număr. În aceste condiţii, un număr redus de joncţiuni determină ca masa musculară uterină să nu mai poată atinge pragul de contracţie necesar atât opririi hemoragiei prezente în sarcina cu *placenta praevia*, cât şi desfăşurării unui travaliu normal. Concomitent, este necesar a fi luat în consideraţie şi rolul oxitocinei în susţinerea contracţiei musculare uterine (3,5,).

6. Aspecte ale eficienţei terapeutice. Studiul a arătat că tratamentul aplicat gravidelor a fost diferit în relaţie cu vârsta sarcinii, starea mamei şi a fătului, valoarea socială a viitorului născut. Astfel, 35 (66,37%) gravide au beneficiat de un tratament medical, pentru 8 (15,09%) cazuri s-a efectuat cezariană de urgenţă, iar 10 (18,86%) au

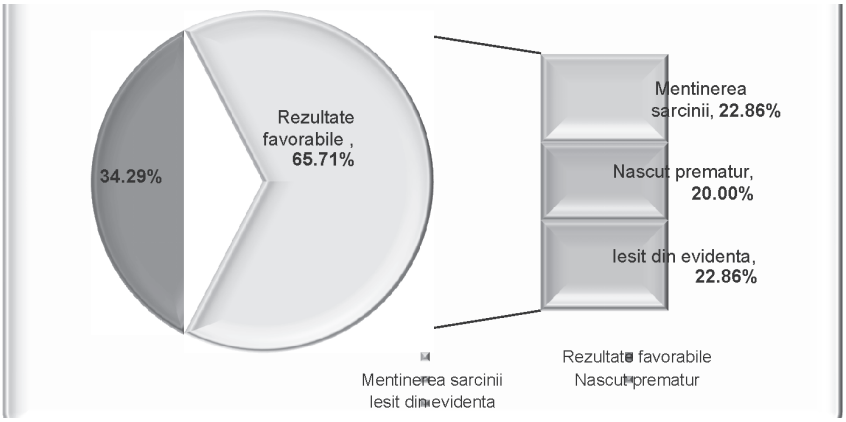

Fig. 6. Rezultatul tratamentului medical

TABELUL II

Parametrii biostatistici estimaţi privind asocierea evoluţia cazurilor cu *placenta praevia* supuse tratamentul medical

(df=1)	Chi-pătrat χ^2	p (*95% interval de încredere*)
M-L Chi-pătrat - χ^2	5,16	0,0231777
Coeficient de corelație (Spearman Rank R)	0,68	0,0359378

TABELUL III

Estimarea parametrilor de şansă şi risc ale evoluţiei cazurilor cu *placenta praevia* care au beneficiat de tratament medical

Parametri	Valoarea estimată	95% interval de încredere	
		min.	max.
Raportul şansei (OR)	2,24	1,05	4,83
Riscul relativ (RR)	2,17	1,09	4,30

născut pe cale naturală (fig. 5).

Dintre gravidele cu *placenta praevia*, tratamentul medical a reuşit în 8 cazuri, cu menţinerea sarcinii până la 39-40 săptămâni, necesitând internări repetate, iar 20% au născut prematur (9, 10) (fig. 6).

7. Analiza statistică a dovedit că tratamentul medical al pacientelor cu *placenta praevia* are şanse semnificative pentru o evoluţie favorabilă ($\chi^2=5,16$; $p=0,0231$; 95% CI) (tab. II).

S-a constatat că tratamentul medical în cazurile cu *placenta praevia* are şanse de 2,24 ori mai mari pentru o evoluţie favorabilă (OR=2,24), dovedind că raportul de risc reprezintă un indicator prospectiv privind eficienţa tratamentului medical, care induce o evoluţie favorabilă a sarcinii (tab. III).

CONCLUZII

Progresele obţinute în stabilirea diagnosticului de *placenta praevia*, cu explorare ecografică de rutină, cu o supraveghere susţinută a gravidelor cu sângerare îndeosebi în trimestrul al III-lea de sarcină şi datorită promovării naşterii prin cezariană, a determinat scăderea mortalităţii materne. Pentru sarcina cu *placenta praevia*, naşterea prematură continuă a reprezenta riscul major.

Investigaţiile histopatologice în *placenta praevia* s-au dovedit utile pentru cunoaşterea factorilor de risc, detectarea fenomenelor de acretizare, stabilirea concordanţei cu examenul imagistic, anticiparea evoluţiei şi promptitudinea aplicării conduitei terapeutice şi, în final, ameliorarea prognosticului.

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MULTI-STAGED ENDOVASCULAR SOLUTION FOR A COMPLEX, GIANT VERTEBRAL-BASILAR JUNCTION ANEURYSM

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MULTI-STAGED ENDOVASCULAR SOLUTION FOR A COMPLEX, GIANT VERTEBRAL-BASILAR JUNCTION ANEURYSM (Abstract): We present a very particular case of a complex vertebral-basilar junction aneurysm in a 34-year-old male patient. The patient benefited from a multi-stage endovascular treatment with a good neurological result at 12 months follow-up. Following an intricate history and a complex diagnosis process, we describe the therapeutic challenges and the steps taken in securing the aneurysm and dealing with the complicated hemodynamic architecture of the aneurysm and vertebral-basilar complex as well as the critical neurological evolution during the multi-staged process. Our case brings a new perspective to a rare and very difficult pathology and contributes to an increasing pool of data regarding the clinical management and the therapeutic options in this type of vascular lesions. **Keywords**: COMPLEX, VERTEBRAL-BASILAR, ANEURYSM, ENDOVASCULAR, TREATMENT

Vertebral-basilar junction giant aneurysms are rare and raise big challenges in terms of management and outcome. We present a case, which was particularly challenging in terms of management not described previously in the literature (1-4). We describe the approach to secure the aneurysm and the imagistic and clinical outcome.

CASE REPORT

The history of the 34-year-old male patient started 3 months prior to presentation in our service with persistent headache and vertigo. Two months later his condition worsened with right side progressive hemiparesis (a deficit score MRC 3/5) accompanied by mild dysphonia and dysphagia. Patient was admitted to a local medical emergency unit where the CT scan (fig. 1 A) showed a well delineated hyperdense mass in the posterior fossa, anterior to the medulla. He received symptomatic treatment and was referred to a specialized neurovascular department. Two weeks later it experienced the first syncope, which determined his presentation to a neurovascular unit where the MRI (fig. 1 B, C) confirmed a giant unrupted vertebro-basilar aneurysm.

The diagnostic angiography performed afterwards defined the aneurysm anatomy and its hemodynamics (fig. 1 D, E, F). Considering the fact that the aneurysm had quite large dimensions the choice of treatment method was not easy. Must be considered

the fact that the symptoms were determined by mass effect on the medulla or the cerebral micro emboli. We decided that, given the localization and the vascular anatomy of the aneurysm, the best way to approach is a multi-stage endovascular treatment.

The first step was a balloon occlusion test of the left vertebral artery (fig. 1 G) with the patient awake. The main reason for this test was the absence of a dominant posterior communicant artery. 10 minutes occlusion resulted in no changes in clinical status. As a result, we decided to permanently occlude the artery which we achieved by coiling PICA close to its origin.

The procedure was performed under general anesthesia with systemic heparinization for the whole duration. We performed the procedure with a 6F introducer and selective catheterization of the left vertebral artery through a 6F Mach 1 guiding catheter on a 0.035" hydrophilic guidewire. We then performed microcatheterization of the left vertebral artery in the neighborhood of PICA at the skull entry with Excelsior SL-10 microcatheter on a 0.014 micro guidewire. 5 GDC-10 coils were introduced and detached until complete vertebral artery occlusion was achieved (fig. 1 H, I). We verified angiographically the patency of the left PICA. We observed a significant difference in the pattern of aneurysm filling as it changed from unidirectional concurrent flow to a circular flow running on the entire surface of the aneurysm wall figure 1 J, compare to figure 1 E.

At 24 hours after the intervention, the patient showed a slight deterioration of the initial symptoms with onset of dyspnea, followed by desaturation and obnubilation, and he was intubated. The control CT showed a mild increase of the aneurysm volume. In this context, we decided to postpone the second procedure after clinical stabilization. One-month post-procedure the patient showed reliable signs of stabilization from a clinical point of view.

The second procedure consisted in a quasi-total spiral embolization of the aneurysm secured with stent, under general anesthesia and heparinization for the whole duration (5000 IU/hr). Double antiaggregating (Fraxiparine 0.6 mg/12 hrs and Aspenter 150 mg/day) was initiated one week prior to the procedure. The same approach was performed on a 6F introducer. Vertebral artery was selectively catheterized using a 6F catheter on a 0.0035 hydrophile guide. Passing a micro-guide from the vertebral artery to the basilar trunk was difficult due to the circular flow in the aneurysmal sac. We managed to pass a 0.010 Transcend micro-guide which was followed by a stent carrying catheter (4.5/50 mm Self-Expandable LEO+). Stent detachment was initiated in the middle part of the basilar trunk to the entry point of the vertebral artery into the aneurysm. With the stent partially detached, we catheterized the aneurysm with an Excelsior SL-10 microcatheter on a 0.014 guidewire. 8 GDC® 360° detachable coils (sizes from 15/30 mm to 6/11 mm) were placed and detached until partial occlusion of the aneurysmal sac. Finally, the spiral delivery catheter was pulled back and the detachment of the stent was finalized (fig. 1 L, M).

The CT, MRI, and Angio-MRI controls at 14 days showed partial thrombosis of the aneurysmal sac, vascular patency within the stent, and an overall reduction in aneurysm volume. At one-year follow-up MRI and angiography showed a stable evolution of the aneurysm and endovascular implants (fig. 1. N, O) but not a significant clinical improvement compared to the discharge status.

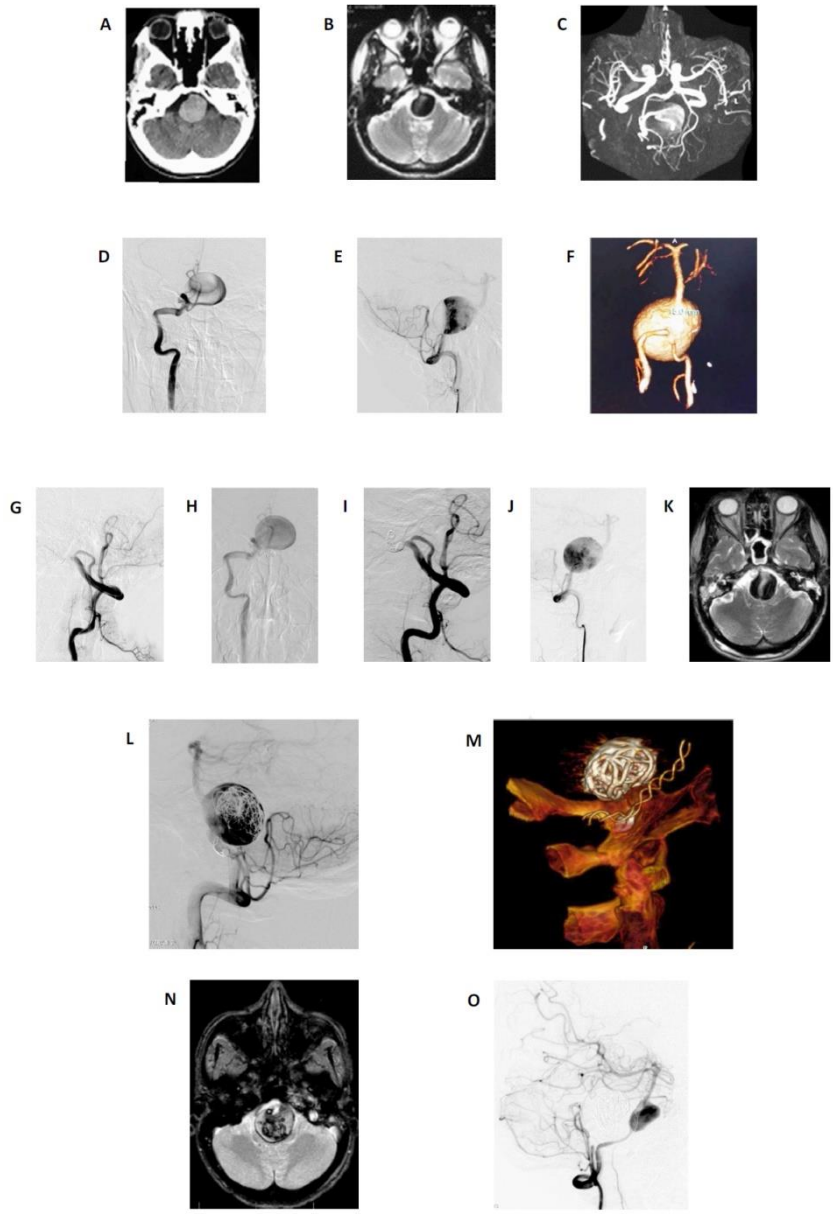

Fig. 1. A) Initial CT, B-C) Initial T2 weighted MRI with Angio-MRI, D-F) diagnostic angiography, showing the flux (D), aneurysm filling pattern (E), and 3D reconstruction with dimensions (F), G) Angiography during balloon occlusion, H) Flux in the aneurysm after left vertebral artery occlusion, I) Detail showing the patency of PICA, J) Aneurysm filling after left vertebral artery occlusion, K) MRI control after vertebral occlusion, L) Final angiographic image after aneurysm coiling and stent placement, M) 3D reconstruction of the endovascular construction, N) MRI at 5 months follow-up, O) Angiography at 5 months follow-up.

DISCUSSION

The giant vertebro-basilar trunk aneurysms are rare and very difficult cases due to their location, complex vascular anatomy, and very intricate hemodynamics. In the case presented, during the angiography, is very well illustrated the turbulent flow at the level of the aneurysm sac. The main vascular flow into the aneurysm is from the right vertebral artery and to a very small extent form the left vertebral artery. For this reason, the decision was reached that closing the left vertebral artery will reduce the flow into the aneurysm.

The wide-necked aneurysms are more effectively treated with stent-assisted coiling. Following the hemodynamic changes manifest after left vertebral artery occlusion, with important changes in the aneurysmal flow, the increase of aneurysm wall pressure and worsening of the clinical status because of brainstem compression, we reasoned that we have to use the technique that will increase the chances of a slow aneurysm thrombus formation.

The first requirement for the stent was to have enough radial force to allow a coil construction with a support from the aneurysmal wall of about 1/3 of the circumference. In addition, the presence of numerous perforator arteries originating from the portion of the aneurysmal wall that supported the stent required a stent of medium porosity (5).

The decision to use a LEO plus stent (and not a laser-cut stent or a flow-diverter) in the treatment of this vascular lesion was based on a number of factors. The LEO Plus stent (Balt) has a design that offers a higher radial force compared to flow diverters (6, 7). We believed that is was needed to insure a better support of the coil's construction. Another particularity is that the LEO (+)'s cells are closed but not predetermined in size and shape and allow for an easier access of a microcatheter for an eventual subsequent recoiling (8). It is also known that the maximal differential pressure inside the aneurysm remains high for high porosity stents (5), which implies that the flow reduction inside the aneurysm is not enough. A more suited pressure could be achieved using LEO+ stent that offers larger cells than flow diverters with less hemodynamic effect on the aneurysm and thus a slower thrombosis (8).

CONCLUSIONS

We present a very particular case that benefited from a multi-stage, complex endovascular treatment with a good result at 12 months' follow-up.

Our case brings a new perspective to a rare and very difficult pathology and contributes to an increasing pool of data regarding this type of aneurysms.

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The authors B. F. Iliescu and A. Chiriac contributed equally to this paper.

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NEWS

IT'S ALL IN THE EYES: ASSOCIATION BETWEEN RETINAL NERVE FIBER LAYER THICKNESS AND RISK OF COGNITIVE DECLINE

The global burden of people with neurodegenerative pathology is increasing and this is why there is a great desire to discover the perfect screening tool for cognitive decline. A group of researchers used the retina as "mirror image" of the central nervous system and conducted a study on 32,038 UK residents aged 40 to 69 who underwent cognitive testing and optical coherence tomography imaging. They discovered that participants with a thinner retinal nerve fiber layer (RNFL) were more likely to fail at least 1 or more of the 4 cognitive tests compared to those who had a RNFL of at least 60.2 μm. Furthermore, a repeated assessment of 1251 participants after 3 years showed that those with a thinner baseline RNFL did worse on at least 1 follow up cognitive test. This is a very exciting study because it opens new directions for research in the prevention and treatment of dementia and it is the largest one of this kind so far. (Ko F, Muthy ZA, Gallacher J, *et al.* Association of Retinal Nerve Fiber Layer Thinning with Current and Future Cognitive Decline: A Study Using Optical Coherence Tomography. *JAMA Neurol*. 2018; 75(10): 1198-1205).

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FUNGII CA AGENŢI ETIOLOGICI EMERGENŢI AI PNEUMONIEI ASOCIATĂ VENTILAŢIEI MECANICE POST-CHIRURGIE CARDIACĂ

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FUNGI AS ETIOLOGIC EMERGENT AGENTS OF THE VENTILATOR-ASSOCIATED PNEUMONIA AFTER CARDIAC SURGERY (Abstract): Evaluation of the fungal involvement in the etiology of ventilator-associated pneumonia (VAP) after cardiac surgery. The study had as target the patients diagnosed with VAP after cardiac surgery at the Institute of Cardiovascular Diseases in Iaşi, between April 2nd 2002 and December 31st 2009. **Material and method**: Our study included only cases with fungal etiology of VAP. We examined quantitatively the tracheal aspirates with cytology quality score Q = 2+: microscopy and cultivation. The isolates with clinical significance (= 10^6 UFC/mL significantly associated to inflammatory cells) were identified by standard methods or based on the API system (bioMérieux) and tested by the breakpoints method for susceptibility to antifungal agents. **Results**: Among the 2167 operated patients, 35 of them were diagnosed with VAP through the clinical pulmonary infection score = 6. Fungi caused 15 of 44 VAP episodes. With a frequency of 30.19%, fungi are on 2nd place within the etiologic agents of VAP after non-fermenting Gram-negative bacilli (45.28%). The fungi involved in VAP were: *Candida albicans* (16,97%), *Pneumocystis jirovecii* (3.77%), while *Candida glabrata*, *Candida sake*, *Candida krusei*, *Geotrichum capitatum* and *Cryptococcus humicola,* amounted 1.89% each. The widespread use of broad spectrum antibiotics led to the emergence of fungi as a common cause of ventilator-associated pneumonia. The involvement in the VAP etiology of *P. jirovecii*, an un-cultivable fungus with special sensitivity to antibiotics, requires a careful microscopy of specimens. **Key words**: ETIOLOGY, FUNGI, VENTILATOR-ASSOCIATED PNEUMONIA, CARDIAC SURGERY

Pneumonia asociată ventilaţiei mecanice (PAV) este cea mai frecventă infecţie nosocomială din unitatea de terapie intensivă (UTI) post chirurgie cardiacă, cu mortalitate de 16-76% (1,2,3).

Agenţii etiologici comuni ai PAV sunt: *Pseudomonas* spp., stafilococi, *Enterobacteriaceae, Acinetobacter* spp., alte specii de bacili gram-negativi multirezistenţi la antibiotice, streptococi şi *Haemophylus* spp. Fungii sunt potenţiali agenţi patogeni ai PAV, dar nu au fost consideraţi comuni în contextul pneumoniei apărute pe perioada ventilaţiei mecanice (VM) (1,4).

Scopul acestui studiu a fost evaluarea implicării fungice în etiologia PAV la pacienţii din UTI post chirurgie cardiacă la Institutul de Boli Cardiovasculare din Iaşi (IBCVI).

TABELUL I
Etiologia PAV în perioada 02.04.2002 - 31.12.2009

Microorganisme	Număr	%
Pseudomonas aeruginosa	10	18,87
Acinetobacter baumannii	9	16,97
Candida albicans	9	16,97
Stenotrophomonas maltophilia	5	9,44
Corynebacterium striatum/amycolatum	3	5,66
Escherichia coli	3	5,66
Klebsiella pneumoniae	3	5,66
Pneumocystis jirovecii	2	3,77
Staphylococcus aureus	2	3,77
Candida glabrata	1	1,89
Candida krusei	1	1,89
Candida sake	1	1,89
Corynebacterium jeikeium	1	1,89
Cryptococcus humicola	1	1,89
Enterobacter aerogenes	1	1,89
Geotrichum capitatum	1	1,89
Total	53	100

MATERIAL ŞI METODĂ

Am analizat retrospectiv pacienţii diagnosticaţi cu PAV în UTI aferentă secţiei de chirurgie cardiovasculară (CCV) la IBCVI în perioada 02.04.2002-31.12.2009. Eligibili au fost pacienţii operaţi pe cord sau vasele mari, cu vârsta ≥ 14 ani, cu VM ≥ 48 ore şi PAV cu etiologie fungică. Această unitate are 12 paturi cu un rulaj mediu anual de 53,95 pacienţi per pat. Studiul a fost aprobat de Comisia de Etică a IBCVI.

Exsudatele căilor respiratorii inferioare au fost prelevate cu ajutorul unei sonde de aspiraţie (lungimea = 50 cm, mărimea 14 Fr) introdusă prin tubul endotraheal, iar secreţiile au fost aspirate fără instilare de soluţie salină. Aspiratele traheale cu scorul citologic de calitate Q ≥ 2+ le-am examinat cantitativ prin citobacterioscopie (frotiuri colorate Gram, eventual Giemsa) şi izolare prin epuizarea diluţiilor 10^{-2} şi 10^{-3} pe medii agarizate (geloză-sânge, agar Mac Conkey, agar Sabouraud cu cloramfenicol) (5). Incubarea a fost aerobă cu citirea culturilor la 24, 48 ore, iar pentru fungi până la 7 zile. Am identificat izolatele prin metode standard şi sistemul automat API pe galerii ID32 C (bioMérieux) şi le-am testat sensibilitatea la antifungice prin metoda punctelor de ruptură pe galerii ATB Fungus 3 (mini API - bioMérieux).

DEFINIŢII

Pneumonia asociată VM a fost diagnosticată prin scorul clinic de infecţiozitate pulmonară (SCIP) ≥ 6. Calcularea acestui scor include 7 variabile: temperatura (≥ 38,5°C/< 36°C), numărul de leucocite (< 4000/> 11000 leucocite/mmc), oxigenarea pulmonară apreciată prin raportul dintre presiunea parţială a oxigenului arterial şi fracţia inspiratorie a oxigenului (PaO2/FiO2), aspectul şi progresia infiltratului pulmonar pe radiografia toracică (Rx), aspectul şi cultura aspiratului traheal (6). Prezenţa unui infiltrat pulmonar nou şi/sau progresiv pe Rx a fost obligatorie (7). PAV este clasificată *precoce* sau *tardivă* în raport cu debutul, = 4, respectiv > 4 zile de VM (1).

Traheobronşita asociată VM (TAV) a fost definită de absenţa leziunilor evolutive de pneumonie pe Rx în prezenţa aspiratelor traheale purulente, febrei şi leucocitozei (7).

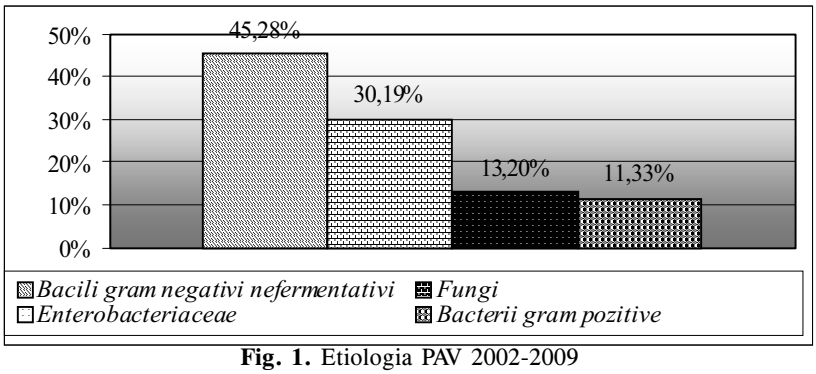

Fig. 1. Etiologia PAV 2002-2009

Cazurile de PAV sau TAV au fost confirmate microbiologic (5) când : fungii izolaţi în cantităţi ≥ 10^6 UFC/mL s-au regăsit semnificativ asociaţi celulelor inflamatorii pe frotiul Gram din aspiratul traheal ; acelaşi izolat din aspiratul traheal a fost regăsit şi în hemocultură ; chişti şi/sau forme trofice ale *Pneumocystis jirovecii* (fung necultivabil) au fost observaţi pe frotiurile colorate Giemsa ale aspiratelor traheale (5).

Colonizarea tractusului respirator inferior : prezenţa unui microorganism în cultura aspiratului traheal în absenţa semnelor clinice de infecţie a tractusului respirator inferior şi asociaţiei semnificative cu celulele inflamatorii (5).

Doze zilnice prescrise (DZP) reprezintă frecvenţa cumulată a dozelor zilnice de agenţi antimicrobieni prescrişi după natura acestora.

Un episod de PAV reprezintă debutul unei pneumonii urmat sau nu de vindecare clinică şi microbiologică la un pacient intubat ≥ 48 de ore.

Ventilaţia mecanică prelungită este VM cu o durată mai mare de 48 de ore la un pacient intubat (1).

REZULTATE

Pe parcursul celor 7 ani de studiu au fost operaţi 2167 pacienţi. Postoperator, 40 au fost suspectaţi de PAV : 35 confirmaţi cu PAV, iar 5 cu TAV. Cei 35 de pacienţi au avut 44 episoade de PAV în care au fost implicate 53 de microorganisme (tab. I). Toţi pacienţii au avut VM prelungită.

Cinsprezece pacienţi (42,86%) din 35 au avut 15 episoade de PAV cu fungi, care au fost : monomicrobiene 10 (66,67%), posibil mixte 5 (33,33%). Acestea au fost asocieri ale fungilor cu o bacterie (*C. albicans* cu *A. baumannii*, cu *E. aerogenes* sau cu *C. striatum/amycolatum* ; *C. glabrata* cu *E. coli*) sau cu alt fung (*P. jirovecii* cu *C. krusei*). Ca debut, cele 15 episoade de PAV au fost : 7 precoce (46,67%) şi 8 tardive (53,33%).

Din 15 pacienţi 10 (66,67%) au avut mai mult de 60 de ani. Dintre comorbidităţi, insuficienţa cardiacă (IC) definită prin criteriile *New York Heart Association* (NYHA) şi clasificată în 4 grade (I, II, III şi IV) a fost prezentă la toţi (33,33% – II ; 46,67% – III ; 20% – IV). Diabet zaharat (DZ) au avut 4 (26,67%), iar 3 (20%) au prezentat boli pulmonare cronice. Intervenţia s-a efectuat cu utilizarea circulaţiei extracorporeale (CEC) la 14 (93,34%) din cei 15 pacienţi. Apariţia PAV a fost precedată în 5 cazuri (33,33%) de : colonizare 1 (6,66%) şi traheobronşită 4 (26,67%). Hemoculturi pozitive cu acelaşi fung izolat din aspiratul traheal au avut 2 (13,34%) dintre pacienţi.

Cei 15 pacienţi au primit antibiotice (214 DZP) : preoperator, 3 pacienţi (20%) au primit terapie antimicrobiană : unul ceftriaxonă (11 DZP), altul ciprofloxacin (5 DZP),

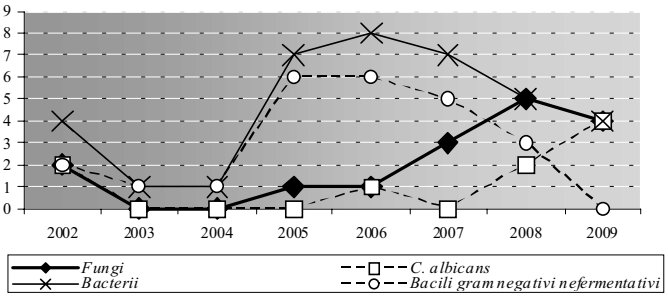

Fig. 2. Prevalenţa microorganismelor în etiologia PAV 2002 - 2009

iar ultimul ampicilină (9 DZP); intraoperator şi postoperator, toţi pacienţii au primit antibiotice până la episodul de PAV (189 DZP) şi toţi au primit antibiotice cu spectru larg.

Din totalul de 214 DZP administrate la pacienţi (preoperator, intraoperator şi postoperator), cele mai frecvente au fost antibiotice cu spectru larg, respectiv 148 DZP (69,16%): cefalosporine de generaţia a III-a 43,93%, fluoroquinolone 18,22% şi imipenem 7,01%.

Cu o frecvenţă de 30,19% fungii ocupă locul 2 între agenţii etiologici ai PAV după bacilii gram negativi nefermentativi (45,28%) (fig. 1). Şaisprezece fungi au fost implicaţi în PAV: 9 *C. albicans* (16,97%), 2 *P. jirovecii* (3,77%) şi câte o tulpină de *C. glabrata*, *C. sake*, *C. krusei*, *G. capitatum* şi *C. humicola* fiecare cu frecvenţa de 1,89% (tab. I).

Prevalenţa implicării fungice în etiologia PAV a crescut în perioada 2002 - 2009 de la 2 la 4 izolate, cu un maxim de 5 izolate în 2008. *C. albicans* a fost cel mai frecvent patogen al PAV în 2009. După ce bacilii gram negativi nefermentativi au fost cei mai frecvenţi agenţi etiologici ai PAV timp de 3 ani (2005 - 2007), cu un maxim de 6 izolate în anii 2005 şi 2006, în 2008 am izolat 3, iar în 2009 au fost absenţi (fig. 2).

DISCUŢII

Microorganismele implicate în etiologia PAV sunt variate. Fiecare UTI are un spectru etiologic propriu. Factorii de risc care favorizează apariţia PAV şi-i influenţează spectrul etiologic pot fi grupaţi în 4 categorii: 1) condiţii care predispun la o creştere a colonizării orofaringelui şi/sau stomacului cu microorganisme (agenţii antimicrobieni, internarea în UTI, prezenţa bolilor pulmonare cronice); 2) factori care favorizează aspiraţia în tractul respirator inferior a secreţiilor orofaringiene sau refluate din tractul digestiv (intubaţia endotraheală, intervenţii chirurgicale toracice şi la nivelul abdomenului superior); 3) situaţii care necesită utilizarea prelungită a VM; şi 4) factori proprii gazdei – vârsta înaintată (> 60 de ani), imunosupresia (8, 9).

Park (4) într-un studiu din 2005 privind etiologia PAV la pacienţi imunocompetenţi din diferite UTI (medicale, chirurgicale, medico-chirurgicale, secţii de traumatologie) au stabilit că patogenii comuni nu includ fungii, dar la pacienţii imunosupresaţi devin cauze importante.

Ashman et al, în 2004 (10) au studiat răspunsul imun în infecţiile fungice: principalul mecanism de apărare este mediat celular de limfocitele T şi o deficienţă a acestui sistem predispune la infecţii invazive cu fungi (*Candida albicans*). Pacienţi imunodeprimaţi sunt şi cei cu boli cronice (DZ, IC) (9). Chirurgia cardiacă cu CEC determină o scădere a imunităţii mediată de limfocitele T. Funcţiile limfocitelor T sunt supresate sever din ziua 1 post-operator cu revenire la valorile preoperatorii în zilele

3-5 după intervenţie (11). Aceste modificări sunt responsabile de scăderea competenţei răspunsului imun nespecific şi specific la pacienţii supuşi chirurgiei cardiace cu CEC (12). Astfel, am putea considera pacienţii post chirurgie cardiacă un grup de pacienţi aparte, cu o supresie temporară a imunităţii.

Studiul nostru a vizat o subpopulaţie de pacienţi, a celor supuşi intervenţiilor cardiace : la majoritatea s-a folosit CEC (93,34%) şi toţi au avut IC (100%). Aceste date ar explica apariţia precoce a PAV fungică (46,67%).

În studiul nostru fungii au ocupat locul 2 după bacilii gram negativi nefermentativi. *C. albicans* a fost cel mai frecvent fung şi a ocupat locul 2 după *P. aeruginosa* (18,87%), pe acelaşi loc cu *A. baumannii* (16,97%) în etiologia PAV. Fungii, în primul rând *C. albicans* au devenit agenţi etiologici comuni ai PAV la IBCVI.

Datele obţinute de noi cu privire la etiologia PAV se corelează cu cele obţinute de Wójkowska et al (13) în 2009 : cei mai comuni agenţi etiologici ai PAV după chirurgie coronariană sunt bacilii gram negativi şi fungii : *K. pneumoniae* 16,7%, *C. albicans* 14,6%, *E. coli* 12,5% şi *P.aeruginosa* 10,4%.

Presiunea selectivă a medicaţiei antimicrobiene are un rol major în selectarea tulpinilor şi speciilor cu rezistenţă intrinsecă (fungi) (1).

Adăugarea presiunii selective a terapiei antimicrobiene (aproximativ 70% cu spectru larg – cefalosporine de generaţia a III-a, fluoroquinolone, carbapeneme) la factorii ce determină imunosupresie în grupul nostru de pacienţi (IC – 100%, DZ – 26,67%) explică apariţia tardivă a PAV cu etiologie fungică (53,33%).

El-Ebiary et al. (14) într-un studiu cu privire la semnificaţia izolatelor de *Candida* spp. din exsudatele tractusului respirator inferior la pacienţi critici fără neutropenie constată că deşi izolarea acestora este frecventă, ele reprezintă mai degrabă colonizări decât pneumonii la pacienţii imunocompetenţi. Toţi pacienţii noştri au avut un anumit grad de imunosupresie prin bolile preexistente (IC – 100%, DZ – 26,67%) (9).

Datele din literatură (1, 4, 5, 15) recomandă o interpretare prudentă a semnificaţiei clinice a speciilor de *Candida* izolate din aspiratele traheale ale pacienţilor imunocompetenţi cu suport ventilator mecanic. Biopsia pulmonară cu evidenţierea fungilor şi/sau pseudohifelor este metoda care poate confirma implicarea *Candida* spp. în etiologia PAV. Hemoculturile pozitive, exsudatul pleural cu acelaşi izolat din aspiratul traheal confirmă etiologia PAV (5, 15). La pacienţii studiaţi de noi nu s-a efectuat biopsie pulmonară, nu am avut exsudate pleurale, iar hemoculturile au fost pozitive cu aceeaşi tulpină de *Candida* la 2 (13,34%) din cazuri.

Implicarea *P. jirovecii* (fung necultivabil) în etiologia PAV post chirurgie cardiacă la IBCVI impune o microscopie atentă (frotiuri colorate Gram, Giemsa) a prelevatelor la pacienţii cu scădere persistentă a saturaţiei arteriale a oxigenului, fără răspuns la antibiotice cu spectru larg şi cu imagini radiologice „aproximativ normale". Se recomandă terapie cu trimetoprim-sulfametoxazol, per os/intravenos, 15-20/75-100 mg/kgc/zi pentru 14 zile (16).

CONCLUZII

1. Emergenţa fungilor drept cauză comună a pneumoniei asociată ventilaţiei mecanice în unitatea de terapie intensivă post chirurgie cardiacă la IBCVI se datorează unui cumul de factori : pacienţii prezintă insuficienţă cardiacă, ceea ce le conferă un grad de *imunodepresie* ; pacienţii post chirurgie cardiacă la care s-a folosit CEC prezintă o *imunosupresie temporară* din ziua 1 până în zilele 3-5 postoperator ; şi utilizarea pe scară largă a antibioticelor cu spectru larg (cefalosporine de generaţia a III-a, carbapeneme, fluoroquinolone) care afectează bariera

ecologică a pacienţilor.
2. Semnificaţia clinică a izolatelor fungice (*Candida* spp.) trebuie interpretată cu prudenţă pentru fiecare caz.
3. Implicarea în etiologia PAV a *P.jirovecii*, fung necultivabil cu sensibilitate aparte la antibiotice, impune microscopia atentă a prelevatelor.

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IN VITRO EVALUATION OF NOVEL FUNCTIONALIZED NANOPARTICLES FOR TERAHERTZ IMAGING

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IN VITRO EVALUATION OF NOVEL FUNCTIONALIZED NANOPARTICLES FOR TERAHERTZ IMAGING (Abstract): **Background**: Early clinical diagnosis of gastric cancer is crucial for the treatment of patients, with increased healing rates being achieved by digestive endoscopy or surgery in patients with early gastric cancer. Magnetic nanoparticles (MNPs) are a major class of nanoscale materials currently under extensive development for improved diagnosis of a wide range of diseases, including cancer, cardiovascular disease and neurological disease. The nanoscale dimensions of MNPs give rise to unique magnetic properties and the ability to function on a cellular and molecular level, these characteristics making MNPs such promising contrast agents in different imaging applications. **Aim:** The research was focused on the development of some new nanoparticles suitable for use as contrast agents for terahertz imaging and assessment of the impact on gastric cell line AGS viability. **Material and methods:** Magnetite/Fe_3O_4 and Gd_2O_3 were functionalized by citric acid and carboxymethylcellulose and were tested on the standardized human gastric cell line AGS for the impact on cell viability by MTT assay. **Results**: Fe_3O_4 nanoparticles have induced a smaller amount of cytotoxicity at the first two dilutions, followed by an increase of the cytotoxic effect with the maxim effect at the dilution of 1:1. Also, the Gd_2O_3 stabilized in carboxymethylcellulose has presented a moderate cytotoxic impact in the range of 1:75 to 1:5 dilutions, followed by registration of a significant toxic effect at 1:1 dilution. **Conclusions**: The results obtained indicate that there is a high degree of tolerance to the tested nanoparticles, pointing out these nanoparticles suitable as contrast agents. **Keywords:** FUNCTIONALIZED NANOPARTICLES, TERAHERTZ IMAGING, AGS CELL LINE, IN VITRO CYTOTOXICITY.

Cancer is a common disease, with a high impact on mortality and the quality of life, and also with psychological and health budget impact. Due to its long asymptomatic character cancer is poorly recognized in the early stages of its development (1).

Among the most common causes of cancer death in the world it is also the gastric cancer (with approximately 738,000 deaths per year, accounting for about 9.7% of all

cancers) (2). Early clinical diagnosis of gastric cancer is crucial for the treatment of patients, with increased healing rates being achieved by digestive endoscopy or surgery in patients with early gastric cancer.

Iron oxides are natural compounds, often find in natural conditions, and easy to synthesized in laboratory conditions. Iron oxide nanoparticles consist of maghemite (γ-Fe_2O_3) and/or magnetite (Fe_3O_4) particles with diameters ranging from 1 and 100 nanometer and find applications in magnetic data storage, biosensing, drug-delivery etc.

Nanotechnology has given scientists new tools for the development of advanced materials for the detection and diagnosis of disease. Magnetic nanoparticles (MNPs) are a major class of nanoscale materials currently under extensive development for improved diagnosis of a wide range of diseases, including cancer, cardiovascular disease and neurological disease. The nanoscale dimensions of MNPs give rise to unique magnetic properties and the ability to function on a cellular and molecular level. It is the combination of these characteristics that make MNPs such promising contrast agents in different imaging applications (3). Among the various MNPs under investigation, superparamagnetic iron oxide nanoparticles (SPIONs) have attracted considerable interest due to their excellent magnetic properties, biocompatibility and biodegradability.

Iron oxide nanoparticles tend to aggregate due to their large surface to volume ratio exhibiting strong superparamagnetic properties. Physiochemical properties such as the particle size, distribution, and concentration are invaluable for adjusting the optical and magnetic properties. Combinatorial therapies assisted by iron oxide nanoparticles are indicated in cancer therapy. Fe_3O_4 nanoparticles can provide targeted drug delivery and enhance imaging and therapeutics through high localization at the target site under magnetic field. Synergistically these nanoparticles also cause tumor cell death via localized hyperthermia under alternating magnetic fields and/or photon application (with therapeutic agents) that generates heat (4).

Multiple techniques are applied for the synthesis of magnetic nanoparticles. Although coprecipitation of iron salts is the most common method, desired size range and nonuniform distribution of the particle are some of the drawbacks (5). Sonolysis, electrospray synthesis, flow injection synthesis, polyol method, sol-gel reaction, hydrothermal synthesis, and microemulsions are just a few of these techniques. Naked iron oxide nanoparticles are highly unstable. This material oxidizes in air due to strong chemical activity from the large surface to volume ratio that leads to loss of magnetism and dispersibility. To enhance the stability of the particles, the particles are often surface modified or coated to protect the nanoparticle as well as to introduce advanced functionality. Both organic and inorganic compounds can be conjugated for surface functionalization (5).

Magnetite powder was obtained in laboratory conditions by co-precipitation. Co-precipitation is the process of preparing wetted magnetite particles and consists in adding a basic solution to a solution containing iron ions (II) and iron ions (III) in stoichiometric proportions to achieve a certain pH value. Co-precipitation has the advantages over the conventional ceramic procedure by simple and rapid preparation of the compounds; reproducibility of the chemical composition; good control over the particles dimensions distribution by

parameters of co-precipitation process. Temperature is an important factor in co-precipitation process, determining the primary size of crystals, specific surface and even the phases that form.

The research focused on the development of some new nanoparticles suitable for use as contrast agents and assessment of the impact of the newly developed nanoparticles on gastric cell line AGS viability, in order to be used for terahertz imaging.

MATERIAL AND METHODS

A. Nanoparticles preparation

The experimental setup for magnetite synthesis. Magnetite obtainment: precise quantities of $FeCl_3x6H_2O$ and $FeCl_2x4H_2O$ were dissolved in degassed water (by nitrogen flux) and the resulting solution was transferred to a flask with 3 neck flasks in a preheated water bath at 75°C. Subsequent, to the cationic iron solutions was added a basic 25% solution of NH_4OH, with the role of co-precipitation agent. After a short time, a black precipitate corresponding to magnetite was observed. The mixture was maintained under stirring for 30 minutes, whole process being realized under controlled atmosphere by introduction of a constant nitrogen flow. The solid product (magnetite) was separated by magnetic decantation, washed with distilled water for the elimination of basic solution excess and dry in oven, at 50°C for 12 hours.

Functionalization of magnetite with citric acid. The dispersion and stabilization of magnetite (Fe_3O_4) in citric acid supposed the dispersion of iron oxide in distilled water and heating of the obtained magnetic fluid at 90°C with continuous mechanical stirring and ad of concentrated citric acid solution. The mixture thus prepared was kept continuous stirring for 1 hour, at 90°C.

Functionalization of magnetite with carboxymethylcellulose. The functionalization of the magnetite with carboxymethylcellulose was accomplished by using a magnetic fluid as that obtained previous for stabilization in citric acid. The magnetic fluid was sonicated for 15 minutes, after which a solution of carboxymethylcellulose was added. The resulting mixture was sonicated for 1 hour at 50°C.

Gadolinium oxide. Dispersion and stabilization of gadolinium oxide (Gd_2O_3) in citric acid was carried out as follows: the oxide powder was dispersed in water by sonication for 10 minutes, followed by add a solution of concentrated citric acid to the dispersion. The prepared mixture was sonicated for one hour at room temperature. The TEM micrograph demonstrates that dispersion and stabilization of Gd_2O_3 in tricarboxylic acid has led to a decrease in agglomeration degree.

Functionalization of gadolinium oxide with carboxymethylcellulose was performed with the same methodology as used for the dispersion of magnetite.

B. In vitro assay

Cell line. AGS (Human Gastric adenocarcinoma cell line, CLS 300408) cells were cultivated in a DMEM medium (Dulbeco's Modified Eagle's Medium, Biochrom AG, Germany), supplemented with 10% fetal bovine serum (FBS, Biochrom AG, Germany) and also with 100 µg/mL streptomycin (Biochrom AG, Germany), 100 IU/mL penicillin (Biochrom AG, Germany). The cell cultures were seeded in a 24 well plates (20,000 cells/well) and maintained in a CO_2 incubator, at 37°C.

C. Viability Assay. Cells were seeded in 24 well plates and were allowed to grow

overnight. After the medium was discarded and cells were washed, the renewal complete medium was supplemented with the tested nanoparticles (C = magnetite/Fe_3O_4 stabilized in diluted citric acid; D = magnetite/Fe_3O_4 stabilized in carboxymethylcellulose; E = Gd_2O_3 stabilized in carboxymethylcellulose) in consecutive dilutions of 0, 1:100, 1:75, 1:50, 1:25, 1:10, 1:5, 1:1. Duration of treatment was of 24 hours and the cell viability was assessed by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetra-zolium bromide) test. After treatment time has expired, the medium was discarded; cells were washed with PBS (phosphate-buffered saline) and covered with 100 µL fresh complete medium. 10 µL of MTT (5 mg/mL) were added in medium and cells were incubated for 3 hours. To solve the formed formazan DMSO (dimethyl sulfoxide, Merck) was used and the absorbances were recorded at 570 nm (1). Cellular viability (%) was calculated according to the formula: % cell viability = [Absorbance]sample / [Absorbance]control x100.

RESULTS AND DISCUSSION

Magnetite characterization

Magnetite was characterized by X-ray diffraction and transmission electron microscopy (TEM). X-ray powder diffractogram of the synthetized material is depicted in figure 1. The identification of the crystalline phases from diffractogram is based on the data of powder diffraction data from JCPDS (Joint Committee on Powder Diffraction Standards) database.

All the diffraction peaks from figure were identified and indexed according to JCPDS card no. 19-0629. Formation of magnetite with spinel structure is confirmed by the exclusive presence of maximum peaks for the plans (2 2 0), (3 1 1), (2 2 2), (4 0 0), (4 2 2), (5 1 1), (4 4 0), (6 2 0), (5 3 3). The presence of the narrowed peaks indicates a very high crystallinity grade for synthetized magnetite.

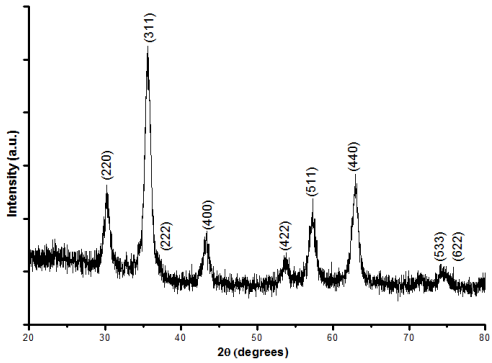


Fig. 1. X-ray diffractogram of magnetite / Fe_3O_4

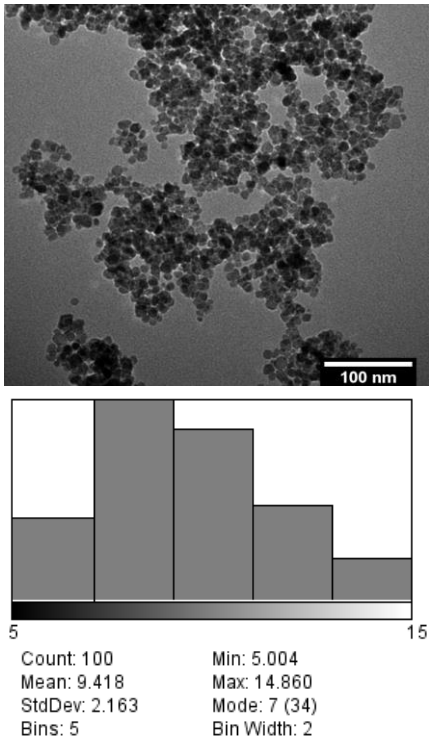


Fig. 2. TEM micrograph of magnetite/Fe_3O_4 and its size distribution

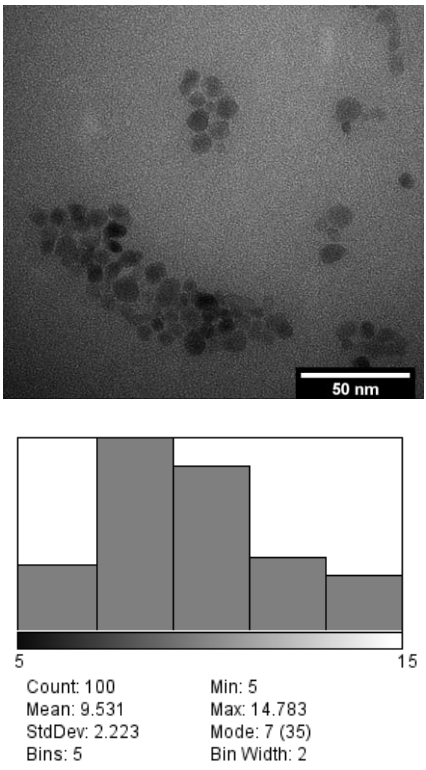


Fig. 3. TEM micrograph of magnetite/ Fe_3O_4 stabilized in citric acid and its size distribution

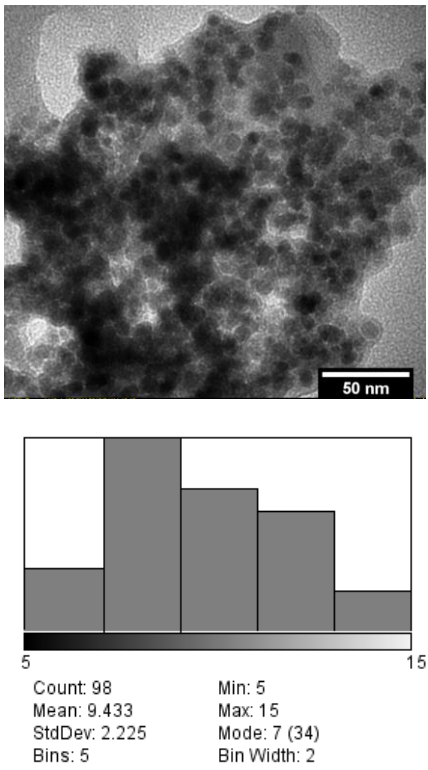


Fig. 4. TEM micrograph of magnetite/ Fe_3O_4 stabilized in carboxymethyl cellulose and its size distribution

Morphology of magnetite was studied by TEM microscopy (fig. 2), the mean size and size distribution of the particles were estimated with a dedicated software and taking in consideration minimum 100 repetitions. Analysis of TEM micrograph and particle size distribution showed that obtained magnetite nanoparticles have presented a regulated shape, almost spherical, with a degree of agglomeration relatively high. The mean size of the magnetite nanoparticles was of 9.418 nm, with sizes from 5 to 15 nm.

Characterization of magnetite functionalized with citric acid by TEM. The materials obtained were analyzed by electronic microscopy. TEM micrograph of stabilized magnetite in citric acid is shown in figure 3. There is a decrease in the agglomeration degree due to dispersion in the tricarboxylic acid. It is important to note that the stabilization process does not influence the mean particle size and the magnitude distribution of the magnetic nanoparticles.

Characterization of magnetite functionalized with carboxymethyl cellulose by TEM.

TEM analysis of the magnetite functionalized with carboxymethylcellulose (fig. 4) highlights the presence of the cellulose derivative in the vicinity of the inor-

ganic component of the system. Similar to the previous case, the functionalization with carboxymethylcellulose does not affect the magnitude particle size distribution or the average size.

Gadolinium oxide. The gadolinium oxide was characterized by electronic microscopy (fig. 5). It was observed that it is in the form of irregular and extremely agglomerated particles. After dispersion and stabilization, gadolinium oxide (Gd_2O_3), TEM analysis has demonstrated that dispersion and stabilization of Gd_2O_3 in tricarboxylic acid has led to a decrease in agglomeration degree.

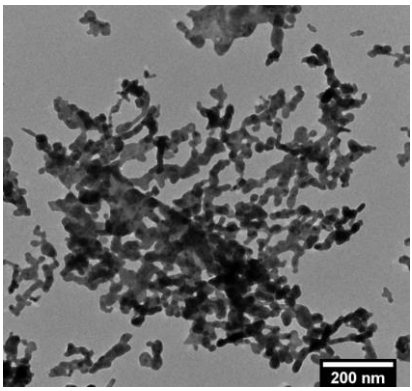


Fig. 5. TEM micrograph of Gd_2O_3

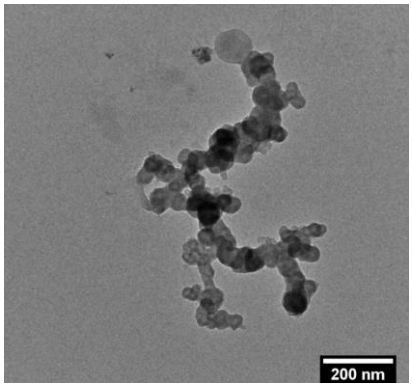
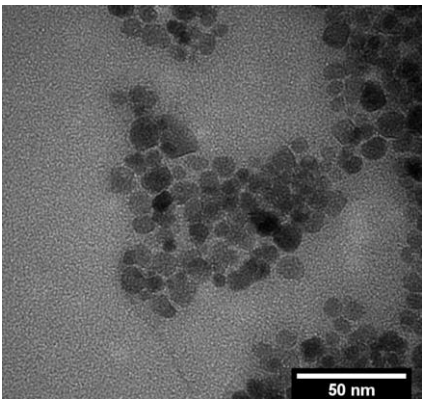


Fig. 6. TEM micrograph of Gd_2O_3 stabilized in citric acid



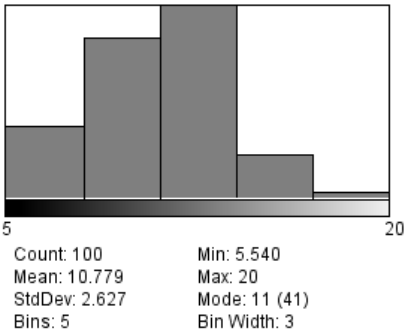


Fig. 7. TEM micrograph of Gd_2O_3 functionalized with carboxymethylcellulose

Characterization of gadolinium oxide functionalized with carboxymethylcellulose by TEM.

The functionalization of gadolinium oxide with carboxymethylcellulose was performed with the same methodology as used for the dispersion of magnetite. From the TEM analysis, we can see that the functionalization process allows deagglomeration of the inorganic particles in the presence of the considered polymer (fig. 6). Thus, the functionalized gadolinium oxide particles have dimensions ranging between 5 and 20 nm, with an average of 10.779 nm (fig. 7).

Cell viability was determined by MTT assay after 24 hours of incubation of AGS

cells in the presence of different dilutions of nanoparticles. The impact on cell survival was dependent by concentration of the nanoparticles added to the media.

TABLE I.
Cell viability of AGS cells as determined after 24 hours from add of nanoparticles C-E in dilutions of 0, 1:100, 1:75, 1:50, 1:25, 1:10, 1:5, 1:1.

		C		D		E	
Dilution	**p**	**Mean ± SEM**	**p**	**Mean ± SEM**	**p**	**Mean ± SEM**	**p**
0		100.00±0.05		100.00±0.04		100.00±0.02	
1:100	NS	90.74±0.04	NS	91.10±0.06	NS	92.47±0.01	NS
1:75	<0.05	89.97±0.04	NS	83.44±0.06	NS	74.70±0.00	<0.05
1:50	<0.01	86.81±0.04	NS	81.16±0.07	NS	71.49±0.01	<0.01
1:25	<0.001	75.61±0.01	<0.01	81.68±0.07	NS	78.51±0.00	<0.05
1:10	<0.001	72.85±0.03	<0.05	73.62±0.07	NS	74.70±0.01	<0.05
1:5	<0.001	83.92±0.08	NS	81.33±0.04	NS	89.46±0.03	NS
1:1	<0.001	57.99±0.01	<0.01	46.01±0.03	<0.01	49.70±0.01	<0.01

Fe_3O_4 nanoparticles (C, functionalized in citric acid, and respectively D, functionalized in carboxymethylcellulose) has induced a smaller amount of cytotoxicity at the first two dilutions, followed by an increase of the cytotoxic effect with the maxim effect at the dilution of 1:1 (42% and respectively 54%). In this case, an important role in the induction of the cytotoxic effect seems to be due nanoparticles themselves, as the presence of different functionalizing agents didn't cause high variations of cells survival. Also, the dose-effect relationship Gd_2O_3 stabilized in carboxy-methylcellulose (E) presented a moderate cytotoxic impact in the range of 1:75 to 1:5 dilutions, followed by registration of a significant toxic effect at 1:1 dilution (50%).

Analysis of the results, as the result of viability quantification at the highest dilution used, shows that C-E nanoparticles functionalized either with citric acid or with carboxymethylcellulose are well tolerated by AGS cells. Also, in the range of 1:100 to 1:5, the impact on cell viability is reduced or moderate, confirming that these types of nanoparticles aren't cytotoxic for the cancerous gastric cells.

There are two factors which could influence the impact of nanoparticles on cell viability: the concentrations of nanoparticles: smaller dilutions will contain higher numbers of the nanoparticles which will be deposit on higher areas of cell culture plate, impairing cell adhesion and subsequently cell survival; concentration of the functionalizing agents (citric acid and carboxymethylcellulose) which could change the normal cell culture environment.

CONCLUSIONS

Tested nanoparticles (C-E) functionalized either with citric acid or with carboxymethylcellulose are well tolerated by AGS cells.

The other studied nanoparticles are well tolerated, but with the reduction of the dilution, the cytotoxic impact on cell cultures is higher.

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NEWS

IMPROVED OVARIAN RESPONSE TO STIMULATION AND EMBRYOLOGICAL PARAMETERS IN YOUNG WOMEN WITH POOR OVARIAN RESERVE IN IVF-ICSI CYCLES AFTER TREATMENT WITH COQ10

One of the major challenges in reproductive medicine is to manage the women with reduced ovarian reserve or poor ovarian response (POR) to stimulation. The most important contributors of poor ovarian response are oxidative stress. In a study published in Reproductive Biology and Endocrinology, the authors evaluated the association on pretreatment with coenzyme Q10 and ovarian response and embryo quality in young low-prognosis patients with POR. The study group included 69 participants (76 treated with CoQ10 for 60 days preceding IVF-ICSI cycle and 93 controls). The results showed that women in CoQ10 group had increased number of retrieved oocytes, higher fertilization rate and more high-quality embryos. These results sustain the role of pretreatment with CoQ10 in improving ovarian response to stimulation and embryological parameters in young women with poor ovarian reserve in IVF-ICSI cycles.(Xu Y, Nisenblat V, Lu C, Li R, Qiao J, Zhen X, Wang S. Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: a randomized controlled trial. *Reprod Biol Endocrinol*. 2018 Mar 27;16(1):29. doi: 10.1186/s12958-018-0343-0).

GINGIVO-PERIODONTAL MANIFESTATIONS IN CHILDREN WITH LEUKEMIA: A LITERATURE REVIEW

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GINGIVO-PERIODONTAL MANIFESTATIONS IN CHILDREN WITH LEUKEMIA: A LITERATURE REVIEW (Abstract) **Objectives:** The purpose of this paper was to review the current literature concerning oral manifestations in children with leukemia. The main signs and symptoms recognized in these patients, emphasizing the gingivo-periodontal manifestations and symptoms, were investigated. **Background:** Leukemias represent about 30% of childhood cancers, acute lymphoblastic leukemia (ALL) being by far the most common form in children. The most frequent oral manifestations of leukemia are gingival bleeding, generalized hyperplasia, gingival erythema, petechiae. Often, these are the first hints for the disease, therefore, the dental practitioner is very much challenged in recognizing the main oral manifestations of this impairment, in order to make an early and efficient diagnosis. **Methods:** Medline database (via PubMed) was searched, using MeSH terms. Moreover, other physical sources, represented by articles and published papers relevant for the proposed theme were used. **Results:** Main oral signs and symptoms highlighted in children with leukemia were noted. The most frequent oral and gingivo-periodontal manifestations observed in this type of disease are gingival edema and erythema, spontaneous gingival bleeding, ulcerations, petechiae, opportunistic infections (Herpes, Candida, bacterial infections), dental mobility, generalized gingival hyperplasia. **Conclusions:** Dental practitioners should be aware of the main oral signs and gingiva-periodontal manifestations that can be present in children with leukemia. Very often, these are the first manifestation of the mentioned disorder, and the dentist is the first to remark them and refer a patient to a complex systemic evaluation and a proper diagnosis. **Keywords:** LEUKEMIA, CHILDREN, ORAL MANIFESTATIONS, PERIODONTAL MANIFESTATIONS.

Worldwide, in children and adolescents, cancer tends to exceed infectious diseases as a mortality cause. For children populations in Europe, North America and other developed regions of the world, incidence rate of cancer is about 140 in one million. In the United States, cancer is the second mortality cause in children, after accidents (1). According to the European Society for Pediatric Oncology (SIOPE), 15,000 new cases are diagnosed every year in Europe, and each year over 3,000 children and adolescents die of cancer, which still remains the leading annual cause of death by disease in Europe

(2). In Romania, an incidence of 7.35 in 100,000 children and a distribution of types of tumors similar with international statistics has been reported (3).

According to the literature data, almost 30% of children cancers are leukemias (4). Moreover, in Brazil, leukemia is the main cause of death by cancer in children (5).

The classification of leukemias is complex, and all the types can be found in detail in the World Health Organization's classification of hematopoietic and lymphoid tissues tumors, published in 2001 and revised in 2008 (6). Leukemias can be thus generally classified based on the primary cell line of origin in lymphoid or myeloid leukemia. Further, according to their evolution, leukemias can be divided into acute or chronic (7). hence, one can distinguish 4 main types of leukemia: acute myeloid leukemia (AML), chronic myeloid leukemia (CML), acute lymphoblastic leukemia (ALL), chronic lymphoid leukemia (CLL) (8).

Acute lymphoblastic leukemia is the most common form in children, consisting in about 75% of the newly diagnosed leukemias and 25% of all malignant diseases of the child (6). The etiology is still uncertain, but a few causal factors have been proposed: viral infections, exposure to ionizing radiation and chemicals (benzoic derivatives, heavy metals, pesticides) and also immunologic factors (9). Some studies indicate environmental factors like: smoking and alcohol consumption in parents, bacterial infections, chemical substances, exposure to electromagnetic radiation and electric fields, as well (10).

The purpose of this paper was to scrutinize the existing literature for the current data regarding the correlation between oral manifestations and leukemia in children, with strength upon the most common gingival and periodontal signs observed in children with leukemia.

MATERIAL AND METHODS

The articles used were accessed from Medline database (via PubMed), using the following MeSH terms: "oral manifestations" and "leukemia" and "children" and "gingival manifestations" and "periodontal manifestations". The literature search continued by hand, for printed articles and publications with the same criteria as with the electronic search.

RESULTS

Historically, the connection between specific oral signs and acute leukemia was first noted in 1964, by Roath (11) in a study which reported 20% of acute leukemia patients also presented with "buccal lesions" observed that 58% of acute leukemia patients studied presented with oral signs of the disease (12). In a study of Tagaki 50% of the 16 patients with acute leukemia studied were first seen by a dentist, for oral problems (13). Stafford reported that dentists were responsible for referring patients to hematological tests, which later led to diagnosing leukemia based on suspicions raised by oral manifestations in a quarter of the patients with acute myeloid leukemia and a third of patients with acute myelomonocytic leukemia (14). All these papers evaluated the correlation between leukemia and oral manifestations, disregarding age.

In present time, modern oncology requires the presence of the dentist in each treatment phase and even before the diagnosis, for the management, maintenance of oral health and life quality of patients with leukemia (15).

The oral cavity represents, to some extent, the interface between oral and systemic health, some oral diseases being able to affect general health, or reflect a manifestation of systemic homeostasis (16). For instance, multiple caries may lead to difficulties in the growth and development of the

child (directly by nutrition, or indirectly by chronic pain and its consequences). Vice versa, it is known that systemic diseases may alter oral health status by defectuous dento-facial development, altering the oral physiology and compromising patient's ability to maintain oral health (17).

Schlosser *et al.* (18) note that the mechanisms of oral pathology in hematological diseases like leukemia may be direct infiltration through abnormal hematological cells, formation and deposit of abnormal proteins, oral ulcerations and abnormal hematopoiesis.

According to Philipone (19), clinical manifestations of leukemia in general, result from loss of normal leukocyte function, suppression of hematopoietic cell lines, or by direct infiltration of leukemic cells in tissues. Thus, systemic signs and symptoms may include fatigue, anemia, lymphadenopathy, recurrent infections, bone and abdominal pain, bleeding and petechiae. Oral manifestations involve the mucosa and may be gingival bleeding, petechiae, ulcerations. Patients are also susceptible to severe viral, bacterial or fungal infections, as a consequence of immunosuppression. Gingival leukemic infiltrate leads to edematous, erythematous and friable gingiva.

McCord *et al.* (8) describe the following main oral signs and symptoms in children with leukemia: neutropenic ulcerations, spontaneous gingival bleeding, opportunistic infections (like: *Candida albicans*), and in chronic lymphoid leukemia – the possibility of developing paraneoplastic pemphigus.

Hou *et al.* (20) have shown that oral manifestations may appear in any type of leukemia but are more prevalent in acute leukemias (as in chronic leukemias) and myeloid leukemias (as in lymphoid leukemias). In his study, McKenna (21) suggests that deep, painful oral ulcerations are common in leukemic patients. Eisen *et al.* (22) stated that oral examination of patients with leukemia may reveal mucosal pallor due to anemia, or bleeding and petechiae in the palate, lingual or labial regions, as a result of thrombocytopenia.

In a recent study, Casamassimo describes several oral manifestations in lymphoid leukemia, as following: mucogingival pallor, purpura, spontaneous gingival bleeding, necrotic ulcerations, herpes infections, candidiasis, bacterial infections, dental mobility, aggressive periodontitis, periapical radiolucency, paresthesia, facial edema, lymphadenopathies in head-neck region. For the myeloid leukemia, the author reports diffuse gingival enlargements and masses of soft edematous tissue in the oropharyngeal region (16).

In children and adolescents, clinical aspects and manifestations of periodontal diseases are different than those seen in adults, one of the major differences being gingivitis (it is more frequently seen in children, whereas periodontitis develops more rarely). Such differences disappear in children with associated systemic diseases, particularly leukemia.

In 2012, Pels *et al.* have studied 156 children (78 with acute lymphoid leukemia and 78 with unaltered systemic homeostasis) from rural and urban regions in Poland (23). They have analyzed oral hygiene status and the results suggested a precarious oral hygiene in children with leukemia from rural regions, compared to leukemic children in urban regions. The oral hygiene was better in children with acute lymphoid leukemia compared to healthy subjects, because of their rigorous oral hygiene regime. However, the gingival indices evaluated (simplified oral hygiene index – OHI, plaque index – PI and gingival index – GI) were more elevated in leukemic children, indicating a higher risk of mucositis, despite a more important preoccupation for maintaining oral hygiene. In a previous study from 2007, the same

authors have reported similar results – they noticed a better oral hygiene and a lower incidence of plaque in children with acute lymphoid leukemia (24).

In 2011, Hedge *et al.* (25) have evaluated periodontal health status in 120 children with and without acute lymphoid leukemia. The authors have noticed the presence of gingival inflammation more often in children with ALL than in healthy subjects. Ponce-Torres *et al.* (26) have conducted a study in 2010 on 49 children with ALL, reporting a prevalence of 91,84% for gingivitis and 16,32% for periodontitis in these children. Literature data are somewhat inconsistent, especially considering the geographical distribution of populations, some reports from the beginning of this millennium indicating a much lower incidence, about 10-17% for these gingival manifestations in children with leukemia (27, 28).

Morais *et al.* (29) have reported, as the most common oral manifestations in children with leukemia, the following: gingival bleeding, hyperplasia, opportunistic infections and bone alterations. Previously, the most common oral manifestations observed were gingival edema caused by leukemic infiltrate, gingival hyperplasia usually being generalized and different in severity (30).

Baliga *et al.* have studied, in 1995, patients with acute lymphoid leukemia, and the most suggestive signs of leukemia they revealed were: lymphadenopathy in the head-neck region, along with gingival bleeding and mucosal pallor (31). In another study of 77 patients with ALL, poor oral hygiene, infections due to medullary and immune system suppression and altered healing responses were reported (28).

In a recent study on 3,789 children under 12 years of age, the authors reported that oral manifestations were a big contributor to the diagnosis of leukemia for 6% of the patients (32). The most common oral signs observed were gingival bleeding, petechiae, ecchymosis, mucosal alterations and fungal lesions. In another study, gingival bleeding was observed as a first symptom of leukemia in children for 17% of acute cases and 4% of chronic forms (33).

The importance of a complex systemic evaluation in the context of suggestive oral signs is also underlined by Safari *et al.* (34) in a case study in 2016. Along other unspecific general symptoms such as bone pain, a 9-year-old patient presented to the dentist for dental pain, dental mobility, erythema and gingival edema. He was later referred to the pediatric and hematology department, where the suspicion for lymphoid acute leukemia was confirmed.

In 2002, Katz and Peretz have reported a case of acute lymphoid leukemia diagnosis based on the presence of dental trismus (35). The trismus was probably caused by the infiltration of leukemic cells in the deep portions of facial muscles. With their study, the authors underlined the importance of clinical and laboratory exams recommended by the dentist.

In a case study in 2014, Kolli *et al.* (36) have identified distinctive signs of acute necrotizing ulcerative gingivitis (AUNG-GUNA) associated to acute lymphoid leukemia: patient presented the classic triad of pain-bleeding-ulceration, along with secondary signs like sub febrile state and decapitated gingival papilla, covered with pseudo-membranes.

In present, there are tendencies in the literature to systematize, in a standardized fashion, the most common oral manifestations in general, and gingival-periodontal manifestations in particular, ascertained in children with leukemia. The dentist should know and differentiate these signs and symptoms during the protocols for clinical examination. This would allow a higher success rate of an interdisciplinary early

diagnosis issued by the cooperation between the dentist and pediatrician/hematologist, for children with leukemia. To accomplish these needs of standardization and systematization, further studies are required.

CONCLUSIONS

Considering the growing prevalence of these diseases, children leukemia must be approached in a complex generalized systemic manner, in which the dental practitioner has a significant contribution. He often is the first one to observe signs and specific symptoms of the disease in the dental office. Among these oral signs, the gingiva-periodontal manifestations are the most common, hence every dental practitioner should be aware of, and able to relate their presence in the oral cavity with the possibility of leukemia.

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CLOSTRIDIUM DIFFICILE INFECTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS. THE EXPERIENCE OF AN INFECTIOUS DISEASES HOSPITAL FROM NORTH-EASTERN ROMANIA

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CLOSTRIDIUM DIFFICILE INFECTION IN PATIENTS WITH TYPE 2 DIABETES MELLITUS. THE EXPERIENCE OF AN INFECTIOUS DISEASES HOSPITAL FROM NORTH-EASTERN ROMANIA (Abstract): *Clostridium difficile* infection (CDI) in patients with other comorbidities such as diabetes mellitus (DM can set special diagnosis and management problems. **Material and methods:** Retrospective study comparing two groups of consecutive patients (group 1: 71 cases with DM, group 2: 336 cases without DM) diagnosed with CDI in the Iasi Infectious Diseases Hospital from January 1, 2016 to January 1, 2017. **Results:** Mean age, the proportion of patients over 60 years, as well as the proportion of cases with community-acquired infections were significantly higher in group 1. Clinical manifestations were similar in both groups, except for the greater amplitude of fever in group 2. Group 1 patients had a slightly higher frequency of the severe forms of CDI and higher serum creatinine levels compared with group 2 patients. The rate of recurrent CDI did not significantly differ between the two groups, but the age of relapsing diabetic patients was higher. Lethality was not influenced by the presence of diabetes. **Conclusions:** Patients with CDI and type 2 DM were older, had more community-acquired infections, some clinical features of the disease compared to nondiabetic patients. **Keywords:** DIARRHEA, FEVER, ANTIBIOTICS, RELAPSE.

Clostridium difficile infection (CDI) is an important issue for both healthcare providers - being recognized today as the most common cause of health care-associated diarrhea (1) - as well as for the patients in the community. *Clostridium difficile* (CD) is widely found in the environment. Human contamination probably occurs by ingestion and can lead to long-term colonization or symptomatic infection due to competition with normal gut flora (2).

Other than a greater amplitude of fever and other undesirable effects (related to resistance, toxicity, etc.), uncontrolled and inappropriate administration of broad-spectrum antibiotics, also causes each time a disruption of the equilibrium of nonpathogenic intestinal flora, which fosters the development of highly pathogenic CD strains (3).

Due to the secondary impairment of the immune system (4), patients with diabetes

are at higher risk for developing infection (urinary, respiratory, skin, bone, sepsis or peritonitis) (5) and thus receive antibiotics more frequently. Numerous studies (6, 7, 8) have shown that the risk of CDI is increased in diabetic patients, in which recurrent infections are more common. In these patients, the use of metronidazole was linked to an increased risk of CDI treatment failure (9). Some authors (10) suggest that metformin treatment seems to have a protective effect against CDI development.

In this study, we aimed to highlight differences between diabetic and nondiabetic patients with CDI in order to optimize the early management of this category of patients.

MATERIAL AND METHODS

This retrospective comparative study included consecutive patients diagnosed with CDI at the Iasi "Sf. Parascheva" Clinical Hospital for Infectious Diseases between January 1, 2016 and January 1, 2017. Diagnosis of CDI was based on the immunochromatographic detection of toxin A only and/or toxins A and B in stool of patients presenting more than 3 loose stools per day. Recurrence of infection was defined as reappearance of diarrheic syndrome with positive test for CD toxins within 3 months of the previous episode.

The patients were divided into 2 groups according to the presence/absence of diabetes mellitus (DM): group 1 - patients with CDI and type 2 DM and group 2 - patients with CDI without DM. The following data were collected: demographics, history of antibiotic use, as well as clinical data related to short- and medium-term recurrences (re-admission within up to 3 months after discharge), laboratory data and etiological treatment.

Statistical analysis. The *Chi-squared* and *t-Student* tests were used for statistical analysis and a p value < 0.05 was considered as statistically significant. The objectives and procedures of this study were approved by the Ethics Committee of the Iasi Hospital for Infectious Diseases. No written consent was given by the participants, as data were collected and analyzed anonymously.

RESULTS

During the analyzed interval a number of 407 patients with CDI were included in the study, of which 71 (17.4%) with DM.

TABLE I

Characteristics of patients with CDI

	Group 1 (+ DM) n=71		Group 2 (- DM) n=336		*p*
	N	%	N	%	
History of previous antibiotic use	62	87.3	286	85.1	0.76
Surgical interventions	14	19.7	60	17.8	0.87
Healthcare facility-onset (HO) CDI;	38	53.5	204	60.7	**0.008**
Community-onset, healthcare facility-associated (CO-HCFA) CDI	20	28.2	109	32.4	
Community-associated (CA) CDI	13	18.3	23	6.8	
Mean age (years)	63±4		58±3		**0.03**
Sex (M/F)	34/37		180/156		0.49
Rural/urban area of residence	31/40		164/172		0.51

Mean age of patients in group 1 was higher than in group 2 (p = 0.03), and more group 1 patients were over 60 years, compared to group 2 (64.8% *vs.* 51.8%, p = 0.04) (tab. I).

The average duration of diarrheal syndrome was of 7.6±1.1 days in group 1, compared to 8.38±1.3 days in group 2 (p = 0.67). Amplitude of fever was higher in group 2-30 (8.9%) patients presented with >39°C, as compared to 2 (2.3%) patients in group 1 (p=0.067) (tab. II).

TABLE II

Clinical manifestations

	With DM		Without DM		p
	N	%	N	%	
Fever	11	15.49	63	18.75	0.52
Abdominal pain	52	73.24	245	72.92	0.98
Vomiting	15	21.13	75	22.32	0.82
Watery stools	40	56.33	211	62.79	0.31

TABLE III

Toxins identified in patients' stool

Group	A		B		A and B	
	N	%	N	%	N	%
With DM (71 - 17.44%)	6	8.45	23	32.39	42	59.16
Without DM (336 - 82.56%)	40	11.9	135	40.18	161	47.93
p	0.403		0.221		0.047	

Diagnosis of CDI was confirmed following the identification of toxins in patients' stool by means of immunoenzymatically assay. In group 1, both toxins were more commonly present (59.16%) as compared to group 2 - 47.93% (p = 0.047) (tab. III).

TABLE IV

Analyzed laboratory variables

	Group 1		Group 2		p
	N	%	N	%	
Urea (BUN) >40 mg/dL	39	54.9	139	38.4	0.02
Creatinine > 1.5 mg/dL	24	33.8	78	23.21	0.04
Leucocytes > 15,000 / mm^3	16	22.5	52	15.5	0.20
ALT > 35 UI/L	26		15		0.49

Severe forms of CDI (according to SHEA/IDSA 2010 criteria) were identified in 26 (36.6%) patients in group 1 as compared to 98 (28.5%) patients in group 2 (p=0.1) (tab. IV).

The same therapeutic approach was

used in both study groups; anti-CD acting antibiotics available on the Romanian market were used, the most commonly used being metronidazole (60.5% of the cases *vs.* 63.1%) (tab. V).

Ninety-six (23.6%) patients presented recurrent CDI - 19.8% in group 1 *vs.* 22.9% in group 2 ($\chi^2 = 0.29$, $p = 0.58$).

TABLE V
Antibiotics used for the treatment of CDI

Antibiotics	Group 1		Group 2		*p*
	N	%	N	%	
Metronidazole	43	60.56	212	63.1	0.68862
Metronidazole + Vancomycin	12	16.9	54	16.07	0.86313
Vancomycin	12	16.9	58	17.26	0.94169
Other	4	5.64	12	3.57	0.41651

TABLE VI
Comparison between patient characteristics in recurrent CDI

	Group 1		Group 2		*p*
	N	%	N	%	
Fever	3	15.79	13	16.88	0.9
Vomiting	3	15.79	13	16.88	0.9
Abdominal pain	16	84.21	61	79.22	0.62
Latency period	5.9 ± 0.8 days		7.35±1.0 days		0.045
Sex ratio (F/M)	11/8		43/34		0.87
Mean age	70 ± 6 years		56 ± 5 years		0.006

Mean age of patients with diabetes who presented recurrence of CDI was significantly higher compared with that of patients without diabetes ($p = 0.006$) and the duration of symptoms was significantly shorter in diabetic patients (group 1, 5.9 *vs.* 7.3 days) (tab. VI).

In patients with recurrent CDI both toxins have been identified in a similar percentage of patients (50.9% *vs.* 49.8%) compared with the patients with initial CDI episode, but these were more commonly found among diabetic patients (73.7% *vs.* 59.2%) (tab. VII).

Lethality was not influenced by the presence/absence of DM (12.7% *vs.* 13.9%, $p = 0.15$) (tab. VIII).

TABLE VII
Toxins identified in patients with recurrent CDI

	A		B		A and B	
	N	%	N	%	N	%
+ DZ (19 - 19.79%)	1	5.26	4	21.05	14	73.69
- DZ (77 - 80.21%)	7	9.09	29	37.66	41	53.25
p	0.58874		0.17219		0.10676	

TABLE VIII

Patient lethality rate in the Infectious Disease Hospital during the studied period

	No patients	No deaths	Lethality rate (%)	p
All hospitalized patients	25598	471	1.8	-
CDI without diabetes	336	47	13.9	$p = 0.91$
CDI with diabetes	71	9	12.7	

DISCUSSION

In our study, the mean age of patients with CDI and diabetes was significantly higher compared to patients without type 2 DM (63 *vs.* 58 years, $p = 0.03$). Contrary to the results published by Lessa *et al.* (11), sex ratio was almost uniform in our patients and did not significantly differ between the two groups.

Exposure to antibiotics, age over 65 years, surgical interventions on the digestive tract, prolonged hospitalization, female sex, presence of diabetes mellitus, administration of proton pump inhibitors are risk factors for CDI quoted by various publications (12, 13). In our study, the average age of patients with CDI and diabetes was significantly higher compared to patients from group 2 (63 *vs.* 58 years, $p = 0.03$). Also, the proportion of patients aged over 60 years of age was higher among diabetic patients (64.8 *vs.* 51.8, $p = 0.04$). We can speculate that this category of patients, with a more frequent medical monitoring, is less prone the antibiotic misuse and overuse and acquire CDI later in life.

The vast majority of patients (87.3 *vs.* 85.1%) in both groups had received antibiotics before the diagnosis of CDI. According to recent studies (11, 12, 13, 14, 15) assessing the origin of CDI, up to 94% of cases are associated with health care (37% with Healthcare facility-onset). In our study, 59.5% of patients had Healthcare facility-onset CDI, 31.7% Community-onset, healthcare facility-associated CDI, and the remaining 8.8% had community-acquired infection. In the group of patients with diabetes, a significantly higher proportion of patients with Community-onset CDI (18.3% *vs.* 6.8%, $p = 0.008$) was identified. This fact could be explained by the more frequent use of antibiotics by persons with diabetes in an outpatient setting, although they may have more frequent contact with the medical system.

There were no significant differences in clinical manifestations between diabetic and nondiabetic CDI patients. Also, the percentage of patients with high fever was higher among the nondiabetic patients (8.9% *vs.* 2.3%, $p = 0.07$) probably due to the lower production of interleukins in response to infection; reduced chemotaxis and phagocytic activity in patients with diabetes (**Error! Reference source not found.**).

There are several criteria in use in assessing the severity of infection with CD (17) without any consensus with regard to the best. According to SHEA/IDSA 2010 criteria, in the group with diabetic patients a greater proportion of severe cases was identified (36.6 *vs.* 28.5%), but the difference is not statistically significant ($p = 0.10$). Among the severity factors most commonly identified in the diabetic patients, we should mention the proportion of patients with a creatinine over 1.5 mg% ($p = 0.02$), but this could this be the sign of a

pre-existing kidney disease.

The main pathogenic mechanism of CDI is the secretion of toxins during the vegetative phase of the germ, most notably toxins A (TcdA) and B (TcdB), that lead to local neutrophil infiltration and systemic inflammatory response (3, 18, 19). We noted a significantly more frequent detection of both toxins in patients with DM, compared with those without DM: 59.2% *vs.* 47.9%, $p = 0.04$.

Patients were treated according to the recommendations and guidelines with metronidazole or vancomycin, alone or in combination. A small percentage of patients received fidaxomicin (3 patients), rifaximin or symptomatic treatment alone. Some studies warn of an increased risk for metronidazole treatment failure in diabetics (9), but in the present study a relatively equal proportion of patients received this medication.

Regardless of the received therapy, patients in both groups had similar relapse rates- 19.8% *vs.* 22.9%, $p = 0.58$. Among patients with relapses, the mean age of diabetic CDI patients was significantly higher (70.1 *vs.* 56.4 years), and the length of the latency period of the development of CDI was shorter in the_nondiabetic CDI patients (5.9 *vs.* 7.3 days) probably due to the immune system particularities of diabetic patients (16).

Mortality rates for CDI were similar among diabetic and nondiabetic patients: 13.9% *vs.* 17.7% and 7.7 times higher than the overall mortality rates recorded in our hospital. For CDI, the figures are similar to those reported by Huart *et al*. and Iordan (20, 21).

The observational, retrospective nature of the study, not allowing any cause-effect conclusions, its one-center extension are the main limitations of the research. We also were unable to monitor all recurrences in patients admitted to our clinic with an initial CDI episode, some of them subsequently presenting to a family doctor who treated them in outpatient conditions or to a Diabetes and Metabolic Diseases Department (if the patient experienced an episode of decompensated diabetes), and thus contributing to a possible underestimation of CDI recurrence rate, especially in group 1 patients.

CONCLUSIONS

In our study, DM cases accounted for a significant proportion of the CDI patients treated in our hospital. Patients with CDI and type 2 DM were older, presented more community-acquired infections, and some clinical features related to lower frequency of fever and shorter latency period of the disease. No significant differences were found in terms of severity, relapse rate or failure of the various classes of antibiotics.

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HEPATITA CRONICĂ VIRALĂ B – O PROBLEMĂ ÎNCĂ ÎN ACTUALITATE LA COPIL

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CHRONIC HEPATITIS B – STILL A PROBLEM IN CHILDREN (Abstract): Vaccination of the newborns and adolescents had impact on the incidence of acute and chronic infection with hepatitis B virus (HBV) in children. After a decade of this national program application, authors proposed to study the epidemiologic dynamics of chronic infection with HBV in this interval, compared with the previous period, prior to vaccination. **Key words**: HEPATITIS B, VACCINATION, CHILDREN

Incidenţa infecţiei cronice cu VHB a rămas încă medie în ţara noastră, deşi din octombrie 1995 se practică vaccinarea nou-născuţilor în primele zile după naştere.

Prevalenţa AgHBs este, după datele epidemiologice recente, de aproximativ 6%. Supravegherea epidemiologică a infecţiei cronice cu VHB în România implică depistarea unor tendinţe epidemice, identificarea contacţilor care au nevoie de profilaxie post-expunere, definirea modalităţilor de transmisie, tratamentul formelor cronice active.

Riscurile de apariţie a copilului infectat sunt însă departe de a fi rezolvate, în condiţiile în care vaccinarea la naştere nu este completată cu măsuri specifice speciale, imunoprofilaxia pasiv-activă.

Progrese semnificative au marcat atitudinea practică a medicilor în abordarea infecţiei cronice cu virus hepatitic B la adulţi şi copii (1,2). Schemele terapeutice aplicate în ultima decadă pentru eficientizarea răspunsului terapeutic antiviral au avut însă rezultate care pot fi etichetate ca dezamăgitoare (maxim 30 % răspuns virusologic susţinut).

La aproape 35 de ani de când dr. Baruch S. Blumberg a fost recompensat cu Premiul Nobel pentru Fiziologie şi Medicină, datorită descoperirilor ***„concerning new mechanisms for the origin and dissemination of infectious diseases"*** şi pentru descrierea AgHBs, vaccinarea nu a rezolvat în totalitate problemele, deşi a avut efecte benefice considerabile în multe zone ale lumii. Primele descrieri ale mecanismului de replicare al VHB au fost publicate de William Robinson în 1970, când în virioni extracelulari s-a descoperit ADN-polimerază. În 1982, Jesse Summer şi Bill Mason au descris ADN-ul circular dublu catenar, revers-transcriptaza. Anul 1986 a însemnat anul introducerii primului vaccin obţinut prin inginerie genetică – vaccin recombinant VHB (3,4,5).

În epoca actuală de aplicare clinică a noilor analogi nucleozidici la adult, există studii limitate de utilizare a acestora şi la copii. Pe plan mondial cercetătorii se concentrează asupra unui aspect esenţial şi insuficient exploatat: utilizarea resurselor imune ale gazdei.

Focalizarea cercetărilor pe terapia imunologică implică şi cunoaşterea perfectă a

nuanţelor răspunsului imunologic al gazdei, pentru a identifica etapele ţintă pentru controlul total asupra infecţiei cu VHB (eradicarea infecţiei) (6,7).

Sistemul imun al copilului are multe particularităţi şi în aceste condiţii infecţia trece prin etape în care toleranţa imună caracteristică în primii ani după infecţie, poate trece în faza de interacţiune organism-gazdă, moment în care leziunile hepatice devin active. Imunosupresia, autoimunitatea, comorbidităţile infecţioase, toxice sau metabolice pot surprinde organismul vulnerabil al copilului (8,9).

Sunt elucidate astăzi modalităţile evolutive (particulare şi variate) în istoria naturală sau în cursul intervenţiilor terapeutice, în anumite etape ale vieţii, în relaţie strânsă în special cu vârsta la care s-a contractat infecţia cu VHB. Hepatologii au o viziune globală asupra diferenţelor evolutive ale infecţiei cronice cu VHB în relaţie cu vârsta la infestare.

În pediatrie, aspectele clinico-evolutive ale hepatitei B se prezintă astăzi sub noi paradigme, în relaţie cu aplicarea terapiei antivirale sau atitudinii profilactice globale (10). În România, eforturile multidisciplinare pot fi evocate ca succese, controverse dar si deficienţe.

Am efectuat un studiu retrospectiv si prospectiv, în Clinica a II-a Pediatrie UMF Iaşi, în perioada 1995-2009, prin care am studiat dinamica epidemiologiei hepatitei cronice virale B şi alte obiective.

OBIECTIVE

Obiectivele studiului au fost :

- prelucrarea parametrilor demografici reprezentativi pentru a caracteriza particularităţile de contactare a infecţiei virale hepatitice B, factorii de risc, populaţia susceptibilă, corelaţii între parametrii edificatori pentru aprecierea dinamicii fenomenului ;
- aprecierea modalităţilor de debut şi circumstanţelor clinico-biologice evolutive utile în elaborarea diagnosticului pozitiv
- perfectarea unui arbore decizional logic în formularea diagnosticului diferenţial şi precizarea comorbidităţilor ;
- aprecieri asupra terapiei, evoluţiei, toleranţei şi rezultatelor terapeutice la intervale variabile ;
- elaborarea unor concluzii generale practice.

MATERIAL ŞI METODĂ

Lotul de studiu a cuprins 159 de copii internaţi în Clinica II Pediatrie a Spitalului Clinic de copii „Sf.Maria" Iaşi în perioada 2004-2009, diagnosticaţi cu hepatită cronică cu VHB.

Criterii de selecţie şi includere în lot :

- Criterii generale : argumente clinice si biologice de diagnostic pozitiv pentru hepatita cronică cu VHB , Ag HBs pozitiv peste şase luni interval.
- Criterii speciale : AgHBe-/AgHBe+, seroconversia HBe sau apariţia tulpinilor mutante, viremia ADN-VHB , puncţia biopsie hepatică (PBH) sau Fibroscan.
- Observarea minim 6 luni şi supravegherea prin internări periodice în clinică.
- Complianţa familiei.

Urmărirea bolnavilor s-a realizat prin studiul retrospectiv al foilor de observaţie clinică şi întocmirea unei fişe cu principalii parametri urmăriţi în dinamica specifică fiecărui diagnostic şi tratament individualizat.

Criterii de diagnostic

Diagnosticul pozitiv a fost stabilit mai puţin pe criterii anamnestico-biologice şi în special pe criterii biologice şi serologice.

Elemente de reper din anamneză :

- motivele internării (astenia, durerile abdominale, anorexia, hepatomegalia, icterul sclero-tegumentar, infecţiile respiratorii recidivante, investigarea sindromului de citoliză descoperit în ambulator, copii proveniţi din mame infectate VHB) ;
- vârsta, sexul, mediul de proveniență al bolnavilor (instituţionalizarea, polispitalizarea) ;

• antecedente personale patologice reprezentate de transfuzii de sânge în antecedente (în special înainte de 1990), tratamente injectabile repetate, intervenţii chirurgicale, tratamente stomatologice, statusul nutriţional, co-infecţii virale, deficite imune, boli metabolice ;

• antecedente heredo-familiale motivate de posibilitatea transmiterii infecţiei cu VHB de la mama infectată sau infecţia intrafamilială (orizontală) (11,12) ;

• hepatita virală acută în antecedente.

Criteriul clinic – s-a apreciat starea generală, febra, astenia, hepatosplenomegalia şi eventualele semne extrahepatice.

Criteriile biologice au fost reprezentate de studiul sindroamelor fiziopatologice hepatice care au permis stabilirea diagnosticului pozitiv şi urmărirea în dinamică a evoluţiei, complicaţiilor şi ameliorării sub terapie.

Probele biologice s-au efectuat la diagnostic, după instituirea tratamentului şi apoi în dinamică, săptămânal în primele 4 săptămâni, apoi lunar. S-au determinat parametrii hematologici (hemoleucotrombograma), enzimele hepatocitare (ALT, AST, LDH) de apreciere a citolizei, gammaglutamiltranspeptidaza, fierul seric, enzimele pancreatice, parametrii sindromului hepatopriv (serinele, colinesterazele, timpul Quick, fibrinogenul), răspunsul imunologic (gamaglobulinele, markerii de autoimunitate) si valoarea bilirubinei, colesterolului, lipidelor şi lipidograma.

Criteriile serologice s-au apreciat prin teste calitative enzimatice ELISA sau RIBA (determinarea AgHBs, Ac anti HBs, AgHBe, Ac anti HBe, Ac anti HBc de tip IgM, şi IgG, Ac anti VHD) (3).

Prin protocoalele individuale s-au urmărit în etape markerii serologici la iniţierea tratamentului, la 3 luni de la începerea tratamentului, la finalul tratamentului şi la 6 luni de la terminarea acestuia.

REZULTATE ŞI DISCUŢII

În perioada 1995-2003 au fost internaţi cu hepatită virală cu VHB şi co-infecţii, 443 copii. În perioada 2004-2009 au fost internate 158 cazuri, ceea ce arată în mod cert beneficiile vaccinării.

În perioada 1979-1994 au fost diagnosticaţi 108 pacienţi, iar în perioada 1995--2003 au fost 356 de cazuri noi. Din prezentul lot de studiu, 69 de copii au fost diagnosticaţi înainte de anul 2004, în peri-

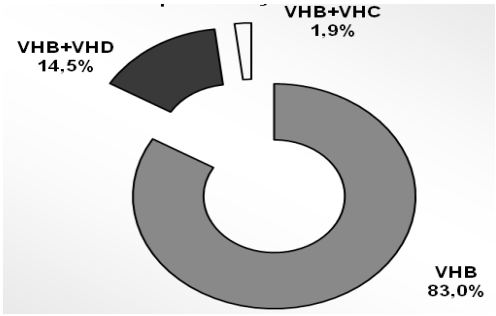

Fig. 1. Etiologia infecţiilor cronice hepatitice

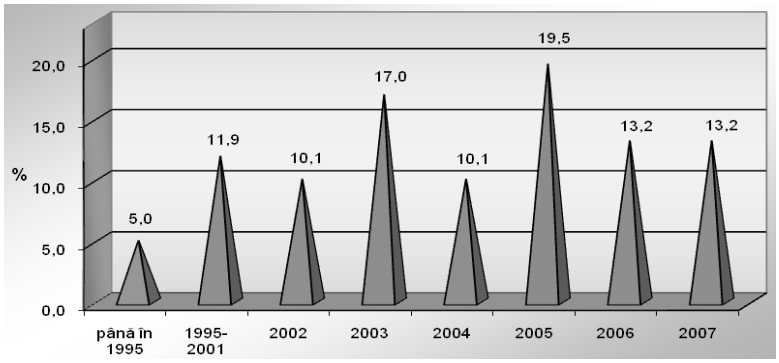

Fig. 2. Repartiţia pacienţilor în funcţie anul primei infecţii

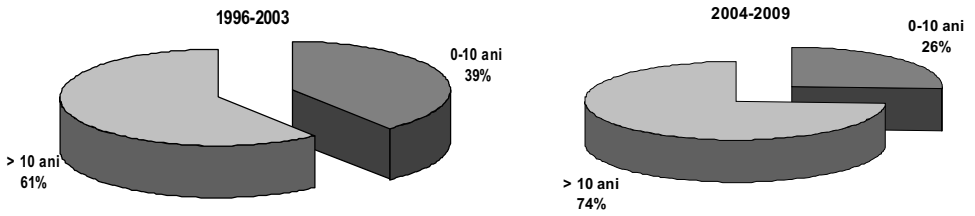

Fig. 3. Infecţia virală B - pe grupe de vârstă

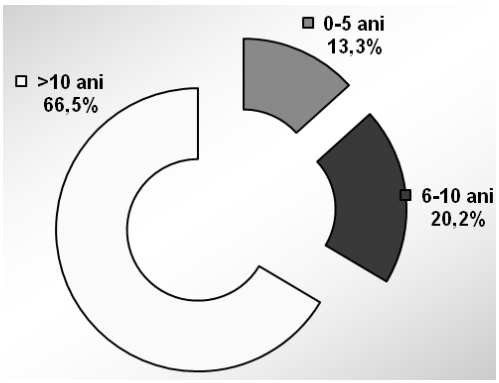

Fig. 4. Repartiţia cazurilor în funcţie de vârsta la diagnosticare

oada 2004-2009 fiind diagnosticaţi 89 de copii. După anul 2005 se remarcă tendinţa de scădere a fenomenului (hepatita cronică B).

Dacă înainte de 2004 predominau pacienţii diagnosticaţi la vârste sub 10 ani, în perioada 2004-2007 cei mai mulţi pacienţi aparţin grupei de vârsta de peste 10 ani.

În legătură cu dinamica grupelor de vârstă se poate remarca o menţinere a infecţiei la vârstă mică prin riscul infecţiei transmise de la mamele viremice.

În funcţie de vârsta la diagnostic se mai poate remarca vulnerabilitatea grupei 0-5 ani, perioada în care, se ştie, există riscul cel mai mare (până la 90%) de cronicizare a contactării infecţiei.

În intervalul de timp 1996-2009, după începerea vaccinării tuturor nou-născuţilor, riscul pentru infecţie la vârstă mică se menţine aparent surprinzător. La cazurile studiate vaccinarea a fost corectă logistic.

Calea de infectare a fost în special de la mamele cu infecţie cronică B ; multe dintre ele nu se ştiau purtătoare de virus.

Din totalul copiilor infectaţi în aceste perioade cazurile prin transmitere verticală reprezintă aproximativ 20%.

În funcţie de dinamica natalităţii se poate estima riscul acestei transmisii, tendinţă care va putea fi stopată doar printr-o profilaxie completă, care implică vaccinarea corectă, administrarea de imunoglobulină specifică şi asta din prima zi de viaţă.

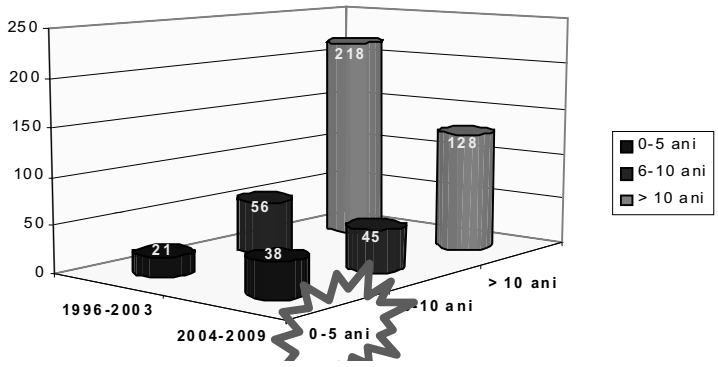

Fig. 5. Studiu cazuistic.

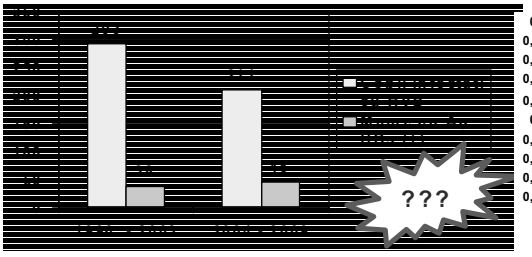

Fig. 6. Cazuri de infecţie cu HVB prin transmitere verticală

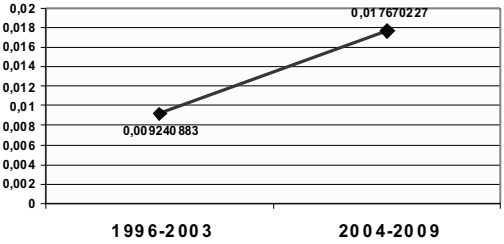

Fig. 7. Creşterea ratei transmiterii verticale a hepatitei cronice cu VHB

CONCLUZII

1. Vaccinarea împotriva hepatitei B la nou născuţi a determinat o scădere semnificativă statistic a numărului de cazuri la vârsta 0-5 ani de la 10,72% la 5,15 % şi la vârsta 6-10 ani de la 34,98% la 21,97%.
2. În ciuda vaccinării, mai există cazuri de infecţie cronică la vârstă mică datorită deficienţelor epidemiologice reprezentate de lipsa programului de depistare a markerilor de infecţie cu VHB la femeile însărcinate şi datorită limitelor programelor de imunoprofilaxie activ-pasivă. După naştere se impune asocierea imunoglobuline specifice la vaccinare la nou-născuţii cu risc, metodă considerată standard pe plan mondial şi care ar putea controla mai eficient infecţia.
3. Interzicerea alăptării a fost mulţi ani o strategie importantă de stopare a transmisiei infecţiei.
4. Academia Americană de Pediatrie recomandă alăptarea după profilaxia pasiv-activă!
5. Seroprotecţia post vaccinală este mai bună la cei alimentaţi natural (Ac anti HBs la un an 80,9% vs. 73,2% vaccin şi 90,9% vs 90,3% vaccin plus IGVB)
6. Nu vor alăpta mamele care primesc terapie antivirală complexă.

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SPONTANEOUS SHRINKAGE OF VESTIBULAR SCHWANNOMA. CASE REPORT AND REVIEW OF THE LITERATURE

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SPONTANEOUS SHRINKAGE OF VESTIBULAR SCHWANNOMA. CASE REPORT AND REVIEW OF THE LITERATURE (Abstract): Vestibular schwannoma (VS), commonly known as acoustic neuroma, is a slow-growing, benign tumor that originates from the transition zone between the central and peripheral myelin of the vestibular branches of cranial nerve VIII. After making the diagnosis, a conservative approach is an option depending on the tumor size and involves the "watch, wait and rescan" (WWR) policy. We present a rare case of a 59 years old woman, who presented with bilateral hearing loss. Routine imaging showed both otosclerosis and a vestibular schwannoma. She was followed-up with annual MRI between 2006 and 2019 showing a complete shrinkage of the vestibular tumor. Studies describe the natural history of a vestibular schwannoma to be slow growing or in small cases spontaneous shrinkage. However, there are still no pathological theories for the tumor regression process. An internationally method of strategies based on size, radiological aspect and symptoms is needed for a standardized care by analyzing a larger series of patients.
Keywords: ACOUSTIC NEUROMA, BENIGN, SPONTANEOUS SHRINKAGE, VESTIBULAR SCHWANNOMA.

Vestibular schwannoma (VS), also known as acoustic neuroma, a tumor with the origin from the transition zone between the central and peripheral myelin of the vestibular branches of the VIIIth cranial nerve, is a slow-growing, benign tumor. It is considered to be the most frequent tumor of the cerebellopontine angle (CPA) (1).

The natural history of these tumors consists in most of the cases of continuous slow growth, followed by stagnation or even shrinkage. Upon diagnosis, the most common attitude involves its treatment through either microsurgery or radiosurgery. However, in some cases, a conservative approach is an option depending on the tumor size and involves the "watch, wait and rescan" (WWR) policy (2).

A review of the literature shows that more than a half of the tumors that are diagnosed do not grow (69%), 16% regress and from those who grow, 70% have a 2 mm/year growth (3). The mean growth rate of VS varies from 0.4 to 2.9 mm/year, and spontaneous shrinkage is observed in 3.8 percent of tumors during observation.

We present a rare case, which presented two independent causes for hearing loss, otosclerosis and vestibular schwannoma on the same side and which showed for the first time in our experience spontaneous regression on the long-term follow-up.

CASE REPORT

A 59 years old woman, after a visit at the ENT doctor because of progressive hearing loss, was recommended a head CT scan that showed that she suffered from otosclerosis. The CT imaging also revealed in the cerebellopontine angle a mass occupying lesion that was further investigated through MRI imaging.

The patient came to us with the head MRI and we concluded that she had a vestibular schwannoma in the left cerebellopontine angle.

Clinical assessment showed a good neurological condition, the cause of the bilateral hearing loss being caused by the otosclerosis.

After discussing the various types of management of this tumor, the patient decided for the "watch, wait and rescan" protocol (WWR). She was instructed to rescan the tumor once in two years or when new symptoms appeared.

The first MRI was performed in 2006, but we only have the written description. The next scan was performed in 2007 and it showed no signs of growth of the tumor with a size stationary at 6.73 mm x 11.33 mm.

Spontaneous shrinkage was confirmed in the next scan, done in 2013, as the tumor size decreased by more than 2 mm. The tumor continued to shrink. The next scan performed in 2016 and 2019, showing a tumor measuring 4.22 mm x 5.48 mm.

The tumor size was measured in coronary, sagittal and axial planes (fig. 1). No additional neurologic symptoms were present during this period. The hearing loss followed the common pattern associated with the ear pathology.

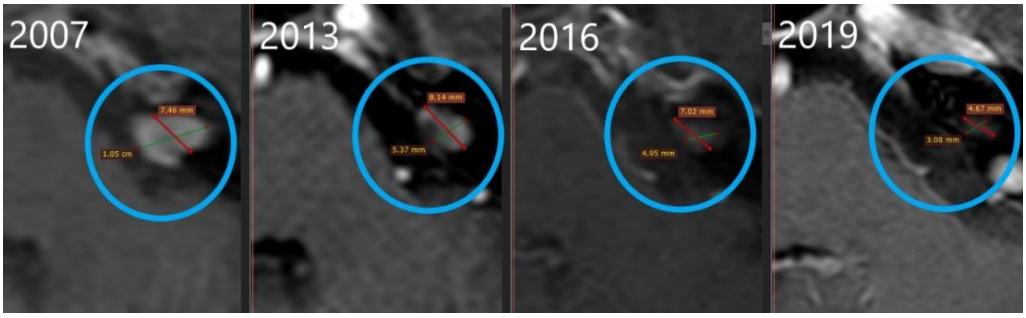

Fig. 1. Vestibular schwannoma in series of MRI with measurements showing the tumor shrinkage in axial view.

DISCUSSION

Spontaneous tumor shrinkage is a rare phenomenon but marked in the literature in some studies with follow-ups as long as 27 years (1, 2).

A review of the studies that mention tumor shrinkage in vestibular schwannoma are presented in the first table, including the current study.

In 27 reviewed studies, between 1998 and 2019, tumor shrinkage has been reported in 229 out of 4,002 patients (5.69%) with a mean follow up of 4.37 years (1-28).

As described by Huang *et al.*, 2013 a

complete regression of a VS from a 14.1 mm tumor was seen during a follow-up period of 12 years (10).

A growth-regression process was described by Yasumoto and Ito (28), in a 75-year-old patient with a right small VS that initial grew from 5.2 to 16.7 mm over 7 years, then spontaneously shrank to 8.2mm with improvement of the tinnitus, dizziness and the other initial symptoms.

TABLE I

Review of the literature – Spontaneous shrinkage of vestibular schwannoma

Author, year	No. of patients with shrinkage/total patients studied (%)	Age of patients (mean)	Mean size of tumor (mm)	mm or % of shrinkage	Follow up years (mean)
Luetjie *et al*., 1988 (13)	1/115	71	13	5,38%	5
Bederson *et al*.,1991 (5)	4/70 (8%)	N/A	N/A	5.70%	2.7
Tschudi *et al*., 2000 (26)	8/74 (11%)	N/A	N/A	16.00%	3
Luetjie, 2000 (14)	6/47 (13%)	66.83	18.75	6.24%	8.46
Shin *et al*., 2000 (22)	7/60 (12%)	63	N/A	N/A	2.6
Hoistad *et al*., 2001 (9)	3/102 (3%)	64	N/A	N/A	2.4
Stipkovits *et al*., 2001 (24)	3/44 (7%)	58.3	19	12.53%	7.6
Mohyuddin *et al*., 2003 (17)	10/50 (20%)	N/A	N/A	N/A	1.65
Slatteri WH III *et al*., 2004 (23)	16/56 NF2 (29%)	N/A	N/A	1-7 mm	4.3
Bozorg Grayeli *et al*., 2005 (6)	7/111 (6%)	N/A	N/A	N/A	2.75
Al Sanosi, 2006 (1)	6/205 (3%)	N/A	N/A	N/A	3.2
Battaglia *et al*., 2006 (3)	6/111 (5%)	N/A	11-25	0,7 mm	3.1
Yasumoto and Ito, 2006 (28)	1	75	16.7	5.51%	4
Penido *et al*., 2007 (2)	2	54	8.5	N/A	3
Mick *et al*., 2008 (16)	2	60.1	N/A	N/A	2.1
Ferri *et al*., 2008 (7)	6/123 (5%)	N/A	N/A	N/A	4.8
Hajioff *et al*., 2008 (4)	16/72 (22%)	61	N/A	N/A	10
von Eckardstein *et al*.,2010 (27)	2 (NF2)	55	28.55	8.36%	6
Pennings *et al*., 2011 (19)	4/47 (9%)	N/A	Intracanalicular	N/A	5.1
Huang *et al*., 2013 (10)	48/1261 (4%)	56.7	12.04	6.25	9.5
Huang *et al*., 2013 (11)	1	67	14	14.10%	12
Romani *et al*., 2016 (20)	2/223 (1%)	28.5	27	6.22%	6
Kontorinis *et al*., 2016 (8)	23/540 (4.3%)	N/A	N/A	N/A	0.97
Sebök *et al*., 2018 (21)	1	61	2.6	0.11 mm	10
Lahlou *et al*., 2018 (12)	14/196 (7%)	60	1.6	0.2 mm/year	5
Tikka T *et al*., 2018 (25)	28/540 (5.2%)	N/A	N/A	3.9 mm	2.54
Amoo *et al*., 2019 (15)	1	41	3.96	3.52 mm	10
Present report	1	59	8.15	4.85 mm	13
TOTAL	229/4002	58.9			

In the largest study on the conservative management of vestibular schwannoma, Huang *et al.* (10), after observing 1261 patients, noted 48 that showed spontaneous shrinkage (4%). The approach of "watch, wait and rescan" (WWR) was analyzed by Kontorinis *et al.*, 2016 in a retrospective analysis of the medical records of 540 VS over a 15 years period, 23 (4.3%) of which demonstrating tumor shrinkage (8). Tikka T. *et al.* in 2018 achieved a direct comparison of MRI of the *internal auditory meatus* (IAM) in a group of patients with sporadic VS demonstrating spontaneous regression compared with a control group of patients with growing VS (25). In our case report the patient showed a linear tumor shrinkage during the 12 year follow up.

The study of Romani R and Pollock J, 2016 made some remarks regarding the mechanism of tumor shrinkage that is not well understood even today. They proposed environmental factors, genetic-molecular and a reduction in vascular supply hypothesis (20).

In a recent study, Lahlou G. *et al., 2018* (12) identified two specific radiologic features in a series of cases of vestibular schwannoma shrinkage. First, it's described a festooned or scalloped appearance, defined by multiple curves in the tumor outline. We found this aspect of scalloped "line" in the first MRI done in 2007, as shown in green (fig. 2).

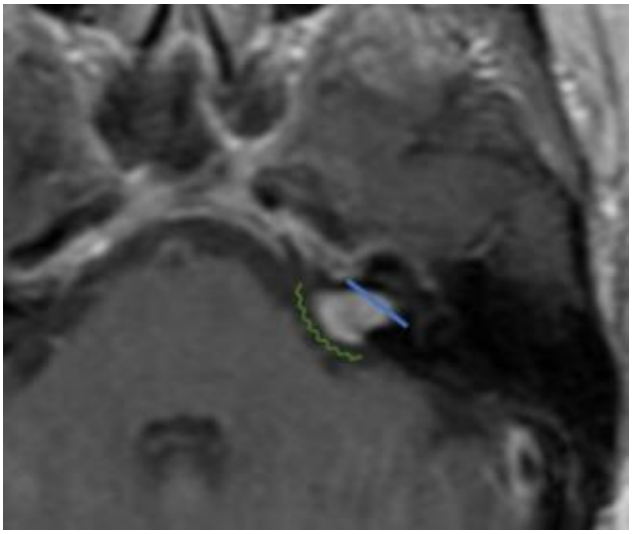

Fig. 2. Vestibular schwannoma in the first MRI:
green line – scalloped/festooned appearance, blue line – CSF infilling of the IAM

Second, on T2 weighted imaging of cerebrospinal fluid (CSF) infilling the intrameatal portion of the tumor, associated with asymmetry of the intrameatal (IAM) size. This feature was recognized in T1 weighted imaging with contrast, as displayed by the blue line, marking the CSF infilling of the IAM.

The study concluded that the two radiological features could allow neurosurgeons and neuro-otologists to easily recognize the vestibular schwannoma that will shrink spontaneous, therefore it is important to look for them and opt for a WWR protocol (12).

CONCLUSIONS

Although spontaneous shrinkage of acoustic neuroma is described as a potential natural evolution in a consistent body of literature, there are still no pathological theories for this evolution. As the genetic variations in schwannoma do not indicate a predisposition for involution, we propose a mechanism involving tumor metabolism. The failure to recruit vascularization might be, for example, an individual mechanism that can lead to tumor shrinkage (as "failure to thrive").

We argue that cases like the one we presented are a strong argument for "watch, wait and rescan" approach (WWR) towards the patients that harbor small sized tumors. The current literature endorses this policy for small or medium sized tumors, with minimal or absent symptoms. The Lahlou G. *et al.*, 2018 study provides evidence for tumor shrinkage even in large tumors (12).

An internationally standardized method of strategies based on size, radiological aspect and symptoms is needed by analyzing a larger series of patients.

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NEWS

EARLY MEASLES VACCINATION AND THE IMMUNE RESPONSE

It is well known that during measles epidemics World Health Organization (WHO) recommends that the first dose of measles-containing vaccine (MCV 1) should be given at 9 months of age. But, globally, there were reported cases on infants who are too young to receive MCV1, and they remain at high risk of contracting measles. This study is the first systematic review to examine the effect of MCV1 administered to infants younger than 9 months on the immunogenicity and vaccine effectiveness of subsequent MCV doses. It was reviewed evidence of humoral and cellular immunity, including seropositivity, geometric mean titres, avidity, T-cell stimulation, and vaccine effectiveness. Their findings suggest that administering MCV1 to infants younger than 9 months followed by additional MCV doses results in high seropositivity(98%), vaccine effectiveness(95%), and T-cell responses, which are independent of the age at MCV1, supporting the vaccination of very young infants in high-risk settings. However, they also found some evidence that MCV1 administered to infants younger than 9 months resulted in lower antibody titres after one or two subsequent doses of MCV than when measles vaccination is started at age 9 months or older. The clinical and public-health relevance of this immunity blunting effect are uncertain (Lochlainn LM, de Gier B, van der Maas N, van Binnendijk R, Strebel PM, Goodman T, de Melker HE, Moss WJ, Hahné SJ. Effect of measles vaccination in infants younger than 9 months on the immune response to subsequent measles vaccine doses: a systematic review and meta-analysis. *The Lancet Infectious Diseases.* 2019 Sep 20.).

FLAVONOIDS AS PROMISING THERAPEUTIC AND DIETARY AGENTS

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FLAVONOIDS AS PROMISING THERAPEUTIC AND DIETARY AGENTS (Abstract): Flavones were an essential anchor for the development of new therapeutic agents. All classes of flavonoids exhibit a variety of biological activities, but among them, flavone derivatives have been considerably explored. Numerous natural, semisynthetic and synthetic flavonoids have been synthesized and evaluated for several therapeutic activities, such as: anti-inflammatory, antimicrobial, antiallergic, antioxidant, antitumor activities. Most metabolic diseases are speculated to originate from oxidative stress and, therefore, it is significant that recent studies have shown the positive effect of flavones on diseases related to oxidative stress. In this paper, we present some fundamental elements for flavonoid therapeutic implications and future uses. **Keywords**: FLAVONOIDS, OXIDATIVE STRESS, THERAPY, PHARMACOKINETICS.

Nature has been the source of medicines for mankind since ancient times. Medicinal foods and herbal medicines, with different composition of secondary and primary metabolites, can play an essential role in the management of complex diseases. Flavonoids can be found in the human diet in fruits, vegetables, legumes, tea, dark chocolate. Some specific parts of these foods are richer in flavonoids than others, such as the peel of certain fruits (1, 2, 3).

Flavonoids are low molecular weight polyphenolic phytochemicals, derived from the secondary metabolism of plants and play an important role in different biological processes. The intake of flavonoid-containing foods may vary depending on the culinary habits of different countries. For example, soybean consumption has historically been associated with oriental crops or tea consumption is higher in countries such as the United Kingdom, Ireland or Turkey, among others. Researches in the field of flavonoids have increased since the French concept of paradox was formulated by French epidemiologists in the 1980s, i.e. a lower cardiovascular mortality rate observed in Mediterranean populations in association with the consumption of red wine and a high intake of saturated fat. Flavonoids are components of a wide variety of edible plants, fruits, vegetables and beverages such as tea, coffee, beer and wine. Thus, *in vitro* inhibition of LDL

oxidation of flavonoids derived from red wine has been demonstrated. Since then, several other beneficial properties of flavonoids have been found, as information on the mechanisms of flavonoids has been scarce (3, 4, 5, 6).

Depending on their structure, the large group of flavonoids can be divided into six categories: flavones, flavanones, flavonols, flavanol, isoflavones and anthocyanidins. Given the immense number of known flavonoid derivatives, their diversity has a direct implication on the molecular targets that have been found to represent important hubs for therapeutics. Moreover, their three-ring carbon skeleton, out of which one is heterocyclic, ensures the flexibility, rigidity and space mobility to adapt to various enzymes and receptors conformation. This is exactly why flavonoids still represent key compounds for therapy and research (5, 6, 7).

Pharmacokinetics

Most of the flavonoids are synthesized by the plants in their O-glycoside or C-glycoside forms, the aglycons being usually the storage form. Their absorption depends on the processing of the vegetal matrix and the chemical structure of each compound. Generally, flavonoids are highly metabolized in the intestine especially by β-glucosidases (acts on monoglucosides) lactase-phlorizin hydrolase (active on glucose and galactose derivatives) and, as a result, their metabolites are transported to the liver. Metabolites formed in the liver can be transported to the target cells, reenter the enterohepatic circulation through bile excretion, be hydrolyzed or excreted through urine or feces. Catechins are usually absorbed rapidly from the intestine, but dimerization leads to a decrease in their absorption. Also, trimers can be metabolized, but larger oligomers can only be transformed by the gut microbiota in the larger intestine (2, 3, 5).

Several studies have focused on improving the bioavailability of flavonoids through different approaches, such as inhibiting enzymes that limit their bioavailability, altering the composition of food matrices, or increasing the rate of dissolution. Moreover, the addition of alcohol (as wine) or proteins (milk) did not influence the absorption of flavonols and catechins (6, 8). For example, hesperidin and its aglycone (hesperetin) are two flavonoids from citrus species that have different biological properties. Hesperidin is transformed into hesperetin by the colonic microflora and it can be directly absorbed by enterocytes, is a cytochrome P450 inhibitor that is capable of affecting the metabolism of other drugs such as diltiazem, verapamil and felodipine (9).

Biologic and therapeutic potential

Flavones have antioxidant, antiproliferative, antitumor, antimicrobial, estrogenic, anti-inflammatory activity and are also used in cancer, cardiovascular disease, neurodegenerative disorders (3, 4, 7, 10, 11). Also, flavonoids affect several mammalian enzymes such as protein kinases that regulate multiple cell signaling pathways and modifications of multiple cell signaling pathways. Apigenin and luteolin are commonly found in a variety of foods such as parsley, celery, artichoke, peppers, carrots, thyme, rosemary, oregano, olive oil, peppermint, salad, pomegranate, cucumber, lemon, beet, cabbage, cauliflower, spinach, and green tea. It is well documented that luteolin acts as an antioxidant, anti-inflammatory, anticancer and inhibitor of prooxidant enzymes, both *in vitro* and *in vivo* (10, 12, 13, 14, 15).

Antioxidant potential

High levels of free radicals in living systems are capable of oxidizing biomolecules, leading to tissue damage, cell death or various diseases such as cancer, cardiovascular disease, arteriosclerosis, neuronal disorders, skin irritation and inflammation (12, 13, 16). Free radicals are highly reactive and therefore can attack membrane lipids, generating carbon radicals and producing radicals that cause lipid peroxidation. Therefore, a single radical can damage many molecules by initiating lipid peroxidation reactions. To counteract the vicious effect of free radicals, the body has several mechanisms of defence of antioxidants in the form of enzymes such as superoxide dismutase and catalase, copper and iron transport proteins, as well as water-soluble and lipid-soluble antioxidants. It has been studied that the imbalance between free radicals and the antioxidant defence mechanism is associated with several human diseases (2, 4).

Antioxidants can act by two mechanisms: preventing the initiation of oxidation or as antioxidants breaking the chain. Preventing the initiation of oxidation occurs by inhibiting the production of superoxide anions, degradation of hydrogen peroxide and chelation or reduction of metal ions, while chain-breaking antioxidants act by eliminating radicals, largely hydroxyl radicals, thus inhibiting the chain of events. Oxidative agents lead to damage of lipid membranes, proteins and DNA. Moreover, oxidative species and free radicals are involved in the pathophysiology of many diseases, such as neurodegenerative disorders, cardiovascular, cerebrovascular, autoimmune disorders such as diabetes, rheumatoid arthritis, psoriasis, etc. Therefore, different natural and synthetic antioxidants are used to eliminate free radicals (2, 3, 14).

Flavones have been reported to have a well-known antioxidant activity and can act in several ways. Therefore, flavones are significantly used in the pharmaceutical and food industry. Flavones, such as luteolin and apigenin, which contain two or three free hydroxyl groups in A / B rings, exhibit antioxidant properties at low concentrations (14, 15).

Some studies, using a QSAR model for different flavones to evaluate the correlation between the structure and the intensity of the antioxidant activity, concluded that as the number of hydroxyl groups increases, the activity is better. Moreover, two neighboring hydroxyl groups have better effects. Different flavone conjugates, such as complexes C_{60}-flavone, have been evaluated for the antioxidant potential in which the flavone phenolic fragment reacts with peroxyl radicals, while the C_{60} part of the molecule acts synergistically by trapping alkyl radicals under reduced O_2 partial pressure. Also, a natural flavone extracted from the tropical plant *Coronopus didymus* was tested for their ability to inhibit lipid peroxidation induced by γ radiation, Fe (III) and Fe (II) radiation and showed a better protective effect (4).

The 5-hydroxyl group in combination with the 4-keto and catechuic hydroxyl groups chelates catalytic active metal ions involved in redox reactions, which may prevent the formation of oxidizing species. The most harmful of the reactive oxygen species generated in the Fenton reaction is the hydroxyl radical, which can induce lipid peroxidation (fig. 1).

$$Fe^{2+} \rightarrow H_2O_2 \rightarrow Fe^{3+} + H\dot{O} + OH^-$$

$$O^{2-} + H_2O_2 \rightarrow O_2 + OH^- + \dot{H}O$$

Fig. 1. Fenton reaction

The hydroxyl groups on ring B donate the hydrogen and one electron to the hydroxyl-, peroxyl- and peroxynitrite radicals, stabilizing them, thus forming a relatively stable flavone radical (fig. 2) (4).

Other studies have shown that inflammation induced by different processes is related to cognitive dysfunction and that luteolin/apigenin treatment can effectively decrease inflammation in the brain (13, 17, 18). Some studies have also reported that the neuroprotective effect of luteolin is related to its antioxidant activity. Improvements in cognitive function due to luteolin treatment have been linked to its antioxidant activity in a streptozotocin-induced diabetes model. For example, neuronal injury and cognitive dysfunction are found in streptozotocin-induced diabetes, and chronic luteolin exposure can reduce neuronal injury and improve cognitive performance. This effect of luteolin is related to its ability to increase antioxidant activity (19, 20).

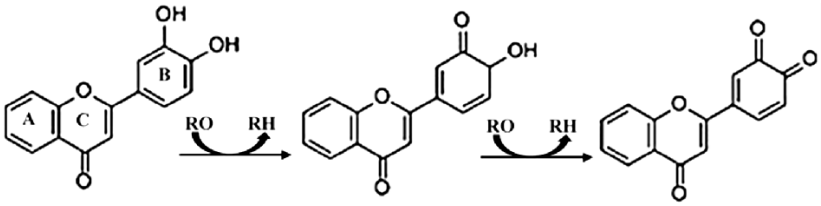

Fig. 2. Changes in the structure of 3′,4′-dihydroxyflavone
(RO – free oxidized radicals; RH – reduced radicals)

Lipid peroxidation is increased, while antioxidant enzyme activities in the cortex and hippocampus are decreased in diabetes. However, chronic luteolin treatment may alleviate such changes induced by diabetes. In addition, apigenin rich extracts have shown to reduce scopolamine-induced neuroinflammation, 1-β interleukin level, acetylcholinesterase, butyryl cholinesterase, lactate dehydrogenase and malondialdehyde activity. Moreover, the same doses (25 mg/kg and 75 mg/kg) restored the normal level of all antioxidant enzymes and the expression of the brain-derived neurotrophic factor (13, 17). These studies suggest that apigenin and luteolin may be beneficial for the prevention and treatment of neurodegenerative diseases, including Alzheimer's, due to its anti-inflammatory activity and other biological functions, including antioxidant activity (2, 5, 13, 17).

Antidiabetic potential

The apathetic lifestyle and energy-rich diet are now aggressively contributing to an astonishing increase in obesity, leading to insulin resistance, to type 2 diabetes along with retinopathy and nephropathy. Various enzymes such as α-glucosidase, aldose reductase, protein tyrosine phosphatases, protein kinase C are associated. The α-glucosidase enzyme is an intracellular enzyme involved in the breakdown of complex carbohydrates, which aids in the absorption of ingested carbohydrates, increases post-prandial blood sugar. Aldose reductase is a key enzyme in the pathway of polyol in which glucose is changed to sorbitol and its accumulation is linked to diabetic complications. It also involves the formation of advanced glycation end products (AGEs). Diabetic complications such as retinopathy, nephropathy, neuropathy are caused by an increase in the advanced

formation of glycation end products and activation of protein kinase C isomers. Among them, AGEs are known to cause ageing, as well as diabetic complications (2, 3, 20).

Numerous scientists have reported different flavone derivatives, of natural and synthetic origin, for the treatment of diabetes and its complications. In one study, various flavone 6- and 7-hydroxy flavone hybrids with aminopropanol were synthesized and evaluated as novel antidiabetic agents. Of all the synthesized hybrids only two were found to be the strongest. These active compounds possess a voluminous lipophilic substitution on ring B of flavone and smaller substituents on the nitrogen atom. Hybrids with tert-butyl and isopropylamine functionality at the N atom are of higher activity (1, 2, 3, 4, 20).

On the other hand, luteolin has inhibited the α-glucosidase enzyme up to 36% at a concentration of 0.5 mg / mL and has the potential to suppress postprandial hyperglycemia in non-insulin-dependent diabetes mellitus. 6-amino-5,7-dihydroxyflavone is a unique flavone having an amino moiety together with the usual 5,7-dihydroxyl substituents, which have been shown to be active against rat intestinal α-glucosidase. Initial studies suggested that 6-amino and 7-hydroxyl groups were essential for activity. The 5-hydroxyl substitution was favorable, while the 8-amino group was unfavorable for activity (21, 22).

Cardiovascular protective activity

It is well known that a healthy cardiovascular system has a positive impact on memory and cognition. Furthermore, most of the risk factors are determinant both for cardiovascular and brain dysfunction. Observations in regard to flavonoid-rich food intake indicated that the best benefit is in at-risk populations. Various cocoa products were administered to a wide range of people and the results indicated a decrease in blood pressure of older generation, especially in those with impaired vascular function. Nevertheless, there are still conflicting studies in which tea and other foods associated with high fat load meals could or couldn't influence the blood pressure. Therefore, further investigations are necessary to elucidate the mechanisms and the real potential of such compounds (2, 3, 6).

Several types of isoflavones such as daidzein, genistein and equol, known to induce hormone-like activity, have been observed in human trials and were noted to have a benefic effect on cardiovascular disorders and osteoporosis. Such flavonoids have shown a positive influence on various parameters associated with cardiovascular complications (atherosclerosis, platelet aggregation, lipoprotein peroxidation). A significant improvement was identified for selective extracts rich in anthocyanins. In this case, several randomized trials indicated no secondary effects and the restoration of the HDL / LDL-cholesterol balance to normal parameters (3, 6, 14).

CONCLUSIONS

Metabolic dysfunctions, cardiovascular problems and neuronal loss represent the most important lifestyle challenges for modern society. Therefore, prevention is essential and dietary choices should be made according to the nutritional and functional value of foods. Vegetable products represent a rich source of pharmacologically active compounds that could prevent ageing deficits by multiple mechanisms. Nevertheless, further research into flavonoids is a common trend that may lead to the synthesis of new therapeutic agents with great benefit for human health.

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EPIDEMIOLOGY OF BURNS IN IAȘI COUNTY, A 10-YEARS STUDY

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EPIDEMIOLOGY OF BURNS IN IAȘI COUNTY, A 10-YEARS STUDY (Abstract): This study intends to obtain a baseline epidemiological evaluation regarding hospitalizations for burns cases in Iași County. **Material and methods**: We performed an epidemiological descriptive study of all hospital admitted burns patients from Iași County, for a period of 10 years, from 2006 to 2015. Data used in this study originates from standard government collected information upon hospital discharge and freely available public demographic information. Statistical analysis was performed using the *Statistical Package for the Social Sciences* (*SPSS*). **Results**: There were a total of 3,065 residents of Iași County hospitalized for burn-related causes in the studied period. Monthly distribution showed an average of 25.5 cases, with peaks in December (30.3 cases/month) and July (29.1 cases/month). Males represented 56% of cases and Male to female Ratio period was 1.27:1. The urban/rural proportion averaged 1:1.55. Average length of stay had a mean value of 9.79 days, while average median length of stay was 7.5 days. A total of 106 deaths were reported. Mortality expressed as deaths-to-cases ratio resulted in 3.54% for the overall period. **Conclusions**: This is the first study upon the epidemiology of burns in Romania in the last 30 years. The studied group presents a strong rural predominance with an increasing trend, and a decrease of annual case numbers combined with an increase in burn-related deaths. Quality of collected data is critical towards a precise overview and specific indicators are needed in reporting burns. **Keywords**: BURNS, EPIDEMIOLOGY, BURN DEATHS, BURN REGISTRY.

Burns represent a common cause of injury and often generate socio-economical losses as a result of disability or death. Decades of experience have shown that a highly coordinated and complex system is needed to manage these kinds of lesions, ensuring the best possible outcomes. Reliable data is paramount in planning such burns management systems and strong epidemiological studies are the foundation of a successful early stage.

Although data is collected from every hospital in Romania after a patient is discharged and burns patients are no exception to this rule, up to this day there has been no report upon annual number of burns, their severity, burn-related deaths or outcomes.

The amplitude of burn care and the necessity of proper burn management systems have periodically occupied the spotlight in Romania during recent years. Ever since the Colectiv night club fire, a mass casualty

disaster in 2015 which claimed the lives of 65 young persons, severe burns often are the subject of media debate. This has steered Romanian politicians towards promising important changes in burn care, most notably building burn centers, but very few changes have actually been implemented to this day.

The purpose of this study is to have a baseline view upon the epidemiology of burns in the second most populous county in Romania, to hopefully provide guidance towards obtaining national epidemiological data.

MATERIAL AND METHODS

This is an epidemiological descriptive study of all hospital admitted burns patients from Iași County, for a period of 10 years, from 2006 to 2015. Included were all patients residing in Iași County, indifferently of where in Romania they received the medical treatment.

Data included in this study was kindly provided by the National School of Public Health, Management and Professional Development, Bucharest (NSPHMPDB) and reflects standard government collected information upon hospital discharge. The analyzed dataset includes following information: main discharge ICD code, urban/rural residence, sex of patient, month of discharge, death (if it occurred), length of stay, mean and median length of stay, patient age category (under 1 year old, 2-4 years, 5-9 years, 10-15 years, 16-19 years, 20-29 years, 30-39 years, 40-49 years, 60-69 years, 70-79 years and over 80 years old).

Demographic data was acquired via TEMPO statistics platform, National Statistics Institute (1). Only residents of Iași County were considered, in the specified timeframe. Total population number, age groups, sex and urban/rural provenience were considered for this study.

Statistical analysis was performed using the *Statistical Package for the Social Sciences* (*SPSS*) software, IBM Corporation (IBM® SPSS® Statistics 25).

RESULTS

There were a total of 3,065 cases of burns patients in the studied period. The year with the highest number of cases was 2012, while the lowest number of cases was reported in 2008. Monthly distribution showed an average of 25.5 cases, with peaks in December (30.3 cases/month), July (29.1 cases/month), followed by November and September (27.4 and 26.3 cases/month).

The incidence of hospitalized burn cases for residents of Iași County was 40.38/100.000 persons. Overall, males represented 56% of cases and female only 44%. Male to female ratio in the whole period was 1.27:1 with a maximum of 1.63 in 2010 and a minimum of 1.05 in 2012. The urban/rural proportion was on average 1:1.55, with a maximum of 1:1.84 in 2008 and a minimum of 1:1.31 in 2012.

Average length of stay varied between 7.56 days in 2011 to 11.61 days in 2009, with a mean value of 9.79 days. Meanwhile, average median length of stay was 7.5 days, with a minimum of 6.93 days in 2008 and a maximum of 8.23 days in 2012.

A total of 106 deaths were reported, with a maximum of 14 deaths/year in 2007 and a minimum of 7 deaths/year in 2011, averaging 10.6 deaths/year. Thus, mortality expressed as deaths-to-cases ratio resulted in 3.54% for the overall period, with a minimum of 2.58% in 2010 and a maximum of 3.9% in 2012. Proportionate mortality for burn cases was an average of 1.26 burn deaths for every per 1000 overall

deaths in Iași County, varying from a minimum of 0.85/1,000 to a maximum of 1.75/1,000.

DISCUSSION

This is the first study on the epidemiology of burns in Iași County and the only such research in the last 30 years in Romania. It is highly reliant on collected data type and quality, resulting in a limited insight in burn severity, survival, reconstructive procedures and long-term outcomes. Also, it only considers discharged cases, as Emergency Department visits, outpatient cases and out-of-hospital deaths are not collected into the same database.

Furthermore, data collection based on discharge documents yields unusable clinical information, as ICD coding is used to report cases. For a quality burns database, the total body surface area of the burn, its degree, anatomical location and etiology are needed. Unfortunately, ICD coding is structured in such a manner that it cannot provide all these data simultaneously, resulting in limited and often truncated clinical information, rendering quality of care studies virtually impossible.

In spite of these limitations, the study exposes important epidemiological data, upon which public health strategies, prevention programs and policy changes can be based upon.

The annual number of hospitalized burn cases shows an overall downward trend, when the whole 10-year period is analyzed. Meanwhile, the total population of Iași County has shown a steady increase, from 853,392 to 916,599 people. Upon first look, this evolution would seem normal in an upper medium income country, member of the European Union, as this decrease in number of burn cases is observed worldwide in such countries. But upon closer inspection, the studied timeframe presents two contrasting periods: a smooth descent in the case numbers curve between 2006 and 2012, followed by a steep rise in 2013, 2014 and 2015. Further analysis of the following years would be interesting in discovering if the trend has indeed reversed and the number of burns cases is increasing.

One of the interesting results is the disparity between the urban-to-rural ratio in the normal population of Iași County and this ratio in the hospitalized burns patients. While in the general population the urban-to-rural ratio averaged 1:0.93 in the analyzed period, this ratio was 40% higher in our studied cases. Moreover, while in the general population of the county this ratio has a decreasing trend, in the burns population it shows an opposite forecast. Considering that the North-East region of Romania is one of the poorest in the European Union, rural areas generally present lower socio-economic indicators and that Iași city is one of the fastest growing hubs of Romania highly driving urbanization through polarization, it seems this disparity will only accentuate with time. An expanding gap could widen, leaving vulnerable population increasingly exposed to events resulting in burn injury unless preventative measures are taken.

Concerning the monthly distribution of burn cases, the observed periods of high afflux follow previously described patterns: a summer peak where usually flame burns occur, and a high plateau during the colder months, where burn etiology is more often related to residential heating and scalding. Quite particular in this geographic region are the flame burns during the beginning of the cold period, resulting from the use of combustion accelerants in stove heaters,

followed by residential fires caused by improvised heating devices. Including burn etiology in the collected data from burns patients would allow precise measurement of the impact on healthcare of each specific cause, enabling tailored prevention programs to be implemented.

Gender distribution showed that males were more frequently burned in each year of the studied period. However, the evolution of the number of male burns cases shows a clear decreasing curve, while the evolution of female burns cases almost is stationary during the studied timeframe. Interestingly, the number of female burns cases follows the same decreasing pattern as the males up to the year 2011, where it reaches a minimum from where it starts a steep ascent in 2013, 2014 and 2015, bringing the male-to-female ratio closer, to 1.1:1. It would be interesting to see if this evolution continues in the same manner and the male-to-female ratio of burns cases will reverse in following years. Also, proper research into burn etiology in women might uncover causes which can be tackled through specific measures, thus reducing overall burn burden.

The evolution of the average length of hospital stay contrasts to the one of the median length of stay: while the former has a slight tendency to decrease, the latter has an opposite increasing trend. While conclusions upon the dynamic of length of stay cannot be drawn, knowledge of this data is a useful planning instrument for healthcare policy developers. More precise studies are needed to clarify length of stay evolution, but other qualitative tools (such as severity indexes) are required for refined answers.

Annual number of deaths of hospitalized burns patients showed a slight expansion, contrary to the trend of case numbers (fig. 1) and international trends. More so, it had the same contrasting segments: an initial steep downwards curve from the beginning of the studied period to a minimum of annual death-related burns in 2011, followed by a similarly, but opposite, steep growth from 2012 to the end of the studied period. Although the number of annual deaths from all causes in Iași County increased each year, proportionate mortality for the burns cases followed an almost identical pattern and its evolution graph is almost identical to the total number of burn-related deaths in hospitalized patients in the studied timeframe. Two different events might be accountable towards an impact on the quality of care in that period: the issue of Health Minister's order (2) concerning transfer of severe burns to facilities in Bucharest and the fact that the only specialized adult burn treatment facility in the County suffered a relocation from where it had uninterruptedly functioned for the previous 25 years to an improperly designed temporary location. Separate studies should be conducted to evaluate the impact of each event.

The average deaths-to-cases ratio representing in-hospital mortality for burns is higher than those found in Taiwan (1.5%) (3), France (2.5%) (4), Norway (2.1%) (5) or the Netherlands (3.2%) (6) in similar years, but lower than the one in Portugal (4.4%) (7) and corresponding to the ones found in China (varying from 0.3% to 7.5%) (8). Data regarding burn-related deaths in Iași County should be however regarded with caution, as it originates from all types of hospitals (primary, secondary and tertiary), in a region with no burn centers, which has traditionally been a referral area for this pathology from neighboring counties but suffered major organizational transitions in the studied period.

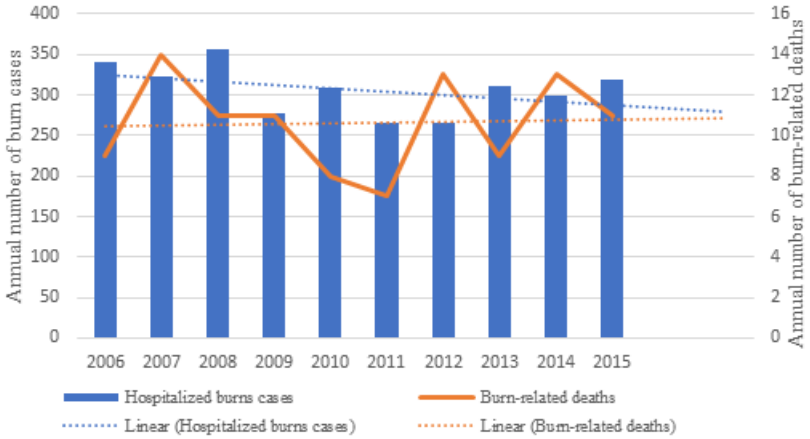

Fig. 1. Evolution of annual numbers of burn cases and deaths, between 2006 and 2015, in Iași County

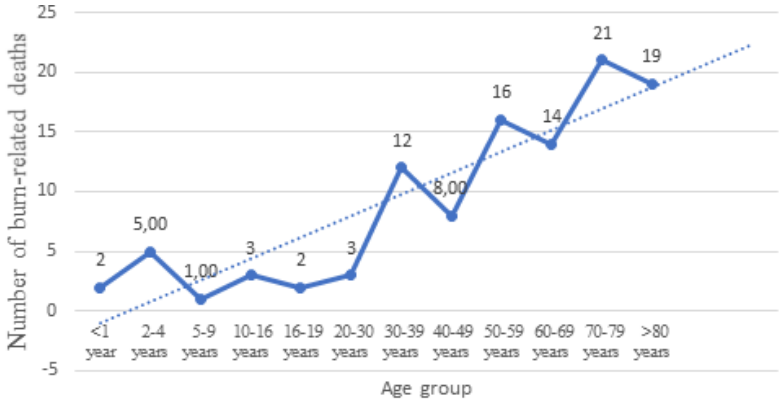

Fig. 2. Distribution of burn-related deaths according to age group, between 2006-2015, in Iași County

Males account for 59% of burn deaths, a higher percentage than in the number of burn cases. This would suggest that males are more susceptible to dying following a burn, but to properly investigate such a claim more precise studies should be performed, to match for burn etiology, size, depth and patient characteristics.

Analysis on the age distribution of burn deaths has been performed and the results show that there is an exponential increase in deaths as age grows (fig. 2). Children under 16 represented only 10.3% of total burns deaths, while adults over 60 accounted for 50.9% of cases. While there are separate facilities in Iași for the care of pediatric and adult burn patients and their results should be evaluated separately, our findings reflect international patterns: children tend to recover better from burns and there are rare cases of pediatric deaths caused by burns, while geriatric patients usually have a higher rate of complications and mortality.

It should be noted that there is a significant number of burn-related deaths which are unaccounted for and evade this statistic, due to the fact that persons who die before reaching the hospital are transported directly to the Legal Medicine Institute of Iași (9). These methods of separate reporting adversely affect the chances of obtaining an accurate image of the burden burns impose on the healthcare system.

CONCLUSIONS

This is the first study upon the epidemiology of burns in Romania in the last 30 years. In the ten-year period studied there has been an annual average of 306.5 burn cases, 10.6 deaths and 3.5% in-hospital mortality. There was a male predominance in case numbers and especially in burn-related deaths. A predominant rural provenience was noted, with an increasing trend. Although annual number of burns cases has been declining, mortality showed a slowly increasing trend line.

Such epidemiological studies provide the foundation upon which lay prevention programs, healthcare policies and government strategies. To this respect, they are indispensable to a nation. However, quality of collected data is critical towards a precise overview. We suggest adding quality criteria (etiology, burn size, depth, location) to government collected data concerning burn patients, preferably in the form of a national burn registry.

ACKNOWLEDGEMENTS

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COLICA NEFRETICĂ LA GRAVIDE. EXPERIENŢA CLINICII UROLOGICE IAŞI

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RENAL COLIC IN PREGNANCY (Abstract): Renal colic in pregnant women is a serious condition, mainly when is associated with fever. **Material and method**: Our retro-prospective study analyzes 111 cases managed conservatively or with endourological procedures for renal colic – insertion of JJ stents and percutaneous nephrostomy. **Results**: Clinical evolution determined the insertion of JJ stents in 60 cases and the failure of this procedure imposed percutaneous nephrostomy in 5 cases. In 56 cases urinary tract infection was associated and in 2 cases, despite all efforts, the patients deceased due to sever sepsis. **Conclusions**: The immediate drainage of the upper urinary tract for renal colic in pregnancy is the recommended treatment, especially when the pain is associated with fever. JJ stens were well tolerated, even when they were replaced after 3 months. Pregnant women with a history of UTI or stone disease should be carefully followed-up. **Key words**: RENAL COLIC, PREGNANCY, HYDRONEPHROSIS, URINARY STONES, JJ STENTS

MYOFUNCTIONAL TREATMENT OF OPEN BITE IN A PATIENT WHO SUFFERED FROM RECURRENT LINGUAL HAMARTOMA. CASE REPORT

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MYOFUNCTIONAL TREATMENT OF OPEN BITE IN A PATIENT WHO SUFFERED FROM RECURRENT LINGUAL HAMARTOMA. CASE REPORT (Abstract): Hamartomas are defined as an overgrowth of improper proportions of tissues indigenous to the anatomic area of occurrence. Hamartomas may occur in any organ of the body and are common in the spleen, liver, kidneys, lungs and pancreas. However, hamartomas of the tongue are extremely rare. The present article aims to present the progress of orthodontic treatment for open bite following tongue enlargement due to recurrent hamartoma in a healthy 7-year-old girl. Multidisciplinary periodontal, pedodontological, and then orthodontic treatment was planned. The patient received a myofunctional device to correct the position of the tongue and to control open frontal occlusion during the eruption of permanent teeth. After 6 months, the patient was reassessed and the maxilla expansion, occlusal status and tongue interposition were improved. **Keywords**: MYOFUNCTIONAL APPLIANCE, OPEN BITE, TONGUE HAMARTOMA.

Myofunctional appliances have been used for many years. There is a definite place for these appliances in orthodontics today because they are simple and economical, but the cases need to be carefully selected, and the operator needs to be well trained in their use (1).

Open bite is considered one of the most challenging malocclusion and its treatment must improve occlusion and oral function producing a satisfactory and stable result. As open bite treatment in adults may frequently require surgical procedures, and malocclusion may lead to the development of temporomandibular disorder (TMD), the earlier this malocclusion is corrected, the better the prognosis will be (2).

The term hamartoma is used to describe a nonneoplastic, abnormal, and haphazard overgrowth of conglomerates of mature cells and tissues indigenous to the anatomic site from which it occurs, often with one predominating element (3, 4). This tumor-like malformation is benign, and most hamartomas are located in the liver, spleen, pancreas, and kidneys (5). However, hamartomas of the tongue are extremely rare (5).

Therefore, the aim of this case report is to present the progress of orthodontic treatment (myofunctional appliance) for open bite following tongue enlargement due to recurrent hamartoma in a healthy 7-year-old girl.

CASE REPORT

A 7-year-old female patient presented to the orthodontic clinic because of aesthetic changes to the incisors (multiple spaces between the teeth). Her medical history revealed that she underwent three surgeries of a recurrent lingual hamartoma.

Extraoral examination revealed the following features: leptoprosopic facial type, enlarged lower face, and reversed lips with short upper lip (fig. 1).

The facial profile was markedly convex. When the patient closed the mouth, the teeth were not visible and there was some degree of lip incompetence. In addition to these dentofacial deformities, there were complex occlusal changes. The patient also reported major functional disturbances: tongue interposition and chewing difficulties.

Intraoral examination revealed moderate oral hygiene and mixed dentition with the following changes: treated and untreated dental lesions, dentoalveolar class II/1 Angle anomaly, anterior open bite canine to canine, bilateral reverse occlusion due to maxillary deficiency, and spaced dentition on the upper and lower arches (fig. 2).

Fig. 1. Extraoral examination

Fig. 2. Intraoral examination

A panoramic radiograph revealed a dentoalveolar disharmony with spacing, and multiple treated and untreated dental lesions (fig. 3).

Cephalometric analysis showed a Class I pattern (ANB, 4°; mean, 0°-4°), although the SNA angle of 77° (mean 82° ± 2°) and the SNB angle of 73° (mean 80° ± 2°) showed a retrognathic condition. The mandibular plane angle was steep with an enlarged gonial angle Sn/GoGn° of 43° (mean 30°± 2°). The upper and lower incisors were buccally inclined +1/Na of 34° (mean 22°) and -1/NB of 29° (mean 25°) (fig. 4).

Fig. 3. Orthopantomography

MEASUREMENT		NORMAL VALUE	VALUE	DIFFERENCE	BIAS
Other					
Angle SNA	°	82	77	-5	
Angle SNB	°	79	73	-5	
ANB	°	3	4	1	
SND	°	77	69	-7	●●●
Interincisal angle	°	140	113	-27	●●
SN/OcP	°	14	23	9	
SN/GoGn	°	30	43	13	
+1/NA	°	22	34	12	
+1/SN	°	103	111	8	
-1/NB	°	25	29	4	
+1i/NA	mm	4	6	2	
-1i/NB	mm	4	6	2	
Pg/NB	mm	N/A	-1	N/A	
Holdaway ratio	%	1	-1078	-1079	●●●
S-Lpoint	mm	51	39	-12	
S-Epoint	mm	22	11	-11	

Fig. 4. Cephalometric analysis

The *treatment objectives* were to (1) improve the patient's oral hygiene and periodontal health, (2) treat the dental lesions, (3) correct the tongue interposition, (4) expand the maxilla, (5) align the arches, (6) close the anterior open bite, (7) correct incisor tipping, and (8) develop ideal overjet and overbite.

Treatment plan. Multidisciplinary, periodontal and pedodontic treatment was carried out. After the improvement of oral hygiene, and treatment of dental lesions, the patient received a myofunctional device, Myobrace for Kids™ -K1 (The Myobrace System ®, Myofunctional Research Co, Australia), to correct tongue position and control the frontal open bite during eruption of permanent teeth (fig. 5). The patient was advised to wear the appliance 1-2 hours during the day and all night to be effective, as recommended by the manufacturer.

Fig. 5. Myobrace for Kids™ -K1

Treatment progress. Patient compliance and cooperation were good. The frequency of follow-up visits was every 2 months, until the eruption of permanent teeth. After 6 months, the patient was reassessed and the maxillary expansion, occlusal status, and tongue interposition were improved (fig. 6). The parents were informed that, depending on the growth pattern, an additional fixed orthodontic treatment with frequent visits and good cooperation will be necessary.

Fig. 6. Treatment progress at 6 months

DISCUSSION

Open bite is recommended to be treated as soon as it is diagnosed. This malocclusion results from an interaction between various etiological factors including bad oral habits, mouth breathing, muscular dysfunctions and skeletal abnormalities (2).

In our case, tongue enlargement due to the recurrent hamartoma determined its interposition between the dental arches, causing the opening of frontal occlusion.

This paper reports a case of open bite associated with posterior crossbite which was treated with a myofunctional appliance. This approach permitted to close the open bite by controlling tongue interposition. Using this appliance also corrected the posterior bilateral crossbite mainly caused by occlusal interference. Thus, the goal of treating this type of malocclusion must be to reinstate an ideal occlusion, improve oral and breathing functions, and properly reestablish normal muscular activity in the mastication, facial and tongue muscles.

Myobrace for Kids™ - K1 (Myofunctional Research Co, Australia) provides habit correction and is made of flexible silicone to adapt to any arch-form and poorly-aligned teeth. Its flexible nature also means that it offers improved retention for night-time use.

This type of functional appliance changes the posture of the mandible into a forward position, and stimulates transverse development, by skeletal and dental improvement in Class II malocclusion (6).

The trainer and similar appliances are claimed to encourage transverse bone growth by acting as a “shield” for the cheeks and bring about muscular relaxation and protection of the teeth and articulations from bruxism, by virtue of the “bite effect” (7, 8). The trainer is claimed to correct a skeletal Class II by an active mandibular force. By distancing the lower lips from the dental alveolar arch, the trainer is claimed to prevent the malposition of the tongue and lower lip during swallowing, thus solving the associated dental open bite (1).

Quadrelli et *al.* recommended this appliance to correct the interposition of lips between dental arches, atypical swallow-

ing, and the centripetal thrust of cheeks upon the dental arches; to discourage oral respiration; to avoid bruxism; and to favor the action of the external pterygoid and thus encourage the active push of the mandible (9-10).

Orthodontic intervention with the eruption guidance appliance in the early mixed dentition is an effective treatment modality for malocclusions with Class II or Class II tendency, excess overjet, deep bite, open bite, crowding, anterior crossbite, or buccal crossbite. During the treatment, the erupting permanent incisors and first molars were guided into their correct positions in the dental arches (11).

Usumez *et al.* (1) reported that this kind of appliances modifies the inclination of both upper and lower incisors. In this case, incisor inclinations were improved in both dental arches bringing their values closer to normal. It may be hypothesized that during the course of treatment the Myobrace for Kids™ - K1 caused positioning of the incisors changing their inclinations, which stimulated modeling of the whole dentoalveolar units (12).

Myobrace for Kids™ , used by the patient during a couple of hours during the day and overnight, was fully accepted with no complaints, and successfully corrected the crossbite, closed the open bite, and discouraged the interposition of the tongue. Therefore, it seems that getting good patient compliance with functional appliances may be more related to the ability of the professional to motivate and encourage the patient to use the appliance than the discomfort, pain, or speech difficulties associated with those orthodontic techniques (13-15).

CONCLUSIONS

Our study shows that although lingual hamartoma is quite rare it can cause major changes in the dentomaxillary apparatus and implicitly in occlusion.

Early functional treatment can correct occlusal changes over time if we cooperate well with the patient.

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NEWS

THE EFFECTIVENESS OF HORMONAL TREATMENT IN PREVENTING ENDOMETRIOSIS RECURRENCE AND IMPROVING WOMEN'S CLINICAL SYMPTOMS AND QUALITY OF LIVE

An observational cross-sectional study was conducted on 185 patients with endometriosis to evaluate the effects of hormonal medical treatment (progestin, GnRh analogs or continuous oral contraceptives) and establish the endometriosis recurrence, the current clinical symptoms and the influence on the quality of life. The group of study consisted in women of reproductive age and they were devised into three groups of patients: Group A (n=34) women who did not undergo hormonal treatment neither before nor after the first endometriosis surgery, Group B (n=76) women undergoing hormonal treatment after the first endometriosis surgery and Group C (n=75) women undergoing hormonal treatment both before and after the first endometriosis surgery. The women have been followed up for at least 2 years after the first endometriosis surgery. The duration of medical treatment was of minimum 12 months before surgery and at least two years after surgery. The results showed a lower rate of endometriosis reoperation and a lower rate of dysmenorrhea among the group C of patients. The study concluded that hormonal treatments represent a valid cornerstone of endometriosis management and it may be useful as alternative to surgery, but also before surgery for a better planning and after surgery in order to reduce the risk of recurrence (Tommaso Capezzuoli, Silvia Vannuccini, Daniele Mautone, Flavia Sorbi, Huixi Chen, Fernando M.Reis, Marcello Ceccaroni, Felice Petraglia. Long-term hormonal treatment reduces repetitive surgery for endometriosis recurrence. Available online 22 September 2020., https://doi.org/10.1016/j.rbmo.2020.09.018).

ASSESSING THE OPINION OF SENIOR STUDENTS ABOUT DENTAL EDUCATION DURING THE PANDEMIC PERIOD

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ASSESSING THE OPINION OF SENIOR STUDENTS ABOUT DENTAL EDUCATION DURING PANDEMIC PERIOD (Abstract): During the pandemic period, many universities reorganized their way of teaching, practicing, and interacting with students. Theoretical and clinical activities were conducted online. **The aim** of the present study is to identify the opinion of senior dentistry students about online activities. **Material and methods:** An online questionnaire was distributed to senior dentistry students enrolled in the Faculty of Medicine and Pharmacy, University of Oradea. In total, 69 respondents were included in the research. Information regarding socio-demographic data, self-reported health status, online activity schedule, and satisfaction with practical and theoretical activity was gathered. Data were analyzed using *SPSS version 23*. **Results:** 49 female and 20 male students, average 25 ± 2.15 (minimum 23 and maximum 35 years old) declared that they sustained only online activities. Most of them connect to online classes from their homes, with 59.42% using laptops and 34.78% connecting from smartphones. Almost half (47.33%) never use video connection in their online classes. Many of them (95.65%) use social media to communicate with colleagues and half of the number of students use the university platform to communicate with their academic teachers. 80% of students preferred practical activities in classrooms and more than 1/3 of respondents considered that there is knowledge that cannot be taught online. The behavior during online classes and the opinion of students regarding the online activity are also presented. **Conclusions:** Despite the present results, there is little evidence about the impact of online medical education on student formation and knowledge. Using their experience as students, seniors do not appreciate online teaching as a consistent option. **Keywords**: DENTISTRY, COVID 19, EDUCATION, STUDENTS, HEALTH, PANDEMIC, UNIVERSITY.

With the advent of the new Coronavirus, the entire world is facing the greatest public health challenge of the 21st century. Although from Spanish Influenza to the present times there have been other viruses that caused acute respiratory syndromes, such as H1N1 in 2009, MERS in 2012 or ZIKA and EBOLA in 2016, none of them had the ability to transmit so rapidly from person to person nor to kill especially the

elderly or people with various comorbidities.

Since its appearance in December 2019 in Wuhan Province, China and until now, SARS COV 2 has infected over 55 million people globally, out of whom 1.34 million have died. (1) Although it was initially thought that the range was only limited, as China took all the measures to limit transmission, because of the migration between countries and continents and due to the ease with which it is transmitted, an explosion occurred in the early 2020 regarding the number of cases and deaths worldwide, which led to the declaration of the SARS COV2 infection wave by the World Health Organization as a global pandemic in March 2020. (2)

In order to try to stop or at least reduce the spread of the disease, in several countries of the world, including Romania, states of emergency or alert have been established leading to restrictive measures by limiting all economic, social, and human activities. This lockdown produced, in addition to affecting the socio-economic environment, a direct problem facing the education system because of the school closures.

Consequently, online teaching has become part of the education system around the world and has taken many countries by storm since there was not sufficient time to adapt to the new teaching style, because everybody was accustomed to traditional, face-to-face teaching and lacked the necessary infrastructure and skills to teach exclusively online. This has, in turn, made it difficult for both students and teachers and affected the educational activity as a whole. These drawbacks in teaching will lead to educational deficiencies in time, and they will be difficult to correct and will implicitly affect the quality of life both socially and economically.

During the lockdown imposed by the COVID-19 pandemic crisis, many European dental schools were rethinking the way they organize their activity of teaching and interacting with students. The face-to-face activities were interrupted, and clinical activities suffered a transition towards the e-teaching processes. Some universities have switched from classroom to virtual activity more easily, and the challenges have been on the part of both academics and students.

During the first months of restrictions, most students returned to their homes. The internet was a powerful resource for reaching millions of students in different geographical regions and the only tool to continue their medical education. As such, it was a great opportunity for dental students and university teachers to build and retain the theoretical and clinical practice through many educational instruments. (3, 4) Also, in a recent survey conducted by Quinn *et al* (5) data were published regarding the immediate changes in the management of dental schools in some European countries: 90% of dental schools reported using online pedagogical software tools, 72% used live or streamed videos, 48% provided links to further online materials while 65% participated in organizing virtual meetings in order to provide more options for students and teachers. Also, data about small working groups and social medical groups were gathered.

Dental students had to face a double wave of stress: on the one hand, it was the fight against the consequences of social distancing and self-isolation (related to the increased rates of depression, anxiety, and fear of infection) and, on the other hand,

the stress of rapidly adjusting to the new way of learning, studying, and being evaluated, as their medical education had to continue in the new framework.

The present study is the first one undertaken in Romania among dentistry students and describes the opinion of senior students. Among all academic years, seniors are more prone to compare different styles of teaching and tools and to identify the strengths and limitations of online teaching and evaluation. The results presented in this paper are part of a larger piece of research work that include dentistry students from all years of study and from many universities.

MATERIAL AND METHODS

Participants and instruments

The study was developed during the last weeks of November 2020. An online questionnaire was distributed among senior medical students from Faculty of Medicine and Pharmacy, Dentistry specialization, University of Oradea, Romania, using *Google Forms*. Students had to answer a succession of items collecting information about their socio-demographic data (age, gender, environment, living conditions, accommodation etc.), medical data related to their health in the last six months (the presence of chronic disease, being under medical treatment, feeling depressed, anxious, bored or having a sleep or appetite disorder), academic activity (online participation, platforms used for didactic activity, their level of satisfaction with the results obtained and with their level of knowledge etc.).

The link to the questionnaire was sent to all senior students by the administrator of the Faculty. All the items were mandatory. The inclusion criteria considered only questionnaires filled in before the deadline.

Ethical approval

The study was approved by ethical committee of the University of Oradea, No.12/20.11.2020. The students were informed about the purpose of the study, the confidentiality of collected data and the fact that they could withdraw from the research with no consequences.

Statistical analysis of data

Collected data was analyzed using IBM *SPSS Statistics, version 23*. For descriptive analysis we calculated means, standard deviations, and frequencies. For comparative analysis we performed Mann–Whitney rank statistic tests to detect and describe significant differences between qualitative variables. The correlation analysis of the data was done using Spearman correlation; a $p < 0.05$ was considered significant.

RESULTS

Socio-demographic and self-reported health-related data

All 69 participants to the research (49 females and 20 males, average 25 ± 2.15, minimum 23 and maximum 35 years old) were enrolled in the final year of study in the University of Oradea, Faculty of Medicine and Pharmacy – Dentistry specialization. The total number of enrolled students is 70, so the response rate was 99.9%.

Most of the participants come from urban and rural areas – counties from Central and Western parts of the country (32 from Bihor, 13 from Cluj, 4 from Salaj, 3 from Suceava, 3 from Alba, 3 from Maramures, 2 from Satu-Mare, 1 from Bistrita, 1 from Brasov, 1 from Bucharest, 1 from Hunedoara, 1 from Mures) and 1 international student. 1/5 of respondents were from rural areas (N = 14, 20.29%),

During the academic year, almost half of them lived by themselves (N = 31,

44.93%), 1/3 with their parents (N = 19, 27.54%), some shared an apartment (N = 18, 26.09%) and only 1 student lived on campus.

More than 1/3 stated that they were smokers (N = 26, 37.68%), 23 (33.33%) were drinking alcohol and 8 (11.59%) used energy drinks, while 2 students declared that they sometimes consume illegal drugs. Several students (N = 7, 10.14%) declared that they have a chronic disease.

Students were asked if they were suspected or confirmed of being infected with COVID 19 - 20 students (28.99%) had been in contact with infected persons with COVID 19 and only 2 were later confirmed of having been infected.

Students had to rate the level of satisfaction with their physical and mental health during the last months of social distance and restrictions related to the pandemic period. The answers were distributed on a 5-points Likert scale from 1 (*totally unsatisfied*) to 5 (*totally satisfied*) (tab. I).

TABLE I

Satisfaction with their physical and mental health during the pandemic

Items	Mean ± SD*
*How satisfied are you with your **physical health** during the restrictions related to the COVID 19 pandemic?*	3.59 ± 1.129
*How satisfied are you with your **mental health** during the restrictions related to the COVID 19 pandemic?*	3.23 ± 1.139

*Mean and standard deviation (M ±) and N (%)

Living for a long period of time inside their homes, houses or rented appartements in the city or on campus determined a lot of changes in their daily lifestyle, particularly related to sleeping and eating behavior and mood. Students were asked to identify if, in the last 6 months, they had experienced sleep or appetite disorders, if they felt depressed, anxious, or bored (tab. II).

TABLE II

Self-rated health

Items	Mean ±SD *
I noticed that during the pandemic, I had sleep disorders	2.58 ± 1.355
I noticed that during the pandemic, I had appetite disorders	2.17 ± 1.414
I noticed that during the pandemic, I felt more anxious	2.77 ± 1.238
I noticed that during the pandemic, I felt more depressed	2.75 ± 1.218
I noticed that during the pandemic, I felt more bored	3.22 ± 1.571

*Mean and standard deviation (M ±) and N (%)

Social relationship and daily activity

Because of the social distancing and, for some of them, the impossibility to see their families due to the travel restrictions, some students felt isolated and lonely. Part of the students returned to their homes, but others had to shelter in their rented apartments or on campus. Social activities were

restrained. Some items questioned students about their level of satisfaction regarding the relationship with family members, colleagues, and friends (tab. III).

During the present pandemic period, online activities forced students to stay inside and to find leisure activities adapted to this situation. Among the most frequent activities that students mention that they have in their free time are cooking (21.7%), reading books (21.7%), watching movies or documentaries (23.2%) and physical activity (10.1%).

TABLE III

Satisfaction with relationship

Items	Mean ± SD*
*How satisfied are you with your relationships with **friends** during the restrictions related to the COVID 19 pandemic?*	2.91 ± 1.210
*How satisfied are you with your relationships with **family members** during the restrictions related to the COVID 19 pandemic?*	3.65 ± 1.293
*How satisfied are you with your relationships with **colleagues** during the restrictions related to the COVID 19 pandemic?*	2.84 ± 1.346

*Mean and standard deviation (M ±) and N (%)

Fear of infection with COVID 19

The fear of becoming infected with COVID 19 was also measured. During practical stages, students are usually in close contact with their colleagues and patients. Among the medical specialties, dentistry is evaluated as having a high risk of infection (with students and dentists being in contact with salivary and blood compounds and exposed to aerosols) (tab. IV).

TABLE IV

Fear of becoming infected with COVID 19

Items	Mean ± SD *
I am afraid that I may become infected with COVID 19 during practical activities at the faculty	3.78 ± 1.371
I am afraid that I may become infected with COVID 19 from patients I meet during internships.	3.84 ± 1.400
I am afraid that I may become infected with COVID 19 from colleagues I meet during practical stages and lectures.	4.09 ± 1.337

*Mean and standard deviation (M ±) and N (%)

Theoretical and practical activity during pandemic period

Immediately after the lockdown, dental schools continued to have online activities and teaching and evaluation were all conducted online. Students were asked *how satisfied they were with the academic results during the restrictions imposed by COVID 19 pandemic*. The answers ranged from 1 (*totally unsatisfied*) to 5 (*totally satisfied*), with the following frequencies: 11.6%, 15.9%, 21.7%, 33.3% and 17.4% respectively.

More than 50% of students stated that their practical training suffered due to the exclusive online activity. Similarly, while

31% agree with the fact that lectures are more interesting online, only 13% believed that the same was true when it comes to the practical stages. From all questioned students, 1/3 stated that their time allocated for individual training increased considerably and 1/3 of respondents considered that there is knowledge that cannot be taught online, with practical stages being mandatory for some disciplines.

Some items wanted to identify the way students attended their online classes and what their most common behavior was (tab. V).

TABLE V
Students' opinion about online activity

Items	Mean ± SD *
I always attended the course / lab in its entirety when I connected online	4.14 ± 1.033
The amount of information taught during online activity is higher	3.13 ± 1.110
I believe that online teaching is as effective as institutional teaching	3.10 ± 1.426
During online activity I prefer to stay connected through video	2.03 ± 1.248
Online student assessment is more objective than face-to-face assessment at school.	3.05 ± 1.290
I believe that exclusive online assessment of students encourages unethical behaviors	2.68 ± 1.266

*Mean and standard deviation (M ±) and N (%)

Comparative analysis

Significant differences depending on the gender of the participants were identified only in an item aimed at reporting health-related data. Thus, a higher score was identified for men (Mean rank = 37.45), compared to women (Mean rank = 34.00), in terms of drug use ($p = 0.026$).

Regarding the comparative analysis based on the students' residential environment, the *Mann-Whitney U Tests* revealed that there is a significant difference between participants identified for the variables of communication with teachers ($p = 0.012$) and cigarette consumption ($p = 0.045$). Higher scores on these variables were obtained by participants from rural areas (Mean rank = 46.14, respectively Mean rank = 43.07), compared to those from urban areas (Mean rank = 32.16 and Mean rank = 32.95).

The comparative analysis performed according to the existence of a diagnosis of chronic disease indicates that there are significant differences for the variables confirming the diagnosis of COVID-19 ($p < 0.001$) and the fear of infection with COVID-19 from university colleagues ($p = 0.037$). In this case, those suffering from chronic diseases obtained a higher score on the variable that refers to the fear of infection (Mean rank = 48.00). Students who do not suffer from chronic diseases obtained a higher score on the variable confirming the diagnosis of COVID-19 (Mean rank = 36.00).

Significant results were identified between variables considering confirmation of COVID-19. Thus, the scores obtained for the variable "How satisfied are you with your academic results during the restrictions related to the COVID 19 pandemic?" are higher in those who received a positive diagnosis for COVID - 19 ($p = 0.036$, Mean rank = 63.50). Students who did not receive a positive diagnosis for

COVID-19 scored higher on the variable of depressive disorder that occurred during the pandemic ($p = 0.042$, Mean rank = 35.82).

Correlation analysis

Our results indicate a statistically significant relationship ($p < 0.001$, $r = 0.514$) between the chronic disease status of students and the confirmation of the diagnosis of COVID-19, in the sense that those suffering from a chronic disease were more prone to become infected.

The level of satisfaction regarding the mental health of the students during the pandemic correlates negatively with the depressive ($p = 0.001$, $r = -0.390$) and anxiety disorders ($p = 0.009$, $r = -0.312$), and the level of satisfaction regarding the physical health correlates negatively with sleep disorders ($p = 0.023$, $r = -0.255$) and boredom level ($p = 0.041$, $r = -0.247$). In this situation, an increased level of depression and anxiety decreases the level of satisfaction with mental health, while a high level of boredom and the occurrence of sleep disorders lead to a decrease in the level of satisfaction with physical health.

Statistically significant relationships were identified between drug use and fear of COVID-19 infection during participation in practical activities at the faculty ($p = 0.016$, $r = -0.290$), fear of COVID-19 infection from patients encountered during internships ($p = 0.012$, $r = -0.299$), fear of COVID-19 infection from colleagues encountered during practical stages and lectures ($p = 0.006$, $r = -0.325$). In this case, fear of infection with COVID-19 decreased in students who used drugs. Also, alcohol consumption is correlated with sleeping disorders ($p = 0.038$, $r = -0.250$).

The variable "Courses are more interesting online" correlates positively with the level of satisfaction of students regarding the relationship with colleagues during the pandemic ($p < 0.001$, $r = 0.476$) in the sense that the more pleasant the relationships between colleagues are, the more interesting online courses become.

At the same time, the use of an electronic platform for teaching activities correlated positively with the idea that online activity is more interactive than that within the learning institution ($p = 0.001$, $r = 0.377$). The fact that online courses are more interesting correlated with an increased level of focus on new information in this environment, as opposed to laboratory attendance ($p <0.001$, $r = 0.692$) in the sense that an interesting online course leads to increased concentration.

The efficiency of online teaching positively correlated with the good functioning of the internet connection ($p = 0.038$, $r = 0.250$), in the sense that the better the internet connection, the more efficient the online teaching is. Also, the efficiency of online teaching ($p = 0.001$, $r = 0.389$) and the good training of teachers to use the online platform and the audio-video means necessary for the activity (p, 0.001, $r = 0.441$) correlated with the full follow-up of the online course / laboratory.

DISCUSSION

The duration of the social isolation and the predominant use of online education is still unpredictable. This research was conducted during the first semester of the second academic year in which the lockdown was imposed.

Even if 1/3 of students were suspected of being infected with COVID 19, only 2.9% of the total respondents were confirmed to have been positive. This aspect is

important because senior students have already achieved the standards of self-protection, they have great experience in working in clinics and they know how to manage their contact with patients, maintaining the required protocols regarding their hygiene and health. This result is encouraging because some studies already presented in specialty literature showed that medical dentistry presented high rates of infection among students due to poor knowledge and insufficient infection prevention. Ghai states that the current dental curriculum does not cover infection control adequately (especially from airborne pathogens). The author states that infection control education needs to be included in the dental curriculum worldwide. Academic teachers must provide adequate knowledge and attitudes related to infection control measures and teach students how to protect themselves and their patients, too (6).

The self-isolation was identified by a few studies published in the first six months of the pandemic period to be responsible for symptoms of depression, anxiety, and high levels of fear of infection. For students, concern about the delay in completing their graduation is an additional trigger for stress and depression, especially for those enrolled in their final year. This context can cause anxiety, poor sleep or short sleep durations, predisposing people to depression and post-traumatic stress disorders PTSD), as Tang *et al.* (7) identified in Chinese students (9.0% for depression and 2% for PTSD). In the present research, 10% of investigated students had frequent sleep and appetite disorders and 1/3 of them felt bored during the last months. The results are congruent with those presented by Emami (8) who pointed out that new perceptions must be incorporated into the new academic scenario, including students' wellbeing, feelings of loneliness, and familiar losses.

Poor physical and mental health negatively impact learning and academic achievement. From all respondents, we identified that only 18% of students were totally satisfied with their academic achievement during pandemic period. A great number of students felt bored, and some of them felt anxious and depressed. Tang *et al*. (7) showed that the psychological consequences of COVID-19 could be serious and psychological interventions are needed to reduce fear for the home-quarantined university students.

Quinn *et al.* (5) gathered data from dental faculties in Europe and identified that all dental schools reported access restrictions to academic buildings and provided online education. On the other hand, the academic risk for all students from clinical years is greater than those from preclinical years. Face-to-face practical classes containing interaction with real patients and simulation models is no longer a valid option for the acquisition of mental skills and manual skills for senior students. In Unites States, Deery (9) identified gaps in dental schools' preparedness for pandemics and this opinion is common for many countries in Europe, too. Also, theoretical, and practical examinations were redesigned but despite the reorganization and resilience of the academic staff, problems arise for both teachers and students: disruption of dental students' activity, interrupted access for students to dental faculty clinics; financial loss etc. (10)

Online tools are replacing face-to-face interaction, but our study revealed that senior students prefer more school-based courses than online ones. In a recent study,

Chang *et al.* (11), interviewed academic teachers from different dental schools and showed that the COVID-19 pandemic affected dental education a lot. It is true that intelligent technology surely has a lot of benefits for the learning process of dental education (12), but this is not enough for seniors' dexterity and practical achievement. In many schools, dental practices have closed or have been significantly reduced, while students were stressed about their grades and professional abilities. In a focus group conducted by Hasan & Bao (13) it was shown that students were afraid of "e-Learning crack-up" and "fear of academic year loss". The study proved that fear of academic year loss is the key responsible factor for psychological distress during COVID-19.

Strengths and limitations of the study

This study is the first one focusing on Romanian dentistry students' personal and academic life during pandemic period. Due to its entirely practical formation, the dentistry specialization needs an interactive and instructive formation, with the present research identifying the fact that dentistry students are very aware of the importance of practical stages and the level of knowledge for their future career. The second strength of the study is reflected by the investigated population: senior students (compared to freshmen or sophomores) are more prone to objectively appreciate the changes in the way of teaching and practicing.

The first limitation of the study is related to the low number of students involved in the research, limiting a stronger analysis of data considering some variables like (gender differences, rural/urban, accommodation). Because of this limitation, results cannot be generalized.

CONCLUSIONS

There is a great impact of the COVID-19 pandemic on dental education. The traditional model of dental education should be innovated to be suitable to different situations and must use diverse and intelligent technologies.

Academic staff should provide both educational and psychological support for senior students to adequately adjust to the new way of learning/teaching/being evaluated and to help them be professionally well-prepared for practicing during the next time frame of continuing pandemic.

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NEWS

IMPACT OF COVID-19 INFECTION AMONG LIVER TRANSPLANT RECIPIENTS

Transplant recipients compared to general population are deemed at a particularly high risk for developing COVID-19 severe infection due to their altered immunological status mainly caused by long term administration of immunosuppressants. Although data on COVID-19 infection among liver transplants are scarce and heterogenous and even though the results show a possible beneficial effect of immunosuppression regimen on COVID-19, the underlying mechanisms remain unclear and trends among transplant physicians are to reduce the immunosuppression dosage, especially of the antimetabolite drugs, carefully evaluating case by case in order to balance the risk of rejection which could potentially be more harmful than immunosuppression itself. (Parente A, Manzia Maria T, Angelico R, *et al.* COVID-19, liver transplant, and immunosuppression: Allies or foes? *Transpl Infect Dis* 2020; 00:e13417. https://doi.org/10.1111/tid.13417).

JOINT DISEASE AND ITS MEDICAL AND SOCIO-ECONOMIC IMPACT ON HEMOPHILIA CARE IN ROMANIA

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JOINT DISEASE AND ITS MEDICAL AND SOCIO-ECONOMIC IMPACT ON HEMOPHILIA CARE IN ROMANIA (Abstract): Hemophilia is a congenital, hereditary, rare bleeding disorder, which fortunately has nowadays an optimal therapeutical solution, namely a life-long replacement therapy (RT) with the missing coagulation factor VIII (hemophilia A) or IX (hemophilia B). Inappropriate treatment results in secondary morbidity, dominated by the chronic hem arthropathy, with high medical and socioeconomic burden. **Objectives**: As in our country, RT has been started only in 1997 and a real prophylactic replacement (PR) only in 2016/2017, we aimed at conducting a study of persons mainly with prophylactic RT and of those mainly with on-demand (OD) regimen for obtaining a comparative evaluation of the medical and socio-economic outcomes. **Material and methods:** This observational cross-sectional patient-reported outcome (PRO) survey included 122 patients with a severe form of the disease, willing to answer to a large questionnaire with 56 items focused on 4 domains: socio-demographic, medical, quality of health and life, and direct costs of care. **Results:** There have been assessed striking, statistically different results, concerning the markers which reveal clinical severity (annualized joint and non-joint bleeding rate, target joints, hem arthropathy), the need for chronic analgesic therapy and orthopedic interventions, frequency of deficiency, disability, handicap and social assistance in the group of persons with OD treatment *vs.* PR. The high costs related to surgical interventions and occurrence of inhibitors have been underlined, related to a secondary morbidity of hemophilia. **Conclusions:** The favorable effects of PR are evident also in our country; its high costs are counterbalanced by the deleterious health and quality of life results of OD treatment, and also by its high costs for the specific secondary morbidity, pleading in the favor of PR, in accordance with the principles of care in Europe. **Keywords:** PRO-SURVEY, HEMOPHILIA, HAEMARTHROPATHY, QUALITY OF LIFE, COSTS OF CARE.

Hemophilia is a congenital, monogenic, hereditary, X-linked disease belonging to the group of rare, often "orphan" disorders. It is an incurable disease, requiring a life-long replacement therapy with the deficient coagulation factor: VIII (hemophilia A - HA) or IX (hemophilia B - HB) (1, 2, 3). It is clinically defined by recurrent bleeding events, the most vulnerable site being the joints. Early introduced, adequate replacement therapy (RT) can prevent its crippling evolution, otherwise resulting in the most important disease-related secondary morbidity, the chronic hemarthropathy (3, 4).

In Romania, the RT with coagulation factors concentrate (CFC) has been introduced in 1997, the regular prophylactic replacement (RPR) with the missing coagulation factors, being really introduced only in 2016/2017 (5). As we accept that "we can't manage, what we don't measure" (Lord Kelvin, 1883), we decided to conduct a patient-reported outcome (PRO), model of survey for obtaining an insight into the real-life of persons with hemophilia (PwH) in our country, looking for a most reasonable, effective and affordable solution according to the European principle of hemophilia care (6).

STUDY DESIGN

It is a retrospective cross sectional observational study of persons with hemophilia (PwH) and its related von Willebrand disease (vWD), diagnosed and treated in 11 neighbor counties from the Western part of Romania; with an acceptance rate of 62.4%, there were 122 patients included in the study. We used a PRO model survey, based on a specific questionnaire administered by e-mail, relative to a time interval of 12 months (1st January - 31 December 2019). All participants expressed their informed consent, and the Institutional Ethics Committee approved its performance.

OBJECTIVES

Our study was focused on 2 subgroups of patients, the assignment being based on their therapy regimen; subgroup 1, mainly with prophylactic replacement (PR) and subgroup 2, with mainly on demand (OD) therapy, having 4 objectives:

- to assess the frequency and severity of joint disease in our PwH, comparatively in the two subgroups of patients,
- to evaluate the impact of joint disease on the health and Quality of life (QoL),
- to estimate the costs related to the joint disease and
- to compare their dimension, depending on the treatment regimen PR or OD replacement.

MATERIAL AND METHODS

The study population consisted of 122 patients with severe (115) and moderate with severe phenotype (7) of hemophilia A (92), hemophilia B (16), and severe form of vWD (14). The cohort was divided into 2 subgroups: subgroup 1, consisting of 39 patients born after 1997 with a history of RT since their early childhood and subgroup 2 of 83 patients born before 1997, the year when RT was introduced in our country, lacking this treatment for a period of time (tab. I).

The study was based on a comprehensive questionnaire, administered to the patients, consisting of 56 items, for recording appropriate information on 4 domains: socio-demographic - 9, medical - 31, quality of health and life - 10 and the direct costs of treatment - 6.

TABLE I

Characteristics of the patients in subgroups 1 and 2

Variables	Subgroup 1 (39)	Subgroup 2 (83)	p-value
Age (years) (X ± SD)	11.3 ± 6.51	39.7 ± 10.79	<0.001
BW (kg) (X ± SD)	38.87 ± 27.02	79.91 ± 17.98	<0.001
HA -moderate	1(2.56%)	4(4.82%)	0.1
-severe	25(64.10%)	62(74.70%)	<0.001
HB -moderate	1(2.56%)	1(1.21%)	-
-severe	7(17.95%)	7(8.43%)	-
vWD -severe	5(12.82%)	9(10.84%)	<0.01
Therapeutic regimen			
-OD	3 (7.69%)	33 (39.76%)	<0.001
-STP	6 (15.38%)	43 (51.81%)	<0.001
-RPR	30 (76.92%)	7 (8.43%)	<0.001
Age at start of replacement (years)	2.22 ± 1.88	18 ± 10.69	<0.01

BW-body weight, HA-hemophilia A, HB-hemophilia B, vWD-von Willebrand disease, RPR-regular prophylactic replacement, OD-on-demand, STP-short term prophylaxis

For the economic evaluation of direct medical costs, the following parameters were considered: specific medicines - coagulation factor concentrates (CFC) and bypassing agents (BPA), biological and imagistic assessments for diagnosis and monitoring, hospital admission, and ambulatory bleed-related activity. All data regarding the real costs were sourced from the administrative departments of the treating clinics and from publicly available data, expressing them in national currency exchanged in EUR at the present rate. For the evaluation of health quality much attention was dedicated to the impact of treatment on the main hemophilia - related secondary morbidity, the chronic hemarthropathy, an important driver of budget consumption; the surrogate markers for its severity like annualized bleeding rate (ABR), annualized joint bleeding rate (AJBR), target joints, need for invasive orthopedic intervention, life-threatening bleeds were used. In connection with quality of health, QoL was evaluated using generic instruments, largely used in hemophilia assessments: EQ-5D and VAS; they aimed at receiving an insight into 5 domains: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Additionally, the International Classification of Functioning (ICF) of WHO for the estimation of function and participation of patients, was used, in connection with some social consequences.

Statistical analysis was performed using *Microsoft Office Excel* 2013 and *SPSS 14.0* for Windows Evaluation Version, calculating the average and standard deviation with significance at $p<0.05$ and correlation coefficient, significant when $r > 0.4$.

RESULTS

This PRO survey brings to our attention the great burden for hemophilia of the secondary age-independent chronic hemarthropathy present in 69.88% *vs.* 20.51% of patients from subgroup 2 *vs.* 1, respectively, being in 69.88 % *vs.* 12.82% with multijoint involvement (tab. II).

ABR and AJBR were significantly higher in patients of subgroup 2, their values being 19.42-ABR and 19.75-AJBR, in

comparison with subgroup 1 ($p<0.001$). Target joint, meaning ≥4 bleeds in the same joint occurring in a period of 6 months, another surrogate marker of severity, was concordant with the AJBR, 55.42% *vs.* 17.95% and multi-articular in 40.96% *vs.* 7.69% in the subgroup 2 *vs.* subgroup 1, respectively.

TABLE II

Joint disease and its impact on health and quality of life

Variables	Subgroup 1 (39)	Subgroup 2 (83)	p value
ABR	4.76±11.20	19.75±17.36	<0.001
AJBR	2.41±7.21	19.42±16.78	<0.001
Patients with chronic hemarthropathy	8(20.51%)	58(69.88%)	<0.001
Polyarticular chronic hemarthropathy	5 (12.82%)	58 (69.88%)	<0.001
Patients with target joints	7(17.95%)	46(55.42%)	<0.001
Polyarticular target joints	3 (7.69%)	34(40.96%)	<0.001
Invasive intervention			
-orthopedic	0	9 (10.84%)	0.001
-non-orthopedic surgery	1 (2.56%)	4 (4.81%)	<0.01
Acute pain	5 (12.82%)	29 (34.94%)	<0.001
Chronic pain	13 (33.33%)	56 (67.47%)	<0.001
Use of analgesics	5(12.82 %)	35(42.17 %)	<0.001
Polypharmacy	0	10 (12.05%)	<0.001
AJBR-Age correlation	r=0.32 p=0.045	r=-0.03 p=0.85	-
AJBR-Target joints correlation	r=0.86 p<0.001	r=-0.04 p=0.80	-
AJBR-Chronic arthropathy correlation	r=0.77 p <0.001	r= 0.40 p=0.005	-

ABR-annualized bleeding rate, AJBR-annualized joint bleeding rate

In patients of subgroup 1 the frequency of hemarthrosis and AJBR were dependent on the age and it is evident the correlation between AJBR, target joints, and the development of the chronic arthropathy; in subgroup 2 the AJBR is not anymore related to target joints and age, remaining in correlation only with hemarthropathy (tab. II).

This depressing history of frequent joint bleedings was naturally accompanied by suffering, chronic pain affecting 67.4% of PwH of subgroup 2 *vs.* 33.3% in subgroup 1, requiring daily analgesic drugs in 42.17% and even polypharmacy (> 5 different drugs at the same time) in 12.05% in the same group of patients, respectively.

It needs to be underscored that joint disease was present in both subgroups of patients, as they all had a period in their life not covered by RT, but the worse outcome of PwH of subgroup 2 is connected with the late start of RT at the age of 18.10±10.0 *vs.* 2.22±1.83 years in subgroup 1 (tab. I).

Beside the locomotor deficiency, disability, and handicap, the pain was the most decisive reason for invasive orthopedic interventions. Out of 14 invasive interventions performed in the analyzed year (2019), 9 (75%) were orthopedic surgeries, mostly arthroplasty (hip - 3 and knee - 4), exclusively performed in patients from subgroup 2.

The impact on health and quality of life was decisive (tab. III). It was impressive in subgroup 2, with an EQ-VAS of 0.63 ± 0.14 *vs.* 0.84 ± 0.19 in subgroup 1, with the association of disability and handicap in 56.98%, with social assistance or sick retirement in

62.82% of the persons of the subgroup 2. Frequent absences from work and loss of productivity for not being active at work are also to be mentioned.

TABLE III

The impact on QoL and social condition

	Subgroup 1 (39)	Subgroup 2 (83)
Quality of life(EQ -VAS)	0.84 ± 0.19	0.63±0.14
ICF classification		
-deficiency	8 (20.5%)	34 (40.96%)
-disability	-	30 (36.14%)
-handicap	-	17 (20.84 %
Sick retirement	-	10 (12,82%)
Social assistance	2 (5.13%)	39 (50%)
Absence from school/ work		
-number of persons	5 (12.82%)	14 (16.87%)
-days/person/year	1.92	7.53

AJBR-annualized joint bleeding rate, EQ-VAS-EuroQol-visual analogue scale, ICF- International Classification of Functioning

All these outcomes were accompanied by the evident high medical and psychosocial burden for our PwH, who missed for years in their young age RT and are still receiving OD RT. From the standpoint of the costs, looking at the expenses of surgical interventions and the other direct costs, we realize the high values, which could have been avoided with an adequate RT (tab. IV). It is to underline that out of 14 patients who underwent invasive intervention in 2019 (the year of our study), 13 belong to subgroup 2, 9 of them having chronic hemarthropathy related orthopedic surgery. The distribution of the annual direct costs/patient, consisting of the expenses for medication, diagnosis and monitoring assays, medical devices, hospital stay, in those 9 patients who needed major orthopedic invasive interventions are presented in the fourth table.

TABLE IV

Distribution of average costs of orthopedic invasive interventions /patient/year (EUR)

PwH	Cost of investigations / monitoring	Cost of CFC	Cost of medical devices	Total cost/patient/ intervention
Average ±SD	568.04± 116.93	44,399.71±17,592.88	1,273.63±158.70	46,241.38

(PwH-persons with hemophilia, CFC-coagulation factor concentrates)

DISCUSSION

The recurrent bleedings have dominated over centuries the clinical image of hemophilia, (1, 4). The introduction of RT with CFC and BPA, since more than five decades ago, has succeeded to control the bleeding events and save lives, but without significantly changing the handicapping outcome of the bleeding-associated joint disease. Only the introduction of PR has brought a revolutionary change in hemophilia care, preventing and /or slowing down the progressive joint damage.

Today, in an era with a large landscape

of CFC, it has been accepted that prophylaxis is the standard of care (7, 8). This decision is based on controlled randomized prospective clinical trials which have proved that ABR and AJBR, as well as joint scores, are significantly improved by this approach.

The ESPRIT study conducted on children reported an ABR of 1.32 *vs.* 5.76 and an AJRB of 0.6 *vs.* 2.88 in the group with PR and that with OD therapy, respectively (9). Another clinical randomized study conducted on children under the age of 30 months, with subgroups assigned to receive prophylaxis (25 IU/kg each other day-EOD) or enhanced episodic OD therapy indicated an ABR of 3.27±6.24 *vs.* 17.69±9.25 and an AJBR of 0.63±1.35 *vs.* 4.89±3.57, undoubtedly better in the first group of patients with PR (10). Another study dedicated to children, comparing two types of prophylaxis regimens (EOD and pharmacokinetic–PK driven) with OD replacement, revealed that the ABR was 43.9±21.9 in the OD group in comparison with 1.6±1.2 with the EOD group and 1.9±11 in the group with PK-driven prophylaxis, respectively, both being significantly lower (11). The same good results are demonstrated for hemophilia B. In a multicenter randomized open-label study the results of RP *vs.* OD were significantly better ($p<0.001$) in both two subgroups of patients with hemophilia B, irrespective of the treatment regimen, 100 IU/kg once/ week or 50 IU x 2/kg /week (11). A comparable situation was found in adolescents and young adults in another randomized study: the AJBR value of 0.63±1.35 *vs.* 4.89±3.57 and the ABR of 3.27±6.24 *vs.* 7.69±9.25 in the group of prophylaxis and that of OD RT, respectively, impacting in a favorable manner also the joint health and the quality-of-life parameters (12).

The high effectiveness of RPR is doubtless, even with slight differences in the lower dosage regimen *vs.* OD. The comparative study between the Dutch and Swedish regimen, with the weekly dosage in IU/kg of 46 (34-55) *vs.* 88 (61-113) respectively, assessed a better AJBR, joint function, and EQ-5D in the higher dosage regimen (13). At the same time, it is worth to be mentioned that even in the situation of very low dosage (10-30 IU/kg/week) prophylaxis (ReCare trial) the ABR rates were in comparison to the baseline significantly lower, the best being the results of primary prophylaxis (14).

There are other studies, showing significantly improved results also even in the case of late initiation of tertiary prophylaxis. In the POTTER study undertaken in adults, stratified into two age groups (12-25 and 25-55 years), the introduction of PR revealed a dropping of AJBR from 16.8 to1.97 and from 16.71 to 2.46 in the two age groups, respectively (15). The delay of starting RP as primary prophylaxis is confirming the statement that "one (joint) bleed is too much", as it can initiate the vicious circle of synovium and endochondral damages. A comparative study performed in 4 countries (Sweden, United Kingdom, Ireland, and France) is highlighting the fact that early joint bleeding can impact the long-term outcome, leading to inferior results. Sweden, the first country which started early RP, has significantly better values for ABR, target joints, EQ-5D, missed days from work than other countries (16). Similar results come from the Canadian observational study performed on two age groups of adults (>18 years of age) PwH, younger and older than 40 years of age, one group which started

PR and the other remaining on OD therapy. In both age groups, the reduction of ABR was significantly better ($p<0.001$) than in the group of OD treated persons, but in the groups of OD treatment, there were no significant differences ($p = 0.86$) between PR and OD (17, 18). Striking differences are assessed also in the northern countries (Norway, Sweden), patients with OD in comparison with PR having a need for invasive intervention in 28.75 *vs.* 4.91, with a proportion of sick leave/early retirement of 36% *vs.*13%, respectively (19). Switching from PR to OD in late teens has also deleterious effects (20). All these studies (15-20) are motivating us to hope for a better future in our country for those who didn't experienced PR and to support the decision of maintaining it for PwH who benefited from it, in accordance with our national program, until the age of 18 years.

We believe that the threshold of reimbursement for PR in young adults or even older in our country would be affordable (21, 22). The limit of our study is connected to the short duration of our survey. The condition is to perform a cost-utility and cost-effectivity study on large horizon, including comprehensively all the direct and indirect costs; incremental cost-effectiveness ratio (ICER) calculated in these circumstances would be certainly in the range of acceptance for our country, like in other European countries (23-25).

CONCLUSIONS

The favorable effects of our short history of PR with CFC are evident in our country, as there are the deleterious effects in its absence, supporting the idea of prophylaxis in severe hemophilia in accordance with the principle of hemophilia care in Europe.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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THE WALKING THRU THE PAST AND THE PRESENT OF HIRSCHSPRUNG DISEASE

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THE WALKING THRU THE PAST AND THE PRESENT OF HIRSCHSPRUNG DISEASE (Abstract): Hirschsprung's disease or congenital megacolon is one of the differential diagnoses of chronic constipation mostly in infancy and may indeed represent a challenge for pediatricians, pediatric surgeons, and pediatric pathologists. The diagnosis relies clearly on the identification of the absence of ganglion cells at the plexuses (submucous and myentericus) of the bowel wall. Hirschsprung's disease) is usually located at the terminal (distal) rectum with potential pre-terminal or proximal extension to the less distal large bowel (sigmoid colon). Astonishingly, there is some evidence that Hindu surgeons of prehistoric India may have been exposed and had considerable knowledge about Hirschsprung's disease), but this disease is notoriously and eponymously named to Dr. Harald Hirschsprung (1830-1916), who brilliantly presented two infants with fatal constipation at the Berlin conference of the German Society of Pediatrics more than one century ago. Hirschsprung's disease has been originally called "Die Hirschsprungsche Krankheit"). More than 100 years following his meticulous and broad description, Hirschsprung's disease is still a puzzling disease for both diagnosis and treatment. Hirschsprung's disease remains a critical area of clinical pediatrics and pediatric surgery and an intense area of investigation for both molecular and developmental biologists. **Keywords:** DR. HARALD HIRSCHSPRUNG, HIRSCHSPRUNG'S DISEASE, CONGENITAL MEGACOLON, HISTORY.

Medical history is fascinating and, since its beginning, it was meant to enlighten scientific issues, booster the medical profession, and celebrate the traditions or tales of particular places. Harald Hirschsprung, who presented two infants with fatal constipation at the German Society of Pediatrics more than one century ago. Is known in medicine for his eponym of intestinal aganglionosis. Events running before and after Dr. Hirschsprung's discovery may drive interest in medical students and doctors to review the diagnostic procedures of this intriguing disease and recognize the enormous input given from microscopy and pathology to this diagnosis.

Hirschsprung disease, first described in detail by Harald Hirschsprung in 1886, is a congenital disorder characterized by the absence of ganglion cells in the myenteric and submucosal plexuses of the gastrointestinal tract, leading to functional bowel

obstruction. Despite its long history, the scientific community is still unraveling the complexities and intricacies that define this disease. As a condition that impacts thousands of newborns globally each year, Hirschsprung disease poses unique clinical challenges that necessitate a multidisciplinary approach involving pediatricians, gastroenterologists, surgeons, and geneticists, among others.

This article aims to walk through the historical landmarks that have shaped our understanding and management of Hirschsprung disease, from its initial identification to the modern era of molecular genetics and targeted therapies. It will explore the diagnostic and therapeutic advancements that have transformed patient outcomes and shed light on the current research frontiers that hold the promise of even better management strategies. Additionally, the article will scrutinize the existing gaps in our knowledge and identify areas where further research is crucial for more effective treatment and improved quality of life for affected individuals.

By bridging the past and present, this comprehensive review provides not only a historical perspective but also an up-to-date overview of Hirschsprung disease, thereby serving as a valuable resource for clinicians, researchers, and medical students alike.

MATERIALS AND METHODS

A comprehensive search strategy was devised to include articles from the earliest available records to September 2023. Keywords and phrases used in the search included but were not limited to: “Hirschsprung Disease”, “Congenital Aganglionic Megacolon”, “Colonic Aganglionosis”, “Ganglion Cells”, “Treatment”, “Diagnosis”, “Genetics”, and “Pathophysiology”.

The databases utilized for the literature search in this review include PubMed, Web of Science, and ScienceDirect. These databases were chosen for their wide scope, accessibility, and credibility within the scientific community.

The studies were selected based on the following *inclusion criteria*: Original research articles, reviews, case studies, and clinical trials focused on Hirschsprung disease / Articles published in peer-reviewed journals / Studies that contributed to the historical understanding or current advancements in diagnosis, treatment, and management of the disease.

The *exclusion criteria included* were: Articles not available in English / Duplicate studies or those that did not contribute new information / Papers lacking methodological rigor, as assessed by the authors.

The data was then organized into themes pertaining to the historical milestones, diagnostic methods, treatment options, and future research avenues in Hirschsprung disease. This thematic organization facilitated the narrative synthesis of existing literature and helped identify gaps for future research.

By adhering to this methodological framework, this review aims to present a balanced and comprehensive overview of Hirschsprung disease, tracing its historical roots and elucidating its present landscape to identify the most promising paths for future research and clinical practice.

BRIEF HISTORY

Young Harald Hirschsprung was born in 1830. He decided not to join the family’s tobacco company to study medicine. Medical studies would become an indirect reason for Hirschsprung’s name to gain immortality. For more than 100 years congen-

ital intestinal aganglionosis, whose macroscopic pathology he described in such detail, has been known worldwide as Hirschsprung's disease (1).

Harald Hirschsprung (1830-1916) was the founder of Danish pediatrics, and during his work as professor and chief physician of the Queen Louise Children's Hospital, he made the hospital a home of Danish pediatric research. Dr. Hirschsprung positioned Danish pediatrics internationally, had close relationships with foreign scientific societies and used to attend pediatrics congresses in Germany and Austria every year (fig. 1).

Fig. 1. Harald Hirschsprung (Source: https://ugeskriftet.dk/videnskab/harald-hirschsprung-og-den-kongenitte-aganglionose)

In 1886, Hirschsprung held the lecture "Stuhlträgheit Neugeborener in Folge von Dilatation und Hypertrophie des Colons" in Berlin at the annual congress of the German Society for Children's Diseases. He was referring to two cases of the disease that Hirschsprung had observed in 1880 and 1885 respectively at the Dronning Louises children's hospital. In the introduction to the lecture, he said: "I take the liberty to present to this erudite assembly two presentations of an unusual nature and at the same time briefly explain the associated medical histories (1)." The first presentation was described as follows: "As you can see, it is a large intestine, but of such a size that it will no doubt surprise you to learn that the intestine came from a child who died only at 11 months of age." In the presentation it was also said that the colon, in addition to being dilated, was also significantly thickened. The dilatation extended into the rectum which appeared normal. The child, who had been constipated since birth, had worsened at eight months of age when Hirschsprung first saw it. Constipation required frequent enemas, the abdomen became strongly prominent, and the child lost weight rapidly. Eleven months later it died. The second case described by Hirschsprung was similar (2).

In the following years Hirschsprung saw several similar cases of the disease and wrote about this condition in one of his works: "It is strange that a form of disease like this which nevertheless produces such prominent symptoms and is of such great importance for the patient is left completely unmentioned in the pediatric literature. However, given my experience, I cannot believe that the condition must really be that rare. But maybe it will happen so often that attention needs to be paid just to make the cases more frequent". This assumption turned out to be correct. In the following years, an increasing number of reports of these cases of the disease, which were already internationally referred to as Hirschsprung's disease, were mentioned.

Hirschsprung was convinced that the

disease was congenital, however, it was not until the late 1940s that basic aganglionosis in the rectosigmoid was detected and thus the basis for rational surgical treatment was simultaneously created (3).

Doctor Harald Hirschsprung

Dr. Hirschsprung, who graduated with a degree in medicine in 1855, showed since his first steps into medical career a strong interest in pediatrics, becoming astoundingly the chief physician of the only children's hospital in Copenhagen just fifteen years later. The appointment as professor of pediatrics came in 1877 and Dr. Hirschsprung became director of a larger children's hospital in Copenhagen from 1979 until 1904, when he retired. The new children's hospital was magnificently named after Denmark's Queen Louise (The Queen Louise Hospital for Children). Dr. Hirschsprung was fond of anatomical variations, peculiar aspects of medicine, and intriguing diseases. He was a brilliant teacher with loyal and faithful students. Between 1880 and 1885, Hirschsprung came across two infants with similar clinical presentation. The first child had bowel problems that persisted soon after birth with absence of spontaneous bowel movements. Daily enemas and laxatives were necessary. The second case had similar bowel distensions with terminal bouts of diarrhea variably alternating with impossibility to evacuate. Notwithstanding the scarcity of information continuous therapy was applied, but, unfortunately, both children eventually died and a post-mortem examination was performed. At the autopsy, the rectum was narrowed, but there were striking dilation of the bowel loops with some ulceration of the mucosa associated with thickening of the bowel wall. In his publication, Dr. Hirschsprung summarized his findings and drew attention to the typical clinical presentation of both cases. In an opinion, which is worldwide shared, his meticulous and comprehensive description is not the first one to indicate details of this disease, but he provided a complete and excellent account of this entity in consideration of his clinical experience and practice in pediatrics. The name "Hirschsprung's disease" came into widespread use shortly before the end of the 20th century and, in 1916, Hirschsprung himself added an additional 10 more cases before his death (2).

Pre-Hirschsprung Era

The examination of the scientific literature seems indicate that approximately 20 similar cases have been recorded between 1825 and 1888. However, there is evidence that Hindu surgeons of prehistoric India had considerable knowledge about Hirschsprung's disease. Sushruta's description of a disease called Baddha Gudodaram is extraordinarily analogous to that of Hirschsprung's disease. Semantically, it seems indicate "abdominal distension due to blocked rectum".

According to Sushruta, Baddha Gudodaram is a type of disease caused by (functional) blockage of the ano-rectal canal. The affected child or, even, young adult may show rectum and distal colon stuffed with gas, "stones" (fecaliths), "hair" (undigested fibers), and, obviously, feces. In the child, there is an abdominal distension, which is characteristically seen between the heart and the umbilicus. Scanty stools are evacuated with greatest difficulty, and, eventually, it was reported that the patient might vomit feculent fluid. However, the pre-Hirschsprung era is not complete, if additional reports are not also mentioned.

The Sushruta Samhita is an ancient volume of Ayurvedic surgery compiled by Sushruta (circa 1200-600 BC). Passages of interest were identified by browsing through the authentic English translation of the old compendium. The accuracy of the translation was verified by comparing it with the original Sanskrit verses with the help of a Sanskrit scholar. A condition called Baddha Gudodaram, described in the Samhita, closely resembles Hirschsprung's disease. There are indications that the ancient Indians even deciphered the etiology as defective vayu alias vata (2).

Although the disease was considered incurable, a palliative operation was discussed.

Descriptive details of the operation match those of sigmoid colostomy. Evidence from the Sushruta Samhita indicates that Hindu surgeons in prehistoric India probably had considerable knowledge of Hirschsprung's disease. Further research corroborating other sources of evidence is needed to confirm this claim (4).

Indeed, Fredericus Ruysch, a Dutch anatomist in Amsterdam (Netherland) in 1691 described a 5-year-old girl with abdominal pain. It seems that the usual treatment to relieve pain was permanently ineffi-cacious and the child continued to pass flatulence. No evacuation was practically possible and the child ultimately died (fig. 2).

Fig. 2. A historical overview of the discovery of Hirschsprung's disease (Source: http://old.semmelweis.hu/wp-content/phd/phd_live/vedes/export/ baradcsillamaria.d.pdf)

Domenico Battini, an Italian physician of the 19th century described a child that he followed up for 10 years with severe constipation. The child died and the autopsy demonstrated severe dilatation of the colon. The published Italian contribution showed

not only a very careful clinical evolution of the child, but also provided a detailed autopsy with examination of the abdominal viscera. Particular mention to the morphological alterations occurred in the large bowel were present in the original Italian publication. Through the examination of the reports, Fiori concludes that Dr. Battini, nearly one century before Hirschsprung, may have originally achieved its target in reporting an archetypal case of megacolon of congenital type. Distinctively, a number of characteristic features, including familiarity and the peculiar selective involvement of "neural layers" of the bowel, that later became characteristic of Hirschsprung's disease, were remarkably well postulated by Dr. Domenico Battini. To increase the controversy between Denmark and Italy as well as other countries, there may be a few more reports that need to be listed (5).

In 1836, Ebers reported a 17-year-old boy with a history of constipation since birth or, perhaps, as toddler, while Jacobi had also described two neonates with intestinal obstruction in 1869. Fragmentary reports of children who died of severe constipation appeared also in the literature in the pre-Hirschsprung era. In fact, Gee reported the autopsy findings of a 4-year-old child with a "spasm" of the sigmoid colon without involving of the rectum in 1884, while Bristowe described the outcome of an 8-year-old girl who died of mechanic ileus after longstanding severe constipation of the bowel (6).

Dr. Hirschsprung died on April 11, 1916, but his supreme legacy to clinical pediatrics, pediatric surgery, and pediatric pathology is unmatched with contributions that go further than severe constipation in children or the disease harboring his name ("Hirschsprungsche Krankheit") (fig. 3).

Fig. 3. A typical case handled by Hirschsprung in his hospital (Source: https://revista.svhm.org.ve/ediciones/2008/1-2/art-3/)

The Danish pediatrician observed and described in detail several diseases, including pyloric stenosis and intussusception. Dr. Hirschsprung indicated guidelines for management of a broad spectrum of pediatric diseases, including contributions in the area of teratology and clinical dysmorphology. These contributions are now part of the didactic activity and scientific investigation of innumerable professors and researchers of Pediatrics, Pediatric Surgery, and Pediatric Pathology. He participated actively to the building of the children's hospital and his dedication to children constant. Queen Louise wanted the wall spaces above doors to harbor biblical quotations for the edification of the sick children, but Dr. Hirschsprung considered more appro-

priate to give the children an environment that could bring some quietness. In consideration of the sensibility of the children, he firmly refused the holy quotations and suggested colorful and beautiful wall decorations of animals and flowers, which in the end were well accepted by the Danish crown to be inserted in the wall spaces above doors (7).

The Post-Hirschsprung Era

Recently, the interest has focused on the diagnosis of Hirschsprung's disease mainly because of the jeopardy of methods used to make the diagnosis and their accuracy. Being a genetic disease quite diverse, main topics on Hirschsprung's disease involve, indeed, the investigation of the best diagnostic marker for Hirschsprung's disease and changes that may take place during ganglion cell maturation. Hirschsprung's disease is now known as the most common cause of neonatal lower intestinal obstruction occurring in 1:5000 live birth newborns. Hirschsprung's disease involves in about three quarters of cases male children, and its incidence is variable according to ethnics (8). The caudal migration of the primordial neural crest cells starts at the upper end of the gut following progressively the vagal fibers distally. A delay or arrest in this migration induces failure of the neural crest cells to reach the distal bowel with consequent congenital abnormal nerve innervation of the bowel. There is a caudocranial severity, which means from the internal anal sphincter extending proximally for a variable length of gut. Pathophysiological, there is a proximal intestine, which is dilated and progresses to an abrupt or, alternatively, gradual transition to a normal calibrated distal bowel. This distal intestinal segment shows typically a funnel like or cone shaped zone in between (the so-called "transition zone"). Moreover, there is a proximal muscle hypertrophy. This anatomic and prominent feature of the colon, located proximal to the aganglionic segment, represents undoubtedly an effort to overcome the partial obstruction. The bowel becomes distended with thickening of its wall, and the degree of dilatation and hypertrophy depends intricately upon both the time and degree of obstruction and obviously, indirectly, to the age of the patient. Clinical presentation settings include failure to pass meconium within the 24-hrs. of life considering that 98% of newborns pass meconium in less than 24-hrs. of age, neonatal intestinal obstruction syndrome (abdominal distension, refusal to feed, and vomiting of bilious type), and recurrent enterocolitis (mainly infants less than 3 months of life), toxic megacolon, spontaneous perforation, and chronic constipation with persistent failure to thrive. Toxic megacolon includes fever, abdominal distension, bile-stained vomiting, explosive diarrhea, dehydration, and shock. History includes failure to pass meconium, painless abdominal distension, and, obviously, constipation. Physical examinations of children with Hirschsprung's disease includes enlarged abdominal circumference with numerous fecal masses (9). Digital or post-evacuation examinations reveal hypertonic anal sphincter, typical empty rectum, and hard fecal mass. Radiology (plain abdominal X-ray both erect and supine as well as contrast enema) typically shows narrow distal segment, funnel-shaped dilatation characteristically localized at level of transition zone as well as marked dilatation of the proximal colon. Moreover, a poor emptying of barium throughout the colon in 24 hours delayed films is also found. A differential diagnosis of "psychogenic" stool is requested and, in this latter case,

the barium generally collects in the distal recto-sigmoid colon. Electro manometry shows absence of the recto-anal inhibitory reflex (RAIR) when the rectum is distended. RAIR is defined as the reflex of relaxation of the internal anal sphincter following rectal distension (balloon). RAIR (+) means normal, while lack of RAIR, RAIR (-), means Hirschsprung's disease. Bedside or outpatient procedures seem to give no complications (10). The test is unreliable if the gestational age plus post-natal age is less than 39 weeks and birth weight is less than 2.7 kg. However, if, at neonatal age, electro manometry is useless, it represents a good screening tool in infancy and childhood. Ultrasonography is important to rule out associated anomalies and a genetic counselling may be considered appropriate according to the familiarity and the phenotype of the patients affected with Hirschsprung's disease. Rectal biopsy is the definitive diagnostic test showing absence of ganglion cells, presence of nerve hypertrophy, and increased acetyl-cholinesterase activity. It may include either a suction mucosal biopsy (at different levels) or a full thickness biopsy. Suction, transmural, and jumbo biopsies are the usual biopsies taken in an infant with severe constipation. It is a general opinion that pediatricians play a major role in diagnosing Hirschsprung's disease and dysmorphic features remain important landmarks that need to be identified first by clinical pediatricians and, later discussed with clinical geneticists. Typically, Hirschsprung's disease is clinically identified as a solitary gastro-intestinal anomaly in a full term, otherwise healthy newborn or infant, but associated anomalies do occur in about 1/5 of cases, including uro-genital system (11%), cardio-vascular system (6%), gastro-intestinal system (6%), and other systemic congenital defects (8%). In as many as 1/10 of children with Hirschsprung's disease, the condition of prematurity has been reported. Trisomy 21 syndrome (Down syndrome) occurs in approximately 1/20 of children with Hirschsprung's disease. The work of the pathologists is impressive and their criteria for diagnosing Hirschsprung's disease are quite straightforward and easy to follow in classic cases. It is important to highlight that not all-severe constipation mean automatically Hirschsprung's disease. Both the lack of ganglion cells and the hypertrophy of nerve fibers are pathologic landmarks of Hirschsprung's disease (8).

The diagnosis and surgical treatments of Hirschsprung's disease have undergone various changes in the last few decades because of establishment of laparoscopic procedures. A nationwide retrospective study conducted in Japan he used the patient data were collected in 4 phases: Group 1, between 1978 and 1982; Group 2, between 1988 and 1992; Group 3, between 1998 and 2002; and Group 4, between 2008 and 2012. The results of the study were that primary operations without laparotomy, including TAEPT and laparoscopy-assisted operations, have become the first choice for the definitive surgical treatment of HD in Japan. The mortality rate has decreased over time. However, the mortality rate of small intestinal aganglionitis is still relatively high. The development of new treatment strategy for small intestinal aganglionitis is called for (11).

In the year 2022 a new surgical procedure successfully addresses a common lingering challenge - incontinence - faced by some families with young children who have undergone surgery for Hirschsprung disease. This first-of-its kind procedure was pioneered by surgeons in the Division of Colorectal and Pelvic Reconstruction at

Children's National Hospital, led by Division Chief Marc Levitt, M.D. The new pilot study shows long-term outcomes for seven patients between the ages of 2 and 18 who underwent the novel technical approach called sphincter reconstruction. Each of the seven were diagnosed with an anatomic or physical issue contributing to their incontinence. It was during a repair procedure for the first patient that Dr. Levitt and his team had the idea of a technique for sphincter reconstruction. After that, six more patients with similar anatomy were offered the procedure.

The study regarding transcriptional Profiling, Functional Analysis, and Organoid Modeling of Intestinal Mucosa in Hirschsprung Disease it had as a conclusion that despite normal ganglionic structure, the section of colon adjacent to the aganglionic region in Hirschsprung disease and patients has perturbed gene expression which resembles the aganglionic segment. Transcriptional and functional changes in colonic epithelium are persevered in the ganglionic colon used for pull-through surgery. This may explain persistence of enterocolitis despite surgical excision of aganglionic colon and subsequent endorectal pull-through performed with aganglionic colon during correction of Hirschsprung disease (12).

Current evidence suggests that transplantation of enteric nervous system stem cells, sourced from easily accessible postnatal gut, could be a viable alternative treatment for Hirschsprung disease and ultimately for a number of other congenital or acquired enteric nervous system disorders.

Hirschsprung disease (HSCR) is a congenital anomaly of the colon that results from failure of enteric nervous system formation, leading to a constricted dysfunctional segment of the colon with variable lengths, and necessitating surgical intervention. The underlying pathophysiology includes a defect in neural crest cells migration, proliferation and differentiation, which are partially explained by identified genetic and epigenetic alterations. Despite the high success rate of the curative surgeries, they are associated with significant adverse outcomes such as enterocolitis, fecal soiling, and chronic constipation. In addition, some patients suffer from extensive lethal variants of the disease, all of which justify the need for an alternative cure. During the last 5 years, there has been considerable progress in HSCR stem cell-based therapy research. However, many major issues remain unsolved. This review will provide concise background information on HSCR, outline the future approaches of stem cell-based HSCR therapy, review recent key publications, discuss technical and ethical challenges the field faces prior to clinical translation, and tackle such challenges by proposing solutions and evaluating existing approaches to progress further.

CONCLUSIONS AND FUTURE DIRECTIONS

Since the clinical presentations by Harald Hirschsprung in Berlin in 1886, the condition that bears his name has had a rich history. The seminal events that influenced progress in the understanding and management of this complex congenital disorder have been briefly covered in this historical review. More than 100 years ago, the condition was considered incurable and uniformly fatal over time (12, 13). Mortality rates continued to be high in the 1940s (70%) and remained high even in the 1970s (25%). By the 1990s, more than 90% of patients survived (14).

Currently, the survival in most ad-

vanced medical environments is greater than 95%, excluding cases with chromosomal disorders or advanced comorbidities. While mortality has improved, there remains much to be learned. Why some patients with HSCR do poorly following operative repair remains an enigma. Similarly, the proper management of many patients with variants of HSCR needs to be more clearly elucidated. Continuing studies of the ENS and the molecular genetics of these conditions may shed further light on these issues and provide a better understanding of the choice of management in the future for affected children. Recent studies have transplanted human enteric neural progenitors into the mouse colon and shown engraftment (15). Currently, researchers are working to develop novel stem cell therapies, whereby stem cells could be transplanted into the aganglionic segment of bowel to replace the missing ENS.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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THE VALUE OF IMAGING INVESTIGATION IN DESIGNING OF AN EXPERIMENTAL STUDY OF BONE TISSUE DYNAMICS IN ORTHODONTIC MINI-IMPLANTS

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A SHORT HISTORY OF THE HISTOLOGICAL PROCESSING TECHNIQUES FOR TOOTH AND SUPPORTING HARD TISSUES (Abstract): **Background and aim**: Mini-implants play an important role in orthodontic treatment. However, the mechanism of bone remodeling associated with these orthodontic devices is not yet fully elucidated. The research focusing on the dynamics of biological changes at the implant site frequently involves experimental animal models. Our study aims to evaluate, by imaging methods, the success rate of implantation process of orthodontic mini-implants, using an original experimental model for the study of tissue biological responses present at the bone – mini-implant interface. **Materials and methods**: The study was conducted on adult male Sprague Dawley rats divided into 9 groups of 6 rats (G1 – G9) in which two mini-implants were surgically implanted in the right tibia. Two rats in each group also acted as controls by inserting a mini-implant in the left tibia without force. After implantation, traction forces were applied between the two mini-implants with closed NiTi helical orthodontic springs. Springs were extended at 2.2 cm, generating a force of 100 gf (G1, G4, G7); at 1.4 cm, the force generated was 75 gf (G2, G5, G8); at 1.2 cm, the force generated was 50 gf (G3, G6, G9). Animals were sacrificed at 7 days (G1, G2, G3), 21 days (G4, G5, G6), and 42 days (G7, G8, G9) from implantation. On the day of harvesting, each rat was examined radiologically by Spectral AMI HTX, Spectral Instruments. **Results**: The overall mini-implant success rate was 94.1%. In 5.9% of the subjects, either distal implant fracture with spring detensioning or distal implant detachment was observed. In control mini-implants, without spring, the overall success rate was 100%. **Conclusions:** Imaging exams are valuable tools for analyzing bone remodeling, density, and in vivo structure of bone tissue. Our study confirms the effectiveness of imaging examination in verifying the stability of the implantation stage, as a mandatory step in the subsequent evaluation of morphological changes in peri-implant bone tissue.
Keywords: MINI-IMPLANT, EXPERIMENTAL STUDY, BONE TUSSUE, RADIOLOGIC EXAM, SUCCES RATE.

INTRODUCTION

Advances in orthodontic practice are directly related to basic and clinical research focused on oral tissue morphology and craniofacial biomechanics (1-3). The basic principle of orthodontic treatment consists in the application of directed orthodontic forces to the teeth, resulting in concomitant remodeling of periodontal ligament and alveolar bone. The review of the literature underwrites several aspects of tissue transformation in the presence of force application (4). Another line of orthodontic research concerns the relationship between bone resorption associated with orthodontic movement and systemic medication (5).

Mini-implants play an important role in orthodontic treatment. The use of mini-implants is based on the development of implantation techniques because of the optimization of dental procedures, imaging studies of the oro-dental region and implant characteristics (design, biocompatibility). Mini-implants are an increasingly used treatment option, mainly motivated by the significant potential to shorten treatment (6). Through their action, they provide skeletal anchorage and prevent unwanted tooth movement. A major advantage is that no healing period is required after implantation, allowing immediate loading (7).

Although their use is on an upward trend and the clinical benefits are evident, the mechanism of bone remodeling associated with these orthodontic devices is not yet fully elucidated (4). This is mainly due to the difficulty in assessing the dynamics of positive and negative biological changes at the implant site. Consequently, translating the analysis of changes in the anatomical structures involved from the clinical, macroscopic level to the cellular and molecular level is a challenge for orthodontic research (8). For this purpose, specialist groups in the field are constantly using in vitro models and systems.

However, there are also opinions advocating the use of experimental animal models, which are superior as a possibility to test the efficacy and safety of application procedures - although the difficulties of extrapolating results to humans must be considered (9). In experimental studies, to complete the overall picture of bone tissue dynamics at the implant site, advanced imaging techniques can provide important information about the bone remodeling and evolution of treatment process. Nevertheless, the bone response is individualized, depending on several specific factors. Therefore, the design of an experimental study gains, in the relationship between methodology complexity and results consistency, by including an imaging assessment step.

Within this general context of knowledge, our study aims to evaluate, by imaging methods, the success rate of implantation process of orthodontic mini-implants, using an original experimental model for the study of tissue biological responses present at the bone – mini-implant interface.

MATERIALS AND METHODS

The study was conducted on 28-week-old adult male Sprague Dawley rats weighing between 340 g and 450 g, provided by the National Institute for Medical and Military Research and Development “Cantacuzino” (Bucharest, Romania). The ethical aspects of the research were strictly respected in accordance with the European Directive 2010/63/EU on the Protection of Animals used for Scientific Purposes, the European Convention for the Protection of Vertebrate Animals used for Experimental

and Scientific Purposes (Council of Europe No. 123, Strasbourg, 1985), and the guidelines established by the National Animal Welfare Authority (approval no. 64/12.09.2023). The study protocol was approved by the University's Research Ethics Committee (approval no. 59/ 23.03.2021).

The experiment was conducted over a period of 6 weeks. The experimental animals were divided into 9 groups of 6 rats (G1 – G9) in which two mini-implants were surgically implanted in the right tibia. Two rats in each group also acted as controls by inserting a mini-implant in the left tibia without force.

The type of mini-implant used was Dual Top Anchor System screws, diameter 1.3 mm, and length 5 mm, made of titanium alloy. After implantation, traction forces were applied between the two mini-implants with closed NiTi helical orthodontic springs, size: 0.008 x 6 mm, this type of springs generating continuous forces.

The forces were determined using the Sauter Digital Force Gauge FH 10, and the resulting distances were 1.2 cm, 1.4 cm, 2.2 cm. Thus, the spring extended to a distance of 1.2 cm generated a force of 50 gf on the mini-implant; at a distance of 1.4 cm, the force generated was 75 gf; at a distance of 2.2 cm, the force generated was 100 gf. Groups G1, G4, G7 were loaded with 100 gf, groups G2, G5, G8 with 75 gf, and groups G3, G6, G9 with 50 gf.

The animals were sacrificed as follows: after 7 days from implantation for groups G1, G2, G3, at 21 days - for groups G4, G5, G6, and after 42 days - groups G7, G8, G9.

On the day of harvesting, each rat was examined radiologically. Radiological exam was performed using Spectral AMI HTX, Spectral Instruments, with the animals in lateral or dorsal recumbence. A medio-lateral or cranio-caudal radiograph of the hind limb was taken for each subject, focusing on the tibial region.

RESULTS

In G1, two subjects showed a misalignment between the two implants, with a normal position of the spring. All subjects had stable implants and no bone lesions were visible.

In G2 only one subject showed misalignment of the two implants, with a straight position of the spring. None of the subjects showed dislodgement of the implant or visible bone lesions.

In G3, only one subject revealed misalignment of between the two implants, with a normal position of the spring. There were no displacement or bone lesions visible in any of the subjects.

In G4 all subjects revealed stable implants with normal position of the springs. No bone lesions were detected in any subject.

In G5, only one subject showed misalignment of the two implants (fig. 1A), with a normal position of the spring. The implants were stable in all subjects and no bone lesions were detected on radiography.

In G6, all implants were stable except one subject. In one subject the distal implant was dislodged, and the spring was unstressed and curved (fig. 1B).

In G7, all subjects except one subject had a correct position of the implant without visible bone lesions (fig. 1C). The affected subject revealed a tibial fracture with displacement at the insertion point of the distal implant (fig. 1D).

In G8, in one subject the implants were not aligned in the same plane however, but the spring was not rotated, and the implants

were stable. There were no abnormalities found in the rest of the subjects.

In G9, a mild misalignment between the implants was detected by radiography, with a normal position of the spring in two subjects. In one patient, a tibial fracture with displacement at the point of insertion of the distal implant was visible.

The overall mini-implant success rate was 94.1%. In 5.9% of subjects, either distal implant fracture with spring detensioning or distal implant detachment was observed. In control mini-implants, without spring, the overall success rate was 100%.

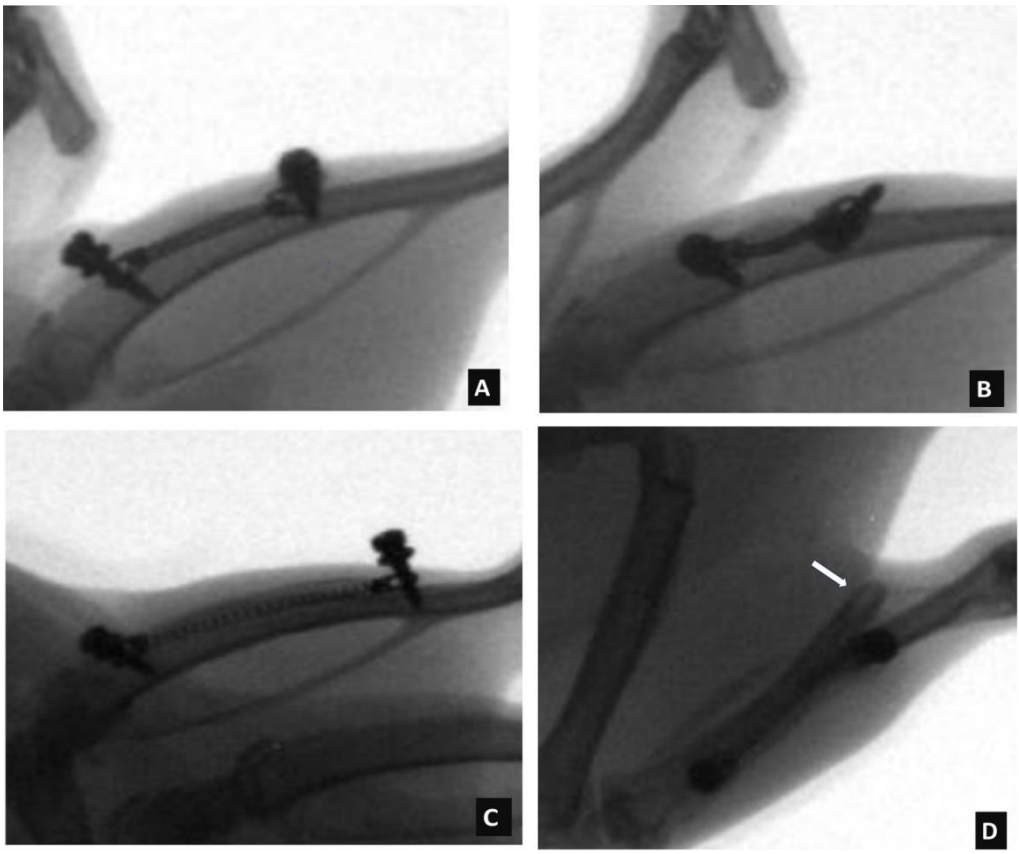

Fig. 1. Radiological images of the hind limb in medio-lateral view in subjects with implants: A. Incorrect alignment of the two implants, but with normal arch position;
B. Distal implant dislocation and arch de;
C. Tibia with implants in normal position and arch under tension;
D. Tibia fracture with displacement, at the distal implant focus;

DISCUSSION

In experimental studies aimed at evaluating orthodontic mini-implants, the use of radiological investigations is relevant both to provide complementary information to other types of examinations (e.g. histological or histomorphometric examinations) and as a single method of analysis (10).

An eloquent example of the value of radiographic examination is the study conducted by the team coordinated by Dhaliwal, which aimed to investigate the osseointegration of mini-implants using histomorphometric analysis. However, after mini-implant placement and suturing, the authors performed radiographs to confirm proper placement of the mini-implants and to observe any bone fractures in the rabbit tibia (10).

Micro-computed tomography (micro-CT) is indicated for further experimental research on the efficacy of mini-implants, as the information on the morphology, structure and density of peri-implant bone tissue is superior to other types of radiological investigations. An experimental study aimed at identifying mini-implant movements in bone and observing the bone remodeling pattern proposes a new experimental animal model, in which mini-implants are inserted into vertebrae, in rats (11). Using micro-CT, the authors recorded volumetric images from the same animal at different times, thus accumulating information on the movements of the mini-implants under the action of orthodontic forces and the changes occurring in the bone structure (11). The results revealed that the mini-implant movements are accompanied by a thickening of the bone, especially in the direction of force loading, which led to a decrease in the mini-implant movement during the experiment (11).

Imaging assessment can also be performed using cone beam computed tomography (CBCT). Recent research in an experimental canine model investigated the role of orthodontic mini-implant placement at different times after lateral incisor extraction (12). The study examined the development of alveolar ridge height, width, and density of the maxillary bone by histological, histomorphometric and imaging analysis. CBCT results showed that mini-implants placed immediately after extraction retained greater alveolar ridge height than mini-implants placed at 6 weeks or in the absence of implant placement (12). Bone density was higher with implants, regardless of when they were placed, compared to bone density in the post-extraction zone without implants (12). Insertion of mini-implants had a positive outcome, preserving post-extraction socket height, increasing bone density and stimulating new bone formation compared to the control area (12).

Not only bone remodeling, bone density or verification of the position of the mini-implant in the bone has been analyzed with radiological studies. Another line of research aimed to assess stability according to the insertion angle of the mini-implant (13). Micro-CT examination of the bone-implant contact area and correlation with the pull-out test showed that stability is influenced by the amount of bone tissue around the mini-implant (13). The obtained data indicated that a maximum amount of bone, i.e. increased stability, can be achieved by inserting the mini-implant at angles between 50° and 70° (1).

The analysis of our results suggests that post-implant fractures, as well as implant dislocation and arch stress relief, occurred because of external factors – namely manipulation or trauma. This assumption is supported by the fact that in most animals the implants remained stable throughout the experiment, without inducing bone damage or dislocation of the implanted system. In addition, all subjects with complications showed a fracture focus at the distal point of implant insertion, an area

where the tibial bone diameter is smaller – which may be explained by reduced bone strength rather than secondary to post-implant osteolysis-type injuries.

CONCLUSIONS

Experimental orthodontic research has the major objective of optimizing orthodontic treatments and increasing the success rate. Imaging exams are valuable tools for analyzing bone remodeling, density, and in vivo structure of bone tissue. Their use, in parallel with histological examination, allows a reduction in the number of animals that will ultimately be sacrificed. Our study confirms the effectiveness of imaging examination in verifying the stability of the implantation stage, as a mandatory step in the subsequent evaluation of morphological changes in peri-implant bone tissue.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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RESEARCH ON THE BIDIRECTIONAL RELATIONSHIP BETWEEN PERIODONTAL DISEASE AND HIGH BLOOD PRESSURE

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RESEARCH ON THE BIDIRECTIONAL RELATIONSHIP BETWEEN PERIODONTAL DISEASE AND HIGH BLOOD PRESSURE (Abstract): As the importance of the connection between periodontal disease and cardiovascular disease becomes increasingly prominent in medical practice, it is essential to explore this relationship and its implications for patients. The **aim** of this research was to investigate the impact of periodontal disease on the development and progression of cardiovascular disease and to establish that periodontal disease represents a relevant risk factor for the occurrence of cardiac pathologies. **Materials and methods:** A retrospective study was carried out, including 138 consecutive patients who addressed the Dental Education Clinical Base, between November 2021 and November 2023. The patients included in the study were divided into two groups, a group composed of 74 patients (group I: n=74) with periodontitis and a group of 64 patients without periodontitis (group II: n=64). **Results:** Research has revealed that the prevalence and severity of periodontal disease are higher in individuals with hypertension compared to those with normal blood pressure levels. Conclusions: our results argue once again for the assessment of the bidirectional correlation between periodontal disease and hypertension. **Keywords:** PERIODONTAL DISEASE, PERIODONTITIS, HIGH BLOOD PRESSURE, HYPERTENSION, BIOFILM, ORAL MICROBIOTA.

Periodontal disease is considered a chronic bacterial infection characterized by persistent inflammation, degradation of connective tissue, and destruction of alveolar bone (1). It includes gingivitis (where inflammation is limited to the gums and is reversible if the patient maintains good oral hygiene) and periodontitis (a bacterial infection characterized by a destructive inflammatory process due to the action of bacteria and their products, which extends and leads to tissue destruction and alveolar bone resorption) (2, 3).

This condition is primarily induced by

bacteria that form biofilms in the periodontium, leading to inflammatory changes in the tissues that support dental units (3, 4). Diagnosing it is complex and is based on clinical examination, the patient's age at the time of diagnosis, the rate of disease progression, as well as systemic and local factors (5). The global prevalence of periodontal diseases is substantial, affecting a significant portion of the population (6). It is considered a public health issue due to its high prevalence worldwide (7).

From a clinician's perspective, defining periodontal disease is fundamental for understanding and addressing this complex pathology. Periodontitis is a slowly progressive disease and tissue destruction is largely irreversible. In the early stages, the disease is usually asymptomatic, but many patients become aware of it when the condition has progressed far enough to result in tooth mobility. Deep pockets occur as a result of further destruction of the fibers of the periodontal ligament (called loss of attachment) and resorption of the alveolar bone that occurs in parallel with the loss of attachment progress.

These diseases are set up by a chronic inflammatory process, starting with bacterial biofilm forming on tooth surfaces, followed by an inflammatory reaction of the host tissues. The complex interplay between pathogenic microorganisms, host immune response and environmental factors leads to progressive tissue and bone changes and damage that can affect oral and general health (8).

Advanced periodontal inflammation is characterized by gingival erythema and edema, gingival bleeding and recession, tooth mobility, pathological malpositioning of teeth, suppuration of periodontal pockets and tooth loss. The condition is very common, with a frequency of 10-15% in adults in most populations studied (9). Moderate periodontitis is even more common, affecting 40-60% of adults, and severe periodontitis has been identified with the sixth highest prevalence of the disease worldwide (10).

In the detailed evaluation of periodontal disease, it is essential to investigate the typologies and stages of this condition for a proper understanding of the mechanisms involved and the development of appropriate therapeutic strategies (11).

Studies show that periodontitis is a chronic inflammatory condition of the periodontal tissue with a multifactorial etiology, caused, among other factors, by the disruption of the oral biofilm. Key pathogens such as *Aggregatibacter actinomycetemcomitans*, *Porphyromonas gingivalis*, *Fusobacterium nucleatum*, *Prevotella intermedia* and *Tannerella forsythia* (12, 13) can disrupt the oral microbiota, inducing dysbiosis and chronic inflammation of the periodontium. These elements are essential in understanding the complex interactions involved in periodontal disease.

Consequently, a detailed understanding of the various types and stages of periodontal disease is fundamental for clinical and therapeutic approaches, highlighting the complexity of this condition and the necessity for personalized interventions (14, 15). At the EuroPerio's eighth edition in 2019, the Congress of the European Federation of Periodontology, a new classification of periodontal pathology was developed (16).

This classification adopted a staging and grading system for the diagnosis of periodontitis, which, on one hand, records different degrees of disease severity, and on the other hand, the anticipated progression of the condition. Therefore, diagnos-

ing periodontal disease also requires a thorough clinical and radiological evaluation, with modern imaging unequivocally contributing to the correlation between periodontal and systemic status (17).

Recent studies highlight the links between periodontal diseases and systemic conditions, including cardiovascular diseases, showing possible common pathogenic mechanisms (18). Over the past two decades, a substantial number of epidemiological studies have been conducted to investigate the relationship between periodontitis and cardiovascular diseases, making this topic one of the major areas of interest in periodontal research (19, 20).

The etiology and microbiology of periodontal diseases have been extensively studied, shedding light on the complex pathogenesis of these conditions (21). The understanding of the molecular mechanisms underlying periodontal tissue diseases has evolved over the years, providing insights into the inflammatory processes, cytokines and matrix metalloproteinases involved in the progression of the disease (21). In addition, the association between periodontal disease and chronic systemic conditions such as hypertension, dyslipidemia, stroke, myocardial infarction, angina pectoris and diabetes has been explored, revealing a higher prevalence of periodontal disease in individuals with these comorbidities (22, 23).

One third of all deaths worldwide are caused by cardiovascular disease, which is thus the leading cause of death worldwide. Moreover, globally, cardiovascular disease causes more deaths than cancer, chronic respiratory failure and diabetes combined (24).

In addition to their impact on the population as a factor increasing mortality, cardiovascular disease is also a major cause of disability and chronic disease, placing a considerable burden on health systems and the economy.

The association between periodontal disease and cardiovascular disease has been intensively investigated and various theories have been proposed to explain this link. One theory suggests a direct invasion of endothelial cells by periodontal pathogens, while another proposes an indirect pathway by which periodontal disease triggers a systemic inflammatory response (25). In addition, a common genetic basis has been suggested as a possible link between these two conditions (25). These theories collectively emphasize the complex interaction between periodontal and cardiovascular status.

Studies emphasize the strong relationship between periodontal and cardiovascular diseases, focusing on two main directions of this association (26). The first direction suggests that bacteria in the oral cavity may directly exacerbate cardiovascular disease or modify systemic risk factors for cardiovascular disease (26). The second direction emphasizes how chronic periodontal inflammation, viewed as a focus of infection, may increase circulating levels of inflammatory molecules, exacerbating cardiovascular disease (26).

As the importance of the connection between periodontal disease and cardiovascular disease becomes increasingly prominent in medical practice, it is essential to explore this relationship and its implications for patients.

This study proposes to investigate the impact of periodontal disease on the development and progression of cardiovascular disease and to establish that periodontal disease represents a relevant risk factor for the occurrence of cardiac pathologies.

MATERIALS AND METHODS

A retrospective study was carried out, including 138 consecutive patients who addressed the Dental Education Clinical Base, between November 2021 and November 2023.

Inclusion criteria. Patients with gingival diseases not induced by dental biofilm, patients without signs of periodontal disease, patients with gingivitis induced by dental biofilm, patients with periodontitis and patients with periodontitis appearing as a manifestation of systemic diseases.

Exclusion criteria. Chronic alcohol users and patients with other cardiovascular diseases other than hypertension (HT) were excluded from the study. According to the World Health Organization (WHO), there is a significant variation in the prevalence of cases with severe forms of periodontitis in the population, which is approximately 10-15%.

Starting from this aspect, we considered an effect size of 11%, representing the reference prevalence for establishing the minimum sample size, which requires obtaining a minimum volume to ensure adequate representativeness of the patient category.

To achieve this prerequisite, we set a 95% confidence interval. The sample size was determined based on the condition of having a confidence interval smaller than the allowable level of error.

Accordingly, we used the equation:

$$n \geq \left(Z_{\left(1-\frac{\alpha}{2}\right)} \right)^2 \cdot \frac{p \cdot (1-p)}{d^2},$$

where Z = 1.96 for a 95% confidence interval and a minimum error of estimate of 5%. We estimated a minimum sample size of 138 cases.

The patients included in the study were divided into two groups, a group composed of 74 patients (group I: n=74) with periodontitis and a group of 64 patients without periodontitis (group II: n=64).

The primary objective of the study was to assess the correlation between periodontitis and hypertension mediated by a number of risk factors.

Hypertension was considered as a dichotomous dependent variable defined by systolic blood pressure of 140 mmHg or more and/or diastolic blood pressure of 90 mmHg or more and/or current use of antihypertensives.

Data were processed in MS Excel and statistical analysis was performed with SPSS v. 29 program (IBM Ireland Product Distribution Limited, IBM House, Shelbourne Road, Ballsbridge, Dublin, Ireland) and STATA 16 software (StataCorp LLC, 4905 Lakeway Drive, College Station, TX, USA).

The existence of significant statistical differences was considered for a confidence interval of 95%, and parametric or non-parametric tests were applied depending on the characteristics of the variables. Student's t-test, Pearson-χ^2, Fisher's test, Levene's test, ANOVA test, logistic regression and multivariate analysis tests were used.

The threshold of significance was p = 0.05 corresponding to a maximum acceptance error of 5%. Descriptive statistical indicators were calculated for continuous variables (mean, standard deviation, standard error, minimum, maximum and quartile intervals).

Within the descriptive statistics, the values of the location indicators (mean,

median, mode), scattering (dispersion, amplitude) were calculated, we determined, as appropriate, the interval of variation with 95% confidence (for the study of proportions) and within the analysis statistics, we applied tests to determine the regression model, comparison of ANOVA means, the Chi square test (χ^2) for comparing proportions and we tested the sensitivities of the diagnostic methods. This allowed the highlighting of associations, relationships and interdependencies between variables of interest.

RESULTS

From the analyzed database, it has been observed that the prevalence and severity of periodontal disease are higher in individuals with hypertension compared to those with normal blood pressure levels.

Demographic characteristics. In the analyzed cohort, the prevalence of periodontal disease was 53.62% (74 cases). In the studied group, 48 patients with periodontitis manifested as a result of systemic diseases were included (tab. I).

TABLE I.
Study Group Structure Based on Periodontal Involvement

	Count	%
Periodontitis absent	64	46.38%
Without signs of periodontal disease	18	13.04%
Gingival diseases that are not induced by the dental biofilm	25	18.12%
Gingivitis induced by dental biofilm	21	15.22%
Periodontitis present	74	53.62%
Periodontitis	26	18.84%
Periodontitis appeared as a manifestation of systemic diseases	48	34.78%

High blood pressure was found in 67.39% of the cases included in the study (93 cases). The distribution of cases according to the degree of high blood pressure showed a frequency of 18.84% for grade 1 high blood pressure, 28.99% for grade 2 high blood pressure, and 19.57% for grade 3 high blood pressure (tab. II).

TABLE II.
Frequency of high blood pressure in the study group

	Count	%
No high blood pressure	45	32.61%
High blood pressure	93	67.39%
High blood pressure grade 1	26	18.84%
High blood pressure grade 2	40	28.99%
High blood pressure grade 3	27	19.57%
Total	138	100%

The evaluation of the association of periodontitis with the presence of high blood pressure revealed a frequency of 89.19% of cases with periodontitis/periodontal disease that associates high blood pressure. The frequency of cases that associated periodontitis and hypertension (89.19%) was significantly higher (χ^2=34.50, p < 0.001) compared to the frequency of cases without periodontitis with hypertension (42.19%) (tab. III).

The analysis of the frequency of cases according to the degree of hypertension noted in table IV.

TABLE III.

Association of periodontitis with the presence of high blood pressure

		Periodontal disease		Total
		absent	present	
High blood pressure	absent	37	8	45
	%	57.81%	10.81%	
	present	27	66	93
	%	42.19%	89.19%	
	Total	64	74	138
Pearson's chi-squared test: χ^2=34.50, p < 0.001 **Spearman correlation: r=0.5338**				

TABLE IV.

Evaluation of the presence of periodontal disease according to the degree of high blood pressure

		Periodontal disease		Total
		absent	present	
High blood pressure	absent	37	8	**45**
	%	57.81%	10.81%	
	grade 1	18	8	**26**
	%	28.13%	10.81%	
	grade 2	7	33	**40**
	%	10.94%	44.59%	
	grade 3	2	25	**27**
	%	3.13%	33.78%	
	Total	64	74	**138**
Pearson's chi-squared test: χ^2=58.61, p < 0.001 **Spearman correlation: r=0.62649**				

In the context in which the specialized literature mentions a series of risk factors for both periodontitis and high blood pressure, demographic and clinical parameters were analyzed as possible associated risk factors. Thus, they were analyzed as risk factors associated with periodontitis in increasing the chance of high blood pressure.

Gender. The gender of the patients did not present a significant association with

periodontitis, an aspect that demonstrates the homogeneity of the group created by the consecutive introduction of cases according to the inclusion and exclusion criteria in the group. In the analyzed group, the presence of periodontal disease showed a frequency of 54.05% in men and 45.95% in women (tab. V).

TABLE V.
Association of periodontal disease with gender of patients

		Periodontal disease		Total
		absent	present	
Patients gender	male	36	40	76
	%	56.25%	54.05%	
	female	28	34	62
	%	43.75%	45.95%	
	Total	64	74	138
Pearson's chi-squared test: χ^2=0.0668, p =0.7959 **Spearman correlation: r=0.02201**				

The descriptive evaluation of the frequency of periodontal disease cases according to the gender of the patients and the association of high blood pressure revealed a significantly (χ^2 = 4.98, p = 0.03198) higher proportion of male cases associating high blood pressure in the case of periodontal disease.

Age. The age of the patients included in the study group showed an average of 48.57 years (SD – standard deviation = 11.53). The minimum age values were 21 years, and the maximum values were 68 years. It was also noted that 50% of the analyzed patients were younger than 51 years old.

Comparing the values of the patients' age according to the presence of periodontitis, a non-normal distribution was noted. The very high frequency of cases with periodontal disease between the ages of 55 and 60 is noted (tab. VI).

TABLE VI.
Statistical indicators of patients' age according to the presence of periodontal disease

Periodontal disease	Average Age	Average		Dev. std	Min	Max	Q25	Median	Q75
		-95%	-95%						
Absent	42.19	39.58	44.79	10.44	21.00	58.00	32.50	45.00	49.50
Present	54.08	51.89	56.27	9.44	28.00	68.00	50.00	56.00	60.00
All Groups	48.57	46.62	50.51	11.53	21.00	68.00	38.00	51.00	57.00
Test statistic: Mann-Whitney U Test: Z = - 6.4193, p < 0.001 Levene Test of Homogeneity of Variances: F=5.5376, p=0.02004									

Residence. Considering the fact that the literature presents socio-economic status as an important risk factor for periodontal disease, we evaluated the rural residential environment as a possible element that can represent a risk for periodontal disease. This aspect can be explained by the limited access to dental care.

The results indicated an inhomogeneous sample in terms of resident environment (U:

R → 90:48). In order to eliminate this particular aspect of the two analyzed groups of patients, the frequency of periodontal disease was evaluated by referring to the number of patients in each group corresponding to the residence environment.

The analysis demonstrated a significant association (p = 0.0126) of the rural environment of rural residence and the presence of periodontal disease in patients from the analysed group (62.5%). In the group of patients from the urban environment, the presence of periodontal disease was found at 48.89% (tab. VII).

TABLE VIII.

Association of periodontal disease with the domicile of the patients

		Periodontal disease		Total
		absent	present	
Residence	**Urban**	46	44	**90**
		51.11%	**48.89%**	
	Rural	18	30	**48**
		37.50%	**62.50%**	
		64	74	**138**
Pearson's chi-squared test: χ^2=12.332, p =0.0126 Spearman correlation: r=0.3707				

The analysis of the correlation between the presence of periodontal disease, high blood pressure and the gender of the patient demonstrated a significant association explained by the high frequency of cases with periodontal disease, high blood pressure coming from rural areas (62.5%) (tab. VIII).

TABLE VIII.

Association of periodontal disease with high blood pressure and gender of patients

	High blood pressure	Urban	Rural	Total
Periodontal disease absent	HBP absent	26	11	37
		28.89%	22.92%	
	HBP present	20	7	27
		22.22%	14.58%	
Total		46	18	64
Pearson's chi-squared test: χ^2 = 7.11272, p = 0.7381 Spearman correlation: r=-0.1823				
Periodontal disease present	HBP absent	8	0	8
		8.89%	0.00%	
	HBP present	36	30	66
		40.00%	62.50%	
Total		44	30	74
Column Total		**90**	**48**	**138**
Pearson's chi-squared test: χ^2 = 8.9718, p = 0.00274 Spearman correlation: r= 0.6225				

DISCUSSION

This result once again argues for the assessment of the bidirectional correlation between periodontal disease and hypertension. The approach in this study of a retrospective analysis will allow the analysis of this correlation only from the perspective of identifying to what extent periodontal disease increases the chance/risk of hypertension.

Over the past twenty years, multiple studies have demonstrated that persons with periodontitis have an increased susceptibility to cardiovascular events, such as myocardial infarction, peripheral artery disease, stroke, and heart failure (HF).

Crucially, pre-clinical research has also corroborated this association. For example, Akamatsu and colleagues showed that periodontal infections caused myocarditis and/or myocardial infarction in mice (17).

While a potential correlation between cardiovascular disease (CVD) and periodontitis has been seen in clinical settings, the specific mechanism that links these two conditions has not yet been fully understood. However, the primary mechanism that explains this association seems to be the spread of oral pathogens into the bloodstream.

For example, bacteremia, which is frequently produced by both non-surgical and surgical dental procedures, is a significant contributor to infective endocarditis in those who are prone to heart disease (18). Therefore, individuals with heart disease who are undergoing dental procedures are offered prophylaxis (18).

The underlying physiological processes responsible for oral health or periodontal disease and hypertension are not thoroughly comprehended. Several studies have indicated that individuals with primary arterial hypertension and poor dental health are more prone to experiencing oxidative stress. This can lead to higher levels of reactive oxygen metabolites, lipid peroxidation, and reduced total antioxidant capacity. This may lead to the deactivation of prostacyclin and nitric oxide (NO). Therefore, an increase in peripheral vascular resistance and hypertension is observed (19, 20).

The correlation between periodontitis and hypertension is crucial, as the advancement of cardiovascular disease is greatly influenced by the level of blood pressure management attained in individuals with hypertension. Fifteen Identifying modifiable risk factors for the advancement of hypertension-induced damage is a top goal worldwide, given its substantial impact on illness, death, and healthcare costs.

Additionally, our investigation was subject to many constraints. The design of our study precludes us from making causal inferences about our findings, as we lack information on the time sequence of the relationship between periodontal disease and blood pressure. Nevertheless, hypertension is unlikely to be a direct cause of periodontal disease.

Additionally, our study was constrained by a small sample size, which resulted in insufficient statistical power for conducting subgroup analyses on specific groups such as diabetics, individuals who have never smoked, and individuals who are former or present smokers.

Although our study has limitations, it clearly shows a potential significant connection between periodontal health and the control of blood pressure. This finding could have a significant influence on public health, particularly among the older popu-

lation. Due to the constraints of our study, additional research is required to validate this correlation in different populations.

CONCLUSIONS

The link between periodontal disease and cardiovascular disease is multifaceted, involving mechanisms such as inflammation, common genetic factors, and systemic responses to oral pathogens.

The results of the retrospective study, together with the findings from various studies, support a significant association between these two conditions, underscoring the importance of considering periodontal health in the management of cardiovascular disease.

Understanding and addressing the interaction between periodontal disease and cardiovascular health is critical to comprehensive patient care and disease prevention strategies.

CONFLICT OF INTEREST AND FUNDING

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PLASMA VITAMIN C LEVELS ASSOCIATED WITH THE DIET AND SUPPLEMENT CONSUMPTION

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PLASMA VITAMIN C LEVELS ASSOCIATED WITH THE DIET AND SUPPLEMENT CONSUMPTION (Abstract): The aim of this study is to show the amount of vitamin C in the plasma as a consequence of lifestyle changes. **Materials and methods:** The level of vitamin C in plasma were determined prior to the study (T1), and after five months (T2). Moreover, a medical record was carried out as well as a questionnaire on general eating habits and specific consumption of food rich in vitamin C. Both of them were completed before and after five months. In addition, related anthropometric analyses were carried out. **Results:** The results of the first determination of vitamin C level in plasma were between 1.0 and 16.0 mg/L and the results of the second determination of vitamin C in plasma were between 4.9 and 14.1 mg/L. Furthermore, it was identified that 50% of the subjects had a low fruit intake. Raw vegetables were consumed by fewer subjects, the intake being higher during the main meals. **Conclusions:** The correlation of plasma vitamin C with the eating habits of the subjects involved in the study points out an interdependence between the consumption of fruit and vegetables and the level of vitamin C in plasma. **Keywords:** VEGETABLES, PLASMA VITAMIN C LEVEL, HUMANS.

INTRODUCTION

Fruits and vegetables are known to possess a plethora of benefits which can improve human health. Observational studies pointed out that a high consumption of fruit and vegetables is associated with a lower risk of developing cancer (1, 2, 3, 4, 5) thus, being beneficial for the prevention of cardiovascular diseases as well (6, 7, 8). The potential benefic role of fruit and vegetables was attributed to the component specific effect, such as vitamins, minerals, food fibers, and a range of secondary metabolites (phytochemicals), responsible for color, flavor, and taste (9).

Previous studies pointed out that the plasmatic concentrations of carotenoids and vitamin C increased both the following and the intake of fruit and vegetables (10, 11).

The correlation between the level of the plasma vitamin C and the eating habits was studied by Wrieden *et al*. in 2000 (12),

concluding that the consumption of fruit and vegetables three or more times a day can significantly increase the level of vitamin in plasma. Furthermore, it was observed that a portion of freshly squeezed citrus juice equals two fresh fruit or vegetables consumed during meal times (13).

The presence of compounds with an antioxidant activity in the composition of fruit and vegetables, which represent the basic food intake of the population living in the tropical area, was studied by Jdeani *et al*. in 2021. They concluded that although these could satisfy an important part of a human's nutritional needs, an increase in population would lead to an increase of the dependence on supplements with synthetic antioxidants, all these with the purpose to reduce terminal and degenerative diseases (14).

The compounds with antioxidant activity present in the composition of fruit and vegetables, such as vitamin C, vitamin A, vitamin E, and carotenoids have a benefic effect, leading to the strengthening of the human immune system and the reduction of risks associated with the development of a chronic disease (14, 15). Furthermore, vitamins C, A, E, carotenoids, fruits, and vegetables are rich in a variety of polyphenols and minerals that exhibit a significant antioxidative activity. These compounds, including flavonoids, tannins, and lignans, contribute to the reduction of oxidative stress and play a vital role in strengthening the immune system as well as in reducing the risk of chronic diseases. Additionally, minerals such as selenium and zinc are crucial cofactors for antioxidant enzymes. Acknowledging the full spectrum of these nutrients underscores the comprehensive health benefits of a diet high in fruits and vegetables.

Vitamin C (ascorbic acid) is a micronutrient with a very important role in the well-functioning of the human organism, being involved in over 300 metabolic functions (1, 2, 3, 5). The sustainability of vitamin C is explained through its capacity to destroy and neutralize free radicals and oxidative stress (16, 17), taking part in a series of redox reaction at cellular level (18). It has an anti-infectious protection role (19, 20), it acts beneficially to combat *Helicobacter pylori* (1); it prevents cancer incidence (19, 21), having anti-inflammatory (21, 22), anti-angiogenic (14, 23-26), and anti-tumoral (15, 19) properties; it participates in the synthesis of the bile acids, it prevents the development of coronary diseases and atherosclerosis (1, 2, 6-8, 14-17, 20-24, 27-31); it diminishes the level of reactive protein C (2, 14, 24, 29-31); it facilitates the elimination of heavy metals from the organism by neutralizing them (1, 6-8, 14-17, 20-24, 27, 29, 31). It plays an important part in stimulating the biosynthesis of stress adjusting hormones, being involved in the production of antistress hormones and of interferon (an important protein of the immune system) (1); it sustains the functioning of the adrenal gland (1), and it participates in the metabolism of the collagen (18-20, 32-35).

The classical diseases determined by a lack of vitamin C is scurvy, which develops due to the role played by the vitamin in the post-translational modification of collagen. An excessive intake of vitamin C can cause diarrhea, abdominal bloating, over-absorption of iron, hyperoxaluria (in dialyzed patients), and hemolysis (in patients with a deficit of glucoses-6-phosphate dehydrogenase). A tolerable upper intake level for vitamin C is equal to 2000 mg/day as it was established by the Council for

Food and Nutrition in 2000 (27).

The recommendations for the vitamin C intake can be found in the Recommended Dietary Allowances (RDAs), developed by the Food and Nutrition Board of The National Academies of Sciences, Engineering, and Medicine, The Recommended Daily. The dose - the average daily intake necessary to satisfy the nutritional need of almost all healthy individuals and which is often used to plan nutritive adequate diets for individuals and which varies according to age and sex - is of 90 mg for men and 75 mg for women (with an additional quantity of 35 mg for smokers). For these doses the bio-availability of vitamin C is of almost 100% (28).

Most vertebrates and invertebrates have the ability to synthesize vitamin C while the human body does not have this capacity. The inability to synthesize vitamin C is due to mutations in the L-gulono-γ-lactone oxidase (GLO) gene.

GLO encodes the enzyme that is responsible for catalyzing the last step in vitamin C biosynthesis (10, 12, 36).

For this reason, the level of this vitamin in the human organism depends entirely on the exogen vitamin sources (1-6, 8, 10, 12-24, 25, 28-32, 34-36) which include fruit and vegetables (20).

Since fruit and vegetables are a primary source in the diet with vitamin C, the plasmatic level of vitamin C is considered a good surrogate or predictor of vitamin C intake. The correlation can be affected / influence by the presence of external factors such as: the bioavailability of the vitamin, the absorption conditions, stress, the food processing degree and the conservation period or even certain errors in the reporting the level of vitamin C (23, 27, 28).

The purpose of the present study was to measure the plasma levels of vitamin C in a group of volunteers, on the basis of vitamin C consumption from diet and / or supplements.

This means increasing the intake of vitamin C-rich fruits and vegetables or using supplementation. The objectives of the study were to: determine the subjects' baseline health and lifestyle; monitor the intake of foods rich in vitamin C ; inform the subjects on vitamin C as well as on the aim of the study; tailor individual diet plans; group subjects by initial vitamin C levels so as to perform targeted interventions; give the subjects autonomy by allowing them to withdraw from the study at any time; measure the impact of the interventions on the levels of plasma vitamin C.

MATERIALS AND METHODS

Subjects and study protocol

In this study, initiated in January 2023 in Arad County, we engaged a large group of subjects via their general practitioners in order to participate in a dietary intervention aimed at optimizing their plasma vitamin C levels through an increased consumption of vitamin C-rich fruits and vegetables or supplementation. The selection of the subjects was a multistage one, beginning with applying a comprehensive questionnaire on health status, psychological, and medical history, lifestyle habits including substance use, ongoing medical treatment, and physical activity levels to gather preliminary information for the study.

Subsequently, we monitored subjects who regularly consumed fruits and vegetables high in vitamin C - such as black currants, kiwis, lemons, strawberries, oranges, tangerines, grapefruits, melons, papayas, and mangoes.

Upon selecting the subjects, we provided them with detailed information about the aims of the study, the role of vitamin C in health, and the effects of its deficiency or excess. After this phase, a part of the selected subjects chose not to proceed due to personal reasons. The remaining subjects underwent anthropometric measurements in order to tailor the diet plans that were to be followed for the subsequent five months. The plans were based on an intake of 2,000 kcal/day, incorporating the necessary vitamin C dosage and macronutrients as advised by a dietician.

Finally, it was pointed out that all group subjects, comprising 20 women and 22 men, aged between 20 and 45 years (mean age of 34.19±7.68), were in good health and not under any medical treatment during the selection phase. They were categorized based on initial plasma vitamin C levels as follows: below the reference minimum value, within the average range, or above the maximum reference level.

Nutritionists, dieticians, and physicians tailored recommendations for each category, leading to the formation of three groups: one on a specific diet, another on slow-release vitamin C supplements 1000 mg per day in the morning, and the third on a combination of both interventions. Subjects had the autonomy to withdraw at any point upon notification.

The second vitamin C measurement occurred approximately five months later, with the timing slightly varying among subjects. Post-second sampling, we conducted individual discussions regarding dietary adherence, supplement intake, and new anthropometric measurements, along with a questionnaire on food consumption during the study.

In the last stage, we analyzed the collected data, ensuring the confidentiality of personal information. This comprehensive approach allowed us to consider both the subjects' therapeutic needs and personal preferences in order to enhance the plasma vitamin C levels effectively.

Criteria for inclusion:

- Age between 20 and 45 years
- Good health condition, not under medical treatment, at the time during selection the selection trail

Exclusion criteria:

- Individuals who opted out after the first stage focused on delivering them information on the study
- Any major health condition that could affect the absorption or metabolism of vitamin C, such as smoking, regular alcohol consumption, supplement intake.

The study was carried out in accordance with the instruction given at the Helsinki Declaration and all the procedures which involve human subjects were approved by the Human Research Ethics Committee. It was agreed to carry out the study and have its results published by the Scientific Research Ethics Committee of the "Vasile Goldiș" Western University of Arad, Romania under No. 4/13.01.2023.

Determination of vitamin C Level in Plasma

The samples were collected either at the general practitioner's private office in Arad, Romania or in a laboratory of medical analysis, located in Arad, Romania, according to the possibility and the preference shown by each subject. The samples collected at the doctor's private office were collected by the staff belonging to the medical analysis laboratory which ensured the optimum transport conditions. The actual determination of the plasma level of vita-

min C was carried out in Germany and the results were delivered after 10 to 12 days after the moment of the sample collection.

The method used to determine the level of the vitamin C in the plasma was the High-Pressure Liquid Chromatography (HPLC) connected to an electrochemical detector by complying to the protocols in the medical analysis laboratories (23, 30, 31).

The volunteers were asked to avoid alcohol consumption 24 hours before the sampling process and do not eat 12 hours prior to the blood collection process. The blood collection process was conducted in the morning (30).

One milliliter of venous blood was collected from the subjects *à jeun* (they subject did not eat anything prior to the blood collection). The blood was collected in vacutainer collection containers which contained lithium heparinate as an anticoagulant. After sampling, the plasma was separated through spinning and it coagulated immediately (it stable one month if kept at temperature of -20°C); the recipients were covered with an aluminum wrap to protect it from light; in the case of the sample collected outside the laboratory, they were transported in the recipient for the frozen samples. The samples, in which a highly hemolyzed, lipemic or unfrozen, unprotected from light specimens were observed, were rejected (30). The reference values are comprised between 4.6 and 14.9 mg/L (30).

Plasma values of < 3 mg/L point out deficiencies of vitamin C and a concentration of < 0.2 mg/L suggest the presence of scurvy. Low plasmatic levels appear in malabsorption, alcoholism, pregnancy, hyperthyroidism, kidney failure, and in smokers.

Limits and interferences may appear after the ingestion of ascorbic acid. The plasmatic values increase rapidly in 1-2 hours and reach a maximum concentration after 3 or 6 hours from consumption. The testing of the subjects who use vitamin supplements may indicate a higher plasmatic level of the vitamin (31).

Statistical analyses

The statistical tests used in this study were selected by taking into consideration data distribution and the specific research questions addressed.

All statistical analyses were performed with the IBM *SPSS Software version 27*.0 (IBM Corp, New York, USA). Discrete variables were presented as medians along with their minimum and maximum values. Continuous variables were reported as means with standard deviations (SDs). Due to the small size of the sample and the distribution of the data, non-parametric tests were used. Specifically, the Kruskal-Wallis Test was used to evaluate the significance of differences between the studied variables, with a p-value of < 0.05 considered indicative of substantial differences.

In addition to these statistical analyses, descriptive statistics for the methodological validation, including the limit of detection (LOD) and limit of quantification (LOQ), were calculated to ensure the reliability and accuracy of the analytical methods used. The LOD was determined on the basis of the lowest concentration of analyte that could be reliably detected but not necessarily quantified, while the LOQ had the lowest concentration that could be reliably quantified with acceptable precision and accuracy.

RESULTS

Two sets of sampling were made in order to determine the plasma vitamin C: the

first sampling was carried out after the selection of the subjects (Time 1) and the second one after an average period of 5 months (Time 2) (figs. 1 and 2).

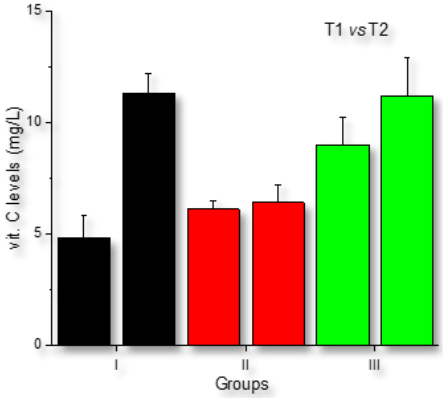


Fig. 1. Subject average vitamin C levels for every group between Time 1 (T1) and Time 2 (T2)

Based on the results of the study, the subjects were divided into three equal groups group, formed of individuals whose plasma vitamin C levels fell on the lower end of the minimum reference values or were below the limit; group II, formed from those subjects whose plasma vitamin C levels were within the normal reference range; and group III, comprising those subjects whose plasma vitamin C levels exceeded the maximum reference range (fig. 1).

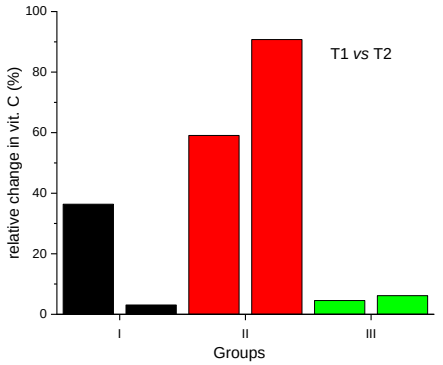


Fig. 2. The changes in plasma vitamin C levels from Time1 (T1) to Time2 (T2) for each group

The questioning of the subjects involved in the study was conducted, both at the time T1 and T2. This questionnaire was completed in relation to the consumption of vegetables and fruit with a high level of vitamin C or other vegetables and

fruit with vitamin C content, consumed frequently, for both moments (figs. 3 and 4).

In figure 3, it can be observed a comparison between the consumption of fruit and vegetables during the first examination and the end of the present study. There were found significant differences between the values at the respective moment ($p< 0.001$).and not found important differences between the values at the end of the present research ($p= 0.560$).

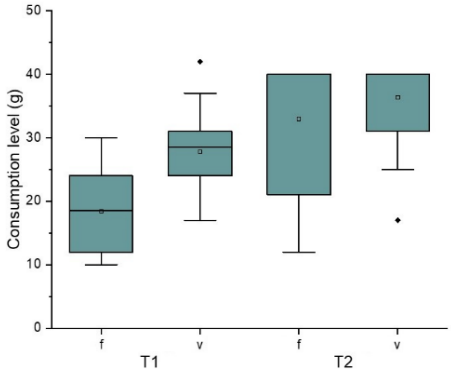


Fig. 3. The ranges related to the consumption of Fruit (a) and Vegetables (b) at Time 1 (T1) and Time 2 (T2), the black line is an indicator of the model

The result of the first collection of plasma vitamin C related to the consumption of vegetables and fruits, from the same period, depending on the group is rendered in fig. 4.

In figure 4, it can be observed a comparative presentation between plasma vitamin C level and the consumption of fruit and vegetables during the first examination. There were found important differences between the values at the respective moment ($p< 0.001$).

The result of plasma vitamin C collection, at the end of the study, compared to the consumption of vegetables and fruit from the same period, depending on the group, is rendered in figure 5.

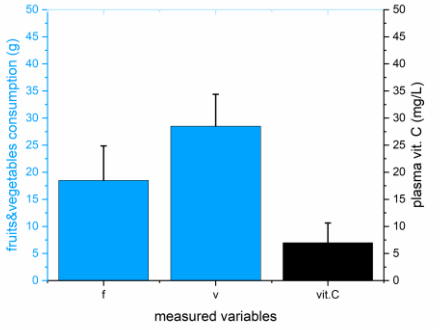


Fig. 4. The consumption of fruit and vegetables as related to vitamin C level at T1 (mode values ± SD)

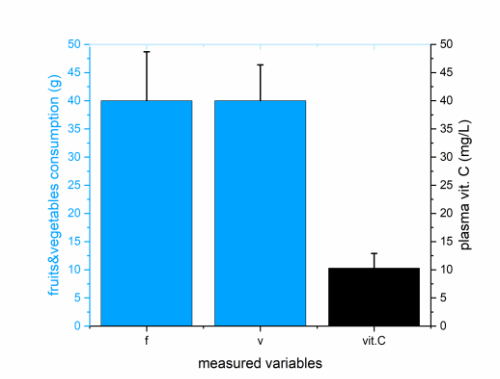


Fig. 5. The consumption of fruit and vegetables as related to vitamin C level at T2 (mode values ± SD)

Figure 5 presents, in a comparative manner, the plasma vitamin C level and the consumption of fruit and vegetables at the end of the study. There were found important differences between the values at that moment ($p < 0.001$).

DISCUSSION

Determination of the plasma vitamin C and its development

The results of the first plasma determination of vitamin C are comprised between 1.0 and 16.0, while the results of the 2nd plasma vitamin C determination are comprised between 4.9 and 14.1 (figs. 1, 2, 4, 5).

The time period between the two analyses is of approximately 5 months, with an insignificant error margin, during which there was a considerable increase in the plasma vitamin C level in 72.73% of the subjects involved, and which was represented by a total increase of 36.59% in the plasma vitamin C level, although 9.09% of the subjects maintained their plasma vitamin C level while 18.18% had experienced a decrease in their plasma vitamin C level.

Ways to consume vitamin C and their subsequent results

It is worth pointing out that 14 subjects agreed to integrate a proper diet into their daily life, other 14 subjects opted for both diet and additional vitamin C consumption, and 14 subjects opted strictly for the consumption of food supplements. During the discussions carried out with the subjects, it was observed a general belief that it is an easier option to solve health problems by consuming food supplements and medicine, than by trying to change one's eating habits and lifestyle.

The maintenance and decrease of the plasma vitamin C level, in most subjects, can also be the result of the fact that the vitamin C content in food is not stable. Most fruit and vegetables are harvested before ripening, so they do not benefit from all the nutritional elements, which would accumulate from soil, rain and sun action. Moreover, groups of vegetables and fruit (especially those consumed in the area and during the period of the study) are grown in greenhouses. Therefore, not enough vitamin C accumulates in the respective food items, or they are stored for a longer period of time. This aspect leads to the loss of vitamin C from the food items.

One of the decreases in the plasma vitamin C level was due to the fact that one person did not administer vitamin C for personal reasons. This aspect was reported

only at T2.

The majority of subjects who opted for vitamin C intake from food supplements had a considerable increase in the plasma vitamin C level. In addition, the subjects who opted for both the diet and the additional consumption of vitamin C from food supplements had considerable increases in the plasma vitamin C level (fig. 1).

This increase is due to a high intake of vitamin C either from food or dietary supplements or both. It can also be the result of the intervention made by nutrition specialists, who gave the right recommendations to each person and offered support throughout the study.

Analysis of eating habits

Habits Related to Fruit Consumption

Although most subjects prefer fruit, their consumption is reduced both at breakfast and lunch. They are usually served as a snack. Fruit was initially consumed a maximum of 1-2 times a day, and 50% of the subjects questioned admitted to have never consumed certain fruit. It was also identified that 50% of those questioned had a low fruit consumption. During the study, fruit consumption increased considerably, due to diet recommendations and explanations provided by the dietician.

Habits Related to Vegetable Consumption

At the beginning of the study, raw vegetables were consumed by a smaller percentage of subjects in comparison with fruit. Furthermore, their consumption was higher during main meals, most subjects refusing to serve vegetables as a snack. The majority of subjects consume vegetables once or twice a day. Among the subjects questioned, 41% have never consumed or very rarely consumed raw vegetables. In most cases, vegetables are prepared for consumption through thermal preparations, an aspect that can lead to the loss of vitamin C content in food.

The subjects' favorite fruit were oranges, tangerines and lemons. The exotic fruit such as papaya and mango as well as strawberries which were out of season (tab. I) registered low consumption values.

TABLE I.

Comparative table on the consumption of fruits with high vitamin C content

FRUIT (g)	Time 1	Time 2
Kiwi	53	76
Strawberries	28	68
Oranges	72	84
Red Grapefruit	52	75
Papaya	3	61
Currants	22	62
Lemons	70	84
Melon	21	68

The preferred vegetables with a high content of vitamin C were bell peppers and tomatoes (mainly consumed raw), potatoes, cabbage, parsley, and peas (consumed in cooked form, included in the main meals), of which parsley and cauliflower were eaten with an increased frequency (tab. II).

TABLE II.

Comparative table on the consumption of vegetables with a high content of vitamin C or with a low content of vitamin C but largely consumed

VEGETABLES (g)	Time 1	Time 2
Cabbage	64	80
Broccoli	34	70
Potatoes	92	93
Bell Pepper	65	82
Parsley	111	95
Cauliflower	49	75
Tomatoes	52	79
Brussels Sprouts	16	67
Spinach	54	77
Peas	75	82

Figure 6-a comparatively presents the consumption of fruits with high vitamin C content between T1 and T2. A significant difference was found between the consumption of the previously mentioned fruits in the two moments (p= 0.009). Figure 6-b comparatively presents the consumption of vegetables with high vitamin C content between T1 and T2. A significant difference was found between the consumption of the mentioned vegetables in the two moments (p= 0.026).

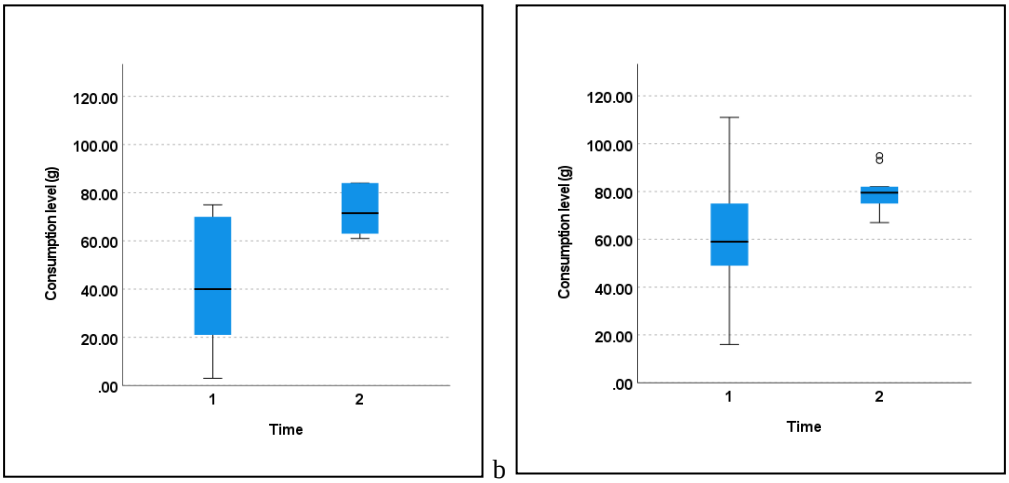


Fig. 6. Comparative consumption of fruits-a / vegetables-b with high vitamin C content between the two questionnaires (the black line is an indicator of the model)

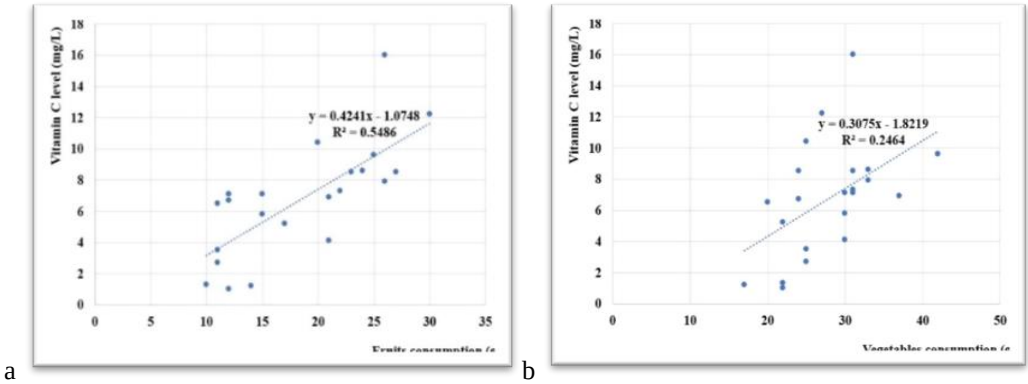
Table III presents a statistical correlation between the plasma vitamin C level and the consumption of fruits and vegetables at the beginning and at the end of the research.

TABLE III.

The values of Spearman correlation coefficient (rho)

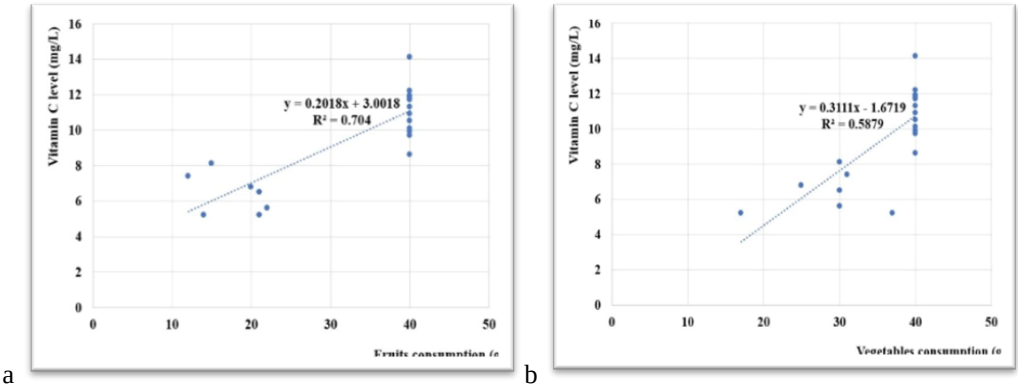
The beginning of study		The end of study	
Fruits *vs.* vitamin C	Vegetables *vs.* vitamin C	Fruits *vs.* vitamin C	Vegetables *vs.* vitamin C
0.791 (p< 0.01)	0.601 (p= 0.003)	0.777 (p< 0.01)	0.795 (p< 0.01)

The values in tab. III present a strong correlation between the consumption of fruits and the level of plasma vitamin C at the beginning of the research and a medium correlation between the consumption of vegetables and the level of plasma vitamin C. At the end of this study, there were found significant strong correlations between the consumption of fruits and vegetables and the level of vitamin C. All these results demonstrate that the consumption of fruits and vegetables which have a high content of vitamin C lead to enhanced levels of this vitamin in human plasma.



a b

Fig. 7. Correlations between the consumption of (a) fruits and (b) vegetables *vs.* the vitamin C level at T1



a b

Fig. 8. Correlations between the consumption of (a) fruits and (b) vegetables *vs.* the vitamin C level at T2

Figures 7 and 8 reveal positive correlations between the consumption of fruits and vegetables and the discovered vitamin C levels.

Some subjects consumed certain fruit and/or vegetables in higher amounts while barely or not at all consuming the others. This aspect changed for most individuals, once with the intervention of the nutritionist, who recommended reducing the consumption of some frequently consumed foods and the intake increase of other fruit and vegetables, thus diversifying the diet of the subjects involved in the study.

It was also observed that women have higher concentration of plasma vitamin C than men. However, the reasons of this observation need further elucidation studies. The results of the study point out that the lower plasma vitamin C concentrations in men compared to women, can be explained due to the higher fat-free mass in men. A special characteristic can be made by a varied series of potential factors such as age, anthropometric variables, the body composition (the total antioxidant status), the intake of vitamin C, lifestyle, the consumption of alcohol, smoking and the phys-

ical activity level (34).

A very slightly increased trend towards the consumption of fruits and vegetables was found at women from Group I and III, but the differences between the men and women was not significantly (p= 0.104). No important difference was also found between men and women in the case of the vitamin C plasma levels for the entire group (42 subjects).

However, our intention was to assess the impact of increased vitamin C intake on plasma vitamin C levels within a real-world setting, within which the subjects were not restricted from their usual lifestyle choices. This approach aimed to assess the practical applicability of dietary recommendations in a non-clinical environment. However, we recognize the limitations of this design and we acknowledge the importance of a control group in clinical research in order to establish a baseline for comparison. Particularly, in our study, this lack was replaced by the following: comprehensive health and lifestyle questionnaires applied in order to establish a detailed baseline for each subject, thus allowing a comparison between the individual outcomes and their own starting points; findings are discussed in the context of current literature in the domain, allowing for indirect comparisons to studies that included control groups. We are planning follow-up studies that will incorporate a control group. This will enable us to validate and compare the results obtained in the current study.

CONCLUSIONS

An important and potential limitation of the current study is the maximum level of fruits and vegetables consumption that was previously established in questionnaires at 40 g.

Although subjects who consumed minimum quantity of fruit and vegetables were chosen for the study, it was identified that the individuals had plasma vitamin C below the reference level (10% avitaminosis C, 28% hypovitaminosis C, 57% in normal limits and almost 5% hypervitaminosis C).

Following the study, a considerable increase in the plasma vitamin C level was achieved, in 72.73% of the subjects. Thus, it was obtained a more appropriate integration within the reference values. This is represented by a 36.59% increase in plasma vitamin C.

The diet group had a smaller increase in plasma vitamin C, and a significant decrease in certain individuals. The group with supplements had a greater increase in plasma vitamin C level and a small decrease in certain individuals. The group following a diet based on supplements had a significant increase in plasma vitamin C levels while no decreases were recorded. In conclusion, vitamin C associated with a proper diet can achieve the therapeutic objectives in a shorter time, than the separate prescription of various diets or supplements, even in healthy subjects. These aspects can indicate that food no longer has the same vitamin C content, raising an alarm signal about the way in which these food items are produced.

Following the study, a capping of the plasma vitamin C level was observed, which may indicate that the surplus of vitamin C is eliminated from the body through urine, but may also highlight a resistance of the body towards the accumulation of the vitamin C. However, further studies are needed in this field.

Other limitations of the present study

include the absence of the anthropometric measurements, the constraints imposed by the small size of the group, the subjects' voluntary retreat from the research, and the methodological challenges.

The plasma vitamin C level is very little influenced by the general daily consumption of products with a high vitamin C content. This aspect that may be due to: the frequency and number of products consumed and which have a high vitamin C content; the eating habits, the way the food is consumed (raw or thermally prepared) as well as the production and supply chain.

Instead, the plasma vitamin C level can be influenced by long-term diet, with regular consumption of food items containing vitamin C. In most cases it can also be influenced by the consumption of food supplements, thus obtaining faster results. However, the best results regarding the increase of plasma vitamin C levels can be obtained by associating a healthy diet with the consumption of vitamin C food supplements.

Since the adequate consumption of vitamin C (and vitamins in general) is very important, it is important to choose suitable food sources that ensure an adequate exogenous intake. In this sense, there is a need to educate the population and its orientation towards local producers and towards products that are as natural as possible, harvested when mature, grown in gardens or open air, but also towards specialists in the field of nutrition and dietetics, who can offer suitable recommendations and necessary support.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study. Written informed consent was obtained from all subjects/patients.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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IMPROVING BREAST CANCER MANAGEMENT: A QUESTIONNAIRE-BASED STUDY ON PREVENTION AND DIAGNOSIS AMONG FAMILY DOCTORS AND SPECIALISTS IN NORTH-EASTERN ROMANIA

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IMPROVING BREAST CANCER MANAGEMENT: A QUESTIONNAIRE-BASED STUDY ON PREVENTION AND DIAGNOSIS AMONG FAMILY DOCTORS AND SPECIALISTS IN NORTH-EASTERN ROMANIA (Abstract): This study **aimed** to analyze the knowledge, clinical practices, and administrative aspects related to breast cancer care among family physicians and specialists in North-Eastern Romania. **Materials and methods:** A structured questionnaire was applied to a total of 200 healthcare professionals, including surgeons, oncologists, radiologists, gynecologists, and general practitioners. Key findings revealed significant deficiencies in screening awareness, interdisciplinary collaboration challenges, and limitations in patient education and reimbursement processes. **Results:** The study suggests the urgent need for a better integration between specialists and primary care providers, the development of enhanced educational programs, and improved access to screening and diagnostic services. **Conclusions:** Addressing these barriers may contribute to more effective breast cancer prevention, early detection, and management in Romania. **Keywords:** BREAST CANCER, QUESTIONNAIRES, PREVENTION.

INTRODUCTION

Breast cancer remains one of the leading causes of cancer-related morbidity and mortality worldwide. In Europe, it is the most commonly diagnosed cancer in women, accounting for nearly 29% of all female malignancies (1). Despite improvements in early detection and treatment, disparities in survival rates persist, with lower survival observed in Eastern European countries, including Romania (2).

The etiology of breast cancer is multi-

factorial, influenced by genetic, hormonal and environmental factors. The increasing prevalence of obesity, physical inactivity, late childbearing, and high-fat diets has contributed to a rising incidence rate (3). Additionally, healthcare disparities, low screening awareness and inadequate funding for national programs further contribute to Romania's high mortality rates (4).

According to the GLOBOCAN 2020 report, breast cancer remains the most frequently diagnosed malignancy worldwide with an increasing incidence trend (5). In Romania, breast cancer ranks among the top causes of oncological mortality, with early detection remaining a major challenge due to a lack of well-structured national screening programs and limited public awareness campaigns (6). Additional barriers in the Northeastern Romania healthcare system-such as inadequate reimbursement policies and poor coordination between specialists and primary care physicians-further delay timely diagnosis and treatment (7).

This study aims to evaluate the current state of breast cancer management in North-Eastern Romania by assessing the knowledge, practices and perceptions of family physicians and specialists while also seeking to identify gaps in early detection, patient education and healthcare infrastructure in order to formulate valuable recommendations for policy improvements.

MATERIALS AND METHODS

A four-step approach was used in this study:

1. *Questionnaire development:* Structured, self-administered questionnaires designed following a literature review and expert input (8). The survey included questions on screening practices, patient management, interdisciplinary collaboration, and administrative challenges.

2. *Data collection:* The survey was distributed to 200 healthcare professionals across various medical specialties, including surgery, oncology, radiology, gynecology, and general practice.

3. *Data analysis:* Responses were systematically categorized and analyzed using descriptive statistics to identify trends in clinical practice, screening recommendations, and interdisciplinary collaboration.

All healthcare professionals provided informed consent regarding data confidentiality and privacy. The data were processed using *Microsoft Excel* 2010.

4. *Validation:* The results were reviewed by experts to ensure accuracy and relevance (9).

The questionnaire assessed various aspects of breast cancer screening, diagnosis, treatment, and follow-up, with a particular focus on healthcare system challenges and opportunities for improvement. The statistical analysis of our study provided a comprehensive understanding of the collected data. Using the descriptive statistics function, we computed key statistical measures, including the mean, mode, skewness, and kurtosis, to assess the distribution and shape of the data.

RESULTS

The study identified several key aspects of breast cancer management among healthcare professionals in North-Eastern Romania.

The demographic structure of the surveyed group highlighted that most participants were young adults (36-40 years old - 32.42%) and women (64.1%). The sample consisted of a diverse group of 100 doctors from various medical specialties, including general and plastic surgery, oncology, radiology, gynecology, and radiotherapy, along with 100 general practice doctors (tab. I, fig. 1)

TABLE I.

Demographic and socioeconomic characteristics.

Characteristics	Frequency %
Age (years)	
31-35	24.27
36-40	32.42
41-45	18.92
46-50	10.84
51-55	13.55
Gender	
Female	64.1
Male	35.9
Specialties	
General surgery	29.4
Plastic surgery	4.7
Medical oncology	27.6
Gynecology oncology	14
Medical radiology	16.6
Radiotherapy	7.7
Family medicine	100

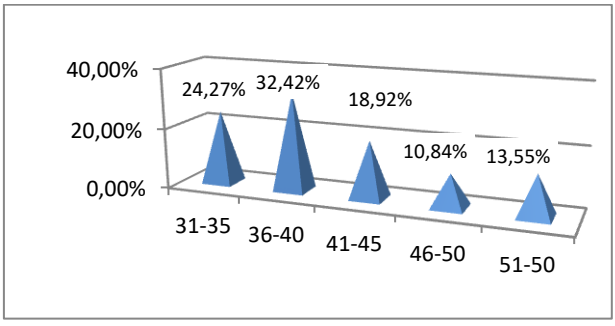

Fig .1. Age histogram

Clinical knowledge and practices among specialists

Most specialists (61.5%) reported frequent encounters with suspected breast cancer cases. However, 76.9% of respondents considered cases in women under 30 to be rare. The most common reasons for referral were the presence of breast lumps (97.4%), abnormal nipple discharge (41%), and a family history of breast cancer (28.2%). In younger patients, breast lumps remained the predominant reason for referral (87.2%).

Regarding diagnostic methods, mammography, breast ultrasound, and biopsy were recommended in over 92% of cases. For younger patients, ultrasound (92.3%) and MRI (74.4%) were preferred over mammography (43.6%). Fine needle aspiration biopsy (48.7%) and core needle biopsy (46.2%) were used with similar frequency.

Knowledge and education among family physicians

Most family physicians rated their knowledge of breast cancer as 'very well-informed' (63.5%) or 'moderately informed'

(35.6%). However, 57.7% had never attended courses or workshops on breast cancer prevention and diagnosis. Additionally, 53.8% of respondents believed that information on breast cancer was not sufficiently available for family physicians.

Diagnosis and recommendations among specialists

The diagnosis of breast cancer following biopsy was reported as frequent or very frequent by 59% of respondents. The most important criteria for monitoring patients included lymph node involvement (97.4%), tumor size (87.2%), and distant metastases (82.1%) (fig. 2).

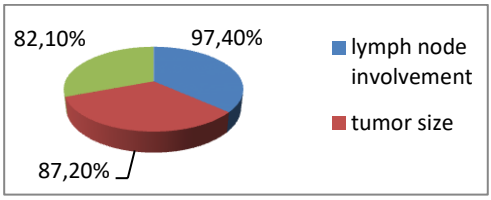

Fig. 2. The most important criteria for monitoring patients

Treatment recommendations were primarily surgical (94.9%), followed by chemotherapy or radiotherapy (92.3%) and hormonal therapy (82.1%).

Screening and diagnostic practices among family physicians

The majority of respondents (46.2%) recommended initiating mammographic screening between 40 and 50 years, followed by 31.7% who supported screening between 30 and 40 years. Breast ultrasound (94.2%), mammography (91.3%), and self-examination (89.4%) were the most frequently recommended screening modalities. MRI was less frequently recommended (13.5%) (fig. 3).

For high-risk young patients, breast ultrasound (89.4%) and self-examination (81.7%) were preferred, with only 39.4% recommending mammography. Screening frequency for moderate-risk women was predominantly every three years (53.8%), followed by every two years (31.7%).

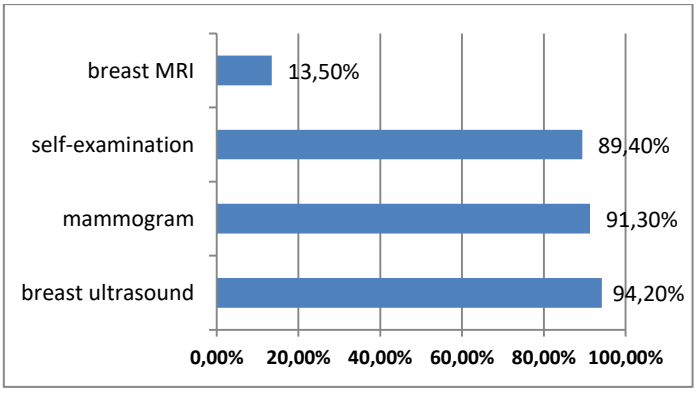

Fig. 3. Recommended types of screening

Challenges in patient monitoring and reimbursement

Among specialists, 76.9% were familiar with reimbursement procedures for breast cancer care. The most common barriers were delays in processing (38.5%), unclear procedures (33.3%), and additional administrative challenges (35.9%). Issues in re-

imbursement were reported as rare by 60.5% of respondents.

Challenges in patient monitoring included patient non-compliance (43.6%), limited access to imaging investigations (41%), and insufficient resources (38.5%). (fig. 4)

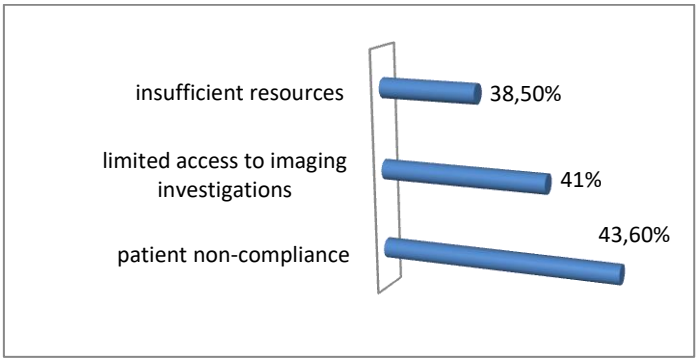

Fig. 4. Challenges in monitoring

Collaboration between specialists and family physicians

Collaboration with family physicians was rated as poor by 43.6% of specialists, while 28.2% considered it acceptable. Proposed improvements included interdisciplinary scientific meetings, training sessions, information bulletins, streamlined referral processes, and enhanced communication channels.

To assess the statistical significance of these differences, several tests were applied. The F-test confirmed significant variance differences between the two groups, justifying the use of a heteroscedastic t-test, which showed statistically significant differences in diagnostic preferences, reimbursement familiarity, and preventive measures. The Pearson correlation coefficient indicated a strong correlation (0.79) between gender and diagnostic recommendations, and a very high correlation (0.92) between mammo-graphy and ultrasound preferences. Conversely, a negative correlation (-0.50) was observed in certain diagnostic choices, indicating contrasting preferences between groups. Cancer frequency perception showed a moderate correlation (0.30) with professional background, while responses within each group were highly consistent (0.99 correlation). Familiarity with reimbursement procedures exhibited a perfect correlation (1.00), demonstrating uniform agreement. A strong correlation (0.95) was found between general practitioners' and specialists' views on prevention, although some prevention strategies showed a negative correlation (-0.38). Professional resource preferences were highly correlated (0.99), with some choices negatively associated with alternative decision-making factors (-0.78). Correlation curves were plotted to visualize these relationships, reinforcing statistical findings.

The correlation coefficient (0.954109) confirms a very strong linear relationship between the two variables, meaning that the responses or recommendations of specialists are highly consistent across the dataset (fig. 5).

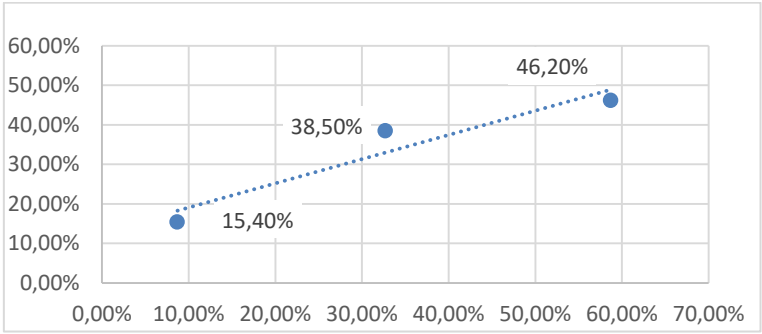

Fig. 5. Correlation between general practitioners' and specialists' views on prevention

DISCUSSION

The results of the questionnaire highlight several critical aspects that influence the management of breast cancer in Romania. One of the main findings is that while there is generally good knowledge of current diagnostic and treatment protocols, there are significant challenges related to collaboration between specialists and family physicians (7, 10, 11, 12-14). This lack of coordination can delay early diagnosis and optimal treatment strategies, which are essential for improving patient outcomes. Establishing a national database for rapid access to imaging investigations, strengthening prevention and screening programs, and reducing the turnaround time for pathological results are key steps that could improve breast cancer management (15-18).

Furthermore, there is a clear need to enhance education and awareness not only among healthcare professionals but also among patients, particularly young women and family physicians (19-22). Improving screening and patient management should involve national information campaigns, better access to diagnostic tests, greater involvement of family physicians in performing breast ultrasounds, and the deployment of mobile medical units in rural areas (23-27). Increasing funding for diagnostic tests, enhancing communication between healthcare providers, and implementing health education programs in schools are also necessary steps to ensure better early detection and treatment adherence.

A major concern identified in the study is the difficulty in reimbursing services and monitoring patients. The complexity of reimbursement procedures and bureaucratic delays further hinder access to timely diagnostics and treatment (16, 18, 28). Establishing a streamlined reimbursement system, along with integrating digital solutions for patient tracking, could alleviate some of these barriers.

Current research and increased awareness are essential to fully understand and respond to evolving trends in breast cancer incidence and patient characteristics. Globally, 90% of observed countries manage breast cancer diagnosis and treatment through multidisciplinary teams that adhere to national guidelines (11). Most European countries align their breast cancer care services with international standards, particularly those established by the European Society for Medical Oncology (12-14). However, Romania remains an exception, with significant gaps in service accessibil-

ity and financing. For example, genetic testing is not widely covered by public healthcare funds, despite its recognized importance in high-risk populations (29).

Survival rates vary significantly depending on the stage at diagnosis, with five-year survival rates ranging from 98% in early-stage diagnoses to only 24% in advanced-stage cases (19). In high-income countries, breast cancer mortality rates have been declining due to advancements in treatment and the widespread implementation of early screening programs. Unfortunately, Romania has one of the lowest participation rates in mammographic screening across the EU, with only 9% of eligible women being screened in 2020, compared to the EU average of 60% (20). A study conducted in Romania demonstrated that education plays a crucial role in influencing women's attitudes toward screening. However, national efforts to raise awareness and implement free breast cancer screening programs have been insufficient (21).

A critical issue remains the lack of studies examining the impact of social media campaigns on public awareness regarding screening importance and the benefits of early diagnosis. Research has shown that social media platforms such as Facebook, Twitter, and YouTube can significantly increase engagement and awareness regarding health-related topics (30). Utilizing targeted digital campaigns to educate younger women on self-examination and early detection strategies may help bridge existing knowledge gaps.

Another significant aspect of breast cancer care is psychological support. Emotional well-being plays a crucial role in patient satisfaction and treatment adherence. Patient advocacy groups and support networks have been shown to provide essential resources for breast cancer survivors, improving their quality of life. In addition, fostering supportive communities and increasing access to mental health services for patients undergoing treatment could enhance overall care standards (31).

To further optimize breast cancer management, Romania must align with European best practices, particularly regarding screening policies. The European Commission's Initiative on Breast Cancer (ECIBC) recommends organized population-based screening programs with biennial mammographic screening for women aged 50 to 69 (26). Additionally, improving national healthcare coverage and implementing more effective strategies for early detection are necessary to enhance survival rates. A systematic review of screening implementation strategies across Europe suggests that access to services, financial incentives, and primary care integration significantly impact participation rates (27).

In conclusion, the study highlights the urgent need to improve interdisciplinary collaboration, streamline reimbursement systems, and enhance patient education. A multifaceted approach-including policy reforms, public awareness campaigns, and greater investments in screening infrastructure-is essential for improving breast cancer outcomes in Romania. Further studies should focus on assessing the long-term impact of targeted interventions on screening adherence and patient survival rates.

CONCLUSIONS

This study highlights significant gaps in breast cancer prevention, screening, and treatment strategies in Romania. While specialists demonstrate substantial expertise, general practitioners face challenges in

accessing ongoing education and navigating referral pathways (19). Improved interdisciplinary collaboration, combined with essential policy reforms, could significantly enhance patient outcomes and reduce mortality rates.

A comprehensive approach that includes increased public awareness, standardized screening initiatives, and systematic healthcare system improvements is crucial to bridging existing gaps and ensuring efficient and timely breast cancer management in Romania (20).

Further research is required to assess the long-term impact of targeted interventions on breast cancer detection and treatment outcomes in Romania.

CONFLICTS OF INTEREST AND FUNDING

All the authors declare no funding received and no conflict of interest.

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EVALUATION OF NEUROLOGIC EFFECTS OF SOME NEW IBUPROFEN THIAZOLIDIN-4-ONE DERIVATIVES

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EVALUATION OF NEUROLOGIC EFFECTS OF SOME NEW IBUPROFEN THIAZOLIDIN-4-ONE DERIVATIVES (Abstract). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as ibuprofen, are widely used to treat pain and inflammation associated with various conditions, including neurodegenerative diseases. The aim of this study was to evaluate the neurological effects of several new ibuprofen derivatives containing a thiazolidin-4-one moiety. **Materials and methods.** The effects of ibuprofen-thiazolidine-4-one derivatives (4a-n) on neuromotor function and locomotor activity in mice were assessed using the Rotarod and locomotor activity tests. The compounds were administered orally by gavage, as a suspension in tween 80, at a single daily dose equivalent to 1/20 of the LD50. A reference group, which received ibuprofen, and a control group, which received tween 80, were also used. **Results:** The ibuprofen-thiazolidine-4-one derivatives (4a-n) did not produce statistically significant changes in neuromotor performance, at any of the three time points assessed (1 hour, 2 hours, 6 hours post-administration). However, a significant decrease in total locomotor activity-both horizontal and vertical movements—was observed with compound 4b. Notably, several derivatives also led to a marked reduction in vertical movements. **Conclusions:** The observed decrease in vertical movements for some ibuprofen thiazolidine-4-one derivatives may indicate a potential anxiolytic effect, though this hypothesis requires validation through further studies. **Keywords:** IBUPROFEN, THIAZOLIDIN-4-ONE, LOCOMOTOR ACTIVITY, NEURO-LOGIC EFFECTS.

INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) is a known class of therapeutics that relieve the inflammation and pain in various diseases as osteoarthritis, rheumatoid arthritis, renal colic, biliary colic, headache, dysmenorrhea and neurodegenerative diseases (1). Ibuprofen (an aryl propionic acid derivative) is a widely prescribed NSAID drug which, besides its known anti-inflammatory, analgesic and antipyretic effects, has some side effects such as gastro-intestinal, hepatic, renal or cardio-vascular toxicity (2). These disadvantages have led to the development of new candidates with a better pharmacotoxicological profile, such as thiazolidin-4-one derivatives.

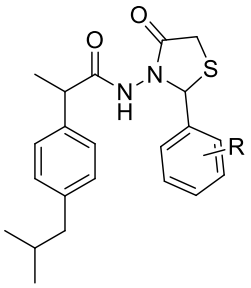
Thiazolidin-4-one derivatives are heter-

ocyclic compounds with various biological effects such as antioxidant, anti-inflammatory, analgesic, antimicrobial or antitumoral (1, 3, 4). Using different methods to evaluate locomotor deficits in memory tasks, some studies evidenced their promising potential against oxidative damage and alterations in the cerebral area (5, 6). Some thiazolidin-4-one derivatives showed beneficial effects in the treatment of neurological diseases associated with neuroinflammation that indirectly support motor function recovery (4, 7).

Also, some clinical trials, have been developed to evaluate the therapeutic potential of NSAIDs, including ibuprofen in neurodegenerative diseases, knowing their promising neuroprotective effects (1, 8). The aim of the study was focused on evaluation of neurologic effects of new ibuprofen derivatives with thiazolidine-4-one structure (**4a-n)**, using two *in vivo* methods: rotarod and locomotor activity assays.

MATERIALS AND METHODS

Ibuprofen thiazolidin-4-one derivatives (**4a-n**) (fig. 1) were synthesized by our research group (3).



4a: R=H 4h: R=4-CH_3
4b: R=4-Cl 4i: R=3-CF_3
4c: R=4-Br 4j: R=4-CF_3
4d: R=4-F 4k: R=4-CN
4e: R=2-NO_2 4l: R=2.6-diCl
4f: R=3-NO_2 4m: R=4-NH_2
4g: R=4-NO_2 4n: R=4-$NHCOCH_3$

Fig. 1. Ibuprofen thiazolidin-4-one derivatives (**4a-n**).

The experiments were performed using Swiss albino mice provided from "Cantacuzino" Institute (Bucharest, Romania). The animals were kept in polyethylene cages with access to water and food ad libitum. The environmental conditions during the study were maintained relatively constant at a 20 ± 4°C temperature, 40-60% relative humidity and a 12 hrs. light/dark cycle. The food and the water were stop 18 hrs. before starting the experiments. The study was approved by the Ethical Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi (approval no. 292). All procedures were carried out in compliance with the European Community Guidelines (Directive 2010/63/EU) and Romanian law (Low no. 43/2014) on the protection of animals used for scientific purposes (1, 9). At the end of the study, all animals were euthanized in accordance with the legislation.

1. Rota-rod performance test

The Rota-rod test is very useful to evaluate the effect of new compounds on neuromotor performance, mainly, on brain function. This test is performed using the Rota-rod apparatus (Ugo Basile, Italy). With the help of five dividers and a revolving cylinder (rod), the device creates four lanes, each of which can hold one animal. A metal plate with a magnet affixed beneath each lane allows the resistance time to be recorded by stopping the timer when

the animal falls (10). The rod speed can be adjusted from the control panel, which features a digital display. According to the experimental protocol, animals were trained on the rotating rod at a speed of 15 rpm, and only those that remained on the rod for more than 60 seconds were selected. The apparatus recorded the time (in seconds) that each animal remained on the rod without falling, with a maximum allowed duration of 180 seconds (11,12). After training, the animals were divided into groups of eight, each receiving the test compounds (4a-n, L1-14), previously synthesized by our research group (3,9). The compounds were administered as a suspension in tween 80, orally by gavage, in a single daily dose of 1/20 of the LD50. To ensure accurate result interpretation, the study included a reference group, which received ibuprofen (L15), and a control group (L16), which received tween 80, used for administration of the tested compounds. The groups structure and administered doses (1/20 of LD50) are presented in first table.

TABLE I.

The groups and administered doses (mg/kg b.w.)

Group	Compound	Doses (mg/ kg b.w.)	Group	Compound	Dose (mg/ kg b.w.)
L1	**4a**	81.25	**L9**	**4i**	86.00
L2	**4b**	81.25	**L10**	**4j**	92.00
L3	**4c**	86.40	**L11**	**4k**	78.25
L4	**4d**	81.70	**L12**	**4l**	79.50
L5	**4e**	91.00	**L13**	**4m**	82.50
L6	**4f**	89.50	**L14**	**4n**	85.00
L7	**4g**	91.00	**L15**	**Ibuprofen**	68.75
L8	**4h**	89.50	**L16/Control**	**Tween 80**	0.1 mL/10 g

At 1 hour, 2 hours, and 4 hours after administration, the mice were subjected again to the test, and the time that the mice remained on the rotating rod was recorded each time. The initial time value, in seconds, was calculated as the arithmetic mean of the values at which all mice remained on the rotating rod at time zero (before the administration of the tested compounds) ± standard deviation.

2. Locomotor activity test

The effect of the tested compounds (4a-n) on spontaneous locomotor activity was analyzed using the IR Actimeter LE-8811 (Panlab, Spain). This device consists of a cage with dimensions of 45 cm (width) × 45 cm (length) × 20 cm (height), equipped with a control unit. The cage is fitted with an infrared frame system that allows monitoring of both vertical and horizontal movements, with multiple sensitivity levels that can be adjusted depending on the animal used. This frame is connected via the control unit to the SeDaCom computer program, which converts recorded data into Notepad formats. During the experiment, three cages were used.

According to the experimental protocol, the animals were divided into several groups (Table I), each consisting of eight mice, and then placed in the cages (one animal per cage). Their horizontal and

vertical movements were recorded for 5 minutes. After this time, the tested compounds were administered orally as a suspension in tween 80, at a dose of 1/20 of the LD50. Measurements were repeated at 1 hour, 2 hours, and 4 hours after administration, with the mice being placed back into the same cage each time. For each tested compound, as well as for the reference group (ibuprofen) and the control group (tween 80), total locomotor activity was determined, representing the total number of electronically recorded movements in both horizontal and vertical planes.

The initial values for horizontal and vertical movements, as well as the total value, were calculated as the arithmetic mean of all the animals studied at time zero (before the administration) ± standard deviation.

The one-way analysis of variance (ANOVA) and T-test were used to determine whether there are any statistically significant differences between tested compounds and controls. The p value < 0.05 was considered to be statistically significant.

RESULTS

1. Rota-rod performance test

The Rota-rod test evaluates the motor coordination of the animals, before and after the administration of tested compounds. For each group were calculated the latency, respectively the time expressed in seconds of how long did the animals lasted on the Rota-rod without falling. The obtained results are presented in the second table.

This test can provide data on motor coordination as well as other aspects of neuromotor function, such as motor learning, which is demonstrated by improved performance after successive trials (13).

TABLE II.

Time (seconds) recorded for animals treated with tested compounds

Group	Time (sec)		
	1 hr.	2 hrs.	4 hrs.
L1	164.29±29.36	162.14±29.70	160.29±25.57
L2	166.50±33.07	165.50±23.81	164.00±25.92
L3	167.60±21.65	164.83±28.15	161.67±27.14
L4	166.50±18.32	165.33±25.41	163.83±27.64
L5	167.67±30.21	166.67±31.21	165.33±30.85
L6	166.80±29.52	166.00±31.30	164.00±20.74
L7	167.00±29.07	166.00±21.91	159.00±25.90
L8	165.17±23.46	164.50±27.60	163.17±28.42
L9	167.00±20.93	165.33±24.39	164.83±28.00
L10	166.83±23.84	165.00±26.77	163.00±28.45
L11	167.33±27.30	164.67±23.89	164.17±24.58
L12	167.83±21.53	166.67±19.66	165.83±18.05
L13	166.67±13.42	165.33±25.55	164.50±25.88
L14	167.50±24.03	166.50±28.45	164.67±28.89
L15	165.80±31.75	156.86±27.22	153.50±30.49
L16	166.33±28.86	165.00±28.11	164.17±30.07
INITIAL	167.20±24.02		

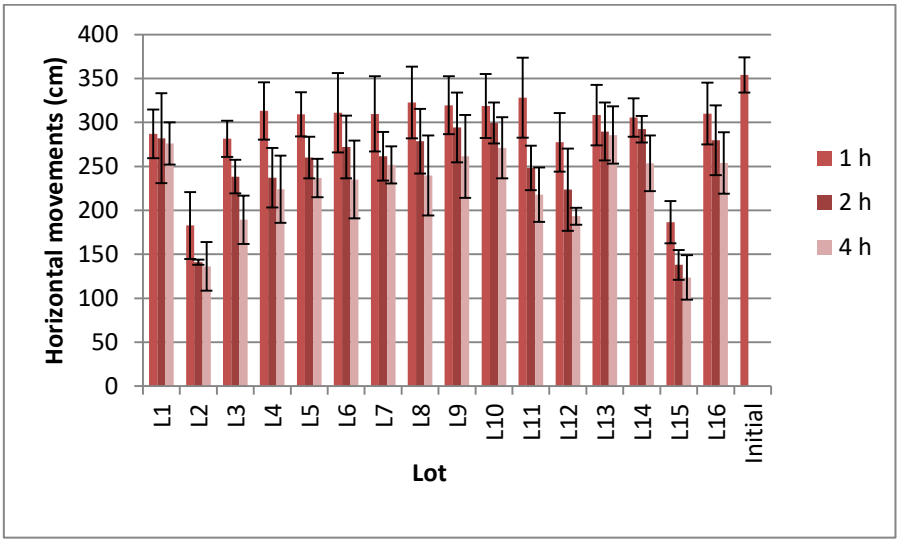
From the analysis of obtained results was noticed that groups treated with tested compounds (L1-L14), ibuprofen (L15) and vehicle (L16) did not display statistically significant differences ($p>0.05$), in all three intervals of time (1 hour, 2 hours, 6 hours). At the same time there were not registered statistically significant differences towards the initial value, before the administration of tested compounds. These observations led to the conclusion that ibuprofen as well as the studied derivatives of ibuprofen with thiazolidine-4-one structure had no influence on motility of the mice, respectively on motor coordination. Also, Singh *et al.* remarked that ibuprofen did not modify the grip strength, distance travelled and time spent on Rota-rod apparatus (14).

Locomotor activity test

It is known that the locomotor activity assay is a behavioral test and is easily influenced by study conditions such as light, cage dimensions or habituation with this space. This test is often used for pharmacological evaluation of new compounds that could be potential therapeutic agents (15). By using this behavioral test, important information relating to spontaneous locomotor activity in experimental animals, in both horizontal and vertical sides, as well as to total locomotor activity, were obtained.

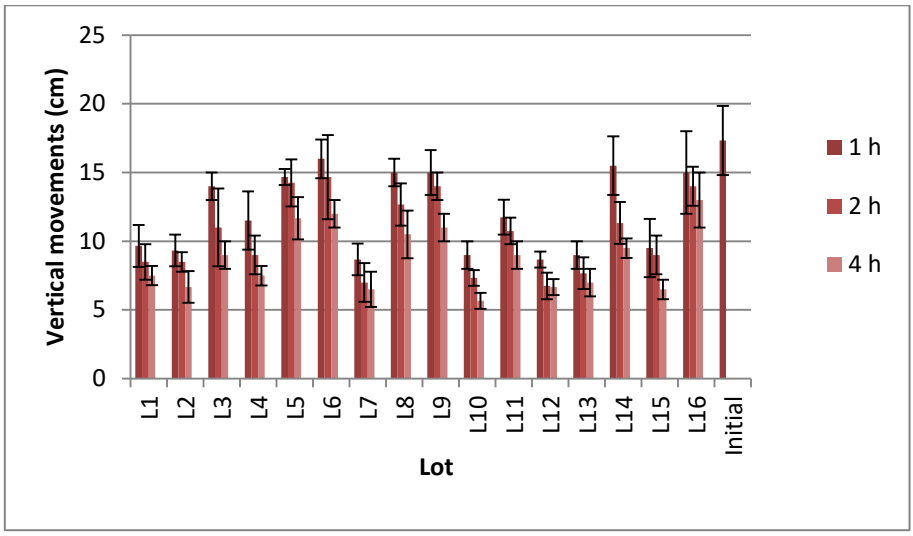
Horizontal movements can provide information relating to the animal's exploration behavior, but also to that of attending to its own hygiene (paw licking, hair and nose cleaning). Vertical movements characterized by the animal's climbing attempt, on the cage's transparent walls can indirectly indicate the animal's fear or anxiety (12).

The obtained results are presented in figures 2-4.



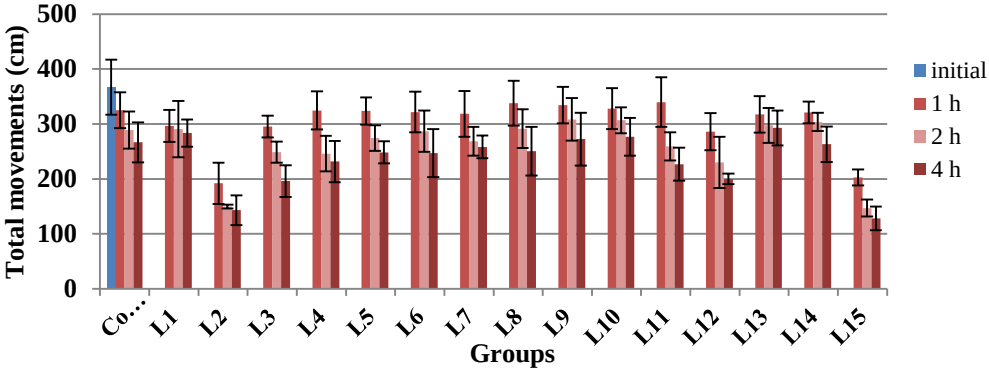
* data represents the average of 8 values ± standard deviation

Fig. 2. Locomotor activity registered in horizontal side of experimental animals treated with studied compounds in comparison with ibuprofen (L15)



* data represents the average of 8 values ± standard deviation

Fig. 3. Locomotor activity registered in vertical side of experimental animals treated with studied compounds in comparison with ibuprofen (L15).



* data represents the average of 8 values ± standard deviation

Fig. 4. Total locomotor activity registered to experimental animals treated with studied compounds in comparison with ibuprofen (L15).

DISCUSSION

Relating to locomotor activity in horizontal side, a significant decreasing of the movements number was noticed in groups treated with ibuprofen (p = 0.006, L15) and **4b** compound (p = 0.004, L2). It seems that the substitution of phenyl ring with chloro may influence the locomotor activity.

The analysis of the locomotor activity registered in vertical side pointed out a statistically significant decline for ibuprofen (p = 0.006) and **4b** compound (p = 0.004) as well as for following derivatives: **4a** (p = 0.003, R = H, L1), **4d** (p = 0.023, R = 4-F, L4), **4g** (p = 0.001, R = 4-NO2, L7), **4j** (p = 0.004, R = 4-CF3, L10), **4k** (p = 0.024, R = 4-CN, L11), **4l**

(p = 0.001, R = 2,6-dichloro, L12) and **4m** (p = 0.001, R= 4-NH2, L13).

From the total locomotor activity point of view, at different time intervals (1 hour, 2 hours, 4 hours), was noticed that the majority of groups did not display statistically significant differences (p>0.05) in comparison to the control group.

A significant reduction in the total numbers of movements (in horizontal and vertical sides) was registered only in groups that were treated with ibuprofen (p = 0.008, L15) and with **4b** derivative (2-(4-chlorophenyl)-3-[2-(4-isobutylphenyl)-2-methyl]acetamide-thiazolidine-4-one, p = 0.004, L2).

Lee *et al*. have been evaluated the effect of ibuprofen in induced stress model with anxiety induced to rats.

Open-field activity was used to evaluate locomotion and exploratory behavior and even if the results were not statistically significant it was observed a decrease in the numbers of crossing in the central zone (16). Another study included salicylic acid and its prodrug that presented some potential of decreasing neuro-inflammation in mice. In the behavioral studies such as open field test, it was evidenced some reduced locomotor activity (9).

CONCLUSIONS

New ibuprofen derivatives with thiazolidine-4-one structure were tested from the point of view of motor coordination (through Rota-rod test) and of spontaneous locomotor activity (behavioral test). The obtained results during the Rota-rod test support the observation that studied derivatives do not influence significantly the experimental animal's motor coordination. At the same time the registered effect for these compounds on the locomotor function was similar to the one observed in case of ibuprofen.

In behavioral tests, derivative **4b** (2-(4-chlorophenyl)-3-[2-(4-isobutylphenyl)-2-methyl] acetamido-thiazolidin-4-one) reduced the total locomotor activity and horizontal movements, exhibiting effects similar to those induced by ibuprofen. The observed decrease in vertical movements for certain derivatives may suggest an anxiolytic effect, though this hypothesis requires validation through further studies.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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FINITE ELEMENT ANALYSIS OF THE EFFECT OF INSERTION ANGLE AND DIAMETER ON STRESS, STRAIN, AND DISPLACEMENT IN ORTHODONTIC MINI-IMPLANTS

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FINITE ELEMENT ANALYSIS OF THE EFFECT OF INSERTION ANGLE AND DIAMETER ON STRESS, STRAIN, AND DISPLACEMENT IN ORTHODONTIC MINI-IMPLANTS (Abstract): Orthodontic mini-implants are widely used as temporary anchorage devices, and their clinical performance depends on biomechanical factors related to implant design and insertion technique. **Materials and methods**: A three-dimensional finite element model of an orthodontic mini-implant inserted into cortical and cancellous bone was developed. Three insertion angles relative to the cortical bone surface (60°, 90°, and 120°) and two mini-implant diameters (1.5 mm and 2.0 mm) were analyzed under standardized orthodontic loading conditions. Total displacement, equivalent von Mises stress, and equivalent linear strain were evaluated. **Results:** Higher insertion angles resulted in increased displacement and strain, with the highest values observed at 120°. Maximum von Mises stress values were comparable among different angles; however, stress distribution patterns and stress localization varied significantly. Mini-implants with a smaller diameter exhibited higher displacement and stress values, indicating reduced mechanical stiffness, while larger-diameter mini-implants demonstrated improved biomechanical performance. **Conclusions:** Both insertion angle and mini-implant diameter significantly influence the mechanical behavior of orthodontic mini-implants. Perpendicular insertion and increased implant diameter were associated with reduced deformation and more favorable stress distribution, suggesting improved primary stability and clinical performance. **Keywords**: FINITE ELEMENT ANALYSIS; ORTHODONTIC MINI-IMPLANTS; INSERTION ANGLE; IMPLANT DIAMETER; BIOMECHANICS.

INTRODUCTION

Orthodontic mini-implants, also referred to as Temporary Anchorage Devices (TADs), have gained significant traction in contemporary orthodontic treatments. The mechanical behavior of these implants is significantly influenced by geometric parameters such as insertion angle and diameter. Understanding these influences is crucial for optimizing the design and application of mini-implants in clinical settings.

TADs demonstrate variable success rates, with studies indicating that failure rates typically range from 9% to 30% (1). Various systematic reviews have reported success rates for TADs from 80% to 94% (2, 3). The relatively high reliability of mini-implants can largely be attributed to their design, which enables stable anchorage even under orthodontic loading conditions, cru-

cial for effective tooth movement (4, 5).

The insertion of mini-implants is a minimally invasive procedure compared to traditional orthodontic anchorage methods, such as palatally installed implants or miniplates (6). The ability to insert these devices without the need for extensive surgical interventions enhances patient comfort and expedites the treatment process (7, 8).

Research has indicated that mini-implants can significantly broaden the range of treatment strategies available to orthodontists. For example, they have been effectively utilized in complex cases, such as maxillary protraction and closure of extraction spaces, allowing for more efficient treatment sequences (9, 10). Their versatility makes them suitable for various applications, including en-masse retraction and space closure procedures (11, 12).

Ongoing research is focused on improving the design and stability of mini-implants. Studies have explored surface modifications to enhance osseointegration and stability (13), and new findings on optimal insertion protocols continue to emerge (14). The stability of mini-implants, both during the healing phase and after mechanical loading, has been a primary focus, with findings suggesting that various lengths and diameters can impact their success rates (15, 16).

While mini-implants present numerous advantages, they are not without risks. Factors that may influence the failure of mini-implants include patient age, oral hygiene, the placement site's bone density, and the amount of force applied during orthodontic treatment (3, 16). Thus, careful patient selection and technique adjustments are crucial for optimizing their performance (5).

The interplay between insertion angle and diameter further complicates the mechanical behavior of orthodontic mini-implants. Wu *et al*. explored how design variables, including taper shape and diameter, jointly influence mechanical stability (17). They found that specific combinations of insertion angle and diameter could lead to optimized outcomes in terms of anchorage strength and resistance to mechanical loading.

FEA is favored in biomechanical studies of orthodontic mini-implants due to its ability to model complex structures and simulate physiological responses accurately. The application of FEA allows researchers to predict stress distribution patterns around mini-implants under different forces, thus facilitating the design of implants that can withstand significant loading without failure (18, 19). By employing FEA, orthodontists can assess the mechanical performance of mini-implants, unveiling insights about their interactions with surrounding bone and tissues that would be difficult to quantify through traditional *in-vivo* experiments (20, 21).

The aim of this study was to evaluate, using finite element analysis (FEA), the influence of insertion angle and diameter on the biomechanical behavior of orthodontic mini-implants. Specifically, the study investigated the effects of three insertion angles (60°, 90°, and 120° relative to the cortical bone surface) and two mini-implant diameters (1.5 mm and 2.0 mm) on total displacement, equivalent von Mises stress, and equivalent strain distributions within the mini-implant.

The objective of this analysis was to identify biomechanically favorable insertion configurations that minimize displacement and stress concentration, thereby contributing to improved primary stability

and providing biomechanical support for clinical decision-making regarding orthodontic mini-implant placement.

MATERIALS AND METHODS

A three-dimensional finite element analysis (FEA) was performed to evaluate the biomechanical behavior of orthodontic mini-implants under different insertion angles and diameters. The modeling process consisted of geometric modeling, material property assignment, mesh generation, application of boundary conditions and loads, and result analysis.

Geometric Modeling. The mandibular model, including teeth and periodontal ligament (PDL), was reconstructed from computed tomography (CT) images, which were digitized and converted into three-dimensional solid models. The periodontal ligament was modeled with a uniform thickness of 0.25 mm based on the external geometry of the tooth roots. Commercially inspired mini-implant models were created according to Leone® and Dual Top Jeil Medical Corporation® (Seoul, Korea) designs.

Two mini-implant diameters (1.5 mm and 2.0 mm) and a total length of 12 mm with a maximum insertion depth of 9 mm were analyzed. Mini-implants were virtually inserted at depths of 5, 6, and 7 mm into cortical and cancellous bone. The insertion site was defined as the interradicular space between the premolar and molar region.

All geometric models were created and assembled using SpaceClaim (ANSYS Inc., USA).

Finite Element Model

The assembled models were imported into ANSYS Workbench (version 2021, ANSYS Inc., USA) and discretized using three-dimensional 10-node tetrahedral elements. A mesh convergence study was performed with element sizes ranging from 0.3 mm to 1.2 mm. An element size of 0.3 mm was selected in the mini-implant insertion region, while a size of 0.8 mm was used for the remaining volume.

All materials (bone, teeth, PDL, and mini-implants) were assumed to be linear elastic, homogeneous, and isotropic. Material properties were assigned based on values reported in the literature. Mini-implants were modeled using Ti-6Al-4V alloy for Dual Top Jeil Medical Corporation® implants and stainless steel 316L for Leone® implants.

Contact Conditions and Boundary Condition. The interfaces between teeth and periodontal ligament were assumed to be perfectly bonded. A frictionless contact (coefficient of friction = 0) was defined between the mini-implant and bone. The proximal and distal surfaces of the mandibular bone were fixed in all directions to simulate boundary constraints.

Loading Conditions. Orthodontic forces ranging from 0.1 N to 10 N were considered. A force magnitude of 2 N, corresponding to the upper limit of clinically accepted immediate loading, was applied to the head of the mini-implant. The force was oriented obliquely in the proximal direction to simulate en-masse tooth retraction and to allow comparative analysis between different insertion angles and implant diameters.

Outcome Measures. The numerical simulations evaluated total displacement, equivalent von Mises stress, and equivalent linear strain distributions within the mini-implant and surrounding bone structures. The results were analyzed to assess the influence of insertion angle and implant diameter on the biomechanical behavior of

orthodontic mini-implants.

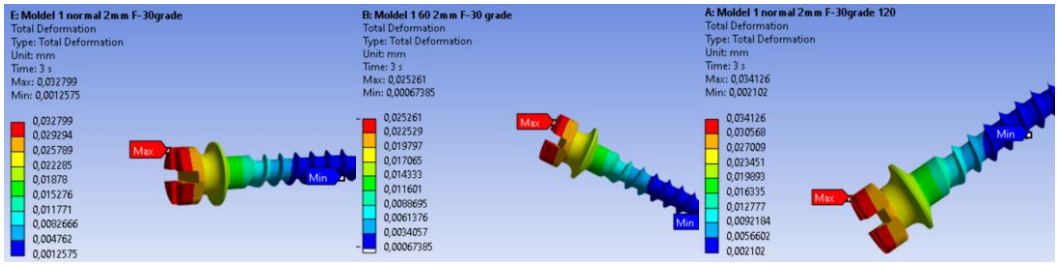
RESULTS

Influence of the mini-implant inclination angle relative to the Y-axis

Total displacements

The total deformation state of the mini-implant as a function of the inclination angle relative to the cortical bone surface is presented in first figure. Higher values are obtained for an inclination angle of 120°. The results indicate that the inclination angle significantly influences the displacement state.

The comparative values of the maximum total displacements in the mini-implant as a function of the inclination angle relative to the cortical bone surface are presented in figure 2.



90º 60º 120º

Fig. 1. Total deformation state of the mini-implant as a function of the inclination angle relative to the cortical bone surface.

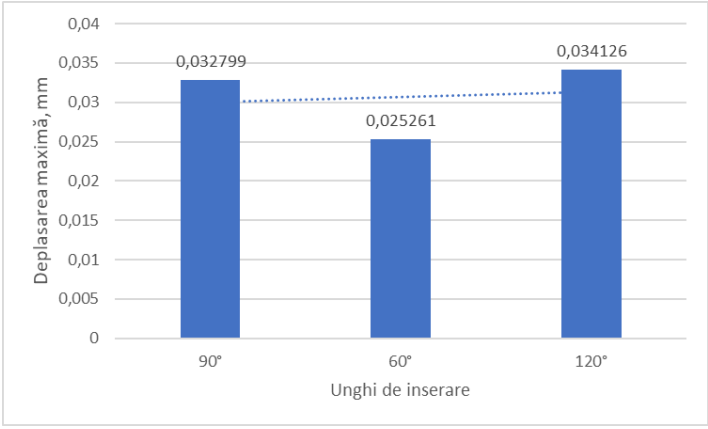


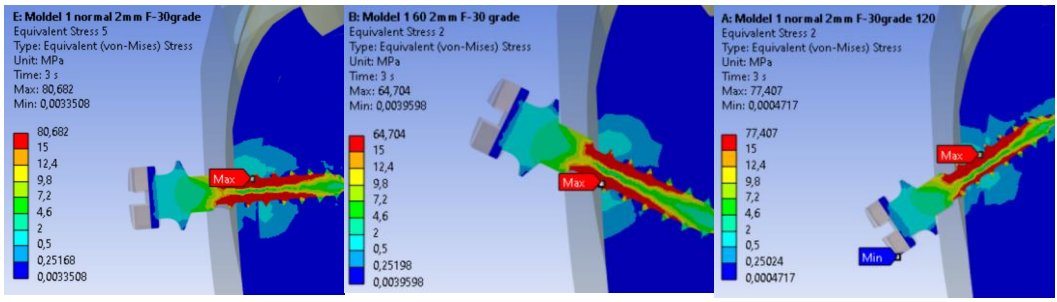
Fig. 2. Comparative values of the maximum total displacements in the mini-implant as a function of the inclination angle relative to the cortical bone surface.

Equivalent von Mises stresses

The stress distribution for the three inclination angles relative to the cortical bone surface, shown in cross-section, is presented in figure 3. The maximum value obtained is 138.92 MPa for the case of an insertion length of 5 mm into the bone. The maximum values for the three analyzed cases are relatively close. The results indicate that the stress distribution pattern differs significantly among the analyzed cases, with the maximum stress

located at a shallower depth for the 60° inclination.

The comparative values of the maximum stress as a function of the inclination angle relative to the cortical bone surface are presented in fourth figure.



90º 60º 120º

Fig. 3. Stress distribution for the three inclination angles relative to the cortical bone surface, shown in cross-section.

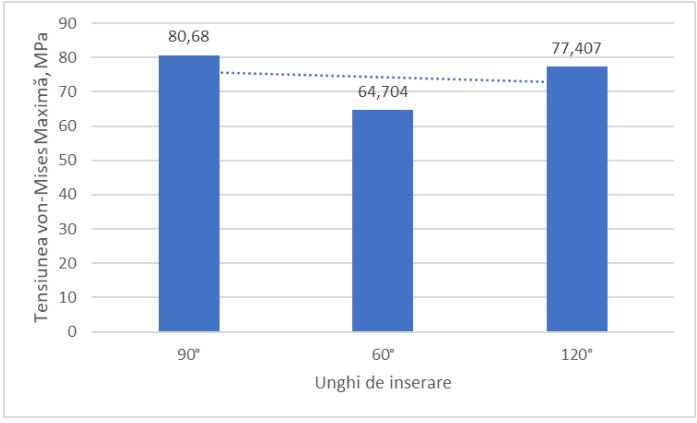


Fig. 4. Comparative values of the maximum stress as a function of the inclination angle relative to the cortical bone surface.

Equivalent strains

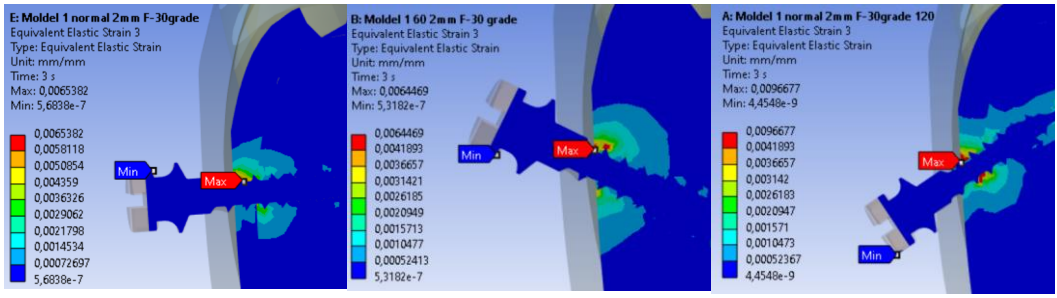
The equivalent linear strain distribution for the three inclination angles relative to the cortical bone surface is presented in figure 5. The maximum value obtained is 0.009677 mm/mm for the inclination angle of 120°. The volume of stressed material is greater for the 60° and 120° cases compared to the 90° case.

The comparative values of the equivalent strains as a function of the inclination angle relative to the cortical bone surface are presented in figure 6

Influence of the mini-implant Diameter. Total displacements

The total deformation state of the mini-implant as a function of the mini-implant type is presented in Fig. 7, with the mini-implant of 1.5 mm diameter shown on the left and the 2 mm diameter mini-implant shown on the right. Higher values are obtained for the

smaller-diameter implant. The results indicate that the diameter significantly influences the displacement state, which is physically consistent, as the bending moment of inertia for a circular cross-section increases with diameter, resulting in a stiffer system.



90º 60º 120º

Fig. 5. Equivalent linear strain distribution for the three inclination angles relative to the cortical bone surface.

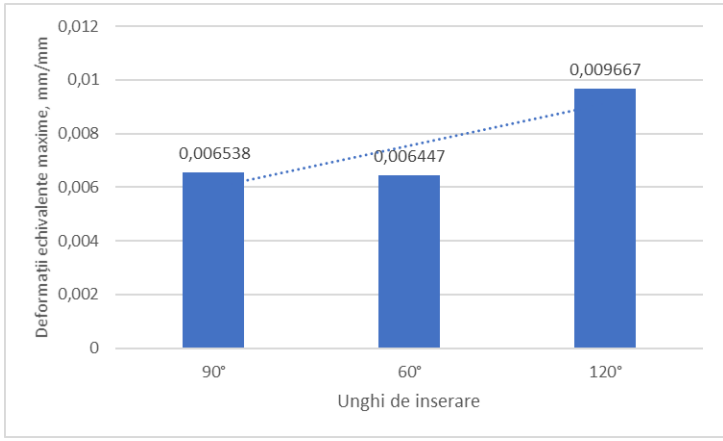
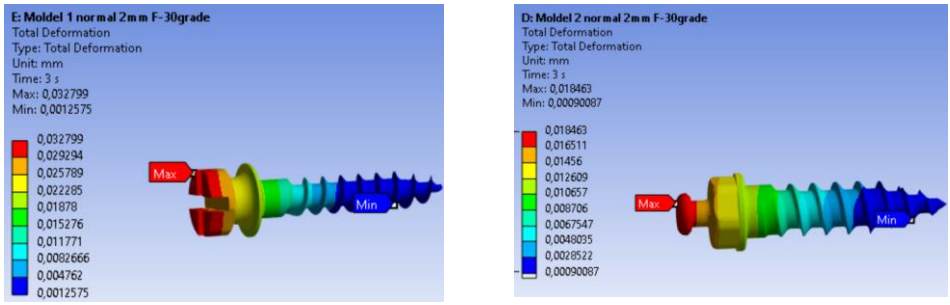


Fig. 6. Comparative values of the equivalent strains as a function of the inclination angle relative to the cortical bone surface.



Model 1 d = 1,5 mm Model 2 d = 2 mm

Fig. 7. Total deformation state of the mini-implant as a function of the mini-implant type.

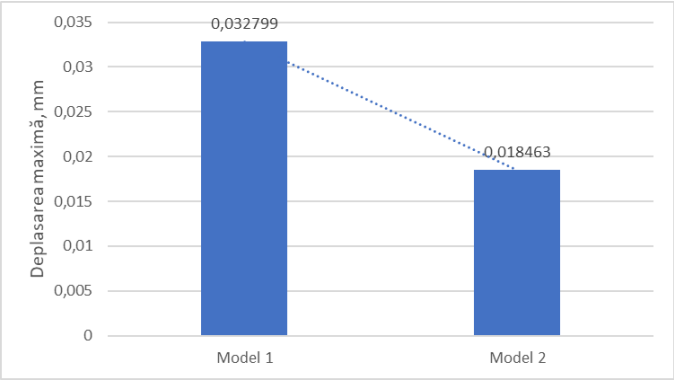
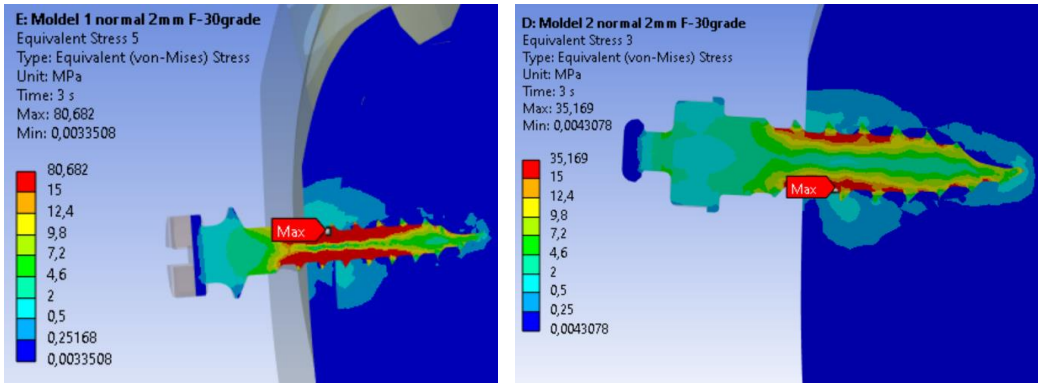


Fig. 8. Comparative values of the maximum total displacements in the mini-implant as a function of its type.

Equivalent von Mises stresses

The stress distribution for the different insertion lengths of the implant, shown in cross-section, is presented in Fig. 9 The maximum value obtained is 80.682 MPa for the model 1 mini-implant with a diameter of 1.5 mm, while the minimum value is 35.169 MPa for the model 2 mini-implant with a diameter of 2 mm. The results indicate that the stress distribution differs significantly between the two analyzed cases. It can also be observed that the volume of affected material is greater for the model 2 mini-implant.



Model 1 d = 1,5 mm Model 2 d = 2 mm

Fig. 9. Stress distribution for the different insertion lengths of the implant, shown in cross-section.

The comparative values of the maximum stress as a function of the mini-implant model are presented in figure10.

Equivalent strains

The equivalent linear strain distribution for the two mini-implant models is presented in figure 11. The maximum value obtained is 0.0066167 mm/mm for model 2. The results indicate that the distribution of equivalent strains differs between the two analyzed cases, with the maximum located

at different positions.

Figure 12 presents the comparative values of the equivalent strains as a function of the mini-implant type.

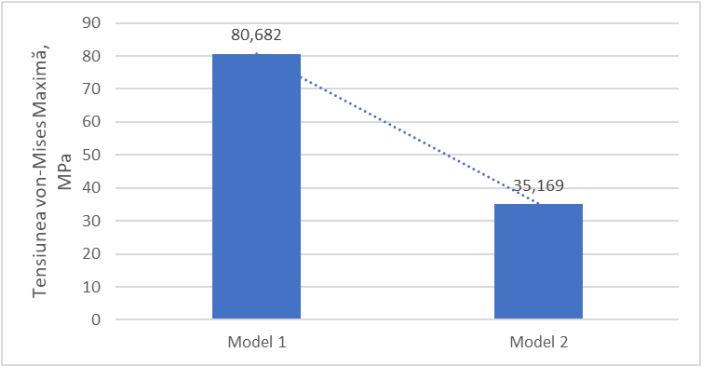
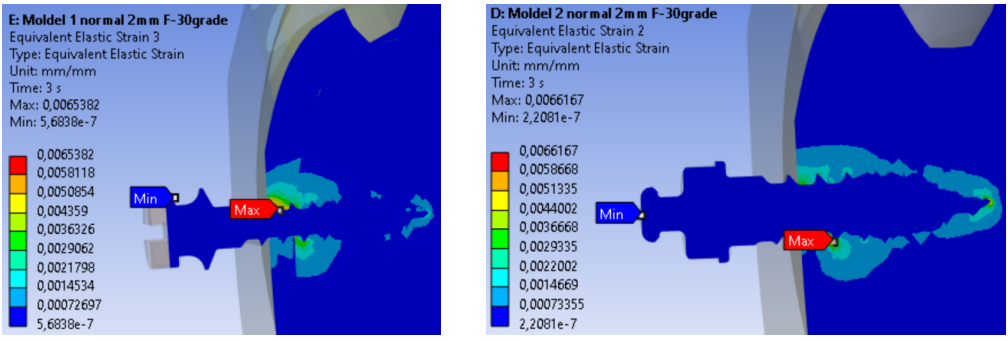


Fig. 10. Comparative values of the maximum stress as a function of the mini-implant model.



Model 1 d = 1,5 mm Model 2 d = 2 mm

Fig. 11. Equivalent linear strain distribution for the two mini-implant models.

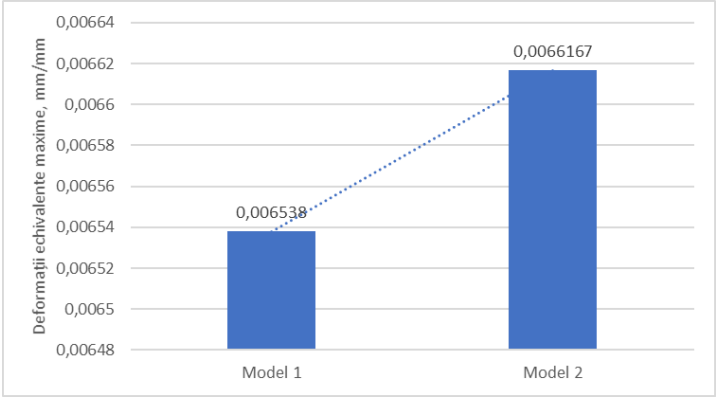


Fig. 12. Comparative values of the equivalent strains as a function of the mini-implant type.

DISCUSSION

Orthodontic mini-implants serve as pivotal anchorage devices in modern orthodontics, enabling effective treatment outcomes through strategic force application. The insertion angle of these implants significantly affects their displacement and overall stability. This article discusses the findings from various finite element studies regarding the impact of increased insertion angles, such as 120°, compared to perpendicular placements at 90° (TABLE 1).

Several studies establish a correlation between the angle of insertion and the displacement of orthodontic mini-implants. For example, a study by Motoyoshi *et al*. emphasizes that increased angles of insertion can lead to heightened stress on surrounding cortical bone, consequently increasing the displacement experienced by the implants (22). This claim is supported by the findings of Sana *et al*., who concluded that oblique angles can create longer lever arms, thereby impacting engagement between the implant threads and the bone (23).

Moreover, a finite element analysis (FEA) study conducted by Xavier *et al*. indicated that an increase in insertion angle from 90° to 45° resulted in greater strain and insertion torque, which was correlated to increased displacement (24).

TABLE I.

Influence of insertion angle on displacement: comparison between present study and literature

Parameter	Present FEM Study	Findings from Literature
Insertion angle	60°, 90°, 120°	45°-120° (22, 25)
Total displacement	Highest displacement observed at 120°; lowest at 90°	Increased displacement reported for oblique angles compared to perpendicular insertion (22, 23)
Mechanical explanation	Increased lever arm and reduced thread engagement at higher angles	Oblique insertion increases bending moments and reduces cortical bone engagement (23, 24)
Clinical implication	Higher angulation may compromise primary stability	Oblique insertion associated with increased risk of micromovement and instability (25)

The primary stability of mini-implants is critical for their success, and the insertion angle plays a vital role in this regard. Wilmes *et al*. report that the insertion angle significantly impacts the primary stability of these implants, with angular placements at 120° being more susceptible to movement than perpendicular placements (25).

A thorough understanding of stress distribution patterns surrounding mini-implants is necessary to evaluate their performance under different insertion angles (tables 2). Sivamurthy and Sundari's study highlights significant alterations in axial load reactions with varying insertion angles (26). The outcomes of this study align with those from Chaddad *et al*., which examined how surface characteristics, including angle of insertion, influence stress on the mini-

implant interface and subsequently affect displacement (27). The correlation between insertion angle and stress allows for deeper insights into how misalignment can increase the risk of failure in clinically modeled scenarios (28).

TABLE II.
Influence of insertion angle on stress distribution

Aspect	Present FEM Study	Literature Findings
Maximum von Mises stress	Similar peak stress values for all angles	Comparable peak stresses reported across angles (26)
Stress distribution	Significant variation in stress localization; shallower stress concentration at 60°	Stress concentration shifts With insertion angle (27, 28)
Risk of failure	Altered stress distribution may increase localized bone overload	Misalignment linked to higher failure risk due to uneven stress (28)

Study limitations

This finite element study is subject to certain limitations.

a. The finite element models assumed all materials (bone, teeth, periodontal ligament, and mini-implants) to be linear elastic, homogeneous, and isotropic, which does not fully reflect the complex anisotropic behavior of biological tissues in vivo.

b. A frictionless contact condition was defined at the bone-mini-implant interface, which may underestimate the effects of interfacial friction and mechanical interlocking occurring in clinical situations.

c. The geometry of the mandibular bone was simplified to reduce computational time, potentially affecting the accuracy of stress and strain distribution in anatomically complex regions.

d. Only static loading conditions were considered, whereas cyclic and dynamic orthodontic forces may influence long-term mini-implant stability.

e. Biological factors such as bone remodeling, osseointegration, and patient-specific variability were not included in the finite element simulations.

f. The study evaluated a limited range of insertion angles and implant diameters; therefore, the results may not be directly generalizable to all commercially available mini-implant designs.

g. The findings are based on numerical simulations and should be interpreted as comparative biomechanical trends rather than absolute predictors of clinical performance.

CONCLUSIONS

This finite element study demonstrated that both insertion angle and mini-implant diameter significantly affect the mechanical behavior of orthodontic mini-implants. Higher insertion angles increased displacement and strain, while larger diameters improved mechanical stability and stress distribution. These findings may support optimized clinical placement of orthodontic mini-implants.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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ATOMIC FORCE MICROSCOPY CHARACTERIZATION OF A THREE-DIMENSIONAL PORCINE COLLAGEN MATRIX FOR PERIODONTAL APPLICATIONS UNDER HYDRATED CONDITIONS

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ATOMIC FORCE MICROSCOPY CHARACTERIZATION OF A THREE-DIMENSIONAL PORCINE COLLAGEN MATRIX FOR PERIODONTAL APPLICATIONS UNDER HYDRATED CONDITIONS (Abstract): The **aim** of this study was to characterize the nanoscale surface topography of a three-dimensional porcine collagen matrix under hydrated conditions, simulating a physiologically relevant environment. **Materials and methods:** Atomic force microscopy (AFM) was performed in hydrated conditions to evaluate surface topography, deflection signal, three-dimensional reconstruction, surface profile, and quantitative roughness parameters. **Results:** The collagen matrix exhibited a continuous, moderately undulating surface without detectable structural defects. Topographic analysis revealed a homogeneous distribution of elevations and depressions, consistent with an isotropic collagen network. Deflection imaging enhanced the visualization of local slope variations, indicating microstructural heterogeneity. Three-dimensional reconstruction confirmed uniform spatial organization with gradual height transitions. Quantitative analysis demonstrated moderate roughness (Sa = 0.1179 µm; Sq = 0.1765 µm) and limited maximum height (Sz = 1.541 µm), suggesting the absence of pronounced irregularities. Height distribution showed a centered dispersion around the mean plane. **Conclusions:** The hydrated collagen matrix presents a stable, continuous, and moderately rough surface morphology, which may support favorable biological interactions and its potential application in soft tissue and periodontal regeneration. **Keywords:** ATOMIC FORCE MICROSCOPY, COLLAGEN MATRIX, SURFACE TOPOGRAPHY, HYDRATED CONDITIONS, ROUGHNESS, BIOMATERIALS, PERIODONTAL REGENERATION, NANOSCALE MORPHOLOGY.

INTRODUCTION

Collagen-based biomaterials are widely used in regenerative dentistry and periodontology due to their biocompatibility, biodegradability, and structural similarity to the native extracellular matrix (1, 2). Porcine-derived collagen matrices, in particular, have been increasingly employed as substitutes for autogenous grafts in soft tissue augmentation procedures, offering advantages such as reduced morbidity and simplified surgical protocols (3, 4). The surface morphology of these biomaterials plays a critical role in their biological performance, influencing protein adsorption, cell adhesion, proliferation, and tissue integration (5, 6). At the micro- and nanoscale levels, surface topography has been shown

to modulate cellular behavior and affect the interaction between biomaterials and the surrounding biological environment (7).

Atomic force microscopy (AFM) is a powerful technique for nanoscale surface characterization, providing high-resolution topographical data and information regarding surface roughness and local mechanical interactions (8). Unlike other imaging methods, AFM allows the investigation of soft biomaterials under hydrated conditions, which more closely resemble physiological environments (9).

Hydration significantly affects the structural and mechanical properties of collagen-based materials, leading to changes in surface morphology, compliance, and topographical features (10). In hydrated conditions, collagen matrices may exhibit a more relaxed structural configuration, potentially reducing apparent surface roughness and altering the visualization of microstructural details (11). Despite the widespread clinical use of collagen matrices, limited data is available regarding their nanoscale surface characteristics under physiologically relevant conditions (12). Most existing studies focus on dry-state analysis, which may not accurately reflect vivo behavior.

Therefore, the aim of the present study was to characterize, using atomic force microscopy, the surface topography of a three-dimensional porcine collagen matrix under hydrated conditions, with particular emphasis on morphological features and rough surface parameters.

MATERIALS AND METHODS

Materials. A commercially available three-dimensional porcine collagen matrix, Mucoderm® (Botiss biomaterials GmbH Zossen-Germany), was used in this study. The material consists of a collagen-based structure designed for soft tissue applications. Prior to analysis, 30 samples were prepared according to standard handling protocols and maintained for 20 minutes in hydrated conditions (sterile NaCl 0.9% solution) according to the manufacturer recommendations. Hydration is an essential step in order to obtain an optimal oral integration in a clinical setting.

AFM images were acquired on synthetic tissue samples in a hydrated state to evaluate surface topography under conditions closely resembling the physiological environment. Hydration affects both the mechanical behavior and the morphological configuration of the surface, inducing structural relaxation of the collagen network and facilitating a more accurate representation of surface irregularities, depressions, and local topographical variations.

Sample Preparation. The specimens of appropriate dimensions (10 mm diameter) placed on a stable support suitable for AFM analysis. Measurements were performed in wet conditions, ensuring the presence of a hydration layer throughout the scanning process. This approach was selected to preserve the native structural configuration of the collagen matrix and to minimize artifacts associated with dehydration.

Atomic Force Microscopy Analysis. Atomic Force Microscopy (AFM) investigations were carried out using an EasyScan 2 system (Nanosurf AG, Liestal, Switzerland), equipped with a highly sensitive PPP-NCHAuD cantilever (NanoAndMore, USA), characterized by a resonance frequency of approximately 330 kHz, a spring constant of 44 N/m, and a length of 125

µm. Image acquisition was performed in Dynamic Force mode (Non-Contact Mode), while data processing was conducted using the dedicated Nanosurf EasyScan 2 analysis software. To ensure stable immobilization of the synthetic tissue and enhance measurement reproducibility, the samples were mounted on commercially available glass slides coated with poly-L-lysine (PLL), which promotes electrostatic adhesion of biological specimens. AFM scans were carried out under hydrated conditions, and prior to imaging, the substrates were allowed to reach thermal equilibrium with the scanning cell environment in order to minimize experimental variability.

The scans were performed over an area of 5 × 5 µm, allowing detailed visualization of surface morphology at the micro- and nanoscale. Several imaging channels were recorded, including: height (topography) images, deflection images, three-dimensional surface reconstructions

Surface Analysis. Topographic images were analyzed to evaluate the overall morphology and spatial distribution of surface features. Deflection images were used to enhance the visualization of local slope variations and microstructural boundaries.Three-dimensional reconstructions were generated to assess the spatial organization of the surface. Surface profiles were extracted along selected cross-sections to evaluate height variations.

Roughness Parameters. Surface roughness parameters were calculated from AFM data, including following parameters:Sa (arithmetical mean height),Sq (root mean square roughness), Sz (maximum height), Ssk (skewness), Sku (kurtosis).These parameters were used to quantify the amplitude and distribution of surface irregularities.

Statistical Representation. Height distribution histograms were generated to evaluate the distribution of surface elevations relative to the mean plane. This allowed the assessment of symmetry, dispersion, and the presence of extreme values within the dataset.

RESULTS

AFM Topography Analysis. AFM topography images acquired on the synthetic tissue in a hydrated state revealed a surface characterized by a non-uniform relief, dominated by heterogeneously distributed morphological variations. An alternation between slightly elevated regions and localized depressions was observed, suggesting a discontinuous spatial organization at the micro- and nanoscale levels (fig. 1).

The surface exhibited a relatively homogeneous appearance in terms of material continuity, with no evidence of abrupt discontinuities or major structural defects. The relief was predominantly defined by rounded features, while transitions between areas of maximum and minimum height were gradual.

The topographical configuration indicated the presence of randomly distributed morphological units, without a clearly identifiable preferential alignment. Furthermore, no filamentous formations or dominantly oriented structures were observed, suggesting a predominantly isotropic character of the analyzed surface.

Overall, the topographic image reflected a continuous surface structure, with moderate variations in relief and without extreme features such as sharp peaks or pronounced depressions.

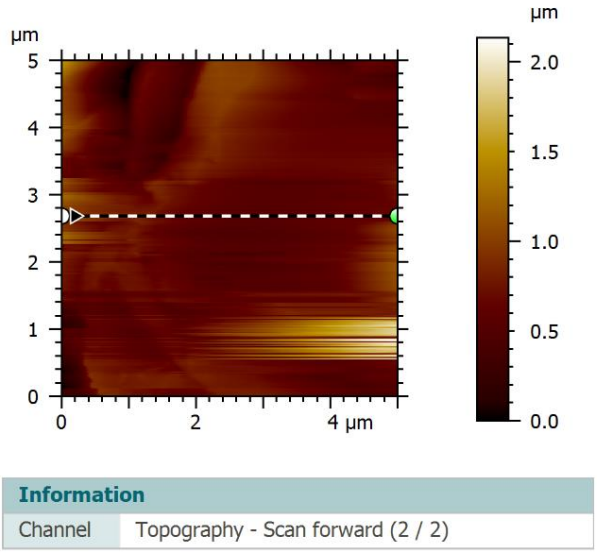


Fig. 1. Surface topography of the synthetic tissue under hydrated conditions as revealed by AFM.

AFM Deflection Image Analysis. The AFM deflection image revealed a pronounced distribution of local contrast, reflecting significant variations in the interaction between the probe tip and the surface of the synthetic tissue. Regions of differing intensity were observed, corresponding to local slope variations and fine morphological discontinuities (fig. 2).

The deflection signal highlighted structural details that were not as clearly visible in the topographic image, particularly at the boundaries between morphological units. Well-defined contours of surface features were identified, along with transition zones where surface inclination changes were more pronounced.

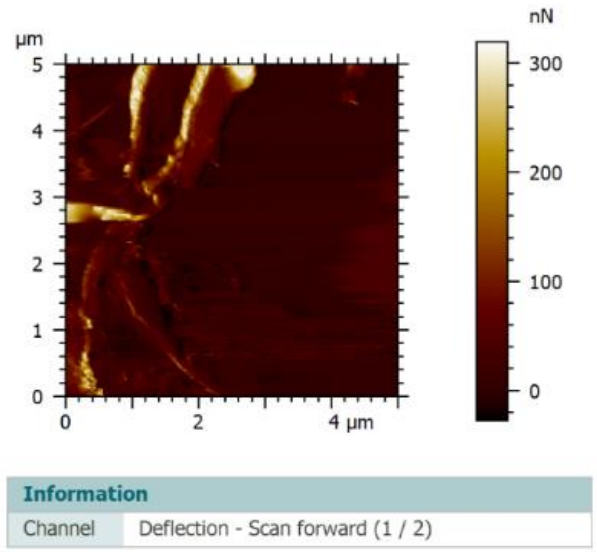


Fig. 2. Representative AFM deflection image of the synthetic tissue under hydrated conditions (5 × 5 µm scan area)

The image exhibited a granular contrast pattern, with an alternation between bright and dark regions, suggesting the presence of micro-scale heterogeneities at the surface level. No major discontinuities or evident artifacts were observed, indicating good scanning stability under hydrated conditions. Furthermore, the distribution of the deflection signal did not indicate a clear preferential orientation, supporting the predominantly isotropic character of the surface structure.

Three-Dimensional Surface Reconstruction Analysis. The three-dimensional reconstruction of the synthetic tissue surface obtained by AFM revealed a relief characterized by continuous altimetric variations, non-uniformly distributed across the analyzed area. The surface exhibited an undulating configuration, with an alternation between elevated regions and depressions, without the presence of abrupt discontinuities or well-defined dominant structures (fig. 3).

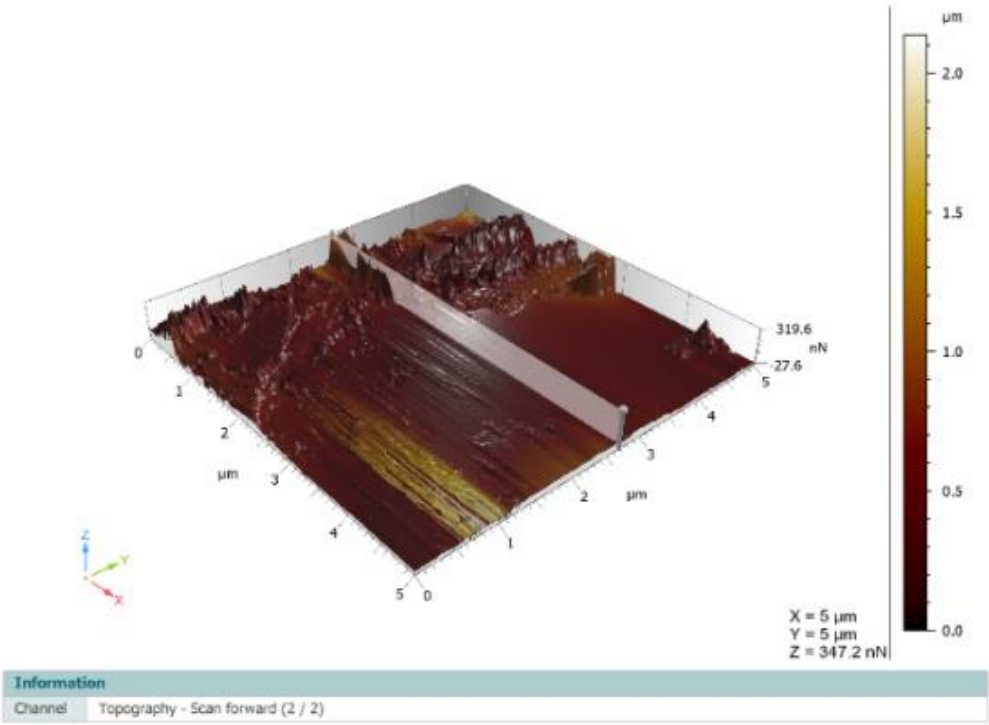


Fig. 3. Three-dimensional reconstruction of the synthetic tissue under hydrated conditions.

The morphological elements appeared to be relatively randomly distributed, without a clearly identifiable preferential orientation, indicating an isotropic spatial organization. The height of the structures varied gradually, and transitions between regions were smooth, suggesting a continuous distribution of the material at the surface level.

The three-dimensional aspect confirmed

the presence of morphological units with rounded shapes, without sharp peaks or deep depressions. The overall relief was characterized by moderate height variations, uniformly distributed, providing a coherent representation of the spatial configuration of the surface. Overall, the 3D reconstruction highlighted a continuous surface with moderate relief and without extreme heterogeneities.

Surface Profile Analysis. The surface profile obtained from a cross-sectional analysis revealed continuous altimetric variations along the analyzed distance, reflecting the undulating configuration of the synthetic tissue surface under hydrated conditions. The profile trace indicated an alternation between regions of maximum and minimum height across the entire length of the section (fig. 4).

Height differences were expressed at the nanometer scale, and the amplitude of the variations was moderate, with no evidence of sharp peaks or abrupt depressions. Transitions between maxima and minima were gradual, suggesting a continuous distribution of the material at the surface level.

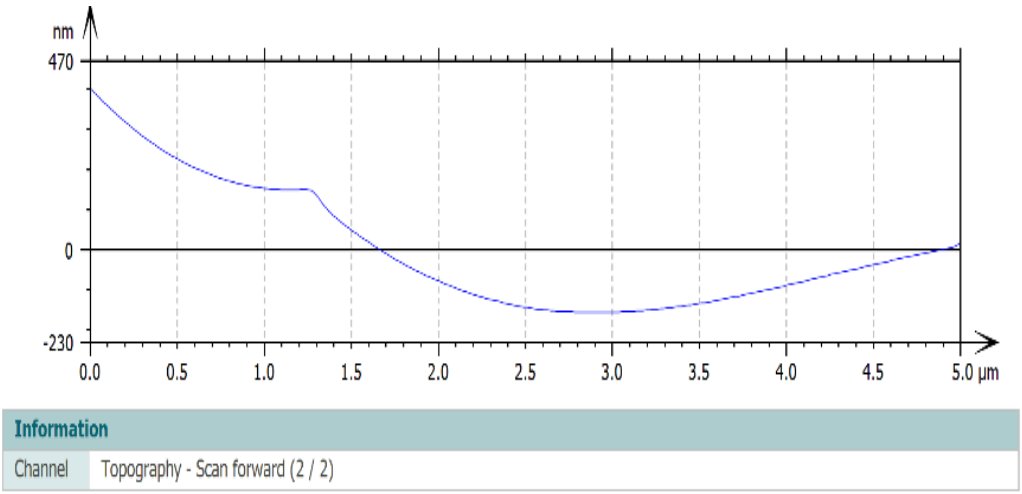


Fig. 4. Representative AFM cross-sectional surface profile of the synthetic tissue under hydrated conditions.

Along the length of the profile (corresponding to the scanned dimension, in the micrometer range), no areas with abrupt discontinuities or localized material accumulations were identified. The profile exhibited a relatively uniform character, with regular height fluctuations, indicating a balanced spatial organization. Overall, the cross-sectional profile confirmed the presence of a moderately rough surface, with uniformly distributed variations and no extreme

Surface Roughness Analysis. Surface roughness parameters derived from AFM analysis revealed a moderate level of altimetric variation of the synthetic tissue surface under hydrated conditions. The amplitude parameters (Sa = 0.1179 µm and Sq = 0.1765 µm) indicated a relatively uniform

distribution of surface heights relative to the mean plane, supporting the continuous nature of the surface relief (tab. I).

The maximum height parameter (Sz = 1.541 µm) reflected the difference between the highest peak and the deepest valley, indicating the absence of pronounced peaks or deep depressions. This observation is consistent with the topographical and cross-sectional profile analyses, where no major discontinuities were identified.

TABLE I.

Surface roughness parameters of the hydrated synthetic tissue

ISO 25178-2 - Roughness (S-L)		
S-filter (λs): None		
F-operation: None		
L-filter (λc): Gaussian, 0.0025 mm		
Height parameters		
Sq	0.1765	µm
Ssk	1.334	
Sku	7.873	
Sp	1.069	µm
Sv	0.4719	µm
Sz	1.541	µm
Sa	0.1179	µm

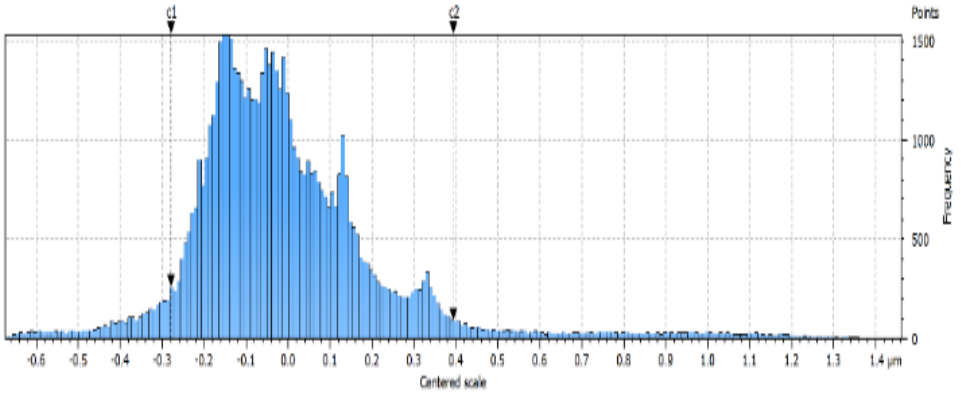
The dimensionless shape parameters of the height distribution (Ssk = 1.334 and Sku = 7.873) suggested a slightly skewed distribution with a tendency toward asymmetry, depending on the obtained values. This indicates a relatively balanced distribution of peaks and valleys, with a possible predominance of either protruding or recessed features. Overall, the roughness analysis confirmed the presence of a moderately developed surface relief, uniformly distributed and without extreme heterogeneities at the level of the analyzed surface.

Height Distribution Histogram Analysis. The height distribution histogram revealed a concentration of values around the central region, indicating that the majority of surface points were located close to the mean plane. The range of height values extended from negative to positive values (expressed in nanometers), reflecting the overall amplitude of altimetric variations (fig. 5).

The highest frequency was observed in the central region of the distribution, where a pronounced peak was identified, suggesting the predominance of height values close to the mean. The distribution exhibited a moderate width, indicating a relatively controlled dispersion of height values.

The shape of the histogram was relatively symmetrical or slightly skewed, depending on the exact distribution of the data, suggesting a balanced or mildly inclined distribution of peaks and valleys. No ex-

tended tails or dominant extreme values were observed, indicating the absence of major surface irregularities. Overall, the histogram confirmed the moderate nature of height variations and their relatively uniform distribution across the analyzed surface.



Information			
Channel	Topography - Scan forward (2 / 2)		
Individual cursors	**Unit**	**c1**	**c2**
Height	µm	-0.279	0.3938
Cursor sets	**c2-c1**	**Unit**	
Height difference	0.6727	µm	
% of points between cursors	90.00	%	

Fig. 5. Height distribution histogram of the synthetic tissue surface as obtained by AFM.

DISCUSSION

The surface topography observed under hydrated conditions reflects the influence of hydration on the morphological configuration of the synthetic tissue. The presence of water has been associated with a redistribution of the material at the surface level, leading to a more uniform appearance and attenuation of topographical contrast. Such behavior is consistent with previous studies demonstrating that hydration can induce structural relaxation and increased compliance in collagen-based materials (10, 13).

The rounded morphology of surface features and the gradual transitions between regions can be correlated with the compliant nature of hydrated soft biomaterials, in which water acts as a plasticizing agent, reducing stiffness and smoothing surface irregularities (14). Consequently, the interaction between the AFM probe tip and the surface is influenced by this hydrated state, resulting in a relatively “smoothed” representation of the surface relief compared to measurements performed under unhydrated conditions (15).

The random distribution of morphological units suggests the absence of a clearly defined directional organization or, alternatively, the masking of an underlying structural arrangement due to hydration effects.

This observation supports the interpretation of an isotropic structural response of the material under the experimental conditions employed, a characteristic commonly reported for collagen matrices lacking preferential fiber orientation (16).

The overall topographical appearance is consistent with a continuous and flexible surface structure, which may have direct implications for interactions with the surrounding biological environment, particularly in the presence of fluids. Surface properties such as roughness and compliance are known to influence protein adsorption and subsequent cellular behavior, including adhesion and proliferation (7,17).

At the same time, potential measurement-related effects specific to wet conditions must be considered. Capillary interactions, variations in contact forces, and hydration-induced changes in tip-sample interaction dynamics may subtly influence the recorded topographical configuration (8). These factors are well-documented in AFM studies performed in liquid environments and should be considered when interpreting nanoscale surface features (18).

The AFM deflection image provided complementary information to topographical data, being particularly sensitive to local slope variations and rapid changes in surface relief. The high contrast observed can be associated with height gradients across the surface, allowing for a clearer delineation of boundaries between morphological structures.

Under hydrated conditions, the deflection response was influenced by the complex interaction between the AFM probe tip and the hydrated surface layer. The presence of water contributes to modifications in contact forces and local mechanical response, leading to an enhancement of contrast in certain regions and attenuation in others (18,19). The well-defined contours observed in the deflection image suggest that, although the overall topography appears relatively smoothed due to hydration effects, local slope variations remain detectable and may even be amplified in the deflection signal.

This observation highlights the increased sensitivity of deflection imaging to fine morphological details, particularly at the interfaces between structural units. The granular appearance of the image can be interpreted as the result of microstructural heterogeneity of the synthetic tissue, combined with tip-surface interaction effects in a liquid environment (18, 20).

The absence of a preferential orientation is consistent with the topographical findings, supporting a predominantly isotropic structural organization. At the same time, it is important to consider that AFM measurements performed under hydrated conditions may be affected by additional factors, such as capillary forces, variations in local adhesion, and changes in tip-sample interaction dynamics (21, 22). These effects can influence the deflection signal and contribute to the observed contrast without exclusively reflecting the actual surface geometry (9).

The three-dimensional reconstruction enabled a more intuitive evaluation of the spatial distribution of height variations, confirming the continuous and relatively uniform nature of the surface observed in the topographic images. The undulating configuration can be associated with the material response under hydrated conditions, where the presence of water influences local material redistribution and

reduces topographical contrast. Rounded surface features and the absence of abrupt discontinuities are consistent with the compliant behavior of hydrated collagen-based structures, in which water induces structural relaxation and attenuates sharp height variations (20, 24).

The relatively random distribution of three-dimensional features further supports the absence of a preferential directional organization, in agreement with the observations from both topography and deflection imaging. Such behavior is characteristic of collagen matrices exhibiting isotropic structural organization under hydrated conditions, as reported in recent biomaterials studies (25).

The 3D reconstruction also facilitated the identification of spatial relationships between elevated and depressed regions, demonstrating that height variations were gradually distributed without the accumulation of critical regions. This configuration is consistent with a continuous material structure exhibiting increased surface flexibility, which may play a role in modulating interactions with the surrounding biological environment (26).

The cross-sectional profile analysis provided a quantitative perspective on surface height variations, supporting the qualitative observations derived from topography and three-dimensional reconstruction. The regular alternation between maxima and minima is indicative of an undulating surface architecture typical of hydrated soft biomaterials (27). The moderate amplitude of altimetric variations suggests a stable surface configuration, without the presence of major local defects. The absence of abrupt transitions further supports a homogeneous material distribution and highlights the influence of hydration in smoothing surface irregularities.

The smooth and continuous shape of the profile can be correlated with the compliant behavior of the hydrated surface layer, where water reduces local stiffness and contributes to the homogenization of the surface relief (28). The relatively uniform distribution of variations לאורך the entire profile length supports the hypothesis of an isotropic surface organization, consistent with the findings obtained from topographic and deflection analyses. Additionally, the absence of localized accumulations of height variations suggests a stable structural response, which may have favorable implications for the functional behavior of the material in wet environments (29).

The roughness parameters obtained were consistent with the observed topographical configuration and reflected the direct influence of hydration on surface behavior. The presence of water was associated with a reduction in altimetric contrast and a homogenization of the surface, leading to moderate values of Sa and Sq. Similar trends have been reported in recent AFM studies of collagen-based and soft biomaterials analyzed under hydrated conditions (30).

The moderate values of the Sz parameter can be interpreted as a consequence of the absence of major structural discontinuities and a stable material response in wet conditions, which agrees with the continuous surface observed in the 3D reconstruction. The shape parameters of the height distribution (Ssk and Sku) provided additional insight into the nature of the surface relief. A near-symmetrical distribution suggests a balanced presence of peaks and valleys, while slight deviations may indi-

cate a modest predominance of either protruding or recessed features. These findings are consistent with the rounded morphology and gradual transitions observed in AFM images (31).

Overall, the results indicate that under hydrated conditions, the synthetic tissue surface exhibits a more uniform and morphologically stable behavior compared to typical dry-state analyses. This surface homogenization may have direct implications for surface interactions, including adhesion phenomena and local mechanical response, which are critical for biomaterial performance in physiological environments (32).

The height distribution histogram further supported the topographical and cross-sectional findings, confirming that the surface was characterized by moderate variations and a relatively uniform organization. The concentration of values around the mean plane indicates the predominance of low-relief regions, typical of hydrated soft materials (33).

The moderate width of the distribution reflects controlled surface variability and suggests the absence of extreme heterogeneities. This behavior can be attributed to the effect of hydration, which contributes to surface smoothing and reduction of altimetric contrast (34). The approximately symmetrical shape of the histogram indicates a balanced distribution of peaks and valleys, consistent with a stable structural configuration.

The absence of extended tails or dominant extreme values suggests the lack of major defects or structural discontinuities, further supporting the continuous nature of the material. Additionally, the observed distribution is influenced not only by intrinsic surface characteristics but also by experimental conditions, including tip-surface interactions and the presence of water, which may contribute to the attenuation of height variations and the clustering of values around the mean (35).

Hydration was associated with an attenuation of topographical contrast and a homogenization of the surface relief, leading to increased compliance of the superficial layer. This aspect is clinically relevant, as hydrated conditions closely mimic the physiological environment in which these biomaterials are typically applied.

The predominantly isotropic character of the surface, together with the uniform distribution of height variations, suggests a predictable material behavior when in contact with soft tissues. Such an organization may promote a more uniform interaction with the biological environment and reduce the risk of localized stress concentrations or uneven tissue integration.

The moderate values of surface roughness parameters indicate a surface that is sufficiently active from a topographical perspective to support biological interactions, without exhibiting extreme irregularities that could compromise mechanical stability or local surface behavior. This balance may be considered favorable for processes such as cell adhesion and the initial stabilization of the biomaterial.

The height distribution, characterized by a concentration of values around the mean plane and the absence of extreme values, further supports the presence of a morphologically stable and homogeneous surface. From a clinical perspective, this uniformity may contribute to more controlled integration and predictable interactions with biological fluids and tissue components.

CONCLUSIONS

The results of the atomic force microscopy analysis demonstrated that, under hydrated conditions, the surface of the porcine-derived collagen matrix exhibits a moderately developed, continuous, and relatively uniform relief, characterized by gradual altimetric variations and the absence of major structural discontinuities.

This morphological configuration reflects a stable structural behavior and a balanced distribution of surface features.

Overall, the identified topographical characteristics suggest that the analyzed collagen matrix exhibits surface properties highly compatible with use in hydrated environments, providing a stable and uniform structural support. These features may have favorable implications for the clinical performance of the biomaterial, particularly in terms of soft tissue adaptation and interaction with the biological environment.

CONFLICTS OF INTEREST AND FUNDING

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CONTRIBUTIONS TO THE CHARACTERIZATION OF BIOCENOSIS COMPLEX OF THE ECOSYSTEM IN THE ORAL CAVITY

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CONTRIBUTIONS TO THE CHARACTERIZATION OF BIOCENOSIS COMPLEX OF THE ECOSYSTEM IN THE ORAL CAVITY (Abstract): Oral biotype includes the local defense factors represented by oral mucosa, reticulohistiocytic system and saliva which opposes biocenosis composed of various microorganisms, normally achieving the state of "biological balance". **Material and methods**: The study included 23 patients with clinical manifestations of oral candidiasis admitted during the period 2014-2016 to the Dental Surgery Clinic of the Iasi "Grigore T. Popa" University of Medicine and Pharmacy. **Results**: Under favorable conditions, many non-pathogenic species become virulent in the host organism. These conditions are created using antibiotics, corticoids, immunosuppressant, antifungal, psychotropic drugs, contraceptives, radiation therapy, hormone treatment, etc. In general conditions caused by metabolic disturbances and decreased body resistance associated with microbial, vitamin and enzymatic imbalances the development of mycoses is favored. **Conclusions:** Diagnosis and correct treatment of mycoses of the oral cavity avoiding complications, oral cavity being the starting point for many endogenous infections, sepsis included. **Keywords**: BIOCENOSIS, ORAL BIOTYPE, ORAL MUCOSA, ORAL CANDIDIASIS.

Oral cavity biocenosis forms an ecological system like other natural biocenoses located in the gingival ditch and dorsal surface of the tongue, where the microbial concentration can be 100 times higher. Of these, 29 microbial species are permanently present in the oral fluid, to which others from the external environment are occasionally added, which make up the fluctuating or temporary flora (1, 2). In case of poor oral hygiene, anaerobic and proteolytic bacteria predominate. Yeasts, microscopic fungi are also present, and in case of poor oral hygiene they are present in high concentration on the dorsal surface of the tongue (3, 4).

Mycoses, diseases produced by the development of microscopic fungi in the tissues, are distinct from mycotoxicosis, fungal allergies, and infections caused by Actinomyces. The most common conditions are candidiasis C, related to the presence of blast spore fungi of the genus Candida. They may be local or general, acute, sub-acute or chronic. Certain mycoses are strictly human, but animal mycoses can be

transmitted to humans by direct or indirect contact with contaminated air and soil.

MATERIAL AND METHODS

The study included 23 patients with clinical manifestations of oral candidiasis admitted during the period 2014-2016 to the Clinic of Dental Surgery of the Iasi "Grigore T. Popa" University of Medicine and Pharmacy. The following were found: one case each (4.35%) of acute oral thrush, glossitis, candidal uranitis, pemphigoid candidiasis, geographic language; 4 (17.39%) cases with exacerbated phenomena and 15 (65.22%) cases with glossitis (tab. I).

TABLE I

Distribution of the study group by disease categories

Disease	No. cases	%
Acute thrush	1	4.35
Glossitis	1	4.35
Candidal uranitis	1	4.35
Pemphigoid candidiasis	1	4.35
Geographic tongue	1	4.35
Exacerbated clinical phenomena	4	17.39
Various forms of glossitis	15	65.22

The results were evaluated by the statistical and mathematical methods included in EPIDEMIOLOGICAL PROGRAMS (EPI-INFO).

RESULTS

In this study the following cases were recorded:

1. A 72-year-old totally edentulous female patient, who, following the extraction of her only remaining molar on the upper left hemi arcade, presented acute thrush, form located on the jugular and especially gingival mucosa in the upper cul-de-sac around the extraction site due to the antibiotic placed in the dental alveolus and on the post extraction wound;

2. In a subgroup of 15 cases of glossitis - of which one case of depapillated median tongue following penicillin treatment; one case with median rhomboid glossitis characterized by a rhomboid lesion with papillated mucosa, located in the middle of the dorsal surface of the tongue, with the great anteroposterior axis just anterior to the V region of circumvallated papillae, placed quite regularly on both sides of the median line of the tongue; a case with chronic glossitis associated with perleche/angular cheilitis, lesion characterized by a depapillated area with irregular margins at the base of the tongue on the right side; healing was obtained only after the discontinuation of antibiotic treatment and administration of a specific antifungal; 2 cases of candidal glossitis, presenting on the mid-dorsal surface of the tongue, jugular mucosa and left commissural lesions of chronic thrush in the form of leukokeratotic deposits in large spots with a carmine red contour in the center of the tongue;

3. Another case is a 61-year-old patient with a diagnosis of gastric neoplasm presenting whitish leukokeratotic deposits with irregular appearance disseminated throughout the dorsal surface of the tongue; the underweight patient was operated 2 months earlier and was undergoing chemotherapy;

4. A female patient with *chronic erosive candidal glossitis* on the whole surface of the tongue, partially covered by white leukokeratosis deposits; laboratory examination made the diagnosis of oral candidiasis;

5. Other cases: *chronic glossitis* in 6 patients, 3 of which had macroglossia and moderately scrotal tongue with superim-

posed infection with Candidal; macroglossia was also recorded in other 3 patients, teeth impressions and scrotal appearance with white Candidal deposits being seen on the margins of the tongue; *chronic erosive candidal glossitis* was diagnosed in a patient with erosive lesions on the whole surface of the tongue, partially covered by white leukokeratotic deposits; glossitis associated with candidal uranitis was recorded in a patient with Kissing "mirror" lesions. The median palatine raphe presented a longitudinal band from where white keratotic granulations come off. On the lateral aspects of the soft palate, close to the tooth cervices, there were discrete erythematous and keratotic placards corresponding to those on the margins of the tongue. Uranitis progressed in parallel with glossitis;

6. The appearance of *geographic tongue* and the smear, culture and positive germ-tube test pleaded for an over-infection with *Candida albicans*. Effective treatment consisted in the administration of nystatin for a period of 12 days concurrently with treatment to balance diabetes and increase body resistance;

7. A 52-year-old male patient with chronic lung disease treated with antibiotics and corticoids for about 30 days showed among others such subjective signs as the inability to masticate and swallow. Thus, besides dryness and burning sensation, the patient complained of pain and even altered sense of taste, worsened in the presence of acid or fermented food. The subjective and objective phenomena disappeared only after sustained treatment for 10 days and discontinuation of antibiotic and corticoid treatment;

8. A male patient with oral candidiasis characterized by large areas of ulcers very similar to pemphigus was diagnosed with chronic pemphigoid candidiasis. The lesion mimicked an oral stomatitis, with, two large superficial ulcerations with slightly irregular contours, bright-red background, partially covered by a white-yellow fibrin layer on the dorsal surface of the tongue. The neighboring mucosa was almost entirely covered by a white parakeratosis bed. The patient complained of troublesome pains and functional impotence;

9. In some patients, chronic oral thrush was characterized by episodes often triggered by the ingestion of sweet foods. The condition is a little troublesome, the general state is good, but is frequently associated with perleche, onychia with perionychia or vulvitis. Intraoral examination revealed stomatitis involving the tongue, soft palate and the jugular mucosa, glossitis being the predominant lesion, or possible associated lesions.

DISCUSSION

It is proven that under favorable conditions many pathogenic species become virulent in the host organism. These conditions are induced by the administration of antibiotics, corticoids, immunosuppressive, antifungal and psychotropic drugs, contraceptives, radiation therapy, hormone treatment, etc. Both in general conditions, favored by metabolic disturbances and decreased body resistance, as well as in local conditions due to microbial, vitamin, or enzymatic imbalances iatrogenic mycoses have been triggered (5, 6).

At present there is a growing incidence of fungal diseases and a diversification of their localization, thus becoming a multidisciplinary concern (7, 8). As the number of patients with such complains is high, there is a need for clarification on "fungal

labeling" of the described situations particularly with respect to the criteria for assessing the pathogenic implications of candida species in the oral cavity (9, 10).

Modern therapeutics, often irrational, excessive and injudicious, and aggressive for the body, gives the chance to some "opportunistic mycetes" to manifest their pathogenicity. Mycology has tracked the evolution of this pathology, bringing solutions to many problems posed by the clinical and biological diagnosis, as well as by therapeutic difficulty (11).

Correct diagnosis and treatment of these conditions is of importance both due to the highest frequency of the species in the oral cavity and the serious complications they may induce, this being the starting point for most endogenous infections (12).

It has been proven that the most common diseases are those caused by the presence of blast spore mycetes of the *Candida* genus. They may be local or general, acute sub-acute or chronic (13).

Mycoses can be distinguished not only by clinical aspect, but also by their geographical distribution, some being ubiquitous, such as *Candida*. Others require conditions and are met in limited geographic areas, being part of the import pathology.

CONCLUSIONS

Our study showed an increase in the incidence of oral fungal infections, important finding because many of them do not remain localized but can disseminate, sometimes leading to septicemic and visceral complications with poor prognosis.

Clinical manifestations in adults and the elderly are extremely diverse, imposing mandatory exams for diagnosis and treatment, as well as dispensing, with frequent recurrent forms.

Etiologically, iatrogenic mycoses ranked first, being the result of antibiotic therapy (27.8%), corticotherapy (4.9%) and psychotherapy (3.1%), thus requiring a more judicious administration of these treatments and their association with long-term nystatin prophylaxis.

Clinical manifestations in adults and the elderly are extremely varied, requiring mandatory exams to make the diagnosis and initiate a treatment, as well as follow-up given the fact that recurrences are common.

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NEWS

SEROTONIN - A POTENTIAL PERIPHERAL BIOMARKER IN SUDDEN INFANT DEATH SYNDROME

Although Sudden infant death syndrome (SIDS) has been related to deficiencies of the central serotonin (5-hydroxytryptamine, 5-HT) localized in the brainstem, recent serum studies have associated the elevation of serum 5-HT levels in the development of SIDS. The authors analyzed the serum 5-HT levels in SIDS infants (n=61) compared with autopsied controls (n=15), using the Enzyme-Linked Immunosorbent Assay (ELISA) test and validated it using High-Performance Liquid Chromatography (HPLC). The test results identified a significant elevation (95%) in serum 5-HT in the SIDS cohort versus control cases. Although the mechanism involving the increased serum 5-HT levels is not well defined or explained, this study clearly indicates/establish that SIDS is associated with peripheral abnormalities in the 5-HT pathway. These results instigate us to believe that high serum 5-HT may be used as a forensic biomarker in autopsied infants with SIDS with serotonergic defects (Robin L. Haynesa,1, Andrew L. Frelinger IIIb, Emma K. Gilesa, Richard D. Goldsteinc, Hoa Trana, Harry P. Kozakewicha, Elisabeth A. Haasd, Anja J. Gerritsb, Othon J. Menae, Felicia L. Trachtenbergf, David S. Patersona, Gerard T. Berryg, Khosrow Adelih, Hannah C. Kinneya, and Alan D. Michelsonb. High serum serotonin in sudden infant death syndrome. *Proc Natl Acad Sci USA* 2017; 114(29): 7695-7700).

Nona Gîrlescu

ACUTE BACTERIAL GASTROENTERITIS AS HOSPITAL-ACQUIRED INFECTION IN PEDIATRICS

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ACUTE BACTERIAL GASTROENTERITIS AS HOSPITAL-ACQUIRED INFECTION IN PEDIATRICS (Abstract): Gastroenteritis as hospital-acquired infection represents one of the most important issue to be managed in pediatric units. **Material and methods**: The descriptive study was conducted on a group of 536 cases with bacterial gastroenteritis as healthcare associated infections (HAIs), admitted in „Sf. Maria" Children's Emergency Hospital, Iasi, during 2013-2016. **Results**: Distribution according to pathogens detected by specific investigations showed that *Campylobacter* (69.77%) was involved in most cases of gastroenteritis, followed by those determined by *Salmonella* (21.45%). Distribution by age group highlighted that children of 0-1-year-old were the most affected and *Campylobacter* was the most involved pathogen in gastroenteritis of that age group, followed by cases with *Salmonella* gastroenteritis. Children from 2-6 and 6-11-years age groups were involved in gastroenteritis with *Salmonella* followed by that with *Campylobacter*. Children of 12-18-years-old were admitted with gastroenteritis due to *Salmonella*, then *E. coli*. *Shigella spp.* was isolated in 10 cases, and *Yersinia spp.* in only 3 cases. **Conclusions**: The issue of gastroenteritis as a hospital-acquired infection remains hereafter the keystone of hospital management, as well as prevention and control strategies. **Keywords**: ACUTE BACTERIAL GASTROENTERITIS, HEALTH CARE-ASSOCIATED INFECTION, PEDIATRICS.

Healthcare-associated infections (HAIs), which were previously referred to as nosocomial infections, are an important public health problem generated by medical practice involving patients, but also medical, paramedical and non-medical staff, which ensure the functionality of hospitals. They have an important impact on the healthcare system, due to the increased morbidity, mortality, and long-term hospitalization costs (1).

The current features of HAIs in pediatric units are determined by a variety of factors: low patient age, prematurity / dysmaturity, immunodeficiency status, alteration of anatomical defense barriers, congenital malformations and/or associated pathologies, prolonged hospitalizations, increasing number of surgical interventions, the multitude of diagnostic and therapeutic procedures, some of them invasive, the abuse of broad spectrum antibiotics, often unjustified, the occurrence of bacteri-

al multidrug resistance to antibiotics (1, 2).

Early detection by screening of patients colonized or infected with multiple resistance bacteria is an important and useful intervention that can be easily implemented and can have a significant impact, increasing the population health. Bacterial screening in carriers, as part of HAIs control programs, allows the implementation of preventive measures to avoid transmission of these infections. To increase the performance of these screening programs, it is necessary to involve the clinical laboratory, hospital management staff (epidemiologist, infectious disease specialist, medical assistant specialized in HAIs control), as well as the involvement of the management team and the hospital administration (1-3).

A screening program for early detection of patients colonized or infected with multidrug resistant bacteria (MDR) at the very hospital admission could achieve several objectives such as: controlling the transmission of MDR bacteria in hospital units; reducing the prevalence of MDR microorganisms and thus reducing incidence or preventing the occurrence of serious infections (sepsis, pneumonia, etc.) in hospitalized patients, increasing awareness of MDR-associated risk among healthcare professionals, standardizing the detection of MDR bacteria in the laboratory; supporting a national strategy to control the transmission of MDR and HAIs with such microorganisms. In the hospital environment, there are many microorganisms that can be the cause of HAIs development and evolution when there is a break in cleaning, disinfection, inadequate unhygienic behaviors. The most common way of transmission of HAIs is represented by the hands of medical staff, so that, a proper hygiene of hands is the basis of any prevention and control program, with an important role in reducing the transmission of infections, as well as the dissemination of resistant germs. Hand hygiene generated many controversies, evidence-based arguments, and research. Improving patient hygiene, access to clean water, keeping HAIs under control might be key elements for reducing antibiotic resistance (1, 2).

MATERIAL AND METHODS

The descriptive study was conducted on a group of 536 cases with bacterial gastroenteritis as healthcare associated infections (HAIs), admitted in "Sf. Maria" Children's Emergency Hospital, Iasi, during 2013-2016. Inclusion criteria were the diagnosis of bacterial gastroenteritis with *Salmonella*, *Shigella spp*, *Campylobacter*, *Yersinia spp*, other bacterial infections, based on laboratory investigations and contracted during the hospitalization; the exclusion criteria were represented only by the diagnosis of viral, food, or allergic gastroenteritis.

The data were collected retrospectively from patient records and HAIs reporting files, subsequently statistically processed using the *SPSS 20.0* software. The age groups cases were processed in accordance with international standards (4).

RESULTS

Most cases of bacterial gastroenteritis diagnosed as HAIs were reported in 2015 (154 cases-28.73% of the total), and the lowest in 2013 (21.26%). There is an upward trend in their incidence (fig. 1).

Gender distribution showed a slight predominance of boys (295 cases-55.03%), only in 2014 prevailing female gender. Distribution according to pathogens detected by specific investigations showed that *Campylobacter* (374 cases-69.77%) was involved in most cases of gastroenteritis, followed by those determined by *Salmonel-*

la (115 cases-21.45%). Distribution by year of study showed that most of *Campylobacter* cases were admitted in 2015 (115 cases-30.74%) and gastroenteritis with Salmonella had an incidence peak in 2014 (43-37.39%) (tab. I, fig. 2).

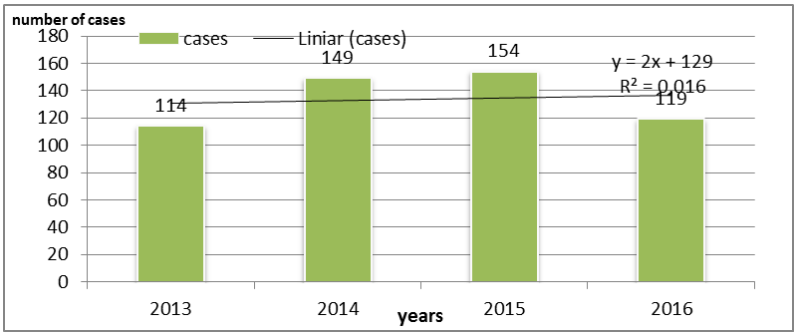

Fig. 1. Distribution of total cases by years

TABLE I

Distribution of bacterial infections cases pathogens by year of study and gender

Years / Pathogens	2013			2014			2015			2016		
	M	F	Total	M	F	Total	M	F	Total	M	F	Total
Salmonella	12	15	27	25	18	43	11	15	26	12	7	19
Shigella	1	3	4	2	2	4	0	1	1	2	0	2
E. coli	0	0	0	4	1	5	5	4	9	2	7	9
Campylobacter	46	34	80	46	49	95	69	46	115	53	31	84
Yersinia enterocolitica	0	2	2	1	0	1	0	0	0	0	0	0
Others	0	1	1	0	1	1	1	2	3	3	2	5
Total	59	53	**114**	78	73	**149**	86	68	**154**	72	47	**119**

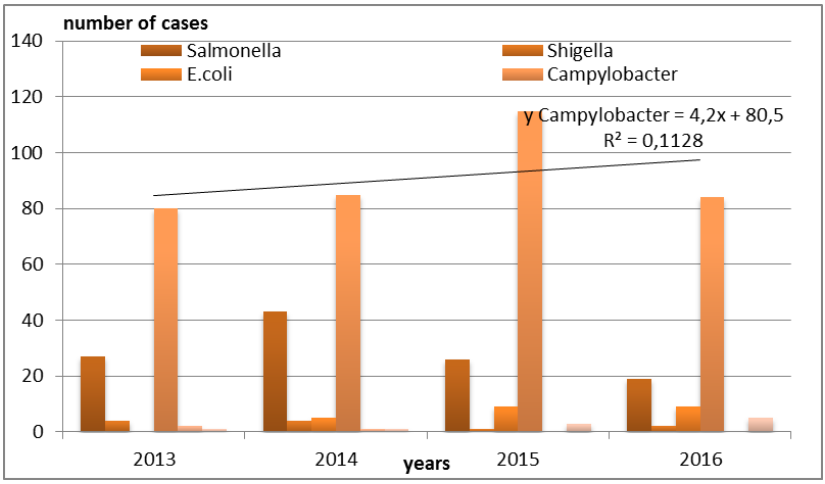

Fig. 2. Distribution of pathogens by year of study

Distribution by age group highlighted children of 0-1-year-old were the most affected (417 cases-77.79%) and *Campylobacter* was the most involved pathogen in gastroenteritis of that age group (337 cases-80.81%), with a peak of incidence in 2014 (107 cases-25.65%), followed by cases with *Salmonella* gastroenteritis (59 cases-14.14%). Children from 2-6-year age group were also involved in gastroenteritis with *Salmonella* (37 cases-53.63% of the total cases from that age group), followed by *Campylobacter* (28 cases-40.57%). Patients of 6-11-years- old suffered from gastroenteritis with *Salmonella* (12 cases-48.00%) and *Campylobacter* (6 cases-24.00%). Children of 12-18 years old were admitted with gastroenteritis due to *Salmonella* (7 cases-28.00%), then *E. coli* (11 cases-44.00%). *Shigella spp.* was isolated in patients with gastroenteritis as HAIs in our study group in 10 cases (1.86%). *Yersinia spp.* was also involved in gastroenteritis etiology, but in few cases (3 cases of our study group-0.55%) (tab. II).

TABLE II

Distribution of bacterial infections cases by age groups

Year	Pathogens	Age groups				
		0-1 years	2-5 years	6-11 years	12-18 years	Total
2013	***Salmonella***	14	10	2	1	
	Shigella	1	0	3	0	
	E. coli	0	0	0	0	
	Campylobacter	71	8	1	0	
	Yersinia enterocolitica	1	0	0	1	
	Others	1	0	0	0	**114**
2014	***Salmonella***	19	13	7	4	
	Shigella	2	1	0	1	
	E. coli	1	0	1	3	
	Campylobacter	84	6	2	3	
	Yersinia enterocolitica	1	0	0	0	
	Others	1	0	0	0	**149**
2015	***Salmonella***	18	6	1	1	
	Shigella	0	0	1	0	
	E. coli	6	1	0	2	
	Campylobacter	107	6	2	0	
	Yersinia enterocolitica	0	0	0	0	
	Others	2	0	1	0	**154**
2016	***Salmonella***	8	8	2	1	
	Shigella	0	2	0	0	
	E. coli	2	0	1	6	
	Campylobacter	75	8	1	0	
	Yersinia enterocolitica	0	0	0	0	
	Others	3	0	0	2	**119**
Total		**417**	**69**	**25**	**25**	**536**

DISCUSSION

Salmonella spp. has gained a distinct status over the last decades, becoming the first amongst pathogens that can contaminate the

food and the external environment involved in sporadic cases of gastroenteritis and outbreaks of food poisoning. Incidence of gastroenteritis with *Salmonella spp*. has increased greatly in almost all regions of the globe. In Western Europe, *Salmonella enteritidis* has become the dominant strain (5, 6).

Morbidity and mortality data are imprecise: there is no reporting system for *Salmonella spp*. isolated in humans, animal or food of animal origin, and the cause of death is not always the salmonellosis etiology. Only the investigation of the *Salmonella* food outbreaks can provide a clear description of this public health problem. Due to the widespread and a high probability of outbreaks and epidemics, several types of surveillance and control programs for *Salmonella* infections were implemented, such as those in Denmark, the United States, Australia and other countries. A Surveillance Program of these diseases has been implemented in the European Union since 2003, focused on food quality (7).

Diarrheal diseases with *Shigella spp*. are endemic worldwide and with extremely high levels of prevalence in undeveloped countries. Shigellosis is a very contagious disease and a frequent cause of death in children under 5 years of age. The United States is currently report a prevalence of 6-10% and the most affected age group is of 0-4 years. In Romania, the incidence of shigellosis had a decreased trend, from 130.6%ooo in 1975 to 31.8 in 1990 and to 6.7 in 2004. *Shigella flexneri* is the most intense circulation strain in Romania followed by *S. sonnei* (especially in Banat and Transylvania regions). *S. dysenteriae* has not been isolated in Romania since 1955. It is noticed that *S. sonnei* circulation is more intense in developed areas. Children were the most affected in regions where the disease is epidemic. Receptivity decreases with the age and epidemiological observations suggest the introduction of specific serotype immunity (8).

Incidence of diarrheal infections with *Campylobacter* tends to have the same position as incidences of infections caused by non-typhoid *Salmonella*. The incidence is higher in the summer season in temperate areas, but there is no seasonality in tropical areas. The morbidity evaluation and management is based on bacteriologically confirmed cases. The United States reported an incidence of 5-6%ooo. Campylobacter infections are hyper-endemic, endemic, or as outbreaks in developed countries with hygienic standards). In Thailand, *Campylobacter* infections seem to dominate the bacterial etiology of diarrheal diseases, and *C. jejuni* serotypes 36, 4 and 11 being encountered at the highest frequency. The highest rates of hospitalization for *Campylobacter* infections (74-78%) were reported by Cyprus, Latvia, Lithuania, Romania, and the United Kingdom. At the same time, Cyprus, Latvia, and Romania have the lowest number of reported cases, pointing out that surveillance in these territories manages to include only severe cases. UK reported 20 deaths of a total of 31 deaths registered in EU, in 2012. In the United States, the incidence of *Campylobacter* infections has declined towards the end of the 1990s and remains at low levels after the implementation of hygiene measures on drinking water disinfection and using chlorinated water in food industry (9-11).

In 40-90% of pediatric patients admitted in intensive care units there is an increased risk of diarrhea as HAI. Infectious diarrhea is usually more severe than non-infectious diarrhea, increasing the frequency of cardiac complications (arrhythmias) due to associated hydro-electrolytic imbalances, cardiopulmonary instability requiring vas-

opressors and mechanical ventilation or intraabdominal complications. An important problem too is the transmission of pathogens from patient to patient, from patient to healthcare worker as HAI outbreaks (12).

When a single case of *Salmonella* infection occurs, it should be checked if the infection is HAI or not. Epidemic investigations should assess food manipulation, inter-patient transmissions, or nurse to patient transmission. Standard detergents for hospitals and alcohol-based hand hygiene products are effective against *Salmonella spp*. Contact precautions are reserved for patients with incontinence or colostomy to control HAI outbreaks (12-14).

CONCLUSIONS

Our study highlighted that gastroenteritis with *Campylobacter* was most commonly reported in pediatric wards, especially in infants of 0-1-year-old and children of 2-6-year-old, followed by cases with *Salmonella*. The issue of gastroenteritis as a hospital-acquired infection remains hereafter the keystone of hospital management, as well as prevention and control strategies.

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PERINEAL EXTERNALIZATION OF LATE JEJUNAL FISTULA AFTER RECTUM AMPUTATION – CASE REPORT

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PERINEAL EXTERNALIZATION OF LATE JEJUNAL FISTULA AFTER RECTUM AMPUTATION-CASE REPORT (Abstract) Digestive fistula represents one of the most severe complications of surgical procedures performed on the abdominal area. We present the case of a 55-years-old female patient, known with a squamous cell carcinoma of the uterine cervix, for which was practiced 16 years ago a Wertheim procedure and afterwards treated according to the oncological medical protocols. In 2015 the patient was admitted in the General Surgery Department of the "Bagdasar-Arseni" Clinical Emergency Hospital from Bucharest for lower rectum tubular adenocarcinoma, diagnosed by clinical exam, radiological findings and histopathology. The patient underwent surgery and required an abdomino-perineal resection of the rectum. The patient had no indication for preoperative radiotherapy. The peculiarity of the case consisted in the development of upper gastrointestinal fistulas lately externalized through the perineal wound. The following surgical intervention could not identify the fistula, but we managed to perform an effective enteral-caecum anastomosis, with the main purpose to achieve a bypass of the intestinal breach. Postoperative evolution consisted in spontaneous closure of the intestinal fistula. **Keywords**: ENTERO-CUTANEOUS FISTULA, RECTAL CANCER, RECTUM AMPUTATION.

Enterocutaneous fistula is an abnormal communications between the gastrointestinal tract and the surface of the skin and can lead to real therapeutic management challenge. With all the progress made by modern medicine, the mortality related to enteral fistulas still remains significantly high (10-30%), especially in neoplastic or septic context (1).

Digestive fistula mainly occur following abdominal surgeries involving gastrointestinal resections followed by an anastomosis, injuries produced by drainage tubes, laborious dissections with subsidiary ischemia of the intestinal wall (2). About 15-25% of spontaneous enterocutaneous fistulas are the result of but inflammatory bowel diseases, corrosions induced by acute pancreatitis, radiation enteritis or diverticular disease (3). Inadequate nutritional support and conse-

quences of septic syndrome are the main factors involved in the morbidity and mortality of patients with enteric fistulas (4).

CASE REPORT

We present the case of a 55-years-old female, admitted to the General Surgery Department of the "Bagdasar-Arseni" Clinical Emergency Hospital from Bucharest for intermittent rectal bleeding, significant weight loss (12 kilograms in the past 2 months). The patient had a history of cervical cancer, treated with radiotherapy followed by the Wertheim procedure about 16 years ago (histopathological findings confirmed a squamous cell carcinoma), as well as a hiatal hernia surgical repair 5 years ago.

Clinical examination performed at the admission concluded with the rectal exam highlights at 3-4 centimeters from the anal sphincter a circumferential tumor with a partially rectal stenosis and bleeding when it is touched. The vaginal examination reveals a vaginal stump without local tumor recurrence.

Under spinal anesthesia was performed instrumental anal dilation and a biopsy was purchased from the endorectal proliferative process in shape of a crater about 5 cm in diameter. Histopathological findings reveal micro-fragments of ulcerated, tubular and papillary adenocarcinoma, with extensive areas of necrosis, fibrosis with granulation tissue, moderate desmoplastic reaction and necrotic debris. With this result the patient is directed to the oncology service, where she received chemotherapy. Radiotherapy is contraindicated due to the maximal doses previously received for the cervical cancer.

After the completion of the oncological treatment, an abdominal and pelvic magnetic resonance imaging (MRI) was performed, identifying asymmetric parietal thickening of the rectum wall (maximum thickness is 19 millimeters in the right lateral wall), intense irregular internal contours and an inhomogeneous structure causing an asymmetric stenosis at this level and invasion of the urinary bladder posterior wall. There were no signs of perirectal fat infiltration, vaginal stump invasion, ascites or pelvic lymph nodes involvement – $T_2N_0M_x$, stage I, Dukes A (fig. 1).

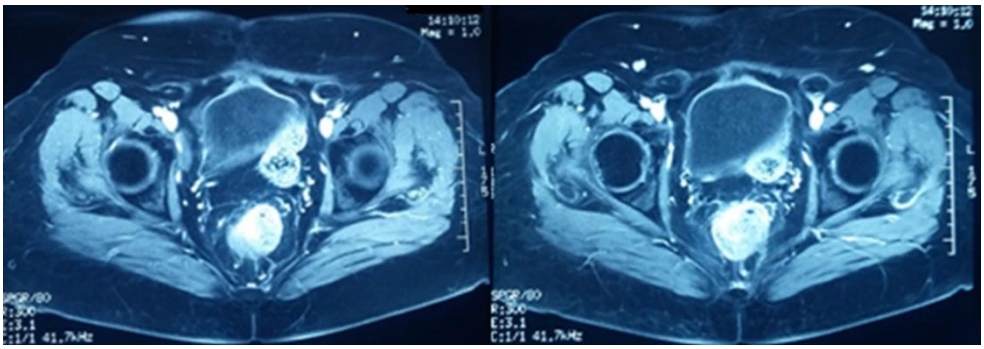

Fig. 1. Pelvic MRI – Invasion of the rectal tumor in the posterior wall of the urinary bladder

The patient undergoes surgery and were identifying a multisacular incisional hernia in the lower abdomen (after the Wertheim procedure), as well as multiple postoperative peritoneal adhesions in the inframesocolic compartment. After sectioning the pelvic peritoneum, the rectal tumor was identified attached to the urinary bladder and the dissection was performed with difficulty. An abdominoperineal resection of the rectum

was performed. The surgical procedure ends with the primary maturation of the colostomy and double drainage: peritoneal and perineal (the last one on a Pezzer tube).

Postoperatively, the patient was closely monitored, with hematological, hydro electrolytic and acid-base status rebalance in the intensive care unit and the resumption of the intestinal transit on the colostomy after 72 hours. The peritoneal drainage was suppressed 9 days after the surgical procedure. On the 10th day was observed an increase of the perineal drainage tube (serous liquid from 100 milliliters (mL)/day up to 300 mL/day). On postoperative day 11, the perineal drainage increased to 700 mL and gained a biliary aspect.

The suspicion of upper digestive fistula was raised (possible jejunal loop injury). This hypothesis was sustained by externalization on the perineal tube of methylene blue previously administered orally. A gastrointestinal transit using a radiopaque substance was performed to identify the fistula but the results were inconclusive. Considering the persistence of a large fistula flow (700-800 mL/day), interruption in intestinal transit and the appearance of an important rash on perineal skin, a new surgical procedure was required.

The accessing into the peritoneal cavity was very difficult due to adherence syndrome and edematous aspect of the intestinal loops with easy bleeding due to dissection maneuvers. In the remnant pelvic area was identified a visceral block, irreducible, adherent to the sacrum and the internal iliac vessels. The repeated attempts to dissect this block failed. Due to the elevated risk of intestinal injury, it was decided to perform an enteral-caecum anastomosis (the jejunal loop was dissected as far away as possible from the Treitz angle – about 150 cm). The enteral-caecum anastomosis was performed lateral-lateral, in double layer, respecting the peristalsis movement directions. The pelvic drainage was repositioned and the perineal drainage was checked out. The abdominal wall was closed using a substitution mesh.

Postoperatively, the patient has slowly recovered, with resumption of the intestinal transit on the colostomy 5 days later. Intestinal content continued to be externalized on the perineal drainage, but the quantity decreased gradually. On the 13th day from the second surgical procedure a gastrointestinal transit using Gastrografin was performed, revealing the small bowels loops opacified, but the anastomosis was not detected and the results were inconclusive (fig. 2).

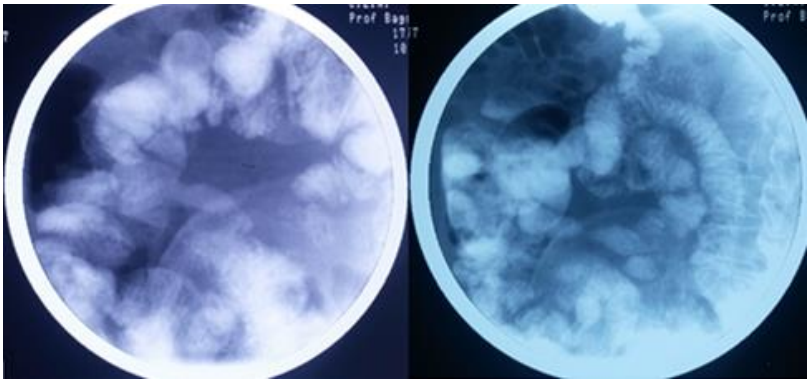

Fig. 2. Gastrografin gastrointestinal transit after the internal by-pass

After one month, the perineal tube was suppressed, the fistula flow being minimal. Wound dressing was performed daily, perineal wound healing being complete 43 days after the second surgical procedure.

The patient is discharged 46 days after the second surgical procedure, with closed intestinal fistula, slender perineal scar, normal bowel movement and discharge on the colostomy. The patient was directed to the oncology service (fig. 3,4).

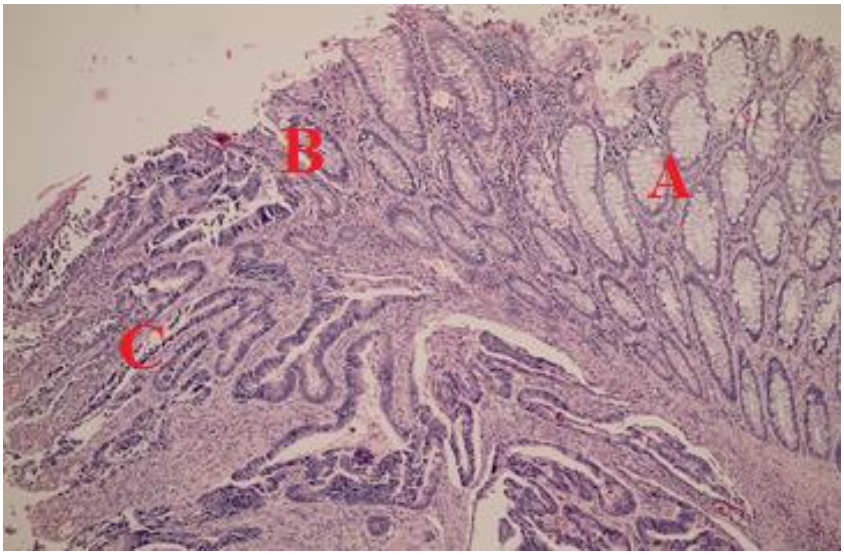

Fig. 3. Rectal tubular adenocarcinoma (A – mucosa of normal aspect;
B – area of impact between the free rectal mucosa and the tumor;
C – rectal tubular adenocarcinoma; Hematoxylin-Eosin staining - 10x

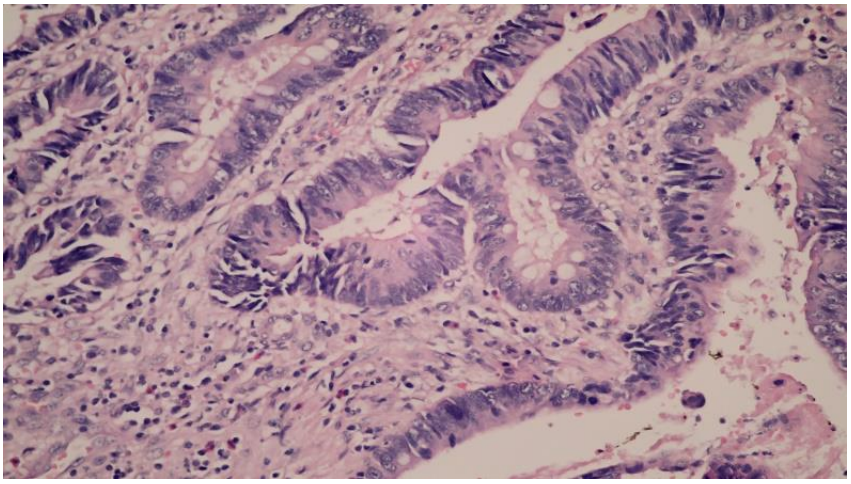

Fig. 4. Tubular adenocarcinoma – tumoral glands with chaotic architecture, mitoses and intratumorally necrosis; Hematoxylin-Eosin staining - 40x

The patient underwent surgery 18 months ago and is still in evidence of the General Surgery Department of the "Bagdasar-Arseni" Hospital, returning to control every 6 months. Following the surgical and oncological treatment, the patients had a good biological status, without clinical and imaging findings suggestive for tumor recurrence.

DISCUSSION

Perineal small bowel fistula is a life-threatening complication of abdomino-perineal resection of the rectum (5). External digestive fistula can occur after any surgical intervention performed into the abdominal cavity and concerns the general surgery departments and intensive care units, in order to overcome septic complications resulting from early intra-abdominal infection, fluid electrolyte imbalance and malnutrition (6). The therapeutic management of the enteral-cutaneous fistulas is complex, involving a balance in the nutrition, sustained drug treatment, protection of the skin against the aggressive digestive content, as well as the psychological monitoring of the patient, all these being accomplished with the help of a multidisciplinary team (7). The prognosis of an intestinal fistula depends on the anatomic location, the flow, septic complications, nutritional status, as well as the other comorbidities of the patient (8).

Choosing the optimal time for surgical intervention has not been well defined, but surgery should be delayed until the intra-abdominal and systemic conditions of the patient are propitious for major surgery (9). Usually, it is expected between 4-6 weeks, during which time about 95% of the fistulas close spontaneously (9,10).

The surgical aims for enterocutaneous fistulas are to restablish the function of the entire bowel, resection of the fistula with end-to-end anastomosis of the bowel and secure abdominal wall closure. Sometimes, previous surgical and oncological procedures such as radiotherapy can lead to a extremely difficult exploration of the peritoneal cavity and to an impossible direct approach of the intestinal fistula. In these cases, one of the few therapeutic solutions is to perform an anastomosis to bypass the fistula (6,9).

In our case, the patient presented since the onset of the fistula a large amount of intestinal output (about 700 mL/day), and lack of response to conservative treatment associated with the modified aspect of the previous irradiated intestinal loops led to performing the surgical procedure.

Regarding our patient, the disease and the changes in the intestinal wall were decisive in the development of the entero-cutaneous fistula and the suspected mechanism was a lesion due to the perineal drainage tube.

Consulting the specialty literature, we found that the externalization of intestinal fistulas predominantly through the abdominal wound or through the drainages is more frequent located on the anterior or lateral abdominal wall and the expression of a jejunal fistula through a drainage located in the perineum represents the peculiarity of this case.

CONCLUSIONS

Our case emphasizes the fact that digestive fistulas represents one of the major complications of abdominal surgery, triggering a major biological imbalance on the patient and requires a complex, interdisciplinary approach, between the surgical and the intensive care procedures. Although

most digestive fistulas close spontaneously after a certain time, there are cases where, due to the large fistulous flow, associated comorbidities or pathological preexisting conditions (neoplasia, irradiation) surgery is imposed.

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ASSISTED REPRODUCTION IN ROMANIA OR RESISTANCE TO CHANGE IN A POST-COMMUNIST COUNTRY?

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ASSISTED REPRODUCTION IN ROMANIA OR RESISTANCE TO CHANGE IN A POST-COMMUNIST COUNTRY? (Abstract**):** Despite the potential role of assisted reproduction technologies (ART) to provide a recognized treatment for the ever-enlarging number of infertile couples and compensate for the Romanian low total fertility rate (TFR), the local legal and clerical community demonstrate not to be ready for cognitive assimilation of ART and for resolving the different bioethical dilemmas. What makes Romania an interesting case to be studied is the very fact that resistance to change has been correlated to notorious bioethical controversies. Therefore, the purpose of this communication was to explore endogenous and exogenous factors that may influence resistance to change, social representation of ART, as well as to observe and understand reasons for the absence of a coherent and systematic law in this field of medicine a law which should address the Romanian social, moral and cultural beliefs and values. Consequently, we have observed that after 42 years of communism and 28 years since the Revolution, the relative apathy at legislative level has been driven by psychosocial factors such as respect for traditions, conformist behavior and fear for the unknown, which have also conducted to social duplicitous behavior having generated undesired international notoriety. **Keywords:** ART, LEGISLATION, BIOETHICS, POST-COMMUNIST DEMOCRACY, RESISTANCE TO CHANGE, ROMANIA.

DECREASING FERTILITY IN ROMANIA–THE NATIONAL AND EUROPEAN CONTEXT

Since 1970, the European statistics have been reflecting a sharp unprecedented fall in the TFR within its 27 European countries. The existent demographic crisis is nothing but a predictor for the imminent economic decline, translated by the accelerated speeding up of population ageing, and implicitly at increased ratio of inactive population relying on the working population (1). The current total fertility rates are situated below replacement level, which is a generalized process all over Europe. The latest projections provided by Eurostat (2, 3) indicate the Central and Eastern Europe and the Baltic states as displaying the lowest total fertility rates. The same low fertility rates are also to be found in certain West European countries, such as Germany (1.36), Spain (1.35), Portugal (1.35).

The main factors for the declining TFR are related to educational attainment, economic security and careers (4). In the ex-communist countries, particularly in Romania, this demographic phenomenon is also a result of the frustrations caused by the deprivation of basic human needs. Thus, the low fertility rate can be regarded as a negative indicator of the quality of its people social life. The decrease of families' income correlated with the increase of the estimated costs for raising a child leads to voluntary and autonomous delaying in planning the first pregnancy until economic stability is possible. On the contrary, in the occidental countries the factors leading to low TFR reflect the fact that the development of these societies has supplied new life opportunities, changing individual values regarding personal goals, family formation and religion. Therefore, in contrast with the Romanian pro-family ideology which promotes family as the fundamental institution for the survival of the society, the individual has become the central element of social life in the West European countries (5).

Moreover, postponing parenthood due to social reasons unfortunately overlaps the biological fertile period and, consequently, diminishes the chances of conceiving. The decline in female fecundity is clinically relevant from mid-30's, when, even assisted reproduction therapy cannot compensate for the decreasing fertility potential associated with postponing childbearing. This delay has led to an increase in permanent involuntary childlessness (6, 7).

In Romania, frustrations related to the social and economic conditions are deeply rooted in the communist era. The infamous 770/1966 Decree has become a referential proof of the propagandistic communist aggressiveness against human rights as the Romanian women were "de-individualized" to the extent that they seemed to have no authority over their own body. They were forced to regard their own womb as belonging to the state and not to themselves (8). Even though the Decree, aiming to increase the fertility rates, had banned the use of any contraceptive methods and any form of abortion, the Romanian women found their own methods of resilience. While in 1967 the fertility rate doubled, reaching the maximum average value of 3.7 children born per woman, in 1973 it declined again. During the dictatorship of Ceausescu, the mortality rate among pregnant women became the highest in Europe. During the period 1966-1989, medical complications caused by illegal abortions resulted in the death of 9452 women (9). As a radical reaction to this death sentencing decree, the very first law promulgated after the 1989 Revolution was The Law-Decree no. 1/26.12.1989 that allowed abortions in the first 3 gestational months.

Therefore, the prevalence of declining fertility has become a general feature of the ex-communist countries having adopted pro-nationalist politics in the communist era. For this reason, Romania disclosed one of the lowest birth rates with an average score of 1.25 children per woman in 2011 (3). Above all, infertility has become a subject of major concern in this country. Under these circumstances, in the post-communist era, the Romanian medical community has been challenged to adjust their professional competencies to the new needs of the society to provide equitable access to optimal care for the Romanian population affected by infertility. Consequently, since 1995, when the first IVF center was officially inaugurated, there have been 24 authorized

IVF centers that have been accredited by the National Transplant Agency, the Romanian Competent Authority. Despite the professional performances achieved by the medical teams of the Romanian IVF centers, the society has demonstrated not to be ready for assimilating the new technologies and resolving the afferent bioethical dilemmas so far.

The current communication provides a retrospective overview of the evolution of assisted reproduction in Romania. Our longitudinal approach is meant to identify the endogenous and exogenous factors that have influenced the resistance to change, the social representation of ART, as well as to observe and understand reasons for the absence of a coherent and systematic law in this field, a law which should address the Romanian social, moral and cultural beliefs and values.

Therefore, the objectives of this communication have been transposed into the following research hypotheses:

1. The poorly understood bioethical challenges raised by the field of medically assisted human reproduction have consolidated resistance to change.

2. The traditionalism specific to the Romanian society has determined a significant delay in the promulgation of a special law regarding medically assisted human reproduction.

Subsequently, to verify the two hypotheses, we have examined the mass-media documents related to ART practice in Romania, the published legislative projects and documents issued by the Romanian Parliament, the current regulations related to ART, as well as the web pages of the Bioethical Commission of the Romanian Orthodox Church, National Agency of Transplant and of the Ministry of Health.

BIOETHICAL CHALLENGES – A TOO LIBERAL PERSPECTIVE ON HUMAN REPRODUCTIVE RIGHTS?

Regarding new reproductive technologies, Romanian contemporary society has not fully embraced the medical innovations in the field. As Serge Moscovici (10) points out, the way that science penetrates society implies a discontinuity which represents a necessary precondition if any innovational scientific knowledge is to enter "the society's laboratory", "where all forms of knowledge acquire a new epistemological status in the shape of social representations." As many social issues, such as organ donation and transplantation, biotechnology, human rights which have been studied in the social representation field have always attracted expansive media coverage and controversy (11), we have analyzed the way ART are reflected in the national media, which has a high exponential degree and is so influential in shaping public opinion. We also have considered the fact that social thinking filtrates and organizes information and events per the confirmation or the invalidation coming from the exterior reality. Regarding this matter, we will illustrate the evolution social representations of assisted human reproduction in Romania as it emerges from the national media that has led to widespread public debates on the social, moral and ethical implications of this kind of scientific progress.

As dynamic elements of knowledge, social representations refer to the ideas, concepts and knowledge shared by the members of the same community and are elaborated in conformity with the phenomena that constitute the center of the social life and represent the object of polemics and changes. Therefore, they represent a set of concepts

generated in the daily life denoting the myths and beliefs systems in traditional societies. Once created, they "lead a life of their own, circulate, merge, attract and repel each other, and give birth to new representations, while old ones die out" (12).

The changes caused by the communist fall have had consequences on the way people perceive their own lives, as well as on the way they perceive the individual and the society they live in. "Change does not display in a spectacular way and is escorted by stamps of resistance to change" (13). That is why change and resistance to change may be considered the two sides of the same coin.

Despite the low TFR in Romania, which could be a representation of the devaluation of the family concept, procreation still represents the center of social life. Accordingly, the increasing incidence of infertility diagnosis among the Romanian population has forced the medical community to direct their professional competencies towards assisted human reproduction field to provide optimal care for affected couples. However, the implementation of innovative technologies of medically assisted human reproduction in Romanian traditional society has determined resistance to change due to specific social, religious barriers. This can be explained by the fact that human reproduction raises important and essential challenges in the social, moral and civic development of the post-communist Romanian society, as "of all the structures composing the human organism, the reproductive system has special societal sensitiveness" (14). Consequently, assisted reproduction technologies have become a controversial medical practice in this country with a traditional Orthodox predominant society. Searching for data in the Romanian and international media documents revealing the public opinion on this matter, we have discovered that most of the articles include information related to bioethical dilemmas, such as the social age deadline for childbearing, the commercial traffic of human cells and mercantile surrogacy.

The most famous cited example related to the use of the assisted reproduction technologies in Romania is the case of Adriana Iliescu, who, in 2005, at the age of 66, gave birth to a child after having received IVF therapy. Thus, she became the oldest mother in the world and her story created a lot of bioethical controversies at that time. The ethical issues residing in this matter are associated with the medical experimental practice, but also with the individual's autonomy, as well as with the individual's right to privacy and reproductive choice. Refusal to assist women requesting ART, even if they are far beyond their natural reproductive limits, could be denial of their decision-making autonomy and of their own reproductive rights. However, recent publications referring to the community attitude towards pregnancy at postmenopausal women indicate insignificant support for this practice. Per most published surveys, the accepted age limit varies between 50 and 55 years of age (14, 15). Furthermore, the public disapproval of the sexagenarian mother may have also induced perception modifications in relation to reproduction, family, and maternity representation.

The "black list" of bioethical deviances can continue with the example of the "reverse traffic" of donor oocytes (16) and with the example of surrogate mothers who rent their uterus in exchange for large amounts of money, all made public on the internet and in the newspapers, clearly

specifying the requested price. To date, surrogacy has not been regulated by law, and thus the proliferation of this highly controversial and "financially profitable" practice.

The commercialization of human cells and commercial gestational agreements on the black market offend a series of ethical principles such as those described by Mykitiuk and Nisker (17), namely "respect for human integrity and dignity through the non-modification and non-commercialization of the person, her or his bodily parts, tissues, substances and processes; protection of vulnerable persons from coercion or inducement; and respect for the patient-physician relationship by avoiding a conflict of interest between the two parties."

On one hand, many of the advances in life sciences and biotechnology field are being a great opportunity to enhance our life through the alleviation and prevention of medical conditions. On the other hand, there has been a high degree of "concern and resistance to some of these developments, particularly about their potential uses (and abuses) of genetic information and genetic transformations" (11).

Romanian women's mercantile decisions regarding their reproductive capacity are an integral part of a more complex process which was explained by Nahman (16) as a response to the repressive probirth increase politics previously presented. Egg donation and surrogacy may also be viewed as "both a continuation of the conscription of women's bodies (by and for science and business) and a break with paternalistic control over women's bodies" (16).

The mentioned bioethical paradigm cases have turned into arguments against the acceptance of assisted reproduction technologies and have contributed to the development of stereotypical responses. Under a similar "halo effect" (18), a cognitive bias by which we make an overall judgment on something in the view of a prominent feature. Public opinion evaluated new technologies through the perspective of the bioethical objections portrayed by the media. "Stereotyping is, by definition, a generalization process which may become prejudicial per the circumstances in which it is used. The categorical labeling may take distance from the reality, even if it derives from it. Stereotypes are reversed, transfixed and rigid social representations. They are constituted in each social and cultural context, determined by a given reality. They are fed by the social memory, traditions and rituals, by mutual symbols" (19). Therefore, negative stereotypes lead to prejudices and become important factors in the development of resistance to change.

TO LEGISLATE OR NOT TO LEGISLATE?

Above all, the controversies generated by these examples are very eloquent for the direct and indirect consequences of the lack of an exhaustive law in the field of ART. These three paradigm cases are impregnated into the social cognition and have drawn attention to the need of an ART specific law. Despite the previous initiated law there was no trigger for local authorities to forward legislative projects and no law has been promulgated so far.

However, ART is not practiced completely outside a legal framework. The existent legislative coordinates represent the result of our adjustment to the European standards and requirements, because Romania becoming an EU Member State in 2007.

Accordingly, under the Law no. 17/2001, Romania has ratified the Convention for the Protection of Human Rights and Dignity of the Human Being about the Application of Biology and Medicine: Convention of Human Rights and Biomedicine, signed at Oviedo on 4th April 1997, and its Additional Protocol, signed in Paris at 12th January 1998. Moreover, Law No. 95/2006 on Reform on Public Health – Title VI: The Storage and Transplant of Organs, Tissues and Human Cells for Therapeutic Purposes, with Annexes I-XIII, and the Methodological Norms for the application of the Law stipulates general provisions referring to the use of the reproductive cells, with the application of the EU Tissue & Cells Directive 2004/23/EC. The mentioned provisions forbid financial payment for donated reproductive cells.

Furthermore, per the Ordinance No. 79/2004, the activity of human organs, tissues and cells is organized and controlled by the Ministry of Health, and the National Transplant Agency was founded to exercise these specific duties such as the coordination of procurement, processing, storage and transportation activities for human tissues and cells based on therapeutic use in Romanian territory. The National Transplant Agency is also in charge of the initiation and management of the National Transplant Register and with the proposal for accreditation to the Ministry of Health of ART units which are authorized to carry out sampling and/or transportation of human organs, tissues and cells for therapeutic purposes, as well as with monitoring their activities in the specified field. The list of the regulations in the field may continue with another two decrees: Decree No. 1242/2007 of the Ministry of Public Health on the approval of standards for the selection and evaluation of tissue and cell donors, the alert systems and emergency procedures, the qualification of the personnel of tissue and cell banks, the quality, import and export of human tissues and cells, and the relationships between tissue and cell banks and third parties. Decree No. 1763/2007 of the Ministry of Public Health provides standards for donation, sampling, testing, processing, preservation, distribution, coding, the traceability rules of tissues and cells of human origin used for therapeutic purposes, and notification of serious adverse incidents and serious adverse reactions occurring during their processing.

Moreover, articles 441-446 of Law No. 287/2009 regarding the new Civil Code encompass provisions related to the third-party reproduction. Firstly, regulated in Romanian law, per article 441, medical donor assisted procreation provides solutions for the heterosexual couples and/or single women who want a child and are not able to conceive naturally, and who shall act as parents of the child so conceived. By providing single women the opportunity to have babies, the new regulations may be considered quite a permissive approach. The new Civil Code also establishes the juridical filiation regime of children conceived through medical assisted reproduction technologies. This way, there will be no affiliation bond between the donator and the child thus conceived, and parents importantly will always be those who have resorted to assisted reproduction technologies. Consequently, the law stipulates the obligatory existence of consent to be given in front of a notary who will explain the consequences of their act related to the future child's affiliation, in terms of highest confidentiality. Until the time of the assisted conception, been undertaken this

consent will remain void in case of death, application for divorce, the separation, or of its revocation by the beneficiaries. The child thus conceived will have the same rights as a naturally conceived baby. However, the new legal framework does not explicitly define the medical techniques specific to third-party conception. Therefore, article 447 stipulates that the third-party donor donation and subsequent use, the juridical conditions, and informed consent related to procedure will be regulated by a special law.

Considering the presented legislation as well as other general regulations related to good practice in scientific research, technological development and innovations, the data processing and protection, the patients' rights, and medical deontology fields, we find that regulation of ART in Romania is fragmented and dissipated in various and numerous norms and regulations, and yet, in spite of this abundant documentation, we ascertain that the challenges of this medical field are still not sufficiently covered, as the current legal provisions in force have a significant delay in comparison with the accelerated scientific medical progresses. Accordingly, because this deficiency and the current high incidence of infertility as a medical condition, beginning with 2003, there were six consecutive legislative projects that entered the legislative circuit, but none of them has succeeded in being promulgated so far.

The first three projects displayed similar characteristics, and were definitively rejected because they proved to contain many unconstitutional articles, a precarious elaboration, with contradictory and inaccurate provisions both in terms of medical accuracy, as well as in terms of legislative technical requirements. Taking into consideration all the past amendments, the fourth project entered the Parliament's circuit with a view of approval in 2011. Therefore, the draft included the themes of gestational mothers, donated gametes, and penalties for commercialization and scientific abuses. It also encompassed provisions related to the equitable access to reproductive health- care services. Although the project seemed to be the best version to have ever entered the legislative circuit, surprisingly, it was withdrawn by the initiator. Simultaneously, the Minister for Health released the National IVF/ET Subprogram, which partially compensated the expenditures for one IVF cycle treatment from the health insurance funds for 800 married couples, annually. Eligibility criteria for obtaining access to Medically Assisted Reproduction (MAR) treatments were established and reimbursement provided only if criteria were fulfilled: the marital status (the couple had to be married for at least two years); the Romanian citizenship (at least one of the partners had to be Romanian, with a stable home address in Romania); the social insurance (at least one of the partners had to be socially insured for at least the last two years); previous children (none of the partners was supposed to have a living child); autologous couple (oocyte, semen donation and surrogate mothers were excluded); maximum age of woman (the woman had to be 24-40 years old), BMI (had to be between 20-30); normal ovarian reserve (determined by the values of FSH and AMH). Per the National Transplant Agency, 11 ART Institutes were included in the National IVF/ET Subprogram, and there were 1499 married couples who subscribed to this program until August 2012 (20). At the time of writing, the Subprogram has stopped, as no

funds are available. To make things worse, the legislative project on health-care reforms, under current public debate, eliminates this compensation for ART treatment.

Meanwhile, after the failure of the four legislative projects, and under the circumstances that Law No.71/2011, of applying Law No. 287/2009 regarding the new Civil Code, imposes the adoption of a special law regarding the third-party donor assisted reproduction, a new legislative draft in the field was put forward. The new proposal did not sufficiently cover the third-party donor reproduction issues. Although the initial form of this project was changed, and another modified draft was approved to be under debate in the Parliament, the current project is on stand-by position now because of the pre-established legal procedures and terms, and because of the recent electoral and political changes in Romania.

ROMANIAN OPINION GATEKEEPERS

To identify the factors leading to change or to resistance to change, we will examine the correlations between the Romanian most influential institutions and people's behavior and perceptions related to procreation. Per Eurobarometer's 68.2 (21) and 72 (22), as for the perception of the national institutions, Romanian people trust the Church and Television with the highest degree of confidence. This way we have the possibility to assume that the two institutions have a powerful social influence on the way Romanian society perceives its surrounding reality.

Having children is a fundamental traditional value in Romanian society, which is one also promoted by the Romanian Orthodox Church. To date, there is no explicit official position related to medically assisted reproduction. The Romanian Orthodox Church's public interventions on this topic are directly correlated with the bioethical deviances generated by the extreme situations mentioned above which gave the Church representatives the opportunity to pronounce themselves against this medical practice. It is noteworthy that the Bioethical Commission of the Romanian Orthodox Church considers the practice of tissue and organ transplantation as one of the highest forms of contemporary medical practice, which transforms pain into hope for more life. It is medical practice that the Church blesses if, by transplant, the crisis determined by the absence of any other healing solutions is resolved and normal life is given back to a person, without taking it away from another person. Nobody should be killed so that somebody else should live (23).

Surprisingly, the same document contains one reference to human embryos as unacceptable, since the embryo, although a living being, is incapable of expressing consent. Therefore, the Romanian Orthodox Church indirectly and tacitly displays a certain degree of tolerance towards new technologies in the field. On the other hand, the Romanian Orthodox Church has taken a firm position against abortion, but, despite this, abortion is legal in Romania (24). Interestingly, abortions rates from this country have declined in comparison with prior decades (2). From a religious point of view, abortion is a capital sin, but, on a more general level, it is also a consequence of the direct effects of the precarious economic and social conditions which do not foster family policies enough. History has taught us that restrictive norms and legislation do not have the expected results.

Consequently, considering the Romani-

an Orthodox Church's relative tolerant attitude towards ART and its very influential power, we assume that the religious traditionalism specific to this country is not a primordial factor leading to the delay of regulation in the MAR field.

On the contrary, the continuous bombardment with ART-related news from the Romanian mass-media devalue these techniques by attributing negative connotations to them, constantly reiterating the bioethical issues related to reproductive tourism, commercialization of human gametes and mercantile gestation. This leads us to assume that public opinion has selectively and distortedly retained mass-media information related to assisted reproduction, and information induces resistance to change.

We have noticed a direct correlation between perception and behavior related to ART. Overall, Romanian society is a conformist and imitative society, so, on an individual level, there is the tendency to fashion one's opinions per the group norms. Therefore, the perception of ART is modeled per the group norm. The clear majority of the population which do not have fertility problems become a reference group who does not have to resort to assisted reproduction services to procreate and for whom "ART may not fit into their ideological definition of family" (25). While the infertile population constitute a dispersed and stigmatized minority, the clear majority of the population is inoculated with the mass-media messages and maintains a non-critical and passive attitude on this kind of information. That is why the public debates launched by the Ministry of Health are practically inexistent, even if the ratified Convention from Oviedo urges that a legislative project in human reproduction field should be accompanied by such debates.

However, the reality is that per the National Transplant Agency, between 2009 and 2011, 10,105 couples were reported to have received assisted reproduction procedures with partner donation, and 898 patients were reported to have received assisted reproduction procedures with the involvement of a donor (26). The increasing number of couples who resort to ART treatments to conceive underlines the fact that the population affected by infertility is becoming conscious of the fact that ART is a pertinent solution to their health problem.

In contrast, Eurobarometer 72 (22) showed the Romanian population perceives national political institutions with the lowest confidence level among other official authorities and demonstrates discontent towards the inability to provide and implement prompt solutions for the current social and economic problems. It is clear the political structures have not sufficiently mobilized themselves to have a law promulgating the assisted reproduction field, in concordance with the existent democratic, ethical and socio cultural values with which ensure equal access to reproductive health-care services.

CONCLUSIONS

In Romania, twenty-three years after the end of communism, the receptivity to the imperative of change displays an asynchronically pattern, often being associated to different reactions of rejection. Respect for traditions, dependence on concepts assimilated through the initial formation, conformist behavior, dependence on majority's opinions, fear for the unknown, refusal caused by ignorance represent the psychosocial factors which block the change path (27).

Nevertheless, about the assimilation of ART treatment into the social cognition, we assume that the technological progress has functioned as a change agent and has modified the social representation of ART, particularly if we take into consideration the increasing number of IVF centers, and implicitly the increasing number of the couples resorting to ART treatment.

On the political level, in the context of a long-lasting transition period, the current situation regarding ART denotes a perpetual procrastination, as well as the adoption of a latent attitude of indifference, reserve and withdrawal towards the demographical decline and towards the increased prevalence of infertility among the population of fertile age. A society without legislation on bioethical problems adopts "the liberal position that every citizen should decide to his or her moral conviction." (28) The delay of a law to democratically and constitutionally regulate the Romanian reproductive rights underlines the fact that in Romania the democratization process has a slow, heterogeneous, evolution, with numerous endogenous socio-economic and political obstacles.

Therefore, considering the moral, spiritual, social and cultural parameters, it is imperative that the next legislative project should meet the specific requirements and bioethical principles, according to the collective values and beliefs, in order to defend every individual's basic rights to procreation, and, implicitly, to decision-making autonomy, human dignity and integrity. Otherwise, ART will continue to receive an unfavorable and controversial social representation instead of becoming a medical treatment for a World Health Organization, (WHO) recognized condition, a medical treatment facilitating what is a basic human right, the right to a family.

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PANCREATICODUODENECTOMY IN PATIENTS WITH HEPATIC ARTERY, ANATOMIC VARIANTS: TAILORING, PERIOPERATIVE CARE AND SURGICAL OUTCOMES

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PANCREATICODUODENECTOMY IN PATIENTS WITH HEPATIC ARTERY ANATOMIC VARIANTS: TAILORING, PERIOPERATIVE CARE AND SURGICAL OUTCOMES (Abstract). **Introduction:** Pancreaticoduodenectomy (PD) is the procedure of choice in patients with tumors of pancreatic head or periampullary region. Hepatic artery anatomic variants (HAav) are frequently encountered during PD. **Aim:** To evaluate the incidence of HAav in a series of PDs and their consequence on technical tailoring and outcome. **Material and methods:** Forty-five patients with HAav were identified in a consecutive series of 140 PDs for periampullary and pancreatic head tumors performed between January 1, 2007 and December 31, 2015. The groups with or without HAav were compared in terms of operative approach, complications and survival. **Results:** All patients underwent Whipple procedure, with right posterior approach for those having HAav. HAav was spared without damage in 41 cases. Four patients in whom the HAav were either sacrificed (2) or damaged (2) required arterial reconstruction. **Conclusions:** HAav are frequently encountered during PD. Its safeguarding is mandatory but in malignancy it can be damaged or sacrificed, hence arterial reconstruction is required. HAav have no negative impact on surgical morbidity and outcome. **Keywords:** PANCREATIC CANCER, PERIAMPULLARY TUMORS, PANCREATICODUODENECTOMY, POSTERIOR APPROACH, VASCULAR ABNORMALITIES.

Pancreaticoduodenectomy (PD) is a challenging operation and the procedure of choice for tumors of pancreatic head and periampullary region but also for some benign lesions (1, 2). However, it is associated with a high morbidity rate of up to 50 % (2). The most feared postoperative complications are anastomotic leakage at the site of pancreatojejunostomy or hepaticojejunostomy and post pancreatectomy hemorrhage (1). Hepatic artery anatomic variants (HAav) are reported to occur in up to 49% of patients and therefore preoperative assessment of liver arterial supply is essential to prevent unnecessary complications during PD (3). Damage or ligation of an aberrant artery may cause bile duct or liver ischemia which can result in breakdown of the bilioenteric anastomosis and liver dysfunction and abscesses (4). Obstinate handling while trying

to spare HAav may result in damage to the vessel adventitia and thus make it prone to pseudoaneurysm formation, so much the more in the presence of pancreatic fistula (5). That involves a higher risk for bleeding. Otherwise, attempts to safeguard the anomalous vessel may also hinder radical oncological resection (4).

Nowadays, Michels and Hiatt international classifications of hepatic artery anomalies are the most common used (6, 7). In both classifications, an aberrant right hepatic artery (RHA) is the most common reported abnormality. Michels makes a distinction between a replaced (Michels type III) and accessory RHA (Michels type VI) arising from the superior mesenteric artery (SMA)(6). This vessel may course posterior to the pancreas, within the pancreatic parenchyma or along the ventral side of the pancreas and its reported incidence vary from 7 to 21 % (8). An aberrant RHA is the most frequently encountered vascular abnormality during PD and due to its course, it is the most susceptible to intraoperative damage and tumor involvement (8). Classically, PD is performed with transection of the pancreatic neck before the retroportal lamina dissection close to the SMA origin. Early right posterior approach PD allows a better assessment of the variant pattern of arterial blood supply to the liver, proper mobilization of the specimen from the vessels before any pancreatic or digestive division and, if necessary, safe venous clamping (9). Our study aimed to underline the implications of an abnormal HA on the PD technical approach, morbidity, the surgical outcome.

MATERIALS AND METHODS

A consecutive series of 140 PDs was performed between January 1, 2007 and December 31, 2015. HAav were identified on preoperative computed tomography. The 16-slice MDCT protocol included bi-phasic examination after intravenous contrast injection (100 mL, 350 mg I/mL, injection rate 3-4 mL/s) and post-processing with MIP and volumetric reformatation. Forty-five patients were identified with HAav and in this group (group 1, no=45; 35%) a right posterior approach PD was performed. The operative parameters, complications and survival were compared with those recorded in group 2 (95 patients without HAav, 65%) (tab. I).

TABEL I

Patient characteristics for the group with or without HAav

Patient characteristic	HAav group		Non HAav- group		p-value
Mean age (SD), years	61		62.2		0.15
Males, n (%)	24	(53.3)	49	(51.6)	0.89
ASA classification n (%)					
I	12	(26.6)	20	(21)	0.30
II	27	(6o)	57	(60)	0.85
III-IV	6	(13.4)	18	(19)	0.27
Co-morbidity, n (%)					
Cardiac	8	(17.7)	20	(21)	0.25
Hypertension	9	(20)	21	(22.1)	0.71
Pulmonary	4	(8.8)	12	(12.6)	0.09
Diabetes mellitus	6	(13.3)	15	(15.7)	0.39
Malignant disease, n (%)	38	(84.4)	81	(85)	0.87
Benign tumor, n (%)	7	(15.6)	14	(15)	0.27

Operative technique. The standard operative procedure for the PD was the Whipple–Kausch operation. All the patients having HAav (group 1) underwent posterior approach PD.

Briefly, after resectability had been assessed, an extensive Kocher maneuver, large opening of the lesser sac, isolation of the superior mesenteric vein at the inferior edge of the pancreas, exploration of the hepatic pedicle, had been carried out. Behind the pancreas, extensive early dissection exposes the origin of the superior mesenteric artery in the angle between the left side of the inferior vena cava and the left renal vein, and highlights the plane between the SMA and the SMV. This exposure enables a safe removal of the retro portal lamina (including connective tissue, perivascular neural plexus, lymph nodes belonging to posterior pancreaticoduodenal vessels), which implies the dissection of the SMA on its right and posterior sides along 3-4 cm. This is the surgical step during which the origin of a RHA (accessory, replaced, totally replaced common HA) arising from the SMA is properly distinguished. Upon discovery, the aberrant vessel is dissected upwards to the hepatic pedicle in the attempt to spare it or to assess tumor involvement. In this case, the vessel can be resected and reconstructed. Reconstruction was performed by retrocolic end-to-side "duct-to-mucosa" stented pancreaticojejunostomy, hepaticojejunostomy and gastrojejunostomy. Octreotide was routinely administrated in the HAav group (posterior approach) and only in cases of soft pancreas or non–dilated Wirsung duct in the non-HAav group.

RESULTS

Patient characteristics in the two groups are summarized in table I. There were no significant differences in terms of age, sex, American Society of Anesthesiologists class or comorbidity. The underlying disease requiring PD was the same in both groups and the malignant disease occurred in 84%, and 85 % cases, respectively. Surgical variables did not differ between the two groups (tab. II).

TABLE II

Surgical variables in the two groups

Operation	HAav group (n=45)		Non-HAav group (n=95)		p-value
Whipple, n (%)	0	(0)	95	(100)	
Posterior approach Whipple, n (%)	45	(100)	0		
Average surgery duration (SD), minutes	310		298		0.94
Median blood loss (SD), mL	1120		1010		0.89
HAav-handling					
Spared	41	(91)			
Accidentally damaged or ligated n (%)	2	(4)			
Sacrificed n (%)	2	(4)			
Reconstructed n (%)	4	(8)			

In four patients with HAav (8%), the aberrant vessel was accidentally damaged (n=2) or willingly sacrificed for oncological reasons (n=2). For the two damaged

arteries, primary closure with 6-0 prolene was performed. The two totally replaced common hepatic arteries sacrificed for oncological reasons required reconstruction using a reversed splenic artery.

Hepatic artery variants. The most common variations were replaced RHA (rRHA) (n=22- 48%) and accessory (accRHA) (n=16- 37 %) - Hiatt type III. A replaced common hepatic artery (rCHA) - Michels type IX, was encountered in 4 cases (8%). Two cases (4%) of rRHA and a left hepatic artery (LHA), and one case (2%) of accRHA and LHA were also disclosed - Hiatt type IV.

Short term outcomes. There were no differences in overall surgical morbidity and in the rates of any of the surgical complications between the two groups (tab. III). The incidence of complications related to the presence of HAav (hemorrhage, hepaticojejunostomy fistula, intraperitoneal abscess), were comparable in both groups. Hospital mortality, relaparotomy rate and length of hospital stay did not differ between the two groups.

TABLE III

Short-term outcome after PD in patients with and without HAav

	HAav group (n=45)		Non-HAav group (n=95)		P-value
Surgical complications, n (%)	24	(53.3)	47	(49.4)	0.14
Pancreatico jejunostomy fistula, n (%)	5	(11.1)	10	(10.5)	0.65
Hepaticojejunostomy fistula, n (%)	3	(6.6)	8	(8.4)	0.23
Gastrojejunostomy hemorrhage	1	(2.2)	3	(3.1)	0.18
Delayed gastric emptying, n (%)	15	(33.3)	34	(35.7)	0.62
Intraperitoneal hemorrhage, n (%)	3	(6.6)	7	(7.3)	0.81
Pancreatic stump pancreatitis, n (%)	1	(2.2)	3	(3.1)	0.18
Intraperitoneal abscess	2	(4.4)	5	(5.2)	0.65
Wound infection, n (%)	4	(8.8)	10	(10.5)	0.42
Other, n (%)	5	(11.1)	12	(12.6)	0.71
Re-laparotomy, n (%)	3	(6.6)	6	(6.3)	0.16
Hospital mortality, n (%)	1	(2.2)	3	(3.1)	0.18
Median hospital stay (days)	16	-	15	-	0.93

DISCUSSION

Pancreaticoduodenectomy (PD) is the treatment of choice for some benign and malignant lesions of the pancreatic head, ampulla, duodenum and proximal bile duct and represents the option of curative treatment in cases of malignancy (2). The first description was given by Whipple et al. (10), and although the classical PD surgical technique has undergone several modifications the morbidity remains high, with a rate close to 40% (11). Regionalization of the surgeries to high-volume centers had a great impact in decreasing the mortality rate.

Because of the continuous decline in mortality rate, PD has become nowadays a routinely performed procedure for tumors of the periampullary region and pancreatic head, with or without invasion of the mesenteric-portal axis (12).

Hemorrhage and pancreatic stump is-

sues remains the major concerns in the assessment of postoperative morbidity. Another predicament in performing PD is the complex anatomy of the peripancreatic vasculature (11). Celiac axis and hepatic arterial variants have been reported in 49% of the patients with pancreatic or hepatobiliary neoplasms (3).

Patient particularities (prior surgery, obesity, enlarged lymph nodes or a biliary stent) can determine difficulties for the accurate intraoperative evaluation of an aberrant hepatic artery.

Variations in hepatic arterial anatomy in the general population are estimated to range from 21 to 49% (4, 11). The most frequently described abnormality is the right hepatic artery arising from the superior mesenteric artery, with an incidence of up to 29% (4, 6). In our series, the distribution of has been of 22 aberrant RHA, 16 accessory RHA, 1 common HA arising from SMA, 2 cases of rRHA and a LHA, and 1 accRHA associated with LHA.

The embryological explanation for the occurrence of aberrant vessels is based on the quantity of signaling molecules and growth factors involved in normal development of any viscera, resulting that an improper signaling and incorrect gradient in visceral growth can cause abnormalities (13).

Nowadays, CT scans are commonly used for the diagnosis of periampullary tumors and to determine the tumor stage and to assess the resectability (4,14). Multidetector computed tomography with angiography is the best method for detection (14).

The detection of an arterial variant involves a technical difficulty that may determine a more conservative resection of the pancreas, especially for retro pancreatic plane, and occasionally inadequate tumor removal (1). Assessment of hepatic arterial anatomic variants in patients who are about to undergo PD is an imperative procedure, allowing the surgeon to minimize such complications as extensive hepatic injury (hepatic necrosis, ischemic biliary injury or anastomotic complications) (11, 15). It is well known that the vasculature of the cranial part of the bile duct is dependent on the right hepatic artery and sparing it is crucial in limiting a breakdown of bilioenteric anastomosis despite the existence of a hiliar vascularization (16)

Is important to recognize a vascular lesion and to proceed to revascularization if there is simultaneously an aberrant right hepatic artery, an accessory RHA with a high flow to the right lobe or an aberrant common hepatic artery (1, 9, 16) Several techniques have been described for RHA reconstruction in case of tumor infiltration or when the intraparenchymal course does not ensure an R0 resection (15, 16). In the literature techniques using interposition of a vein or prosthetic graft have been described (16).

In our study, we found a slight but statistically insignificant increase in surgery duration in the HAav group. No group differences were also found in the amount of packed red blood cells transfused intraoperatively. Bleeding and complication rates were similar in our two groups, findings supported by other studies. The posterior approaches allowed an early control of the retro pancreatic vasculature with more accurate hemostasis in case of bleeding lesions (pancreatic pseudo aneurysm), early assessment of resectability, and sparing of an aberrant hepatic artery originating from the superior mesenteric artery (11).

CONCLUSIONS

A good preoperative assessment of hepatic artery variants allows surgeons to perform the PD in conditions of safety, preventing liver damage and a higher rate of complications like performing the surgical procedure in patients with standard anatomic hepatic arterial disposition. Further prospective studies are required to determine the best surgical approach in cases of aberrant hepatic artery disposition, since there is no worldwide consensus.

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A THIN AND REGULAR ENDOMETRIUM IN ENDOMETRIAL CANCER - ENDOVAGINAL ULTRASOUND ASSESSMENT

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A THIN AND REGULAR ENDOMETRIUM IN ENDOMETRIAL CANCER-ENDOVAGINAL ULTRASOUND ASSESSMENT (Abstract): **Aim**: Our study had as a major objective the highlighting of more objective criteria in establishing the morphological diagnosis and the evaluation of prognosis elements in endometrial hyperplasia and endometrial carcinoma, representing a specific pathology for the premenopausal and postmenopausal women. Endometrial adenocarcinoma is a malignant tumor, rare in women under 40 years of age, but the incidence increases after menopause, gradually reaching a maximum between 70-79 years. **Material and methods**: The study included 291 patients with endometrial adenocarcinoma. The patients were admitted in 2005-2010 to "Cuza-Voda" Obstetrics and Gynecology Hospital in Iasi. The study group was diagnosed and investigated on the basis of the clinical examination and the specialized complementary explorations. The histopathological diagnosis was obtained by the processing of the hysterectomy specimen. **Results**: The results of the study that we conducted highlight the fact that nearly half of the patients diagnosed with endometrial adenocarcinoma were 50-59 years old. In 78.01% cases the uterus was of normal size, between 4-6cm, in 10.31% cases was increased over 6 cm and 11.68% it was of small size, less than 4 cm. **Conclusions**: The study recommends surveillance of cases with endometrial hyperplasia especially if are associated with incriminated risk factors in the etiology of carcinoma of the uterus.
Keywords: ADENOCARCINOMA, ENDOMETRIUM, ULTRASONOGRAPHY.

In the current gynecological practice there are many uterine lesions, many of which may be associated with abnormal uterine bleeding, and for this reason they should be excluded before diagnosing a dysfunctional uterine bleeding. In pre- and postmenopausal women, the most frequent morphological substrate of abnormal uterine bleeding is the hyperplasia of the endometrium and the endometrial carcinoma (1)

The noninvasive diagnosis of such bleeding can be done through endometrial cytology, endovaginal ultrasound and tumor markers (2).

The endometrial cytology has limited value because it is an indirect method, the results of which are encumbered on the one hand by false-negative results due to the

fact that the malignant endometrial cells rarely exfoliate spontaneously and if such phenomenon occurs, they undergo a number of changes during the passage through the genital tract. If endometrial cytology is sampled directly with a brush or by washing, the results are more precise (3).

The tumor marker is not specific to the endometrial carcinoma - CA 125 and, in the early phase of the disease, it is not increased so it did not constitute a diagnostic indication (4).

The endovaginal ultrasound (EEV) is currently accessible, non-invasive and painless and it is used frequently in the screening, assessment and monitoring of metrorrhagia in pre- and postmenopausal women (5).

Endometrial adenocarcinoma is a malignant tumor, rare in the Caucasian women under 40 years of age, but the incidence increases after menopause, gradually reaching a maximum between 70-79 years. The women of the black race, the maximum incidence of the endometrial adenocarcinoma is reported in premenopausal, with a rate of 85 cases per 100,000 women annually.

EH is considered to have increased risk of endometrial carcinoma. It is a common condition and it includes a wide spectrum of histological changes, from the simple aggregation of glandular proliferation with normal appearance, to changes difficult to distinguish from endometrial carcinoma. The EH diagnosis raises three issues: the first relates to the reproducibility of less than 50% between observers; the second is the finding that around 50% of the cases with atypical hyperplasia (AH) have also carcinoma lesions, and the third stems from the fact that the data about the progressive risk of evolution of EH towards the endometrial carcinoma are limited. Current data shows that, in the long term, the risk of developing endometrial carcinoma is less than 5% for women with simple hyperplasia (SH) or complex hyperplasia (CH) but it is of up to 30% for those with atypical hyperplasia (AH).

MATERIAL AND METHODS

Endometrial adenocarcinoma is a malignant tumor, rare in women under 40 years of age, but the incidence increases after menopause, gradually reaching a maximum between 70-79 years.

The study included 291 patients with endometrial adenocarcinoma. The patients were admitted in 2005-2010 to "Cuza-Voda" Obstetrics and Gynecology Hospital in Iasi. The study group was diagnosed and investigated on the basis of the clinical examination and the specialized complementary explorations. The histopathological diagnosis was obtained by the processing of the hysterectomy specimen. We used the data recorded in the observation charts and the computerized system.

The data collected as a study material and considered refer to: the total number of cases of endometrial carcinoma, diagnosed and operated, the annual distribution of the cases, the repartition of cases according the age groups, the type of the invasion of the tumor tissue myometrium, distribution of cases according to the length of the uterine cavity.

Even though the number of cases hospitalized and operated on different gynecological diseases was on the rise at our hospital between 2005-2010, this was not seen in the case of endometrial adenocarcinoma. The maximum number of cases was, at the beginning of the study, years 2005-2007 (96 cases), 2,87% of the surgeries during that period.

In the studied cases, the results of the endovaginal ultrasound exam are relevant.

For the 291 patients with endometrial carcinoma, the local vaginal exam combined with the abdominal palpation and correlated with the endovaginal ultrasound established the size and thickness of the endometrium of the uterus.

Our study, consisting of the two groups of endometrial adenocarcinoma, shows that between the endometrioid and the non-endometrioid adenocarcinoma there are significant differences regarding the invasion in the myometrium.

RESULTS

Of the total number of patients included in the study, 25% are between 33 and 53 years old, and 50 % are up to 58 years old. Most cases of endometrial adenocarcinoma, in our study, have been reported in patients aged 53 (modal or dominant value).

The results of the study that we conducted highlight the fact that nearly half of the patients diagnosed with endometrial adenocarcinoma were 50-59 years old. Among the 291 patients, 268 (92.1% of the total) were postmenopausal.

In the study that we conducted, the diagnosis of admission was neoplasm of uterine body in 197 (67.7%), endometrial polyp in 6 (2.06%), uterine fibroid in 59 (20.27%) and hemorrhages in peri climax in 29 (9.97%).

Another important factor of the prognosis for the patients with endometrial adenocarcinoma refers to the invasion of the tumor tissue myometrium.

Among the 291 studied cases, in 9 patients (3,09%) the tumor tissue has not infiltrated the myometrium; in 148 (50,85%) the myometrium has been infiltrated in a proportion of not less than 50% (the infiltration was limited to the internal half of the myometrium), while in 134 (46,06%) the myometrium showed a tumor infiltration of more than 50% cases.

The results obtained in our study reveal that over half of the cases showed evidence of the presence of lymphatic invasion in the vessel-lymphatic area (157; 53.95% of the studied cases). By age groups, the invasion of the vessel-lymphatic area was at its maximum for the patients falling in the range of 50-54 years (43; 14.78% of the patients included in the study).

Depending on the distribution of the endometrial adenocarcinoma according to the length of the uterine cavity in over 60% of the cases, the uterine cavity size was over 4 cm, and in a quarter of cases studied by us it was of 4 cm. Uterine cavity has a normal length of between 3-5 cm. In the cases included in the study, the uterine cavity length, assessed according to the hysterectomy specimen, was in average 5.019 ± 1.494 cm. Most often we found a value of 4 cm of length of the uterine cavity, but the limits of variation for the studied cases were very high. Therefore, the smallest length recorded was 2 cm, and the maximum recorded value was of 14 cm.

In our study, from the 291 cases of endometrial adenocarcinoma, in 205 (70.44%) by way of ultrasound it was reported the coexistence of hypertrophic or athrophic changes of the endometrium. Adenocarcinoma cases were included in two groups, the first group having 43 patients (20.98%) with endometrial adenocarcinomas associated with hypertrophy that histologically have proven to be hyperplasia and the second group represented by 162 (79.02%) with adenocarcinoma developed onto an atrophic endometrium.

In 86 (29.55%) cases there was not any ultrasound identification of hypertrophic or atrophic aspects of the endometrium.

The histopathological diagnosis was specified on histological sections taken

from the pieces of the surgical resection. In all cases the histological grading was established, along with the invasion of the myometrium.

The value of the endovaginal ultrasound in the diagnosis of the endometrial carcinomas is relevant. Depending on the size, the size of the uterus was divided into three categories: normal size, reduced size and increased volume. The size of the uterus was established following a clinical exam correlated to the endovaginal ultrasound. (fig. 1).

In 227 (78.01%) of the cases the uterus was of normal size, between 4-6 cm; in 30 (10.31%) cases its volume was increased, over 6 cm, and in 34 (11.68%) cases it was of small size, less than 4 cm.

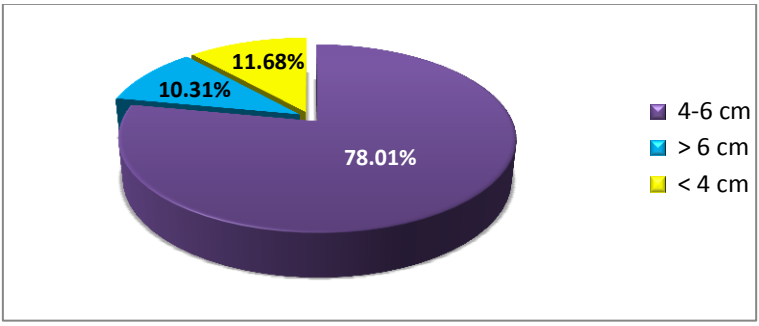

Fig. 1. Distribution of cases depending on the size of the uterus

The total thickness of the endometrium has been measured in the sagittal plan, endovaginal, including any area of focal thickening. The presence of areas with high echogenity, which interrupted the normal trilaminar structure of the endometrium pointed out to an endometrial pathology (fig. 2).

Fig. 2. Endometrium thickness determined by endovaginal ultrasound

In 275 (94.50 %) patients with endometrial carcinoma from our study, the endometrium thickness determined by endovaginal ultrasound was averaging 15 mm, with limits between 5 and 65 mm. In 16 (5.5%) patients with endometrial carcinoma, the

endometrium thickness determined by endovaginal ultrasound was under 5 mm, with limits between 5 and 4.5 mm (fig. 3).

In the case of patients with endometrium thickness under 5 mm, studying the observation sheets, the anamnesis (clinical history and menstrual status) revealed that in the last 3 months, prior to admission, these patients have undergone uterine cavity curettage, that all the patients were between 60 and 69 years old, they were in postmenopausal, and the histopathological examination has identified different types of endometrial carcinoma.

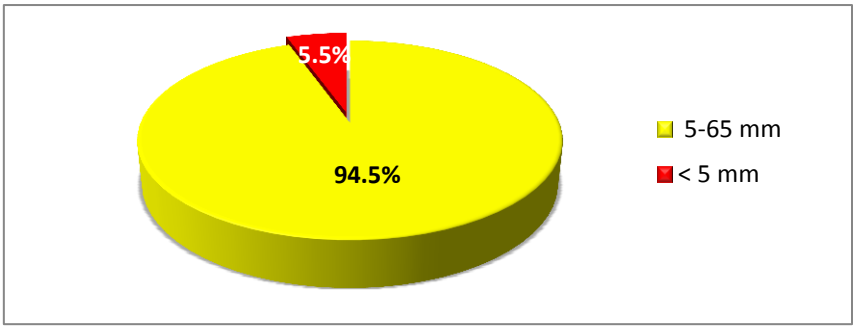

Fig. 3. Distribution of cases depending on the size of the endometrium

DISCUSSION

From the clinical and ultrasound study of 291 endometrial adenocarcinomas, there are a few issues that need to be highlighted and discussed in the light of the recent data from the medical literature.

Our study proves that endometrial hyperplasia are a common diagnosis in pre- and postmenopausal patients, they are often identified as a result of investigations for abnormal uterine bleeding; these are issues that should be considered by the gynecologist during the routine consultations and it is subsequently required an endovaginal ultrasound for the assessment of the thickness of the endometrium.

Most authors (6) consider that measuring the thickness of the endometrium through the EEV is a method with high specificity for the diagnosis of the endometrial carcinoma, especially in postmenopausal women. According to the results in the literature (7), the normal limit thickness of endometrium in postmenopausal women is 5 mm, and above it the endometrium biopsy with histopathological interpretation is required, but some results concerning the linking of the over 5 mm thickness of the endometrium to EEV with severe endometrial pathology would be of only 4% (8). Studies on the histology of a 6 mm thick endometrium at EEV showed a specificity of 98%, but a sensitivity of only 17% which leads to the conclusion that EEV is not an appropriate method for the screening of the endometrial carcinoma (9). Another study published results showing that using for EEV a limit value of 4 mm thickness of endometrium in women with postmenopausal bleeding both for those who used hormonal substitute treatment (estrogen and progesterone) and those without such treatment, the sensitivity in detecting the endometrial carcinoma was 100% and the specificity of 60% (10).

Normal endometrium has a thickness of under 5 mm in postmenopausal women and its borders must be regular and visible throughout the uterine cavity. For the interpretation of the thickness of the endometri-

um, in our statistics (10) we took into account the literature data, which show that the endometrium with a thickness greater than 5 mm in postmenopausal women is associated with an increased probability of the existence of a hyperplasia or an endometrial carcinoma, and any focal lesion of the endometrium, revealed at endovaginal ultrasound, regardless of the thickness of the endometrium, requires endometrial biopsy.

Our results confirm the data from the literature (11), showing that the endometrial adenocarcinoma in postmenopausal patients can be associated with a thin endometrium, with a thickness under 5 mm.

However, such cases published in the literature are rare. Thus, until 2007, founds in the literature only 14 authors who reported cases of endometrial adenocarcinoma in postmenopausal patients, where the endovaginal ultrasound showed a thickness of under 5 mm of the endometrium (12,13).

With the help of the endovaginal ultrasound with pulsed Doppler it is identified, highly accurately, the presence of the endometrial adenocarcinoma, which determines the decrease of the number of false-positive results (14).

The studies published in the literature specify that tumor infiltration in the myometrium is associated with a poor prognosis. Adenocarcinomas limited only to the endometrial mucosa have a survival rate of over 95% at 5 years (15).

CONCLUSIONS

Our study revealed that the endovaginal ultrasound assessment of the thickness of endometrium, is absolutely necessary in the diagnosis of the endometrial lesions and it has an important role in the screening of endometrial adenocarcinoma. The endovaginal ultrasound can provide orientation in more than 90% of the cases, the existence of a proliferative lesion of the endometrium in postmenopausal women, where the thickness of the endometrium is greater than 5 mm.

Also, our study had as a major objective the highlighting of more objective criteria in establishing the morphological diagnosis and the evaluation of prognosis elements in endometrial hyperplasia and endometrial carcinoma, representing a specific pathology for the premenopausal and postmenopausal women.

If the endovaginal ultrasound reveals the endometrium with a thickness of less than 5 mm and regular layout, in patients that are in pre- and postmenopausal at increased risk and abnormal uterine bleeding, who underwent a previous biopsy, within a period of less than three months from the date of the first submission, the uterine curettage is necessary for a more accurate histological diagnosis.

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NEWS

PERFORMANCE OF MOLECULAR TESTS IN THE DETECTION OF *TRICHOPHYTON* SPECIES

Dermatophytosis caused by *Trichophyton* and *Microsporum* species represent the most frequent mycoses worldwide. Molecular detection methods are highly sensitive and allow for rapid and accurate identification of dermatophytes from clinical specimens. Kupsch *et al* investigated the sensitivity of different molecular methods compared with conventional diagnostics (culture) in the detection of dermatophytoses caused by *T. rubrum* and *T. interdigitale* in 375 clinical samples of nail, skin and hair. The compared molecular methods included already published PCR-ELISA assays, qRT-PCR as well as a newly developed PCR-ELISA targeting the internal transcribed spacer region. In 39.5 % of cases a positive result was obtained in at least one molecular test or by culture. The results shoed that the most sensitive test was a PCR-ELISA targeting a microsatellite region which identified 81 *T. rubrum*-infections followed by an ITS PCR-ELISA (60), qRT-PCR (52) and a topoisomerase II PCR-ELISA assay (51); significantly, cultivation detected *T. rubrum* in only 37 samples (Kupsch C, Ohst T, Pankewitz F, Nenoff P, Uhrlaß S, Winter I, et al. The agony of choice in dermatophyte diagnostics - Performance of different molecular tests and culture in the detection of *T. rubrum* and *T. interdigitale*. Clin Microbiol Infect. 2016 May 30. pii: S1198-743X(16)30142-2. doi: 10.1016/j.cmi.2016.05.015).

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INFLUENCE OF GLAUCOMA ON DIABETES-INDUCED CHANGES IN THE ANTERIOR OCULAR SEGMENT

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INFLUENCE OF GLAUCOMA ON DIABETES-INDUCED CHANGES IN THE ANTERIOR OCULAR SEGMENT (Abstract). **Aim**: To monitor the influence of glaucoma on the changes caused by diabetes in the anterior ocular segment. **Material and methods**: Prospective, comparative clinical study, which included the patients with glaucoma and diabetes assessed at the Iaşi "Sf. Spiridon" Hospital, "Euro Medi Center" Clinic and "Oftaprof" Clinic. The study included a number of 142 patients, divided into two groups: 67 patients with glaucoma and diabetes (study group) and 75 patients with diabetes mellitus (control group). Upon enrollment the patients were subjected to a complete assessment of their ophthalmologic and metabolic status. Monitored aspects: prevalence of lens changes, extent of ocular surface disease and identification of the changes in corneal biomechanical parameters in the patients with glaucoma and diabetes. **Results**: In both monitored groups, the cortical lens changes prevailed, both at the beginning and at the end of the study, with no statistically significant differences between groups ($p>0.05$). The risk of developing lens changes reaches 50% in the patients who have had diabetes for 20 years. In the patients in the study group, tear secretion was significantly lower at the end of the study (Schirmer's test values 11.61 vs. 10.67 mm, $p=0.045$). Tear film instability was present in 37.3% of the patients. Corneal hysteresis (CH) and corneal resistance factor were significantly reduced in the group with glaucoma and diabetes, as compared to the group with diabetes ($p=0.001$). **Conclusions**: There is significant ocular surface disease in the patients with glaucoma and diabetes, as compared to those with diabetes alone. Glaucoma and the number of antiglaucoma drugs are associated with a significant reduction in tear secretion. The association of glaucoma resulted in the reduction in CH and corneal resistance factor in the patients with glaucoma and diabetes as compared to the healthy ones. **Keywords**: POAG, CH, CRF, SCHIRMER'S TEST, OSDI QUESTIONNAIRE.

Diabetes and glaucoma are two chronic conditions with a significant impact on visual function, both determining degenerative changes at ocular level. In the patients with diabetes and in those with glaucoma, the vascular factor has an important role, in both conditions abnormalities in retrobulbar vascular flow and retinal microcirculation being present (1). These are a problem mainly in the developed countries with high life expectancy. Most complications involve both the anterior and the posterior segment of the eye, but numerous clinical studies only describe the presence of changes caused by diabetic retinopathy. A range of other ocular changes at the level of the lens and cornea are not well documented and that this study aims to highlight them (2). Studies in the

literature have shown that lens changes are a cause of severe visual impairment in diabetic patients, the most frequent changes occurring at cortical and posterior subcapsular level, the risk increasing with diabetes duration and poor glycemic control (3,4). Ocular surface changes associated with diabetes have been carefully studied over the last decades. Clinical studies have shown that diabetes is associated with lacrimal gland dysfunctions, reduction in reflex tear secretion and tear film break-up time of the (5,6). Other studies aimed to demonstrate the role of diabetes and glaucoma in the changes in ocular biomechanical parameters, mostly with contradictory results. It was found that these properties determined by ORA (Ocular Response Analyzer) are affected by glucose level, hyperglycemia determining dysfunctions of the corneal endothelium (7,8,9). This study aimed to assess the influence of glaucoma on the diabetes-induced ocular changes in the anterior segment (lens, ocular surface, corneal biomechanics).

MATERIAL AND METHODS

This is a prospective, observational and comparative clinical study carried out in the Ophthalmology Clinic I of the Iaşi "Sf. Spiridon" Hospital in collaboration with the "Euro Medi Center" and "Oftaprof" Clinics. The study was carried out in accordance with the ethical principles of the Declaration of Helsinki, the protocol being explained in detail to each patient, and obtaining the informed consent for the participation in the study. *The inclusion criteria* were: age over 35 years, confirmed diagnosis of primary open-angle glaucoma (POAG) and normal-tension glaucoma (NTG), open camerular angle (III-rd, IV-th degrees) according to Schaffer's classification, associated mixed type 1/type 2 diabetes, assessed based on blood sugar and glycosylated hemoglobin levels. *The exclusion criteria were:* patients with very advanced glaucoma, secondary glaucoma (pseudoexfoliative, pigmentary, neovascular, cortisone-induced), absence of DM and patients not attending the subsequent checkups. The participants were therefore divided into two groups: study group (including patients with glaucoma and diabetes) and control group (patients with diabetes). After enrollment the patients were assessed every 3-6 months.

The protocol for patient assessment included a comprehensive eye and vision examination: visual acuity, intraocular pressure (IOP) measured by Goldmann aplanotonometry, biomicroscopy of the anterior segment, central corneal thickness (Tomey ultrasound pachymeter), computerized recording of visual field by using the glaucoma programs of Humphrey perimeter, indirect gonioscopy with Goldmann three mirror lens, eye fundus exam with Volk lens and retinal photography with Zeiss Fundus Camera. The identification of changes in the retinal nerve fiber layers and the C/D (cup/disc) ratio were carried out by using the Zeiss Cirrus HD OCT device. Lens status after pupil dilation was assessed by biomicroscopy of the anterior segment and by photographic documentation using a Topcon biomicroscope provided with a digital camera, three images being obtained per eye and the type and extension of lens opacity was recorded using as a rough guide the LOCS (Lens Opacities Classification System), the most frequently used system in clinical studies. Both upon enrollment and at the end of the study, every study patient was subjected to Schirmer I test (without anesthesia), BUT (break-up time) and to fluorescein staining. At the end of the follow-up period, the patients filled in an OSDI (Ocular Surface

Disease Index) questionnaire, used to obtain various aspects related to the ocular surface disease. The OSDI includes 3 subscales: ocular discomfort (5 questions), ocular symptoms during daily activities (4 questions) and environmental factors (5 questions); the OSDI total score is assessed on a scale of 0 to 100: 0-12 normal, 13-22 moderate, 33-100 severe. By means of the ORA (Ocular Response Analyzer), the corneal biomechanical parameters have been assessed: CH, corneal resistance factor (CRF), IOPcc (corneal-compensated intraocular pressure) and IOPg (Goldmann- correlated intraocular pressure). Diabetes was assessed by determining the serum level of blood sugar and glycosylated hemoglobin (HgA1c). Diabetes and glaucoma duration and the number of antiglaucoma drugs were noted. The statistical analysis of the data was performed by using the SPSS 18.0 statistics software. Of the used statistical tests, we mention: the ANOVA test, t student (statistically significant at $p<0.05$), chi^2 test, Pearson correlation, Krushall –Wallis correlation, ROC curve (prognostic factor) and the Kaplan –Meier curve (probability, hazard).

RESULTS

The study group included 142 patients with DM, divided into two groups: group I - 67 patients (47%) with diabetes and POAG and group II (controls) - 75 diabetic patients (53%) without glaucoma. The statistical analysis utilized data from one eye only, the most affected one. Gender distribution showed a prevalence of females in both groups. Mean age was 65.58+/- 7.87 years, the groups being homogenous in this respect. Diabetes duration was of 11.01+/- 8.28 years in group I and 13.32+/-9.94 years in group II. Glaucoma duration was of 5.75+/- 4.54 years. According to Hodapp classification, depending on the Mean Deviation level, the patients in group I presented moderate glaucoma both at the beginning and at the end of the study. Only one case was classified as advanced glaucoma (MD> -12db) and was not taken into account in the final evaluation. Of the total number of patients, 22.38% had NTG and 77.62% POAG. At the beginning of the study we found that 68.7% of patients in group I and 57.3% of patients in group II had statistically significant cortical lens changes ($p=0.036$). The other changes (subcapsular, nuclear, corticonuclear) were present in much lower percentages (12%, and 14.7%, respectively).

At the end of the study, in both groups, the percentage of cortical lens changes had decreased, but there was an increase in the percentage of cortical nuclear opacities, the changes at nuclear level determining cataract worsening and impaired visual function (fig. 1).

In the studied cases, multiple linear regression analysis revealed that gender, age, diabetes and glaucoma duration are not good predictors of lens changes ($p>0.05$), a positive cumulative correlation being found in only 3.4% of the patients. At the end of the study the glycosylated hemoglobin level was significantly lower in the patients with cortical changes both in group I (7.03 vs. 6.61%, $p=0.02$) and in group II (7.30 vs. 6.91%, $p=0.025$). Kaplan Meier curve highlighted an increased risk of developing lens changes, more than double in patients suffering from diabetes mellitus for almost 20 years. The probability of developing lens changes reaches the level of 50% in the patients suffering from diabetes mellitus for 10 years or from primitive open-angle glaucoma for 5 years (fig 2).

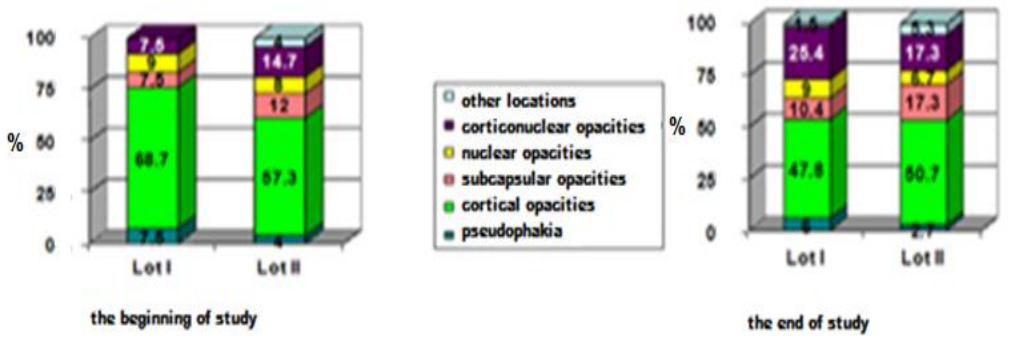

Fig. 1. Group structure according to lens modifications at the beginning and at the end of the study (Group I=glaucoma and diabetes; Group II=diabetes)

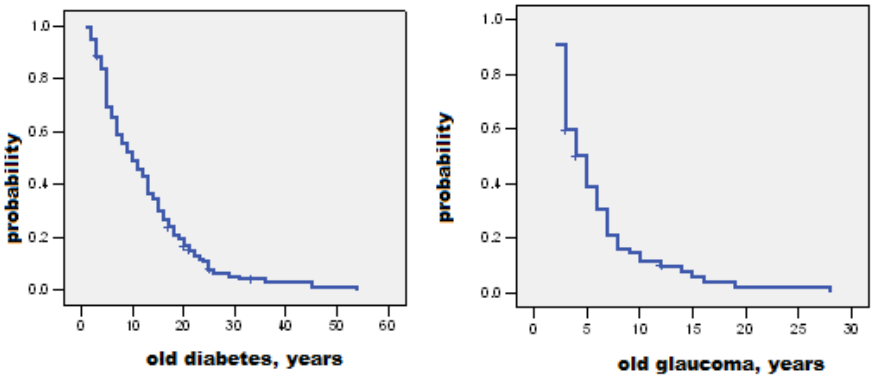

Fig. 2. Probability of lens changes depending on diabetes and/or glaucoma duration

Tear secretion test (basal Schirmer's test) values ranged from 3 to 25mm, a severe value (3 mm) being recorded in one patient (0.7%) low tear secretion values (5-10mm) in 35 patients (26.4%). The average values of Schirmer's test had significantly decreased towards the end of the study in the group I patients (11.61 vs. 10.67; p=0.045) as compared to group II. Tear film break-up time (BUT) ranged from 3 to 21 seconds, tear film instability being recorded in 37.3% of the patients (fig. 3). The ocular surface disease index (OSDI) ranged from 8.33 to 39.58, highlighting a severe damage in 6% of the cases and moderate in 16.4%. Similarly, to tear secretion, in group I patients there was a significant decrease in BUT (9.33 vs. 8.57, p=0.028), but without significant differences in group II, the control group.

The number of glaucoma drugs ranged from 1 to 3. There was an association of 2, 3 drugs in 40% of the patients in the study group. At the end of the study, tear secretion test revealed significantly lower values in those on 3 or more glaucoma drugs (p=0.037). In case of BUT and OSDI there were no statistically significant differences according to the number of glaucoma drugs. The multivariate analysis highlights that Schirmer's test, BUT, OSDI questionnaire and the IOP level are good predictors in the determinism of the degree of ocular damage (p=0.014):

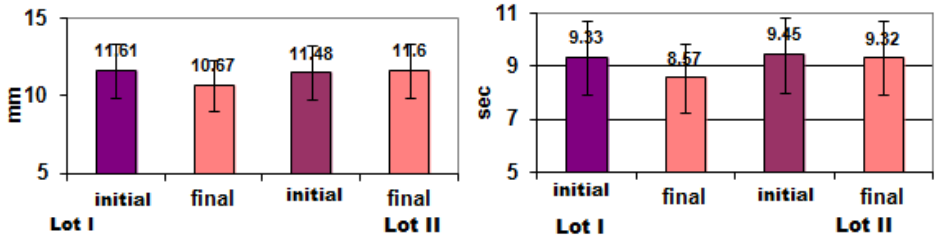

Fig. 3. Average values of the Schirmer's test and BUT by study groups

The assessment of the changes in corneal biomechanical parameters included 156 subjects, 49 subjects with diabetes and glaucoma (90 eyes) selected from group I and 17 subjects with diabetes mellitus (34 eyes) selected from the control group (group III), who were able to be assessed by ORA. These groups of subjects were compared to a group of 46 subjects (92 eyes) with primary open-angle glaucoma (group II) and a group of 48 normal subjects (96 eyes) (group IV). The statistical analysis took into account the measurements in both eyes. Assessment of central corneal thickness (CCT) highlighted no statistically significant differences among the study groups in the right eye (p=0.561) or in the left eye (p=0.455). Goldmann-measured IOP, Goldmann-correlated IOP, corneal-compensated IOP had significantly higher values in groups II and III as compared to group IV (p<0.005), with no differences between the two eyes. Both in the right eye (p=0.001) and in the left eye (p=0.001), CH values were significantly lower in the patients in group I and II, as compared to group IV. The CRF values were significantly lower in the patients with G+/- DM as compared to the other groups (p=0.011), with no statistically significant differences between the two eyes (fig. 4).

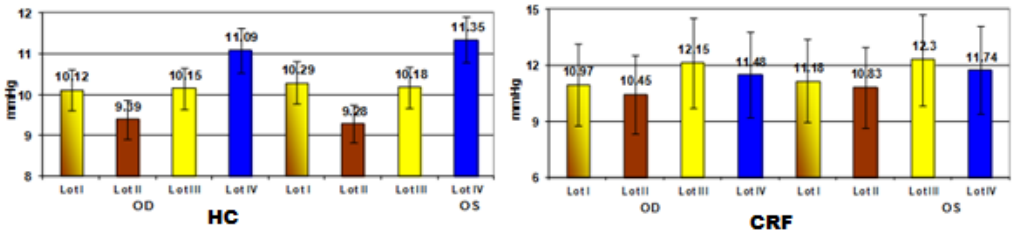

Fig. 4. Average values of corneal hysteresis and corneal resistance factor (mmHg) by study groups (Group I= glaucoma and DM; Group II= glaucoma; Group III=diabetes; Group IV=healthy)

The comparison between CH *and* IOP revealed statistically significant, direct moderate correlations only in group III. The comparison between CH *and* the CCT highlights direct correlations (if the value of the correlation coefficient is closer to +1 there is an all the higher dependence of the two parameters, direct correlation, and if closer to -1, it shows that there is also a very high dependence between the parameters, but an indirect one) with moderate intensity, statistically significant in all groups (r=+1,

$p<0.05$). The comparison between CRF and Goldmann-measured IOP shows that there is a direct, moderate, statistically significant correlation in group II only ($r=+0.579$, $p=0.001$). Moreover, the comparison of CRF and CCT values shows direct, moderate, statistically significant correlations in groups I, II, IV ($r=+1$, $p<0.05$) and indirect correlations in group III ($r=-1$, $p<0.05$). In the diabetic patients with POAG (group I) as compared to those without POAG (group II), we noticed significantly lower values of IOP measured by the Goldmann tonometer and of CRF in both eyes. The level of glycosylated hemoglobin did not significantly influence the values of corneal biomechanical parameters in any of the groups ($p>0.05$). The multivariate linear analysis highlights age, CCT, CH and CRF as good predictors in the determinism of glaucoma+/- diabetes mellitus.

DISCUSSION

The common epidemiological and pathogenic features, the complications involving the ocular structures with irreversible effect on vision have determined the serious assessment of diabetes and glaucoma in numerous studies, some of them with contradictory results. The most controversial result is the one of the Ocular Hypertension Treatment Study (OHTS), the authors concluding that diabetes is a protective factor for glaucoma, a low percentage of the monitored patients with diabetes developing glaucoma (3.1%) (10). Other studies find associations between diabetes and glaucoma, suggesting that diabetes is a risk factor for glaucoma (11,12). A retrospective study carried out on 46 patients with glaucoma and diabetes (92 eyes), highlighted a (marginal statistically) significant influence of the presence of non-proliferative diabetic retinopathy in glaucoma progression (13). This risk is explained by the vascular factor, as the long evolution of diabetes can compromise the vascular, glial and retinal neuronal elements, being associated to a high risk in the development and progression of primary open-angle glaucoma (14, 15). However, most of the studies in the literature only assess the role of diabetes in developing glaucoma; to our knowledge, there are no studies assessing the risk caused by glaucoma on the frequent changes induced by diabetes at ocular level. Knowing that diabetic retinopathy and cataract are the most frequent causes of visual impairment and decreased quality of life in the patients with diabetes, glaucoma association could additionally influence the progression of these changes. The studies in literature have shown that lens changes have an increased prevalence in diabetics (3,4). The results of this study are mostly similar to those in the literature. The groups were homogenous in terms of age (65), the female gender prevailed, the average diabetes duration was of 11 years and of the glaucoma of 5 years. The statistical analysis of the studied cases showed an increased prevalence of cortical lens changes over the study period (68.7% in group 1 and 57.3% in group 2) with statistically significant differences between groups ($p<0.05$). These changes seem to be rather related to diabetes (average duration - 11 years), but they can also be related to age (average age: 65). There were no statistically significant differences between the analyzed groups; therefore, glaucoma did not significantly influence the results. The assessment of ocular surface disease showed a significant degree of ocular surface disease in the patients with G+DM vs. those with DM alone, tear secretion and

tear film break-up time being significantly lower at the end of the study. Glaucoma and the number of glaucoma drugs were associated with a significant decrease in tear secretion (p=0.037). Schirmer's test, BUT, OSDI questionnaire proved to be good predictors of the degree of ocular involvement. Similarly to the studies in the literature comparing the degree of ocular surface disease in patients with glaucoma as compared to those without glaucoma, we found a higher degree of ocular damage due mostly to glaucoma therapy (2,16). The studies in literature assessing the biomechanical properties in patients with diabetes report controversial results. Some studies state that diabetes causes a CH decrease as compared to healthy subjects, others that CH and CRF are significantly increased in patients with poor blood sugar control, as compared to the healthy subjects with good control (8,9,17). CH decrease is explained by the alteration of collagen components, thus reducing the corneal dumping phenomenon, and CH increase is due to the rigidity of collagen structure of the cornea in diabetics (20). The reasons for such contradictory results are probably the differences in age and CCT, but also the diversity of DM types and their severity (in some studies most patients had type 2 diabetes, while others had a similar number of patients with type 1 and 2 diabetes). Similarly, the results obtained in our study show that glaucoma association determined a reduction of CH in the diabetics with glaucoma as compared to those without glaucoma and healthy control subjects. This is explained by the known fact that in glaucoma there is a reduction in corneal viscosity and corneal dumping phenomenon. The altered collagen structure may be additive to the effect of a thicker cornea, thereby further increasing the measured IOP, which can lead to an overestimation of the true IOP with Goldmann aplanotonometer in the population with diabetes (17).

The present study has some limitations. The obtained results cannot be extrapolated to global level mainly because of the small number of patients included in the study and of the short monitoring period. The absence of a comparative OSDI (ocular surface disease index) score in the group with DM is another limitation. In the groups assessed for changes in corneal biomechanical parameters, there are no data regarding the level of HgA1c, blood sugar upon enrollment and at the end of the study, and degree of glaucoma in the control groups.

CONCLUSIONS

In our study the cortical lens changes prevailed at the beginning of the study in both groups. The probability to develop lens changes is of 50% in the patients with 10-year duration of DM or 5-year duration of the POAG. There was a significant degree of ocular surface disease in the patients with G+DM as compared to those with DM alone. The presence of glaucoma and the number of glaucoma medications are associated with a significant decrease in tear secretion (p=0.037). The association of glaucoma determined a decrease in CH and CRF corneal biomechanical parameters in the diabetic patients with glaucoma as compared to those without glaucoma and healthy control subjects. Age, CCT, CH, CRF and Goldmann- measured IOP are good predictors in the determinism of glaucoma +/- diabetes. In the future, a longer monitoring period and database extension could lead to the improvement of the results obtained in this study.

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TREATMENT OF PUBIC DISJUNCTION IN YOUNG-BURGUESS TYPE II AND III PELVIC RING FRACTURES

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TREATMENT OF PUBIC DISJUNCTION IN YOUNG-BURGUESS TYPE II AND III PELVIC RING FRACTURES **(**Abstract). **Aim:** To present the personal experience in assessing the treatment of pubic disjunction in Young-Burguess type II and III pelvic ring fractures. **Material and methods**: Included in the study were 30 patients with type II and III pelvic ring fractures, aged 32 to 76 years. Of these, 18 patients were treated with external fixation, and 12 patients were treated surgically: type II fractures were treated with open reduction and internal fixation (ORIF) of pubic disjunction and type III fractures underwent additionally posterior fixation**. Results**: Mean follow-up was 1.6 years. After external fixation we found the persistence of diastasis (1.5 to 4 cm), which was asymptomatic in all cases. Complications included implant failure in 3 patients, and postoperative infection in 2 patients. Among the 5 patients with type II lesions the clinical and radiological scores were excellent in 3, good in 1, and poor in 1. Among the patients with type III lesions the clinical outcomes were excellent in 5 and good in 2. **Conclusions**: External fixation has proved a good treatment option for patients who cannot tolerate a more extensive surgery. There is no conclusive clinical evidence favoring the use of two perpendicular plates vs. 1 plate for pubic symphysis fixation. In osteoporotic patients the use of 2 plates is recommended. **Keywords:** PELVIC RING FRACTURE, PUBIC DISJUNCTION, ORIF.

Pubic disjunction result from high-energy trauma, and occurrs in 14 - 20% of pelvic ring fractures (1). They are frequently associated with multiple other lesions, such as blood vessels rupture with massive hemorrhage, this association having a mortality rate of approximately 15-35% (1, 2). The major sources of bleeding are supposed to be the presacral and retroperitoneal blood vessels. (1).

The conservative treatment of these fractures has proved ineffective. An early firm stabilization and anatomic reduction are recommended to decrease mortality and allow early mobilization (3).

To stabilize these fractures several methods are available. External fixators can be used for the temporary stabilization of all types of pelvic ring fractures or for a definitive one in some cases. Anterior internal fixation can be used as a definitive treatment for pubic disjunctions if the sacroiliac ligaments are intact. Anterior internal fixation may be associated with posterior fixation in patients with posterior pelvic ring injuries (especially in APC type

III) to improve overall stability (4).

MATERIAL AND METHODS

In the interval 2008-2014 in the orthopedics and traumatology units of the Iasi "Sf. Spiridon" Emergency County Hospital and Botosani "Mavromati" Emergency County Hospital 30 patients with type II and III pelvic ring fractures, aged between 32 and 81 years (mean age 55 years) were treated. The mechanisms of fracture were: motor vehicle accidents (17: 56,66%), falls from height (10: 33,33%) and 3 (10%) by crush injury (3). The injuries most commonly associated with these fractures were: head trauma, craniofacial trauma, thoracoabdominal trauma, splenic rupture, bladder rupture, lower leg fracture, forearm fracture, fractured clavicle and humerus.

Eighteen patients were treated with external fixation (fig. 1) and 12 patients were treated surgically: type II fractures were treated with ORIF of pubic disjunction and type III fractures underwent additionally posterior fixation. Pubic disjunctions in type II lesions were fixed with one 3.5 mm reconstruction plate and 4 screws (2 cases/16.6%) and with two perpendicular plates (3 cases/25%); those in type III lesions were treated with one 3.5 mm reconstruction plate and 4 screws (3 cases/25%) and two 3.5 mm reconstruction plates (4 cases/33.33%) (fig. 2).

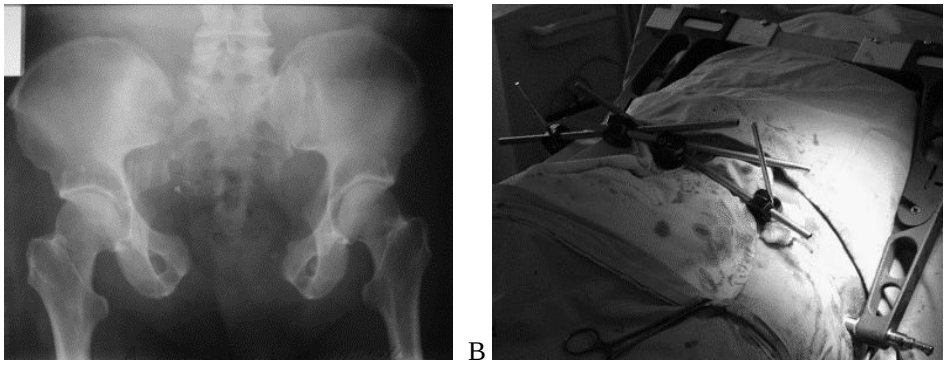
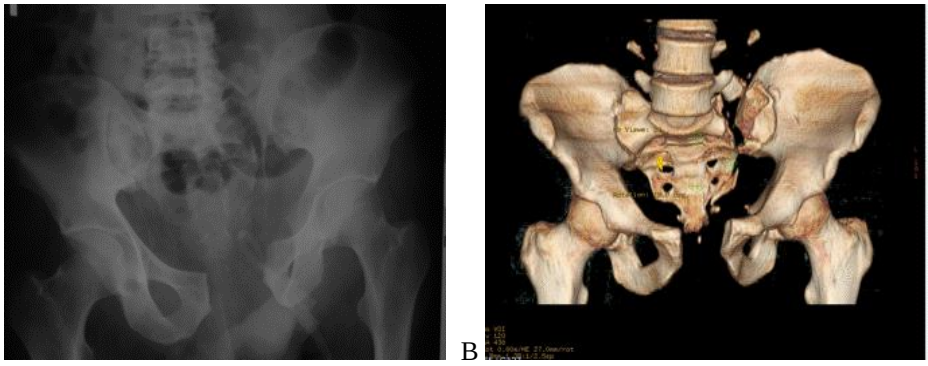


Fig. 1. – patient B.I., 49 years old, victim of motor vehicle accident. A – preoperative X ray – APC type III injury, B – intraoperative view – posterior stabilization with „C clamp" and anterior with supraacetabular external fixation.



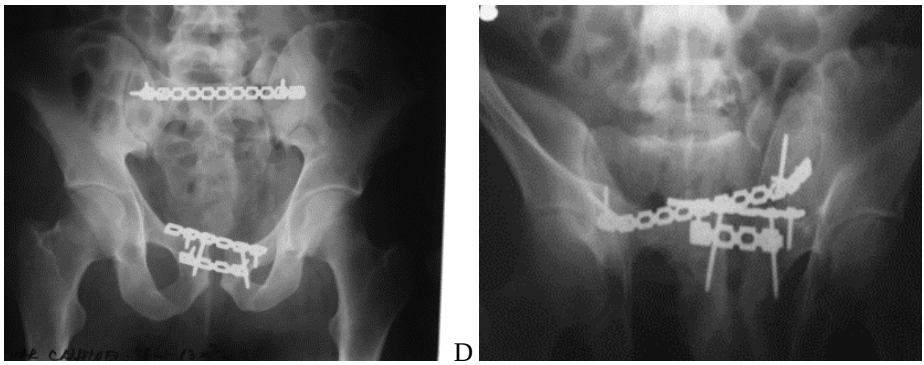


Fig. 2. – patient L.M., 55 years old, victim of motor vehicle accident. A – preoperative X ray – APC type III injury, B – 3D image - APC type III injury, C–postoperative anterior X ray – fixation with 1 plate posteriorly and 2 plates anteriorly, D– postoperative "outlet" X ray – fixation with 1 plate posteriorly and 2 plates anteriorly

RESULTS

Mean follow-up was 1.6 years. The radiologic score and Majeed clinical score were used to assess the outcomes. After external fixation we found the persistence of diastasis (1.5 to 4 cm), which was asymptomatic in all cases (tab.I, II).

TABLE I

Interpretation of the radiologic score

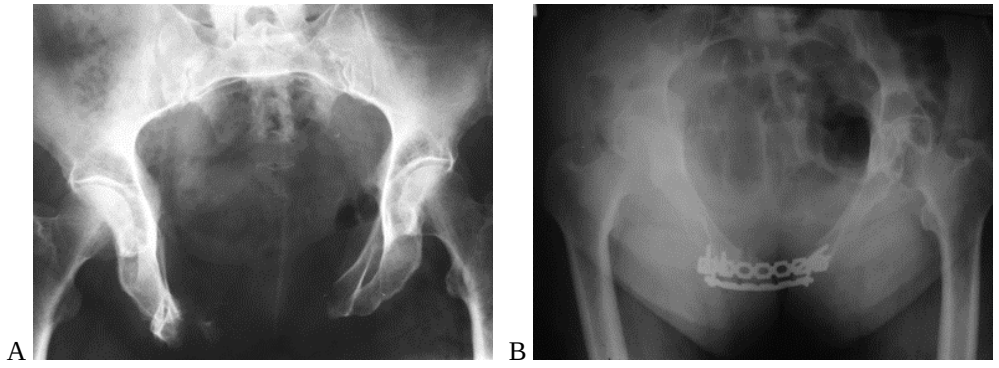
RESULT	RESIDUAL DISPLACEMENT
Excellent	0-5 mm
Good	6-10 mm
Fair	11-15 mm
Poor	>15 mm

TABLE II

Interpretation of the clinical score

RESULT	TOTAL SCORE
Excellent	78-80
Good	70-77
Fair	60-69
Poor	<60

Among the 12 patients treated surgically (ORIF) the complications included construct failure (3:25%) (fig. 3) and postoperative infection (2:16.6%). In the 5 (41,66%) patients with type II lesions, clinical scores were excellent in 3 cases (60%), good in 1 (20%) and poor in 1 (20%).



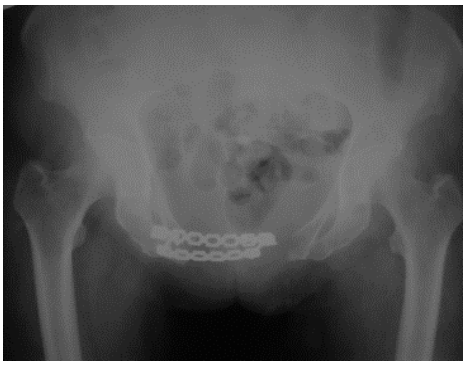
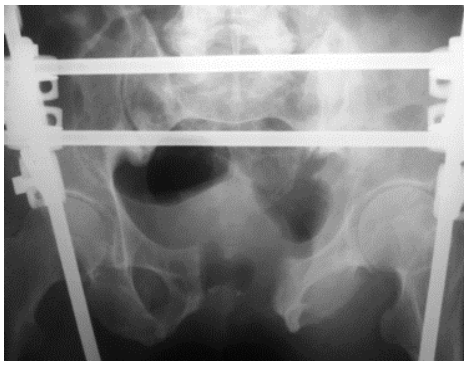
C  D 

Fig. 3. – patient M.E., 76 years old, victim of motor vehicle accident. A – preoperative X ray – APC type II injury, B – postoperative "inlet" view – fixation with 2 plates anteriorly, C– X ray on postoperative day 7 – loss of reduction, D– postoperative X ray – stabilization with external fixator.

Radiological scores were excellent in 3 (60%) cases, good in 1 (20%), and poor in 1 (20%). Of the patients with type III injuries 5 (71.43%) had excellent clinical outcomes and 2 (28.57%) good. X-ray results were similar to those in type II injuries (tab. III).

TABLE III

Profile of the patients treated surgically by open reduction and internal fixation.

No.	Age	Injury type	Mechanism	Fixation	Radiologic score	Clinical score
1	32	APC II	Vehicle accident [passenger]	Anterior [1 plate]	8 mm	70
2	38	APC III [sacral fracture]	Vehicle accident [motorcyclist]	Anterior [1 plate] + Posterior [1 plate]	5 mm	78
3	61	APC III [sacral fracture]	Vehicle accident [passenger]	Anterior [1 plate]+ Posterior [2 screws]	10 mm	70
4	40	APC III [ligament injury]	Fall from height	Anterior [2 plates] + Posterior [2 screws]	4 mm	77
5	48	APC II	Vehicle accident [passenger]	Anterior [1 plate]	5 mm	80
6	55	APC III [sacral fracture]	Vehicle accident [passenger]	Anterior [2 plates] + Posterior [1 plate]	4 mm	78
7	34	APC II	Vehicle accident [passenger]	Anterior [2 plates]	4 mm	77
8	38	APC II	Vehicle accident [motorcyclist]	Anterior [2 plates]	5 mm	78
9	67	APC III [sacral fracture]	Vehicle accident [pedestrian]	Anterior [1 plate] + Posterior [1 plate]	17 mm	74
10	35	APC III [ligament injury]	Vehicle accident [motorcyclist]	Anterior [2 plates] + Posterior [2 screws]	5 mm	79
11	76	APC II	Vehicle accident [pedestrian]	Anterior [2 plates]	36 mm	56
12	36	APC III [ligament injury]	Fall from height	Anterior [2 plates] + Posterior [1 screw]	5 mm	78

DISCUSSIONS

With the progress made in the emergency care system, prompt diagnosis, resuscitation methods, better management of visceral lesions and improved imaging techniques, the orthopedic surgeon plays a key role in the treatment of these patients (3).

In cases of stable fractures without disjunction and stable ligamentous structures a conservative treatment is accepted with good outcomes. Unstable lesions usually require surgical treatment of the anterior and posterior ring, if the latter is injured (4).

In our study external fixation proved to be a good treatment option for patients who cannot tolerate a more extensive surgery.

Management of APC type II pelvic ring fractures with external fixation is effective, relatively simple, minimally invasive, with less intraoperative blood loss and shorter surgical time and allows earlier patient mobilization (5).

A retrospective study of 64 supraacetabular external fixators to stabilize anterior pelvic ring showed iatrogenic lesion of the lateral femoral cutaneous nerve (4.5%) which reversed within 1 year, no pin site infection, no secondary displacements, perforation of the pin into the small pelvis requiring no treatment in 3% of cases, and pseudoarthrosis of the pubic and ischial rami requiring surgical treatment in 1 case (6). The temporary use of an external fixator is safe and effective, but its use for a definitive treatment is associated with high infection rate and secondary displacements according to the study conducted by Mason (7). Galois L (8) says that anterior inferior placement of the external fixator showed a potential for increased stability.

Pubic disjunctions > 2.5 cm reflect rotational instability and benefit from anterior stabilization, internal fixation being preferred to external fixation. There is no universally accepted protocol on the type of plate (dynamic compression plate *vs.* pelvic reconstruction plate), plate thickness (3.4 *vs.* 4.5 mm) or number of plate screws. There is conflicting evidence on the benefits or need to use a 2-hole plate. It has been agreed that additional posterior instability requires a surgical posterior stabilization procedure (4).

In our surgically treated patients we thought that in case of APC type III injuries anterior and posterior stabilization is mandatory. As to the stabilization of pubic disjunction, no differences between the number of used plates (1 plate *vs* 2 plates) and clinical and radiological scores were found. However, it is worth mentioning that in osteoporotic patients using a single plate does not provide a satisfactory stabilization.

Initially based on biomechanical studies it was considered that adding a second plate can lead to better stabilization, theoretically being indicated in unstable fractures (9).

In a retrospective study Bagchi K. (4) found no conclusive evidence favoring the use of two plates *versus* one plate to stabilize the pubic symphysis. Several clinical and biomechanical studies describe the use of a single plate as appropriate for all types of pelvic ring fractures.

Although plate fixation of pubic disjunctions is the recommended treatment, the incidence and consequences of fixation failure remained a concern. Some of these are due to osteosynthesis material failure and low bone density. However, these do not seem to affect the clinical outcome, because few patients require surgical reintervention (2).

A study evaluating locked versus non-locked plate osteosynthesis of the pubic symphysis showed that locked plates together with the required screws have no advantage over non-locked plates, but further studies are needed (10). On the other hand, Pizanis (11) suggested a biomechanical advantage in using anatomically contoured plates with locking screw capabilities in case of large displacement or obese patients. A biomechanical cadaveric study conducted in 2013 revealed that locked plate osteosynthesis at the pubic symphysis has no advantage over non-locked plates (12).

Lange (13) compared osteosynthesis with a two-hole anterior plate versus two plates (one superior and the other anterior) for pubic disjunction. Double plate osteosynthesis offers a more rigid fixation, but the procedure requires extensive dissection which may cause a significant loss of blood. Single plate osteosynthesis is less rigid and helps accommodate the normal movement of the pubic symphysis without loss of reduction and with a limited dissection.

Simonian (14) and Dujardin (15) in their studies concluded that osteosynthesis of the anterior and posterior pelvic ring is optimal for APC II and III injuries as compared to single plate anterior pelvic ring fixation.

CONCLUSIONS

The results of this study allow us to state that external fixation has proved a good treatment option in patients who cannot tolerate a more extensive surgery. There is no conclusive clinical evidence favoring the use of two perpendicular plates *vs.* 1 plate for pubic symphysis fixation; in osteoporotic patients the use of 2 plates is indicated regardless of lesion type. A residual diastasis below 1 cm is accompanied by good clinical outcomes.

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NEWS

HEART RATE VARIABILITY IN OVERWEIGHT HEALTH CARE STUDENTS: CORRELATION WITH VISCERAL FAT

Overweight and obesity are due to abnormal or excessive fat accumulation that may impair health and it is because of an imbalance between calories consumed and expended. The global epidemic of overweight and obesity is the major public health problem in developed as well as developing world. Abdominal obesity, defined as increased waist circumference is one of the components of the constellation of metabolic abnormalities collectively called as the metabolic syndrome (MS). The latest definition of MS by the International Diabetes Federation (IDF) has included abdominal obesity as one of the essential components. Among the different available non-invasive techniques for assessing the autonomic status, Heart Rate Variability (HRV) is a simple method to evaluate the sympathovagal balance at the sinoatrial level. Increased sympathetic activity, decreased parasympathetic activity and sympathetic imbalance (SVI) has been reported in obese individuals. However, the SVI and its association with visceral fat in overweight health care students have not been explored. Therefore, in the present study, was observed the sympathovagal imbalance due to increased sympathetic activity and its association with visceral fat in overweight individuals (Chintala KK, Krishna BH, Reddy MN. Heart Rate Variability in Overweight Health Care Students: Correlation with Visceral Fat. *Journal of Clinical and Diagnostic Research*. 2015, Vol-9(1): 6-8).

Doina Butcovan

MANIFESTATIONS OF SELF-HARM IN RELATION WITH BINGE DRINKING TO STUDENTS FROM TIMIŞ COUNTY, ROMÂNIA

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MANIFESTATIONS OF SELF-HARM IN RELATION WITH BINGE DRINKING TO STUDENTS FROM TIMIŞ COUNTY, ROMÂNIA **(**ABSTRACT): Aim: The research investigated the feeling of sadness, suicidal thoughts and plans associated with binge drinking among students in Timis County, Romania. **Material and methods**: We applied a transversal population study to the representative sample of 2076 students, 62.49% girls and 37.51% boys with a mean age of 21.09 years with SD 1.48. **Results**: The boys practice binge drinking significantly more often than girls, the difference is small to medium: prevalence of 22.1% for boys and 8.7% girls, 1-2 times in the last month. **Conclusions**: Only girls who practice this behavior, increased frequency of engaging in binge drinking is associated with feelings of sadness, suicidal thoughts and suicide plans in a small measure, but significant.
Keywords: STUDENTS, BINGE DRINKING, SELF-HARM.

Binge drinking, the consumption of increased amounts of alcohol on one occasion, typically at age teenager and young adult, has exclusively negative effects on health depending on the amount and frequency of practicing this behavior (1) and it is the cause of various social problems (2. 3).

There is a demonstrated association between the problems of excessive alcohol use and depression symptoms. Results of a longitudinal study (4) which followed a group of 20.728 from teenagers to young adulthood, indicated that excessive alcohol use and depressive symptoms showed a positive association during the period of adolescence to young adulthood. Elevated levels of depressive symptoms were associated with increased baseline levels of alcohol consumption, especially in women, but with faster growth in alcohol consumption in men.

In a review (5) on the same subject, meaning the relationship between symptoms of major depression and inappropriate consumption of alcohol, Boden stated that the presence of either conditions will double your risk of other problems. The two disorders can not be fully explained by common factors that influence them, but it appears to be causally related disorders. Other evidence suggests that causal association between alcohol and depression is one in which alcohol consumption increases the risk of depression, rather than vice versa. Potential mechanisms underlying causal links include neurophysiological and metabolic changes resulting from exposure to alcohol.

Other authors (6) found that alcohol abuse, depression and suicidal behavior in young people presenting biochemical and genetic correlation. Alcohol consumption is shown to be a distal and proximal cause of the attempted suicide among young people. People with alcoholism and depression that have attempted suicide, often had significantly higher levels of aggression and impulsivity. These factors may have been caused or influenced by the underdeveloped coping skills, and comorbid psychiatric disorders. Such behaviours could be a consequence of childhood abuse, social pressure, low self-esteem and / or delinquency. Some studies suggest that the depression encourage the use of alcohol in the form of self-medication, which then leads to suicide, while others require that the starting alcohol consumption is responsible for increasing the symptoms of depression and suicide thoughts among adolescents.

We proposed a study on some manifestations of self-harm (feelings of sadness, suicidal thoughts and plans) associated with binge drinking among students in Timiş County, România.

MATERIAL AND METHODS

The representative sample of students in the study totaled 2076 students from higher education institutions from Timiş county, 62.49% (1296) girls and 37.51% (778) boys. The average age was 21.09 years with SD 1.48, ages ranging from 18-25 years old.

The method used was the transversal population study based on the use of CORT Questionnaire 2004 on health risk behaviors in adolescents and young people. The questionnaire was validated by the Ethics Committee of the University of Medicine and Pharmacy "Victor Babeş" Timişoara.

The study was conducted with the written approval of the higher education establishments from Timiş. Inclusion of young people in the study carried out only after their expressed consent of each participant in the study, with respect for all the individual rights.

The processing and interpretation of data was done by using modern statistical methods and advanced medical PASW 18 software (SPSS 18) 2010. The value of statistical significance was set at $p < 0.05$, except in cases where the Bonferroni correction was applied, the acceptable threshold level was stated in the text. For ordinal data comparisons we used Mann-Whitney and Kruskal test-Wallis. Chi-square test was used for ordinal data / nominal.

RESULTS

1. Binge drinking. By practicing binge-drinking, or increased consumption of more than 5 servings of alcohol on one occasion, we have found: for boys group 11.6% (89) have practiced binge drinking more than 3 times in the last month, 22.1% (170) have practiced 1-2 times, and 66.3% (510) did not practice binge drinking in the last month; 2.5% for the group of girls (32) have practiced binge drinking more than 3 times in the last month, 8.7% (112) have practiced 1-2 times, and 88.8% (1145) have not practiced binge drinking last month. The frequency of practicing binge drinking is significantly higher in boys than girls, $U = 381\,799$, $z = -12.63$, $p < 0.001$, $r = 0.27$, the size difference is considered small to medium based on the Cohen's criteria (fig. 1).

2. Feelings of sadness. A percentage of 16.6% (127) of boys and 25.1% (323) of girls had experienced feelings of sadness for a longer period of 2 weeks during the last year. Girls had these feelings significantly more often than boys, $U = 450454.5$,

z = -4.49, p <0.001, r = 0.09, the size difference is small.

In the group of boys, 33.6% (32) of those who experienced feelings of sadness practiced binge drinking, and of those who have not experienced these feelings 34.0% (226) have practiced binge drinking. Among boys, we found no significant differences between the frequency of binge drinking and experiencing feelings of sadness or hopeless situations more than two weeks in the past year, p = 0.932 (fig. 2).

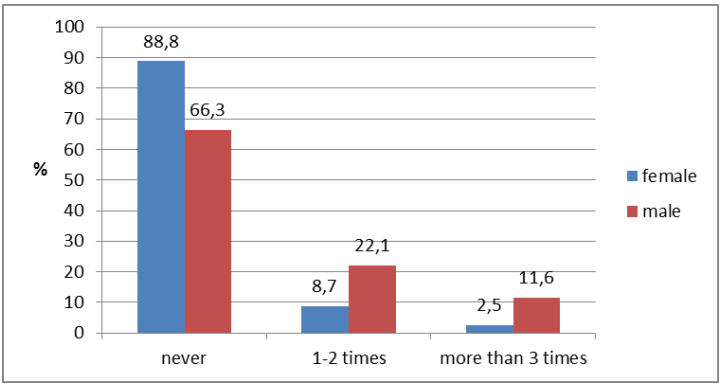

Fig. 1. Percentage distribution of students according to frequency of binge drinking practice last month, by gender

In the group of girls, 17.2% (55) of those who have experienced feelings of sadness practiced binge drinking, and of girls who have not experienced these feelings 9.3% (89) have practiced binge drinking. We found a significantly higher frequency of binge drinking among the girls who have experienced the feeling of sadness, compared to the group that did not experience this feeling U = 141897.5, z = -3.95, p <0.001, r = 0.11, the difference is small in size (fig. 3).

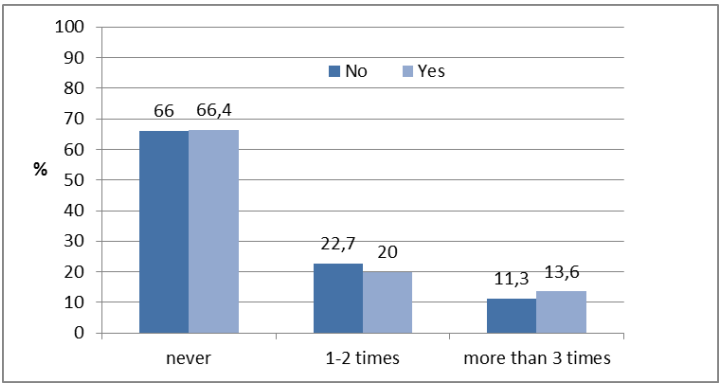

Fig. 2. Percentage distribution of students according to the experience of feelings of sadness or hopeless situations more than two weeks in the past year and frequency of binge drinking practicing during the past month among boys

3. Suicide thoughts. A percentage of 7.2% (55) of the boys and 8.9% (114) of the girls had suicidal thoughts during the last year. Among the girls and the boys we did not find significant differences regarding suicidal thoughts, p = 0.171.

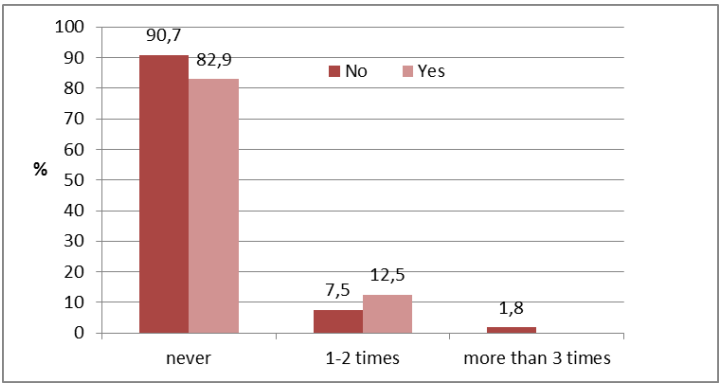

Fig. 3. Distribution of students according to the experience of feelings of sadness or hopeless situations more than two weeks in the past year and frequency of binge drinking practicing during the last month among girls

In the boys group, 34.5% (19) of those who had thoughts of suicide practiced binge drinking, and of those who did not have these thoughts 33.8% (239) have practiced binge drinking. We found no significant differences between the frequency of binge drinking and the existence of suicidal thoughts in the past year, p = 0.873 (fig. 4).

In the group of girls, 19.5% (22) of those who had thoughts of suicide practiced binge drinking, and of those who did not have these thoughts 10.5% (122) have practiced binge drinking. We found a significantly higher frequency of binge drinking in the group who had suicidal thoughts, compared to the group who did not had suicidal thoughts in the past year U = 60 154, z = -2.78, p = 0.005, r = 0.08, the difference is small in size (fig. 5).

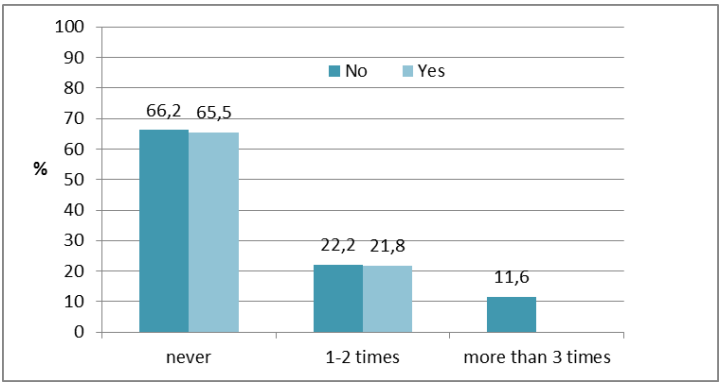

Fig. 4. Percentage distribution of students according to the presence of suicidal thoughts during the past year and frequency of binge drinking practicing in the past month among boys

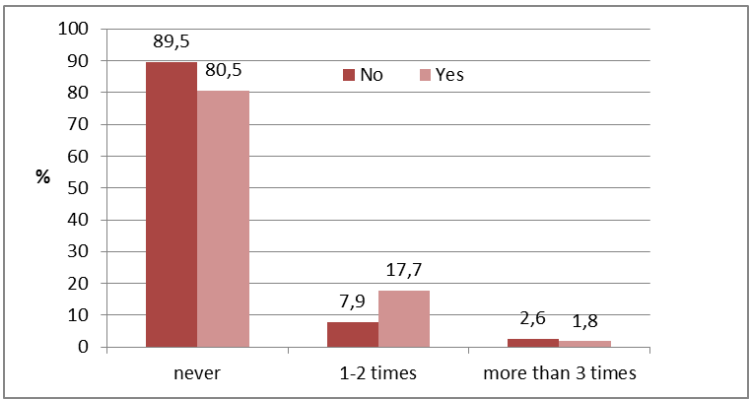

Fig. 5. Percentage distribution of students according to the presence of suicidal thoughts during the past year and frequency of binge drinking practicing in the past month among girls

4. Suicide plan. A percentage of 3.0% (23) of the boys and 2.8% (36) of girls had suicidal plan last year. We did not found significant differences in the presence of suicide plans between girls and boys, p = 0.800.

In the group of boys, 43.4% (10) of those who had suicide plan practiced binge drinking, and of those who did not plan, 33.5% (248) have practiced binge drinking. In boys, we found no significant differences between the frequency of binge drinking and the existence of a suicide plan in the past year, p = 0.343 (fig. 6).

In the group of girls 27.8% (10) of those who had suicide plan practiced binge drinking, and of girls who had no plan, 10.8% (134) have practiced binge drinking. We found a significantly higher frequency of binge drinking in group who had suicidal thoughts, compared with the group who had no suicidal thoughts in the past year U = 60 154, z = -2.78, p = 0.005, r = 0.08, the difference is small difference is small in size (fig. 7).

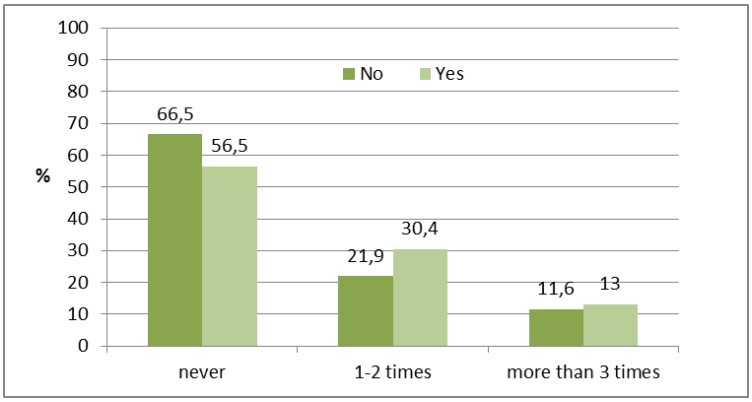

Fig. 6. Percentage distribution of students according to whether they had a suicide plan last year and the frequency of binge drinking practicing during the past month among boys

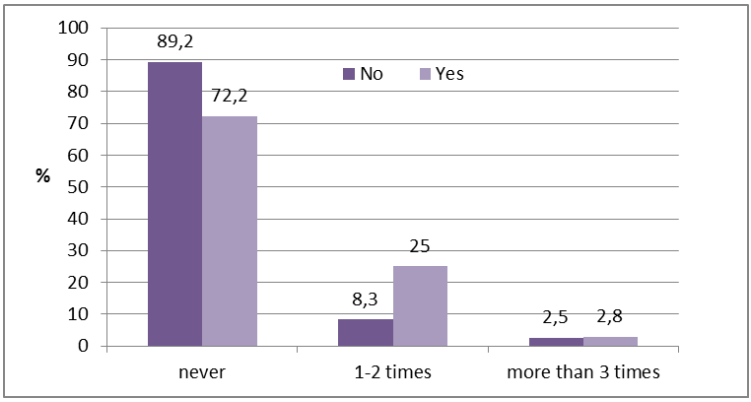

Fig. 7. Percentage distribution of students according to whether they had a suicide plan last year and the frequency of binge drinking practicing during the past month among girls

DISCUSSIONS

Alcohol consumption was associated with depression, but the studies made by Spear (7) failed to determine whether adolescents and young people consume alcohol because of depression or depression occurs due to the toxic effects of alcohol. Research carried out on people who abuse alcohol, found a smaller number of neurons in the *locus coeruleus* from the stem cells of the brain, compared to people who did not use alcohol, suggesting that alcohol could alter brain structure and trigger such a behavior response (8).

Other authors (9) have shown that chronic alcohol consumption in adolescents may increase feelings of depression, due to lower levels of serotonin. The young people involved in the inappropriate consumption of substances, 60% were diagnosed with psychiatric pathologies, according to a study by Armstrong and colleagues (10).

Galaif et al. (11) reported a 100% increase in rates of suicide between 1980 and 1999 in children of 10-14 years and an increase of 14% in those aged between 15-19 years old. They argued that the diagnosis of major depression was the most common reported comorbidity in suicide and alcohol abuse, and there is a mutual synergistic relationship between depression and alcohol abuse. They could not prove a causal relationship between the two diseases.

Groves's study (9) estimates that alcohol would increase the likelihood of suicide 17 times for boys and 3 times in girls. It is possible that adolescents who abuse alcohol have a greater risk of suicidal behavior because each of those two factors, depression and alcohol increase the risk of suicide independently, and together their action is likely to be synergistic.

CONCLUSIONS

In our study, the prevalence of practicing binge drinking 1-2 times in the last month is 22.1% for boys and 8.7% girls and more than 3 times in the last month is 11.6% for boys and 2.5% girls. The boys practice binge drinking significantly more often than girls, the difference size is small to medium.

A percentage of 16.6% of boys and 25.1% of girls had experienced feelings of sadness for a period longer than 2 weeks during the last year. Girls had these feel-

ings significantly more often than boys, the size difference is small. In boys, we found no association between the frequency of binge drinking and experiencing feelings of sadness or hopeless situations more than two weeks in the past year. In girls, the increased frequency of engaging in binge drinking is associated with feelings of sadness, a far small but significant percentage.

A percentage of 7.2% of boys and 8.9% of girls have had suicidal thoughts in the last year, no significant differences between genders. 34.5% boys respectively 19.5% of the girls who practiced binge drinking had thoughts of suicide. In boys, we found no association between the frequency of binge drinking and suicidal thoughts in the past year, but in girls, increased frequency of engaging in binge drinking is associated with suicidal thoughts in a small measure, but significant.

With no differences between sexes, 3.0 percentage of boys and 2.8 of girls have had suicidal plan last year. 43.4% boys respectively 27.8% of the girls who practiced binge drinking had suicidal plan. In boys, we found no association between the frequency of binge drinking and suicide plans in the past year, but in girls, increased frequency of engaging in binge drinking is associated with suicide plans, in a small measure, but significant.

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TIME-LAPSE IMAGING AND ITS POTENTIAL TO IMPROVE EMBRYO SELECTION IN IVF

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TIME-LAPSE IMAGING AND ITS POTENTIAL TO IMPROVE EMBRYO SELECTION IN IVF (Abstract) Time-lapse imaging has given birth to a new concept in the IVF laboratory, termed morphokinetics. Being a combination of the morphological appearance of the embryo and the events leading to a certain stage of development, morphokinetics offers the embryologist the possibility of correlating certain cleavage parameters with the implantation potential of the embryo. The use of time-lapse imaging offers the possibility of following the embryos in a closed system and detecting the developmental anomalies, making it easy to select the best embryo to be transferred. Studies are being made for a better correlation of the parameters offered by time-lapse imaging with a successful embryo selection. In this literature review we are summarizing the studies that determined and examined the markers of embryo development *in vitro* by time-lapse imaging and the way these markers provide proper information about the chromosomal status of the embryo, its ability to reach the blastocyst stage and the implantation potential. **Keywords**: TIME-LAPSE, MORPHOKINETICS, EMBRYO SELECTION.

In 2010, an estimated 48.5 million (45.0 million, 52.6 million) couples worldwide were infertile. One in every four couples in developing countries had been found to be affected by infertility (1). Infertility has numerous causes, approximately 16% of the individuals with reproductive disorders being affected by chromosomal abnormalities (2). In Romania, as in all modern societies, one cause of population decline is postponement of births (3).

In the last 36 years, since the first successful *in vitro* fertilization (IVF) procedure, significant progress was made in improving the procedure in terms of ovarian stimulation protocols, but also in IVF laboratory technology. Even though success rates have very much improved with time, they are still considered to be low, approximately a 33% clinical pregnancy

rate per transfer (4).

An important role for a high pregnancy rate in IVF procedures is attributed to the selection of the best embryos to be transferred. The selection is usually made using morphological systems that are relying on zygote pronuclear scoring systems, cleavage-stage scoring systems and blastocyst scoring systems. This type of assessment is considered to be subjective because it is associated with intra-observer and inter-observer variability (5). Also, different clinics use different scoring systems, making difficult to compare the results.

In the standard procedure, in order to be able to score the embryos, they have to be taken out of the incubator daily, exposing them to variations in temperature, pH and light which can be detrimental for their future development (6). But because only a few moments of embryo development are observed, a great part of its evolution cannot be seen, so the probability of not observing an abnormal process, for instance the direct cleavage to three cell stage, is high.

The importance of choosing the best embryo for transfer comes also from the fact that multiple pregnancy is considered a complication of IVF procedure. As a result, many clinics have adopted the idea of single embryo transfer. So, obtaining a good pregnancy rate and also avoiding multiple pregnancy needs an improved embryo selection.

Because the present embryo grading systems are applied by the embryologist and are subjective, alternative methods were required for a more objective evaluation that can improve the selection process. Among these, preimplantation genetic screening (PGS) is used for the selection of genetically normal embryos. This is an invasive method that requires the aspiration of the first polar body before the fertilization of the oocyte or of one or two blastomeres from the embryo on the third day after fertilization or by trophectoderm biopsy (7). More recently, blastocyst biopsy and array comparative genome hybridization (array CGH) offer a more complete chromosome analysis and results available within 24 hours (8). Even though these techniques give reliable results, it remains the inconvenience that biopsy could affect the development of the remaining embryo (9) and it is also an expensive technique.

Time-lapse monitoring systems have gained a lot of attention in the past few years. With these systems new biomarkers that could be used in clinical settings for embryo selection are identified. The main advantage of time-lapse markers is their non-invasiveness and the possibility of correlating them with embryo parameters such as their quality, development and implantation potential (10).

This review gives brief consideration to the use of time-lapse biomarkers for human embryo selection, with respect to their correlation with the development of the embryos, implantation potential and the genetic status of the embryos.

TIME-LAPSE SYSTEMS CURRENTLY USED IN THE IVF CLINICS AND SAFETY EVALUATION

Currently there are two main commercially available time-lapse technologies for use in IVF clinics: a miniaturized microscope that can be placed into the incubator and that will capture the entire embryonic development or equipment that includes both the incubator and the image capturing device, each one having specific software

for analyzing the acquired images. Each device has a specific culture plate that can hold 9, 12, 16 or 25 embryos, monitored individually either by moving the slide into the optical field of view or they are cultured in multiwell plates, the embryos being all seen in the same field of view (10). The microscopes used for time-lapse monitoring systems have dark field illumination systems or Hoffman modulation contrast illumination. The problem regarding the safety of time-lapse for the embryos is mentioned because they are repeatedly exposed to light at determined time intervals.

In a study by Wong et al. (10) the embryos were exposed 1 second every 5 minutes for 5 or 6 days. The total light exposure time calculated was approximately 24 minutes, at a power of 0.3 mW, equivalent to a light exposure time of 1 minute (60 seconds) under classical IVF laboratory conditions. Nakahara et al. assessed the fertilization rate, cleavage rate and embryo quality by comparing these parameters for the oocytes of the same patient, fertilized by ICSI, cultured in a conventional incubator and in the same incubator that had an integrated microscope. In the time-lapse systems the embryos were exposed every 15 minutes to 0.1 W LED light. They did not find significant differences between the two groups of embryos (11). When comparing the ongoing pregnancy rate for embryos cultured in a time-lapse monitoring system with that of embryos cultured in a conventional incubator, Rubio et al. (12) found in a prospective randomized, double-blind controlled study that there were significant differences between the two groups (51.4% and 41.7%, respectively).

A randomized clinical trial conducted by Kirkegaard et al. evaluated the safety of time-lapse monitoring by comparing the proportion of 4-cell stage embryos, as a primary endpoint, the proportion of 7-8 cell stage embryos and that of blastocysts, as secondary endpoints, in a time-lapse monitoring system and in a conventional incubator. This study showed no significant differences between the two incubators regarding the proportion of 4-cell embryos, 8-cell embryos and blastocysts. Also there was no significant difference regarding the pregnancy and implantation rate (13).

Although improvements can be done to time-lapse monitoring systems, they appear to be safe for use in IVF laboratory.

CLEAVAGE KINETICS OBSERVED WITH TIME-LAPSE SYSTEMS - BIOMARKERS FOR EMBRYO FATE

Cleavage kinetics observed with the use of time-lapse systems began to gain a lot of attention in the search for non-invasive indicators of embryo implantation potential. Embryos are monitored immediately after the fertilization process and information about the timing of pronuclei appearance and disappearance, cleavage divisions, their synchronicity and the time intervals between these divisions are being measured expecting a correlation with the "normality" of the embryo (14).

There is abundant debate regarding the duration of culture (2, 3, 5 or 6 days) and the optimal day for embryo transfer. The advantage of a shorter culture period, with a day 2 transfer after fertilization, would be the shorter exposure of the embryos to the *in vitro* environment. Prolonged *in vitro* culture could determine epigenetic disorders or obstetrical complications, like preterm delivery (15). Wong et al. (16) laid

the basis of an evaluation system using time-lapse imaging for the best embryo selection, before embryonic genome activation. The time-lapse system captured images every 5 minutes with microscopes with dark-field illumination. The parameters established as being predictive for the potential of the embryo to develop to the blastocyst stage were a duration of 14.3± 6.0 minutes for the first cytokinesis from which two cells result, a duration of the two-cell stage of the embryo, from the first mitosis to the beginning of the second mitosis of 11.1±2.2 hours, a time interval between the second and third mitosis (from three to four-cell stage or synchronicity) of 1.0±1.6 h.

The embryos that have a shorter time interval for the two-cell stage, dividing from two to three cells in less than 5 hours are considered of very poor prognosis for implantation. This time interval is required for DNA replication so if this interval is much shorter there is not enough time for complete genome replication, resulting in embryo aneuploidy (17).

Meseguer et al. (18) also used a time-lapse system to determine specific morphokinetic parameters for embryos with good implantation potential. Image acquisition was done every 15 minutes using optics with modified Hoffman contrast for low intensity red light. The most important parameters for embryo implantation potential seemed to be the time between the 2-cell stage and 3-cell stage (<11.9 hours), synchrony of the embryos, the time from the 3-cell stage to 4-cell stage (<0.76 hours) and the time interval between the injection of the spermatozoa and the formation of a 5 cell embryo (48.8-56.6 hours). The implantation rate for the embryos categorized as A according to the time-lapse parameters was 67%. With the help of time-lapse imaging system it was possible to observe that there are embryos that divide directly to three cells, these embryos having a limited implantation potential. Rubio et al. (19) found that 6.6% of the embryos from their cohort cleaved directly to 3-cell stage with only 1% chance of implantation.

Dal Canto et al. (20) made further research regarding morphokinetic parameters until the blastocyst stage, and correlated the cleavage times from 2 to 8-cell stage with the ability of the embryo to form a blastocyst. The system used for image acquisition was similar to that in the previous study. They found that the time needed for the embryos to get to 7 and 8-cell stage was shorter for those embryos that form blastocyst than for the others (56.5±8.1 h compared to 58.8±10.4 h for 7-cell stage and 61.0±9.4 h compared to 65.2±13.0 h for the 8-cell stage).

The prediction of the ability of the embryos to develop to blastocyst stage was also studied by Cruz et al. (21) as a continuation of the study by Meseguer et al. A hierarchical classification of the morphokinetic parameters was used. They concluded that early embryo cleavage is an indicator for the blastocyst stage potential of the embryo. They also considered synchronicity as an important indicator for the blastocysts with a good morphology.

Desai et al. (22) compiled all the kinetic markers established by previous studies and used them for determining the potential of the embryo to form a blastocyst and to implant. They took into consideration time to syngamy (tPNf), time to 2 cells (t2), 3 cells (t3), 4 cells (t4), 5 cells (t5), 8 cells (t8), morula formation, start of blastulation, time to blastocyst and expanded blastocyst

formation. They also recorded cleavage anomalies and multinucleation. The study showed that the most important parameters were tPNf, time to two, four and eight cells, the synchronous divisions and the duration of the third cell cycle, these being significantly different for the embryos developing to blastocyst stage. The study also showed that 56% of the binucleated embryos and 48% of the embryos with more than three nuclei developed into blastocysts that met the freezing criteria, but still without information regarding the implantation potential.

Even though a final embryo evaluation system using time-lapse based biomarkers hasn't been established yet, there is important progress in this direction that could allow a consensus and application in current practice.

THE CORRELATION BETWEEN TIME-LAPSE BIOMARKERS AND THE GENETIC STATUS OF THE EMBRYO

There are studies showing that early-stage embryos are affected by chromosomal abnormalities which determine a negative evolution of the embryo consisting either in developmental arrest, implantation failure or, if they do manage to implant, various genetic complications of the fetus (23). The incidence of aneuploidy is high regardless of the meiotic (gametes) or mitotic (after fertilization) origin (24). The frequency of chromosomal abnormalities increases with age. A systematic review by van Echten-Aremds et al. (25) regarding the prevalence of chromosomal mosaicism in human preimplantation embryos indicates that 73% of the embryos are mosaic, with two categories: 59% diploid-aneuploid mosaic and 14% aneuploid mosaic. This high rate of mosaic embryos raises the question if PGS really helps improving embryo selection and live birth rate (24). Blastocysts appear to show a smaller proportion of aneuploid cells compared with day 2-3 embryos (25).

Recently, with time-lapse imaging development, the idea of using time-lapse morphokinetic parameters as indicators for the genetic status of the embryos was suggested.

The study of Chavez et al. (26) correlates the cell-cycle parameters of embryos until 4-cell stage and the ploidy status by a-CGH. Their analysis showed that the majority of chromosomal errors have a mitotic origin. The embryos chromosomally normal needed 14.4±4.2 min form the first cleavage furrow to the appearance of two blastomeres, 11.8±0.71 h from two to three cells and 0.96±0.84 h from three to four cells. The aneuploid embryos needed a larger time interval for the appearance of the third cell.

In the classical follow-up, in which embryos are observed only at certain time points, the degree of fragmentation is an important criterion in embryo selection. Chavez et al. indicate that this evaluation has to be done in a dynamic way and has to be correlated with the analysis of cell-cycle events and blastomere asymmetry, using time-lapse imaging. Cellular fragmentation was found in the majority of aneuploid and triploid embryos.

Basile et al. (27) analyzed in a retrospective study the development of embryos cultured for 120 hours in a time-lapse imaging system and correlated this with a-CGH results from day 3 biopsy in order to develop an algorithm for non-invasive selection of chromosomally normal embryos. They found that a large proportion of normal

embryos form 5 cells within 47.2-58.2 hours, the duration of third cell cycle is 11.7-18.2 hours and the interval between the formation of 2 and 5 cells >20.5 hours, the last two parameters being the most relevant for chromosomally normal embryos.

Another study of Campbell et al. (17) followed the embryos until the blastocyst stage, designing an aneuploidy risk model based on the information received from trophectoderm biopsy. These information were compared with the morphokinetic variables determined with time-lapse analysis, classifying the embryos into three aneuploidy risk classes - low, medium and high risk - depending on the time from insemination to the start of blastulation (tSB) and the time from insemination to the formation of a blastocyst (tB) (28). The embryos included in the three classes have a probability of aneuploidy of 0.37, 0.69, and 0.97, respectively. For the low risk class tSB was less than 96.2 hours after insemination and for medium risk class was higher than 96.2. Regarding the necessary time for the formation of a blastocyst, the first two classes needed less than 122.9 hours after insemination for this, while the high risk class required more than 122.9 hours to form a fully blastocyst (17). This study was then applied by the same team in order to analyze the application of this aneuploidy risk model on fetal heart beat rate and live birth rate (28). The results for the embryos included in the low risk class were 72.7% for fetal heart beat and 61.1% for live birth rate indicating that the model offers important information regarding the ploidy state of preimplantation embryos using only morphokinetic parameters observed with a time-lapse system.

Another interesting study of Yang et al. (29) followed implantation and pregnancy rate for sibling embryos cultured in a time-lapse system and in a conventional incubator until the blastocyst stage, all being tested using whole genomic amplification and a-CGH. Even though the first morphokinetic parameters set by Meseguer et al. (18) seemed to be the same for both euploid and aneuploid blastocysts, the pregnancy rate and the implantation rate were significantly higher for the embryos cultured in the time-lapse system because this offers advantages like continuous single culture medium, a better temperature and pH control and a minimal light exposure.

Even though time-lapse imaging systems present numerous advantages, such as the possibility of observing every stage of embryo development, a limited exposure to out of incubator environment and to variations in pH, temperature, light, they also have some limitations. Most studies using time-lapse monitoring of embryos were done on ICSI cycles because these allow the exact determination of fertilization. This cannot be achieved for classical IVF. For these cycles the monitoring can be done after denuding the oocytes the following day, being more difficult to assess time parameters from post-insemination time.

CONCLUSIONS

Time-lapse imaging and the potential correlation between the morphokinetic parameters of embryos and their implantation potential represent an attractive domain for IVF specialists trying to find new methods for selecting the best embryos using noninvasive markers. Currently, in Romania only few fertility clinics use time-lapse systems. There is a need for establishing an algorithm that can be applied in all IVF laboratories with certain parameters referring to embryo development to be used

in embryo selection. At this time we cannot replace PGS with morphokinetic selection of embryos, but substantial progress has been made. A noninvasive alternative to PGS would provide important advantages, with PGS use being prohibitive in terms of availability, costs and legislation. With continuous efforts, time-lapse imaging could be used in the future as a non-invasive alternative to PGS.

Embryo morphokinetics can be correlated with the potential of the embryo to develop to blastocyst stage, the potential to implant, to determine a live birth, considering the cleavage pattern.

Time-lapse imaging provides useful information on in vitro embryo development and progress is still being made in finding biomarkers that can be used in current practice for a better embryo selection.

Our opinion - reviewing current results regarding time-lapse based embryo selection and considering the difficulties that we face in current practice of selection by conventional embryo culture and monitoring pleads for the use of this technique with certain non-inferior results and important potential advantages.

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A TODAY VICTIM OF SECOND WORLD WAR

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A TODAY VICTIM OF SECOND WORLD WAR (Abstract): Emergency medicine as a medical specialty has to deal with all kind of emergency situations, from medical to post traumatic acute events and from new born to the elderly persons, but also with particular situations like explosions. In Romania nowadays these are accidental explosions and rare like frequency, but may be dramatic due to numbers of victims and multisystem injury that may occur. We present a case of a single victim of accidental detonated bomb, a projectile from the Second World War, which unfortunately still may be found in some areas. The management of the case from first call to 112 until the victim is discharge involves high professional team work. We use these opportunity to make a brief review of the mechanism through the lesions may appear and also to renew the fact that the most impressive lesion may not be the most severe, and we have to examine carefully in order to find the real life threatening injury of the patient. **Keywords:** EMERGENCY, EXPLOSIONS, HEMOPERITONEUM, HEMORRHAGE, AMPUTATION

Although rare, nowadays there are still unexploded projectiles of the Second World War on Romania's territory, mainly found in rural area, near to or in the forests. Even if they look like antiques and appear to be inoffensive, these projectiles may be very harmful in inexperienced hands.

CASE REPORT

At 12:30 of 21.05.2014 there was a 112 call for a teenager of 17 years old who was found bleeding after he manipulated a projectile found on a wild field. The dispatch sent an ambulance and the helicopter and also the fire workers and special intelligences. At 12.40 the emergency team found an conscious but confused victim, Coma Glasgow Scale 14, with opened airway and spontaneous breaths, hemodinamically stable by appearance, BP=150/96 mmHg, HR=100/min, SaO_2=90%, with partial amputation of I-IV fingers of left hand, nasal stopped bleeding and multiple superficial wounds of the thorax and abdomen.

The emergency team provides the first professional aid by fixing a cervical collar, monitoring vital signs, getting two i.v. lines of 18 G and putting a compressive bandage on the fingers of left hand and also a splint for sustaining the forearm. The patient was transported by helicopter sitting on a stretcher, while he was perfused with normal saline and analgesics (Fentanyl 100 mcg i. v.) (fig. 1).

Fig. 1. Multiple superficial wounds on the thorax and abdomen

In The Emergency Department he was continuously monitorized, blood and urine samples were taken and he got an antitetanic vaccine and antibiotics. Left forearm and hand X-rays were done, as well as a contrast computer tomography of the head, neck and toraco-abdominal-pelvic areas which revealed no pathological signs on cranio-cerebral and neck level, but found multiple solid foreign bodies (shrapnels) on thoracic wall without lung or pleural post traumatic sings of injury; there were found pneumoperitoneum and hemoperitoneum mostly in the right hemi abdomen, liver lacerations in segments V-VI, shrapnels in segments II, III, VI, VII and VIII of liver and interhepatogastric, between the intestines and in the abdominal wall and right hemoscrotum. It's worth mentioning than even from body topogram foreign bodies were identified (fig. 2,3).

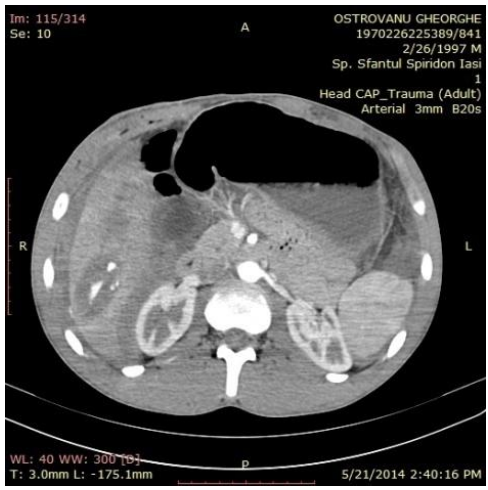

Fig. 2. Liver laceration with active bleeding

Fig. 3. Body topogram showing multiple foreign bodies (shrapnels)

A multidisciplinary medical team (emergency physicians, radiologists, general surgery, orthopedics, ear, nose and throat doctors, oro-facial and plastic surgeons) gathered and decided to do immedi-

ately general surgery intervention to stop the internal bleeding.

Median xifoumbilical laparotomy was performed and when entering in the abdominal cavity hemoperitoneum was found (about two liters of blood). An infracentimetric liver laceration (segment III) and foreign body (shrapnel) with adjacent sub capsular hematoma were identified. The shrapnels were removed, the hematoma drained and afterwards a simple hepatorrhaphy was performed. Further inspection of the abdominal cavity detected multiple transfixiant lesion of the liver (segment V and VI), one of them involving the liver bed with active bleeding unresponsive to haemostatic measures which implied performing tactic cholecystectomy and liver wedge resection (V and VI liver segments). The exploration of the abdominal cavity was continued with dissection of the gastro colic ligament and inspection of retro gastric cavity and mobilization of the superior duodenal flexure, inspection of the bowels and spleen without any source of bleeding. Right persistent patent *processus vaginalis* was found with blood exteriorizing when the hemiscrotum was compressed. A vertical incision was made through the skin of the right scrotum and underlying tissues that found a hematoma that was removed and normal aspect testicle. An orchidopexy was performed and the scrotum was closed. Three abdominal passive drains were placed (right subphrenic space, subhepatic and Douglas) and the abdominal wall was closed in anatomical layers.

Post operatory evolution was good, and the patient remained in intensive care 3 days being monitorized and perfused with normal saline and colloids after he was transfused with plasma during surgery intervention. He also got antibiotics, antiemetic and analgesics.

Although there were no other lesions and the post operatory evolution was good, the teenager remained without first phalanx of fingers I, II, III of the left hand.

DISCUSSION

Even if we live peaceful times, there are rarely cases of accidental blast injuries in Romania and this is the reason why we decided to present a classification of the lesions produced by the explosions and blasts.

In order to understand the mechanism of lesions it is important to know that there are two major types of explosives: high order explosives, which produce a supersonic over-pressurization shock wave (HE) and usually these are the modern military weapons (C-4, Semtex, TNT, nitroglycerin, dynamite, ANFO), and low-order explosives (LE), which develop a subsonic explosion (gun powder, petroleum based bombs, like Molotov cocktails) (1).

Primary mechanism of blast injuries (blast wave) involves intense overpressurization impulse due to detonated HE explosives and affects organs filled with gas (lungs, middle ear, and gastrointestinal tract). The injuries that will be developed are: pulmonary barotraumas, tympanic membrane rupture, eye destructions, brain damage, abdominal hemorrhage and bowel perforation, mesenteric shear injuries, solid organ lacerations, testicular rupture (2, 3).

Secondary mechanism of blasts derive from flying objects hitting the body (parts of the bomb or other adjacent objects) and the subsequent lesions may be eye penetration and blunt or penetration trauma of limbs or body (4).

Tertiary mechanism involves the blast wind which throws away the victims and any part of the body may be affected by blunt or penetrating trauma, fractures and

amputations (5).

Quaternary mechanism is due to all kind of injuries from the explosion like burns, crushes, toxic gases inhaled, asphyxia and complications of chronic pathology (asthma, angina, hypertension, and hyperglycemia) (6).

In our case, was not about HE projectile, so we cannot talk about blast wave, but about blast wind, which caused penetrating and blunt abdominal trauma and secondary mechanism of flying fragments of bomb causing penetrating injuries – shrapnels into the thoracic wall and abdominal wall and cavity and also partial amputation of the left hand fingers.

CONCLUSIONS

Even if explosions are rare circumstances in normal peaceful times, victims of these events usually have multiple traumas and life threatening condition may underlay visible hemorrhage.

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NEWS

SAPHENOUS VENOUS GRAFT PSEUDOANEURYSM

Saphenous vein graft (SVG) pseudoaneurysm is a rare and serious complication of coronary artery bypass (CABG) surgery. The complication can vary from an incidental finding on an imaging study to a life-threatening emergency. In the two reported cases, SVG pseudoaneurysm reflects the extreme variation in presentation and natural history, and highlights the need for adapted management. A review of literature indicates that almost one third of patients are asymptomatic at detection and a history of operative complications and need for re-exploration after the initial surgery may serve as useful predictors for the development of this rare complication. Finally, a conservative management may be considered, especially in the asymptomatic patient with multiple co morbidities and limited life expectancy (Smer A, Alla V, Chandraprakasam S, et al. Saphenou Venous Graft Pseudoaneurysm: A Review of the Literature. *J Card Surg* 2015; 30: 70–73).

Doina Butcovan

INTUSSUSCEPTION IN INFANTS – STORM FROM A CLEAR SKY

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INTUSSUSCEPTION IN INFANTS – STORM FROM A CLEAR SKY (Abstract): Intussusception is one of the most common causes of acute abdominal conditions requiring surgical therapy in infants and babies. Due to the breakthroughs of pediatric intensive care and imaging methods, which may sometimes replace therapeutic methods, this condition, which used to be associated with high morbidity and mortality rates, is now more easily detected and treated immediately, even by non-surgical means. Nevertheless, there still is a high percent of cases in whom the diagnosis is set relatively late and who require laborious surgical procedures and extensive intestinal resections. These cases have high morbidity rates, the condition sometimes has a negative course and the patients die. We conducted an analytical retrospective study on a group of 49 patients diagnosed with intussusceptions, hospitalized and treated in the Pediatric Surgery Ward of the Children's Hospital of Iași City during 5 years. Although the demographics and clinical presentation of our patients is largely in agreement with literature data, we note that 69.4% of the patients came to the hospital 24 hours after the onset of their symptoms, which delayed diagnosis setting, ruled out non-surgical therapy and thus led to a high rate of postoperative complications (24.5%) and also to a high mortality rate (6.6%). **Key words:** INFANT, INTUSSUSCEPTIONS, SURGERY

Intussusception is one of the most common causes of acute abdominal conditions requiring surgical therapy in infants and babies, with an incidence of 0.66-2.24/1000 children (1). It frequently occurs between the age of 6 and 24 months, and it is considered idiopathic for this age group, being supported by the intestinal peristalsis increase. In other children and adults, the condition usually has a mechanical cause, which is the starting point of intussusception (Meckel's diverticulum, colon polyp, hemangioma and tumor). Most intussusception cases are ileocecocolic, despite the fact that the condition may also have any other location, and they are the result of the ileum and cecum being telescoped inside the colon (fig. 1). Especially when it occurs in an eutrophic infant, who apparently is in a good health condition, with sudden onset consisting of extreme restlessness, reflex vomiting and blood in the stool, spontaneously or on digital rectal exam, the diagnosis is mainly clinical, as imaging methods only confirm it. Due to the breakthroughs of pediatric intensive care and imaging methods, which may sometimes replace therapeutic methods, this condition, which used to be associated with high morbidity and mortality rates, is now more easily

detected and treated immediately, even by non-surgical means. Thus, its early diagnosis and adequate therapy have considerably improved the prognosis of this condition, and the rates of mortality due to intussusception are currently below 2%. Nevertheless, there still is a high percent of cases in whom the diagnosis is set relatively late and who require laborious surgical procedures and extensive intestinal resections. These cases have high morbidity rates, the condition sometimes has a negative course and the patients die (2).

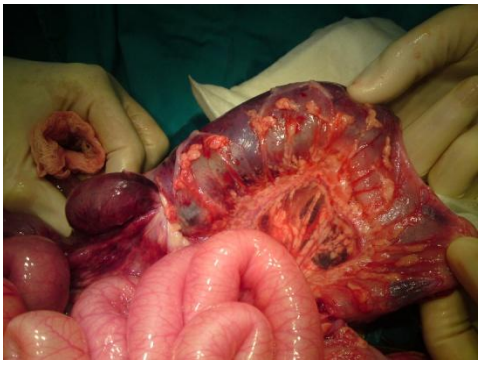

Fig.1. Ileocecocolic intussusception

MATERIAL AND METHODS

We conducted an analytical retrospective study on a group of 49 patients diagnosed with intussusception, hospitalized and treated in the Pediatric Surgery Ward of "Sf. Maria" Children's Hospital of Iaşi City between January 2009 and December 2013. The patients were identified according to the illness code entered into the hospital's computerized data network, the patients' records were identified and retrieved from the clinic's archives, and the following parameters were analyzed: age, sex, clinical signs and symptoms, prior conditions, time elapsed before the patient's coming to hospital, biological and paraclinical tests, conservative or surgical therapy and course of these patients, length of hospitalization.

RESULTS

Two patients whose records were incomplete and two other patients whose intussusception was a postoperative complication of laparotomy performed for other surgical conditions were excluded from the study. 45 patients remained eligible for the study.

Annual incidence ranged from 11 cases in 2009 to 5 cases in 2013, with a mean of 9 cases / year (fig. 2).

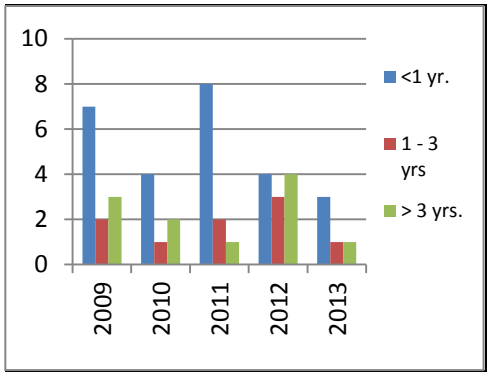

Fig.2. Number of cases per year depending on age.

The average age was 13 months (ranging from 1 month to 17 years), with an incidence peak between the ages of 6 and 8 months. Then the frequency of intussusception decreases with age (tab. I).

TABLE I

Distribution of intussusception cases on age groups

Age	Number of cases	%
0 – 1 year	26	57,7
1 -3 years	9	20
>3 years	10	22,2
TOTAL	**45**	**100**

The gender ratio was 1.5 in favor of boys. Of the 45 children, 19 patients (42.2%) lived in urban areas and 26 patients (57.7%) inhabited rural areas. The time elapsed between the onset of the first symptoms and the patients' hospitalization in our clinic ranged from a few hours to more than 3 days, yet most patients came 24 to 48 hours after symptom onset. The patients were hospitalized in our clinic as follows: 12 cases came directly to us, 17 cases were hospitalized in other clinics (8 cases of acute rhinopharyngitis, 9 cases of gastro-enteritis) and 16 cases were referred to us for suspected surgical conditions (9 cases of intussusception, 3 cases of appendicitis and 4 cases of acute abdominal conditions). The symptoms of the intussusception patients hospitalized in our clinic were dominated by the advent of a state of restlessness due to the child's abdominal pain, vomiting and blood in the stool (spontaneously or on digital rectal exam), and they are shown in table II, which also proves that the classical symptomatic triad occurred in 24 patients (54%).

The physical examination included a digital rectal exam in 50 patients.

Blood was detected in 29 of the cases (66.6%). Intussusception rim palpation during the digital rectal exam combined with abdominal palpations occurred in 5 cases, i.e. 11.1%.

An extremely important element in the study we conducted was related to the time elapsed from the onset of the condition and to the beginning of the treatment, shown in table III, according to which 69.4% of the patients saw a doctor 24 hours after the onset of the symptoms.

Native abdominal X-ray examination was performed in 35 patients and it revealed the results in table IV.

TABLE II

Symptoms of patients with intussusception

SYMPTOMS	Number of cases	%
Abdominal pain	38	85
Vomiting	36	81
Rectorrhagia	32	72.8
Symptomatic triad	24	54
Fever	14	32.2
Diarrhea	2	8
Constipation	3	6.7
Dehydration	2	8
Coughing	1	3.3
Poor general condition	29	66.6
Patient refuses to eat	11	26.6
No transit	3	10
Hematemesis	2	6.6
Abdominal tumor mass	4	13.2
Spasms	1	3.3

TABLE III

Time elapsed from disease onset to the beginning of the treatment

Time	Number of cases	Percent %
< 24 hrs.	13	30,6
24 – 48 hrs.	26	59,4
> 48 hrs.	6	10

TABLE IV

Elements revealed by a simple abdominal X-ray

ABDOMINAL X-RAY	Number of cases	%
Hydro-aeric levels	12	26.6
Dilated loops	10	22.2
Opacity in the right abdominal quadrant	15	33.3
Normal image	17	37.7
Not performed	10	24.4

The intussusception images generated by abdominal ultrasound scanning, which was performed in 40 patients, are illustrated in table V.

TABLE V

Elements revealed by ultrasound scanning in intussusception

ABDOMINAL ULTRASOUND SCAN	Number of cases	%
"Rosette-like" image	26	57.7
Peritoneal collection in Douglas and Morrison space	4	8.8
Abdominal meteorism	10	22.2

A patient whose ultrasound scan and X-ray were not conclusive underwent abdominal CT scanning, which revealed a thickened intestinal wall and the specific "rosette-like" images. As concerns treatment, only three of the 45 patients underwent pneumatic reduction under X-ray control (two were successful and one was a failure). Subsequent surgery was also performed on this latter case.

The surgical procedure consisted of midline laparotomy and manual desinvagination in all 43 cases (95.5%), also followed by bowel resection and termino-terminal anastomosis in 16 cases. Bowel resection was necessary because of the marked ischemia, extended delustered areas due to desinvagination and even intestinal perforation in 12 cases. In four cases (8.8%) bowel resection was aimed at removing the factor that determined the intussusception in the first place, namely two cases of Meckel's diverticulum, one case of intestinal tumor and one case of intestinal polyp (fig. 3).

In all the cases that required bowel resection, the patient saw a doctor 18 hours after the onset of the symptoms. The ileocecal valve was preserved in all the cases. Incidental loose stub appendectomy was also carried out in four patients, due to the alterations in the appearance of their cecal appendix. 34 (75.5%) of the 45 patients had a positive course due to the therapy administered. There were 11 postoperative complications (24.5%), i.e. two anastomosis displacements, two intestinal perforations upstream from the anastomosis, two cases of sepsis originating in the digestive system, one case of kidney failure and one relapse. Three patients (6.6%) died because of generalized peritonitis and severe sepsis, with multiple organ failure (two cases) and generalized metastasis (lymphoma), in one case.

Fig. 3. Meckel's diverticulum (starting point for intussusception)

The average hospitalization time was 3 days for patients undergoing pneumatic reduction, 8.7 days for manual reduction patients and 20.2 days for those who suffered both resection and intestinal anastomosis.

DISCUSSION

The frequency of intussusception detected in our study was 9 cases per year, as compared to 7 up to 49 cases per year reported in other researchers (1, 3). The series-dependent difference in the number of cases is due to variations related to recruitment, expertise and hospital reputation for intussusception diagnosis setting. The highest number of cases was diagnosed in

renowned pediatric surgery centers. In any event, in order to achieve statistical significance, the number of cases reported each year in each specialized service should also be compared with the general population where these cases originate. The intussusception onset age ranged from one month to 10 years in our study, with only few exceptions over this age. Most cases occurred during the first two years of the baby's life, the highest frequency being between the aged of 6 and 9 months. 57.7% of the patients were younger than 1 year, which confirms the predisposition of this age group to intussusception in almost all published series (1, 4). Regardless of the intussusception onset age, the male gender is more affected both in our study and in literature (4). The diagnosis setting time varies depending on the time elapsed from symptom onset to seeking medical attention, and also on the time spent examining the patient until diagnosis setting, which ranged from a few hours to 4 days in our study. Literature reports shorter times in developed countries and similar or even longer times in developing countries (1, 2). This is due to the poor socio-cultural and economic status of disadvantaged inhabitants, who prefer resorting to traditional remedies before seeking medical attention. On the other hand, insufficient training and absence of modern medical examination means lead to misdiagnosing this condition. Thus, all the digestive symptoms are erroneously mistaken for acute gastroenteritis, rhinopharyngitis or other more common pediatric conditions. However, it is true that these pediatric conditions may coexist and may actually constitute a factor promoting intussusception, although most cases occur in eutrophic infants in apparently good health conditions, due to marked peristalsis caused by a more diversified nutrition or by an age-dependent increase in size of Peyer's patches. Other disease-promoting factors are rotavirus vaccinations (5), and other triggering factors in children are colon polyps, hemangiomas, ascaris, Meckel's diverticulum, benign or malignant tumors (6, 7), detected in about 3% of the cases (1), as compared to 8.8% in our study. The symptomatic triad including abdominal pain, vomiting/abdominal tumor burden and rectorrhagia defines the classical for of presentation of intussusception. 54% of the children in our group had all three signs, as compared to 29-33% in literature (1). A recent study argues that the presence of a palpable abdominal mass, crying, vomiting and paleness are the most important clinical signs of intussusception. The association of all these four signs indicates a diagnosis with a 95% probability (8). Digital rectal exam complements the patient's physical examination and it should be done systematically, to support the diagnosis. Digital rectal exam revealed blood in 13 cases (28.8%), and an intussusception rim was felt in 5 cases (11.1%) on digital rectal exam. Imaging examination is merely an additional confirmation of the diagnosis, which is generally a clinical one, relatively simple to set. Abdominal ultrasound scanning or X-rays and sometimes even abdominal CT scanning are absolutely necessary only in older children, in patients that delayed their seeing a doctor or in relapses.

As far as the choice of the most adequate treatment is concerned, it is dependent on age, clinical form; time elapsed from disease onset to diagnosis setting. A conservative therapy is usually preferred. A retrospective study conducted in India reported a 71% rate of surgical therapy and a 0% mortality rate

(4). Unfortunately, only three of the 45 patients underwent pneumatic reduction under X-ray control (two were successful and one was a failure). Subsequent surgery was also performed on this latter case. Unlike literature, which reports success rates that exceed 70% for pneumatic or hydrostatic reduction under ultrasound scanning control (9, 10), the surgical therapy rate was the highest in our study, which suggests that the diagnosis was set late, when the other reduction methods could no longer be used, and the condition was complicated. Early diagnosis is important, since it increases the non-surgical reduction chances and reduces complications risks. Depending on the various series published, the mortality rate has been continuously decreasing lately and is currently below 1% in developed countries (1, 2), as compared to 6.6% in our study.

CONCLUSIONS

Long hospitalization, postoperative complications and their consequences may be prevented by an early intussusception diagnosis setting. Its treatment may be simple and efficient, as it consists of desinvagination by enema or air blowing under ultrasound scanning or X-ray control. Nevertheless, there still is a high percent of cases in whom the diagnosis is set late and who require laborious surgical procedures and extensive intestinal resections. These cases have high morbidity rates, the condition sometimes has a negative course and the patients die.

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SURGICAL OPTIONS IN THE TRATAMENT OF PERIIMPLANT FEMORAL FRACTURES

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SURGICAL OPTIONS IN THE TRATAMENT OF PERI-IMPLANT FEMORAL FRACTURES (Abstract) **Aim**: Hip fractures tend to increase as a global number-approx. 300,000/year. Life expectancy of the population is increasing, implicitly the diseases associated with the advanced age are increasing. At the same time, the number of peri-implant fractures (PIF) is increasing. These types of fractures usually have their headquarters at the base of the implant where poor quality bone comes into contact with the rigid implant. Our aim is to identify the factors that influence the production of these types of fractures and to optimize their treatment. **Material and methods:** We analyzed for 6 months, 4 patients with peri-implant fracture of the femur in terms of age, sex, place of origin, type of fracture, treatment type. The surgical treatment applied was different from case to case with favorable postoperative evolution. **Results:** The results from surgical interventions were optimal, assessing fracture stability, restoring mechanical stability, respecting the biological environment, and choosing the right fixation device. **Conclusions:** Peri-implant fractures are a distinct topic in fractures. The long-term implant can alter the bone structure, and may complicate the healing or placement of a new implant, making treatment a challenge for the surgeon. **Keywords:** HIP FRACTURE, PERI-IMPLANT FRACTURE, IMPLANT, SURGICAL TREATMENT.

PIF represent an important problem for the health system due to their increased incidence, the high average age of the affected population and the exorbitant costs that the treatment implies. As the number of implants used in orthopedics increases, it is inevitable that associated fractures become even more common (1, 2).

The trochanteric fractures that generate a large number of PIF account for about half of the total fracture of the upper femoral extremity, although there are also estimates that their number may be 4 times greater than that of the cervical fractures (3). Trochanteric fractures generally occur as a result of high intensity and energy trauma caused by height drops or traffic accidents in young people or hip failure in elderly patients with osteoporosis (4).

The PIF are due to the same forces that cause fracture without the presence of an implant, but on the background of a damaged bone tissue (5) and usually have their headquarters at the base of the implant, where poor quality bone comes into contact with the rigid implant (6).

The treatment of these fractures depends on the age of the patient, the pres-

ence of multiple associated co-morbidities influencing the prognosis, conservative or surgical treatment.

MATERIAL AND METHODS

We analyzed 4 cases of PIF admitted to Orthopedic Clinic of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi between January - June 2017. The data were obtained from the general clinical observation sheets. We analyzed: demographics characteristics of patients: gender, age, origin, type of fracture, interval between first surgery to second fracture (tab. I).

TABLE I

Data analyzed: demographics characteristics, type of fracture, interval between first surgery to second fracture

	Gender	Age (years)	Place of origin	Type of fracture	Second fracture (months)
S.M.	Female	76	Urban	Intertrochanteric fracture	1
E.I.	Female	97	Rural	Trochanteric fracture	84
T.V.	Male	64	Rural	1/3 middle femur fracture	24
C.C.	Female	59	Urban	Intertrochanteric fracture	3

CASE REPORTS

Case 1: S.M., Left intertrochanteric fracture operated. Treatment: hardware removal and fracture fixation with a long Gamma nail.

Fig. 1. Peri-implant fracture

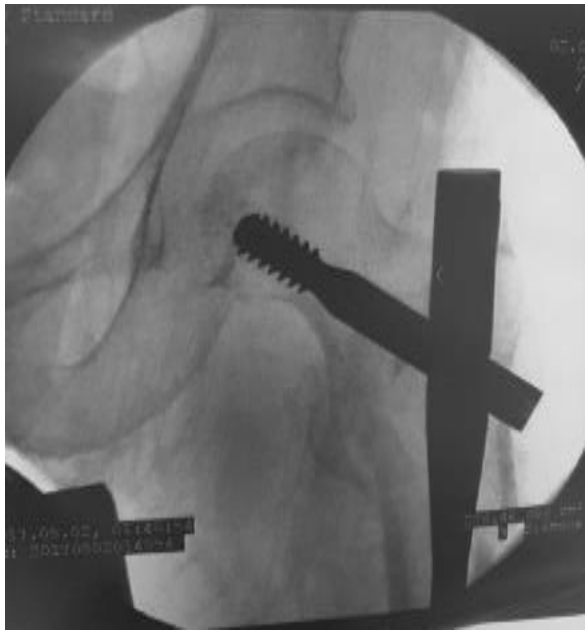

Fig. 2. Postoperative radiographic

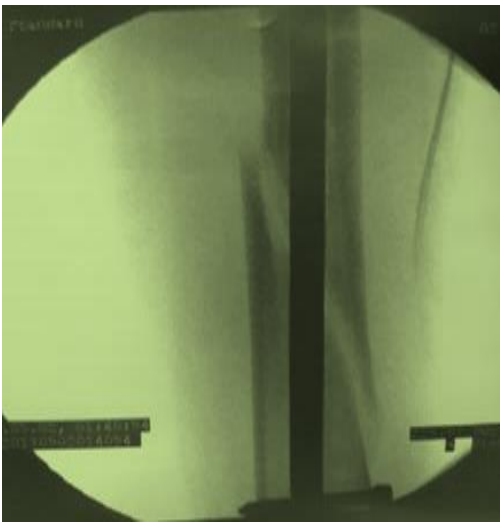

Fig. 3. Postoperative radiographic.

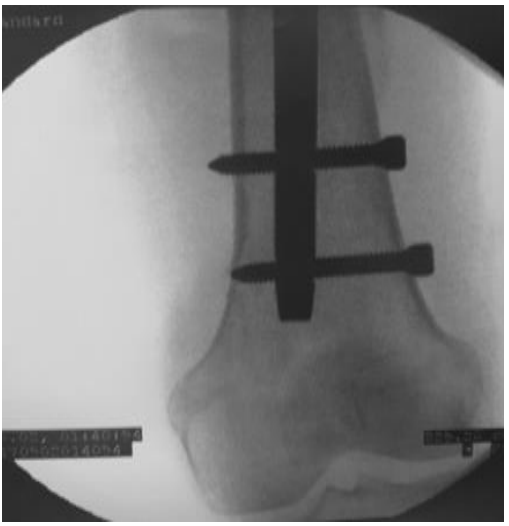

Fig. 4. Postoperative radiographic

Case 2: E.I., Right trochanteric fracture operated, viciously healed. Treatment: hardware removal and fracture fixation with a femoral nail.

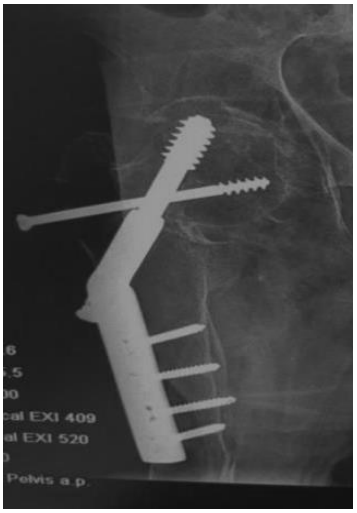

Fig. 5. Right trochanteric fracture, viciously strengthened

Fig. 6. Peri-implant fracture

Fig. 7. Postoperative radiographic

Case 3: T.V., 1/3 middle right femur fracture. Treatment: hardware removal and fracture fixation with a DCS and 3 Dall-Miles Cables.

Fig. 8. Peri-implant fracture

Fig. 9. Peri-implant fracture

Fig. 10. Post-operative anteroposterior view

Fig. 11. Intramedullary nail fixation is present

Case 4: C.C., Left intertrochanteric fracture operated. Treatment: hardware removal and fracture fixation with long Gamma nail.

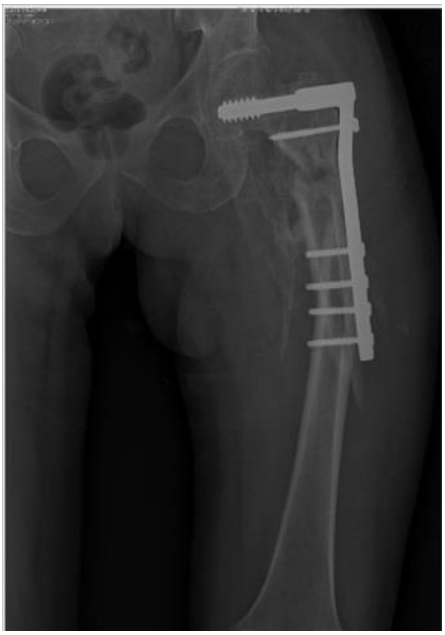

Fig. 12. Peri-implant fracture

Fig. 13. Postoperative radiographic

DISCUSSION

Fractures around implants pose unique fixation challenges. The original placement of the implant may predispose to later fracture, the long-term presence of the device may change the structure of the bone and increase susceptibility to fracture, and the implant itself may interfere with healing or the placement of other fixation devices (7, 8).

The techniques for treating PIF may be more difficult, with more limited options and more frequent complications than the techniques used in treating fractures without the presence of an implant.

Treatment of PIF requires strict adherence to the basic principles of treating any fracture. The surgeon must restore the biomechanical integrity of the bone. This requires restoration of a biologic environment in which the bone can heal and a mechanically stable construct to give the bone a chance to heal.

Biology is maintained by strict soft-tissue and indirect reduction techniques, when possible, to preserve periosteal or endosteal blood supply. The surgeon should minimize periosteal stripping, avoid dead space, and consider bone grafting if the biological environment is compromised. The patient's medical condition should be optimized. The patient should be encouraged to stop smoking when applicable.

Mechanical stability is obtained by restoring the anatomic integrity of the bone and by following AO principles with adequate fixation distal and proximal to the fracture (9).

Essentially, all PIF require treatment, and surgical options include the following: a) Revising the implant by placing a new implant, which also stabilizes the fracture; b) Fixing the bone around the implant which include intramedullary devices (rods, nails) and extramedullary devices

(plates, screws)

CONCLUSIONS

Fractures were usually located at the base of the implant, where poor quality bone comes into contact with the rigid implant, due to the difference in resistance between the osteoporotic bone and the implant used for osteosynthesis of the primary fracture.

In our cases PIF represent a challenge because they have presented technical difficulties in fixation and all cases have required surgery. In all cases the stabilization of the fracture focal point was sought, either by implant replacement or by keeping the implant and stabilization.

In geriatric patients with low bone mineral density, we think that diaphysis equal length centromedulary implants offer proper transfer of mechanical stress, preventing peri-implant fractures. Particularly, in proximal hip fractures in geriatric patients we think that long Gamma Nail offer better stability and protection against peri-implant fractures.

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OBSTETRICAL TRAUMA - THERAPEUTIC DILEMMAS?

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OBSTETRICAL TRAUMA - THERAPEUTIC DILEMMAS? (Abstract): Obstetrical trauma is frequent among newborns alive and could represent a cause of perinatal death. The aim of this paper consists of presenting the authors experience regarding the diagnosis and treatment of skeletal and thoracic-abdominal organs related to traumatic birth. **Material and methods**: Between 2000 and 2010, 33 patients with trauma at birth were included in the study. The type of the lesion and the therapeutical approach and results were analyzed. **Results:** Two categories of results were clearly differentiated: excellent for obstetric trauma involving limbs and soft parts and negative, disappointing results with 10 deaths in 12 cases treated for obstetric trauma of abdominal organs. **Conclusions**: Bone and soft tissue birth trauma are relatively easy to identify and treat, but the medical approach of thoraco-abdomnial organs birth trauma need clinical experience and technical possibilities. **Keywords:** OBSTETRICAL TRAUMA, DIAGNOSE, PERINATAL.

Obstetrical trauma occurs with a frequency of 6-8% among newborns alive and totals approximately 2% of all causes of death in the perinatal period (1).

Risk factors for obstetric trauma are known, and depend on the mechanism of birth, fetal organomegaly, the existence of a tumor or malformation, the maternal constitution (2). There have been important advances in the detection of obstetric trauma predisposing situations especially by obstetrical ultrasound (3, 4). The method allowed the identification of risk factors for obstetrical trauma and caused the modification of pregnancy monitoring and childbirth decision. Improved birth assistance techniques, increased frequency of "Caesarean" interventions applicable to the cases with difficult expulsion decreased the number of cases that required the use of forceps.

Unfortunately, there is "spontaneous" birth trauma, in case of mechanical uncomplicated births influencing the natural passage of the fetus or births by "Caesarean" section (5).

The aim of this paper consists of presenting the collective experience of Pediatric Surgery and Orthopedics Department, "Sf. Maria" Emergency Children Hospital of Iasi regarding the diagnosis and treatment of a category of injuries - skeletal and thoracic abdominal organs - related to traumatic birth.

We started from the premise that the diagnosis and treatment of obstetrical lesions is encoded and accessible to the authors. In the cases presented were recorded different types of situations where the application of classical precepts of diagnosis and treat-

ment were not enough, those situations generating discussion on therapeutic strategy but also ethical controversy.

MATERIAL AND METHODS

Between 2000 and 2010, were admitted to the Pediatric Surgery Clinic Iasi 182 newborns of which, 33 patients were included in the study. The inclusion criteria were trauma at birth resulted in bone fractures or various obstetrical injuries suspected in the clinical context of laborious birth (skin lesions, chest or abdominal trauma).

The 33 cases had obstetrical trauma:

- Skeletal: clavicle fracture 2 cases, femur fracture 5 cases, and humerus fracture 2 cases.

- Obstetrical brachial plexus palsy 6 cases

- The soft tissue: skin dilacerations 2 cases, hematoma and ecchymosed 4 cases.

- Internal organs: chest-spontaneous pneumothorax in 6 cases, esophageal perforation in one case and abdominal: liver rupture 3 cases and ruptured spleen 2 cases.

Treatment differed according to the character of the lesions. For bone and peripheral nerve lesions, conservative treatment was performed with different ways to immobilize the affected segments according to classical schemes, specific to each type of lesion. For cases with peripheral nerve injury medical rehabilitation was utilized and soft tissue lesions were treated with simple wound care treatment.

The 12 cases of thoracic or abdominal trauma had the following treatment:

- six cases had chest drainage: 5 for pneumothorax and one for esophageal perforation.

- one case of pneumothorax received ventilatory assistance.

- four cases of hemoperitoneum: 2 cases with liver dilacerations and 2 cases of splenic rupture have undergone surgery: laparotomy, hemostasis in cases with liver dilacerations and splenectomy in patients with splenic rupture.

- one case of hemoperitoneum by liver dilacerations received fluids compensation treatment, transfusions and haemostatic treatment.

RESULTS

There were two clearly differentiated categories of results: excellent cure for obstetric trauma cases - limbs and soft parts and negative disappointing results with 10 deaths in 12 cases treated for obstetric trauma of abdominal organs.

Two patients with thoracic injuries resulted in pneumothorax (one patient with esophageal perforation and one patient treated with ventilatory assistance) evolved favorably.

DISCUSSION

Fetal trauma during birth must be clearly distinguished from fetal trauma produced during pregnancy. Far from being a rarity, diagnosis and treatment of fetal trauma involves saving two lives-the mother and fetus (6). It is recognized that the extent of injury suffered by the mother does not correlate with the degree of fetus damage (7).

The diagnosis of obstetrical trauma is considered simple and does not require sophisticated explorations. In addition, prenatal ultrasound that can detect risk factors for obstetrical trauma occurrence proved extremely useful.

Our experience, revealed by the cases presented here, clearly distinguished two categories of obstetrical trauma: the skele-

tal and soft tissue lesions and the internal organs (spleen, liver). The first group received standard treatment: immobilization, recovery, local care and have very good results with patients healing. The second group received conservative - resuscitation, transfusions - or surgical treatment - laparotomy, surgical hemostasis (8, 9). Disappointing results, 10 deaths recorded in 12 patients with parenchymal organ damage raised the question of choosing the best treatment strategy beneficial to the patient. We believe that clinical experience is to decide when laparotomy is performed in a newborn with haemoperitoneum. Although there is a trend to lower incidence of obstetrical trauma, unexpected maneuvers applied in the attempt of fetus resuscitation by chest compression can cause liver or splenic rupture and hemoperitoneum. Subcapsular ruptured trauma is less severe than liver dilacerations.

Treatment strategy in cases of abdominal parenchymal lesions referred to prompt volume replacement, correction of coagulation deficits and even surgery by laparotomy, blood evacuation from the peritoneal cavity and liver dilaceration suture. Neonatal liver suture is extremely difficult and only the products for haemostasis improvement -TachoComb, Tachosyl - proved effective. We thought that if the patient is hemodynamically balanced, simple clinical surveillance, hematologic and ultrasound is the most practical approach (8).

Another category of obstetrical trauma was the immediately after birth installed pneumothorax, due to a spontaneous esophageal perforation (10) or hyperventilation due to high pressure in infants who required resuscitation. In these cases air leakage collection was evacuated or mechanical ventilation with positive end-expiratory pressure and minimizing the inspiratory pressure peak applied (11).

Skeletal lesions registered at birth as a result of obstetrical trauma generally have good prognosis and complete healing of the lesions was observed (6). Knowing these facts, would prevent dissemination in the media of subjects without medical support! In addition, pediatric neonatologist together with the surgeon are required to distinguish between obstetrical fracture happened in extracting the fetus and postnatal fracture. Practical experience of the authors says that this is difficult and only in case of a fracture injury diagnosis the so publicized "malpractice" could come in discussion.

CONCLUSIONS

The authors report two distinct categories of obstetric trauma: the skeletal and soft tissue and the abdominal and chest.

Bone and soft tissue lesions are more common, easily diagnosed and treated only conservatively, healing without sequelae.

Parenchymal organ injuries are serious, with a high mortality rate. Treatment strategies are multiple and only clinical experience can determine the best choice.

Medical practice must differentiate lesions produced during birth of those produced during fetal life or postnatal.

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NEWS

COMPARATIVE EVALUATION OF SERUM C-REACTIVE PROTEIN LEVELS IN CHRONIC AND AGGRESSIVE PERIODONTITIS PATIENTS AND ASSOCIATION WITH PERIODONTAL DISEASE SEVERITY

C-reactive protein (CRP) is an acute-phase reactant and has been proved to be a significant predictor of future cardiovascular events. Recent studies have demonstrated a correlation between periodontitis and elevated C-reactive protein levels. Most of the studies have focused on chronic periodontitis and very few studies are done in patients with aggressive periodontitis. The aim of a study realized by a group of indian researchers was to determine and to compare the relative levels of serum CRP in aggressive and chronic periodontitis patients. A total of 75 systemically healthy subjects were divided into three groups: group I (non-periodontitis subjects); group II (chronic generalized periodontitis patients) and group III (generalized aggressive periodontitis patients). All participants were subjected to quantitative C-reactive protein analysis using enzyme-linked immunosorbent assay. Mean CRP levels were significantly greater in both group II and III as compared to group I and group III having greater level than group II. CRP levels positively correlated with the amount of periodontal destruction as measured by probing depth and clinical attachment loss. The study indicates a positive correlation between C-reactive protein and periodontal disease severity with particular concern in younger individuals that could be a possible underlying pathway in the association between periodontal disease and the observed higher risk for cardiovascular disease in periodontitis patients (Goyal L, Bey A, Gupta ND, Sharma VK. Comparative evaluation of serum C-reactive protein levels in chronic and aggressive periodontitis patients and association with periodontal disease severity. *Contemp Clin Dent*, 2014 ; 5 (4) : 484-488).

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SERTRALINE INFLUENCE ON MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS

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SERTRALINE INFLUENCE ON MORPHINE-INDUCED CONDITIONED PLACE PREFERENCE IN RATS (Abstract): Serotonine reuptake inhibitors are an important pharmacological arsenal for treating major depression, a severe disease with poorly understood pathogenic mechanisms. Also, little is known about the action of antidepressants on reward system, the function of which is severely affected in this disorder. **Aim:** To assess the influence of sertraline on brain reward system by conditioned place preference technique in rats. **Results**: Both 3 and 5 mg/kg doses of sertraline determined a significant rewarding effect, whereas only the 5 mg/kg dose increased the morphine-induced rewarding effect (in the morphine-only group time spent in the conditioning chamber increased by 184.92±21.43% post-conditioning vs. pre-conditioning, whereas the increase was 195.56±18.3% in the group treated with morphine and sertraline 5mg/kg, $p<0.05$). **Conclusions**: The stimulant effect of sertraline on brain reward function might be involved in its therapeutic efficacy. **Keywords**: REWARDS SYSTEM, MORPHINE, SERTRALINE, CONDITIONED PLACE PREFERENCE.

Major depression (MD) is a disease which produces lots of disturbances in brain functioning. MD mechanisms are still insufficiently known. Antidepressant medication is one of the most frequently used in psychiatric practice. This medication has multiple action mechanisms. Most patients diagnosed with major-depression are nowadays treated with tricyclic antidepressants or with serotonin and/or norepinephrine reuptake inhibitors. Sertraline is one of the most often used serotonin-reuptake inhibitors in patients with major depression and bipolar disorder (1, 2, 3). There are also other important therapeutic uses for sertraline, including social phobia (4), panic disorder (5), or posttraumatic depression.

Brain reward system is essential for human and animal behaviour. Disturbances in its regulation are involved in the pathogenic mechanisms of several psychiatric diseases. One of the most important problems is the way in which different classes of antidepressant medication influence the brain reward system. The data on sertraline actions on reward system or any of is structural components are very scarce (in both humans and animals) (6). The aim of the present study was to investigate the effects of sertraline on reward system and its influence on morphine-induced conditioned place preference in rats.

Conditioned place preference (CPP) is an experimental protocol used to evaluate reward (drug or natural reward) in laboratory animals. The methods explores the associations between the rewarding effect of the addictive substance and other stimuli

(usually from the external environment) arbitrarily chosen.

MATERIAL AND METHODS

The present experiments were performed on adult male Wistar rats (180-270 g) hosted in individual cages; water and food were provided *ad libitum*, except for the behaviour testing periods. Animals were exposed to a 12/12- hour light-dark cycle; testing was performed during the light period. The conditioning apparatus (Panlab, Barcelona) consists of three chambers: two main chambers, distinguished by different wall and floor patterns, situated to the edges of the apparatus (one with black the other with white walls), and one smaller intermediary chamber. Communication between chambers may be either open or closed (by inter-changeable guillotine-like separations, suitable for a 180º movement). Illumination conditions were standardized by placing bulbs above each chamber. Prior to pre-conditioning, animals are subject to habituation sessions (2 sessions of 30 minutes daily, 2 days and one day before pre-conditioning). To induce morphine CPP we used the method described by Tzschentke (7).

1. *Pre-conditioning* (day 1) - natural animal preference towards one of the main chambers was determined. The rat spent 15 minutes in the apparatus (open communication between chambers). Time spent in each chamber was measured; the main chamber where it spent more time was referred to as *preferred* chamber, and the other as *non-preferred*. 2. *Conditioning* (days 2 – 9). Communication between chambers was closed. Days 2, 4, 6 and 8: the rat was restricted for 40 minutes to the preferred chamber, immediately after morphine (3 mg/kg, subcutaneously) administration. Days 3, 5, 7 and 9: alternatively, the rat was restricted for 40 minutes to the non-preferred chamber, immediately after the administration of 1 mL/kg saline solution. 3. *Post-conditioning* (day 10) - preference to main chambers was again measured for 15 minutes (open communication between chambers). Pre-conditioning, conditioning and post-conditioning stages took place in standard illumination conditions, during the same period of the day, in the absence of other stimuli that might have an influence on behavior (8). Pre-conditioning was initially applied to 80 rats. Animals showing a high preference towards a certain chamber (those spending more than 60% of the pre-conditioning time in any of the main chambers, or more time in the intermediary chamber than in any of the main ones) were excluded from further testing. Thus, 12 such animals (15% of the initial test group) were excluded. Of the remaining 68 animals, by applying homogeneity criteria 60 rats divided into 6 groups of 10 rats were selected for further tests.

According to the treatment received on experiment days 2, 4, 6 and 8 during the conditioning phase, the groups received: SS: saline solution (NaCl 0.9%), 1 mL/kg; Morph: morphine, 3 mg/kg; Sertr 3: sertraline, 3 mg/kg; Sertr 5: sertraline, 5 mg/kg; Morph + Sertr 3: morphine, 3 mg/kg + sertraline, 3 mg/kg; Morph + Sertr 5: morphine, 3 mg/kg, + sertraline 5 mg/kg; injected solutions were prepared so that each animal received 1 mL solution/kg at every administration. Doses were adjusted to animal weight changes during the experiment. On days 2, 4, 6 and 8 of the experiment, the rats received the previously described treatment, and then were placed in the non-preferred chamber. Morphine was administered subcutaneously, immediately before placing the animal in the non-preferred chamber; *sertraline* was administered intraperitoneally 2 hours before mor-

phine. On experiment days 3, 5, 7 and 9 all animals received saline solution, 1 mL/kg, and were placed immediately in the preferred chamber. Saline solution was administered intraperitoneally. Interpretation: a significant increase in time spent in the non-preferred (conditioning) chamber (preconditioning vs. post-conditioning) indicates that CPP was induced (change in preference). In such a case, it is believed that this is due to the fact that the animal associates reward (pleasant, euphoric effect of morphine administration) with the morphine-associated chamber.

The obtained results were statistically interpreted (p-value, t-test: paired – to compare post-conditioning vs. pre-conditioning time in each group; independent – to compare variance between groups in which CPP had been induced).

RESULTS

As expected, morphine determined a significant increase of time spent in the conditioning chamber (form 218.9±24.73 seconds – preconditioning, to 466.3±73.46 seconds, post-conditioning, $p<0.001$), indicating that a conditioned place preference was obtained. However, a significant conditioning effect was obtained with both sertraline doses (3 and 5 mg/kg), the effect of the 5 mg/kg sertraline dose being significantly higher compared to the 3 mg/kg dose (tab. I).

TABLE I

Time spent in the conditioning chamber (pre-conditioning vs. post-conditioning)

		Time spent in the conditioning chamber (pre-conditioning) (seconds)	Time spent in the conditioning chamber (post-conditioning) (seconds)	Statistical significance (preconditioning vs. post-conditioning)
Morphine	Average	218.9	466.3	$p<0.001$
	Standard deviation	*24.73*	*73.43*	
Sertraline 3mg/kg	Average	217,5	254.8	$p<0.05$
	Standard deviation	*23.42*	*41.01*	
Sertraline 5mg/kg	Average	238.7	286.9	$p<0.01$
	Standard deviation	*40.01*	*36.11*	

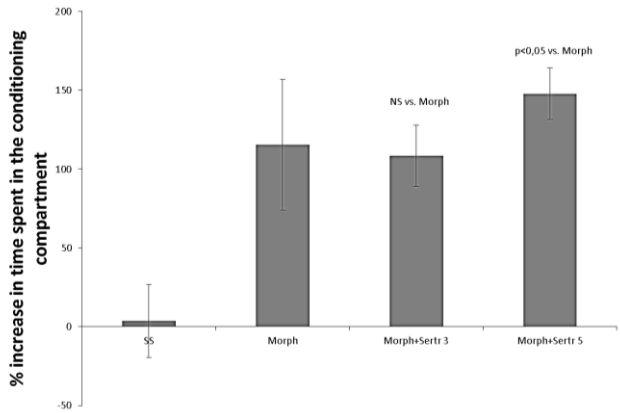

Fig. 1. Variation of time spent in the conditioning chamber (% post-conditioning *vs.* pre-conditioning)

The conditioning effect of sertraline slightly, but significantly potentates the conditioning effect of morphine, but only if higher sertraline doses are used (fig. 1).

DISCUSSION

Addictive substances, as well as natural rewards (water, food, copulatory opportunity) may induce CPP. Bardo and Bevins, (9) enumerated the substances able to induce CPP. Morphine is one of the main addictive substances with rewarding effects proven by this paradigm. Brain reward system represents a system with important involvement in human and animal behavior. This system normal function is also impaired in major depression.

There are very few data regarding sertraline effects on brain reward system in both humans and animals, as well as its effects on different structures belonging to this system. Sertraline is an antidepressant, serotonin reuptake inhibitor, used in the treatment of major depression. This drug has the advantage of relatively few heart side effects (1, 2). Sertraline reduces anxiety and panic, having long-term effect. On the other hand, it has been shown that selective serotonin reuptake inhibitors (SSRI) have important anxiogenic effect. For example, fluoxetine (2.5-10 mg/kg, i.p.) and sertraline (15 mg/kg, i.p.) induce anxiety-like effects (decrease in time of total social interaction and increase in self-grooming compared to vehicle) under low-light, familiar arena test conditions. All these effects were reversed by pretreatment with the highly selective 5-HT2C receptor antagonist. SSRIs also produce anxiogenic effects in animals tested by the elevated plus-maze test (10, 11). We believe that sertraline effect on reward system is also due to receptor-mediated action. Our results are in agreement with other authors, showing that sertraline (2.5-10 mg/kg) induces CPP (12).

CONCLUSIONS

Sertraline, both 3 and 5mg/kg, induced a significant place conditioning. Also, sertraline 5 mg/kg significantly potentiated the rewarding effect of morphine, evaluated by conditioned place preference. We believe that the action of sertraline of stimulating brain reward function might be, at least partially, involved in its antidepressant action and explains its therapeutic efficacy.

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NEWS

LAWSONIA INERMIS-MEDIATED SYNTHESIS OF SILVER NANOPARTICLES

Lawsonia inermis mediated synthesis of silver nanoparticles (Ag-NPs) has been studied by a group of researchers led by Gupta, in order to demonstrate its efficacy against *Candida albicans*, *Microsporum canis*, *Propioniabacterium acne* and *Trichophyton mentagrophytes*. In this study, a two-step mechanism for bioreduction has been proposed, as well as the formation of an intermediate complex leading to the synthesis of nanoparticles and an antimicrobial gel for *M. canis* and *T. mentagrophytes*. The synthesis of Ag-NPs was done using the leaft extract of *L. inermis* in the presence of 1 mM AgNO3 and the compounds were further characterized by Ultraviolet-Visible spectrophotometer and Fourier transform infrared spectroscopy. The size of Ag-NPs was determined by transmission electron microscopy, nanoparticle tracking and analysis sytem. The antimicrobial activity was determined using disc diffusion method. The study concluded that Ag-NPs, due to their biogenic nature, may represent a better candidate drug and an answer to the drug-resistant microorganisms. (Gupta A, Bonde SR, Gaikwad S st al. Lawsonia inermis-mediated synthesis of silver nanoparticles: activity against human pathogenic fungi and bacteria with special reference to formulation of an antimicrobial nanogel. *IET Nanobiotechnol*. 2014;8(3):172-8. doi: 10.1049/iet-nbt.2013.001.

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PARAGANGLIOMA OF THE CAROTID BODY: CASE REPORT

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PARAGANGLIOMA OF THE CAROTID BODY: CASE REPORT (Abstract): With an incidence of 0.03% of all tumors, carotid body tumors (CBTs) are extremely rare. We present the case of a 63 year old female patient with an asymptomatic right sided neck mass of 3 cm diameter. Doppler ultrasound and contrast-enhanced CT confirmed the clinical suspicion of carotid body tumor. The patient underwent complete surgical excision of the Shamblin group II tumor and an adjacent lymph node, without preoperative embolization. The histopathological examination diagnosed a benign CBT. There were no postoperative complications at 1 and 6 months follow-ups. **Keywords**: PARAGANGLIOMA, CAROTID BODY TUMOR, SURGICAL TREATMENT.

Carotid body tumors (CBTs), also known as paragangliomas (PGLs) or glomus tumors, are very rare, with only a few vascular surgeons having encountered such pathology during their career. However, they are the most common para-gangliomas of the head and neck (1) The very few studies on PGLs cited by the literature report contradicting male to female ratios (2-5), but agree the tumors are predominantly unilateral, with more than 50% on the right side of the neck. The mean age of presentation is between 49-55 years and recurrence rate is low (2, 3, 4).

The usual clinical presentation is an asymptomatic, slow-growing neck lump with Fontaine's sign (horizontally mobile, vertically fixed) (1, 2, 4, 5). In few cases, the CBT can cause various pressure symptoms: pain, cranial nerve palsy, dysphagia, odynophagia, hoarseness, dysphonia (1-4) or carotid sinus syndrome like symptoms: palpitations, fainting, falling (4, 5, 6).

Different imaging modalities are used preoperatively, most patients having multiple investigations. Doppler ultrasound (DUS) offers a non-invasive modality of diagnosing CBTs, which appear as solid, well-defined, hypervascular masses causing splaying of the carotid arteries. One series of patients reported CTA as the primary imaging modality, although most of those patients have been followed with DUS as well4. MRI is also effective for diagnosis, as is PET scan for tumors that are less than one cm in diameter (1). Historically, angiography has been advocated as mandatory for preoperative management of CBTs, as it

was shown to reduce the tumor mass and blood flow making surgical resection easier (3, 7, 8). More recent studies did not confirm the difference in blood loss and morbidity between embolized and non-embolized groups (9, 10).

CBTs can be classified as sporadic, familial or hyperplastic, with the former being the most frequent type. The familial type is encountered in only 20% of the cases and follows an autosomal dominant pattern of transmission (1, 19). Malignancy has been reported in less than 5-6% of all tumors (4, 11). It is generally accepted that malignancy is defined only by confirmation of metastasis in non-neuroectodermal tissue (12).

The recommended treatment of CBTs is complete surgical resection, with or without vessel reconstruction (1). Adjunctive therapy such as radiotherapy is reserved for metastatic disease, including patients with locally advanced extension that makes curative resection impossible. To aid preoperative surgical planning, Shamblin introduced a classification scheme that divides CBTs in three groups according to size and local extension. First group consists of tumors that are small and easily removed. Group II includes tumors adherent to or partially surrounding vessels. Group III consists of tumors surrounding or encasing the vessels (13).

The classical surgical technique starts by ligation of the external carotid artery feeding branches, followed by dissection of the tumor from the carotid bifurcation towards the cranial side, in a sub adventitial plane. However, it has been shown that blood loss and cranial nerve injury can be improved with a modified technique that describes dissecting the tumor in a craniocaudal direction, following the course of the ascending pharyngeal artery, suggested to be the tumor's main blood supply (14).

Although the most frequent postoperative complication after CBT resection is cranial nerve palsy that resolves with time (15), a number of other complications have been described: uncontrollable hypertension16, unilateral blindness (17) and persistent Horner's syndrome (18). Mortality rates are between 0 and 3% and stroke rates between 0 and 8% (1).

Routine follow-up consists of clinical evaluation after 4-6 weeks and duplex ultrasound each year. CT or MRI is performed if new pathological findings occur on ultrasound (3).

CASE REPORT

A 63 years old female patient was admitted in our Vascular Surgery Department in May 2013 complaining of a 3 cm asymptomatic right sided neck mass. Her previous medical history included right sided breast cancer 35 years before, surgically treated with right mastectomy combined with axillaries clearance, and non-Hodgkin's spleen lymphoma 6 years before, surgically treated with splenectomy followed by chemotherapy. At the time of admission, she was also being treated for high-risk grade III hypertension. As the patient was diagnosed with a sporadic CBT, no family screening was necessary.

Doppler ultrasound of the mass revealed a highly-vascularized tumor splaying the internal and external right carotid artery and also partially compressing the right internal jugular vein. A lymph node cranial to the tumor was also identified.

Contrast-enhanced CT of the neck and thorax revealed a 3 cm diameter mass located in the right carotid area, partially calcified in the lower segment, with intense

annular contrast intake and post contrast hypo dense center, without evidence of extension to adjacent structures. The tumor had the same size compared to the preview CT scan performed 10 months earlier (fig. 1, 2).

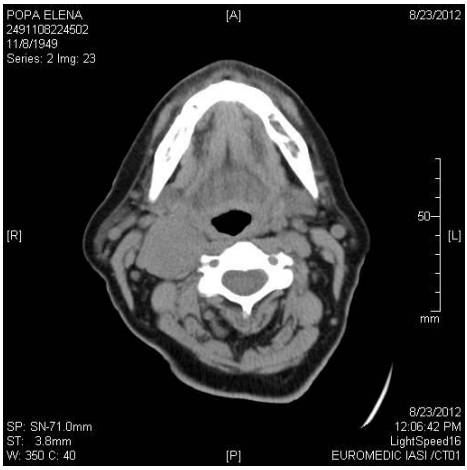

Fig. 1. Native CT

Fig. 2. Contrast CT

Complete surgical removal of the tumor was performed under general anesthesia. The standard incision for carotid endarterectomy on the inner edge of the sternocleidomastoid muscle was undertaken. The common and internal carotid arteries were isolated with vessel loops, while the external carotid artery was clamped. Sub adventitial dissection of the tumor was then carried out from the carotid bifurcation in a cranial direction.

Excision of an adjacent lymph node completed the surgical procedure. The tumor was classified as belonging to Shamblin's group II (fig. 3, 4).

Histopathological examination of the excised tumor and lymph node, characterized the mass as a benign CBT. The lymph node had normal architecture and sinus histiocytosis (fig. 5, 6).

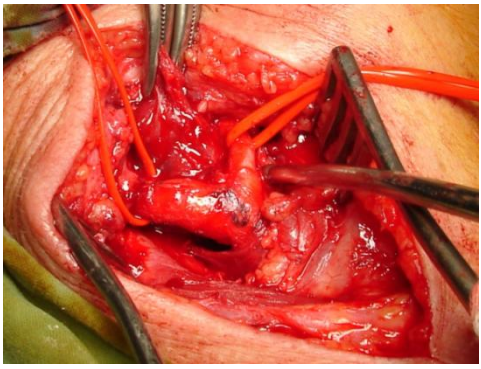

Fig. 3. Intraoperative image of the glomus tumor

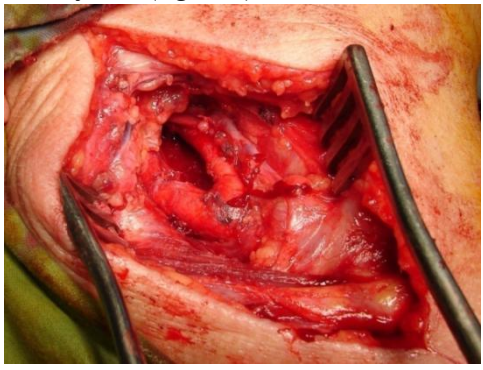

Fig. 4. Intraoperative appearance after the complete resection of the tumor

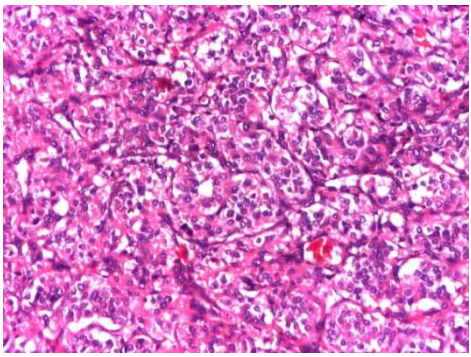

Fig. 5. Encapsulated nodular formations with insular or trabecular architecture consisting of polygonal cells with vesicular monomorphic nuclei, having nucleoli with fine chromatin and pale eosinophilic vacuolar cytoplasm. Stoma highly vascularized, with areas of edema and hemorrhage. (HE staining, 10X magnification)

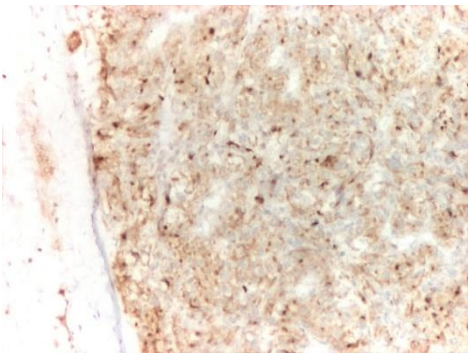

Fig. 6. Chromogranin- positive tumor cells (IHC, Ac anti-chromogranin, magnification 10X)

There were no immediate postoperative complications. The patient remained asymptomatic and complication free at clinical and Doppler ultrasound follow-up after one and six months postoperatively.

DISCUSSION

In patients presenting with swelling of the lateral neck, cranial nerve palsies, voice changes or auditory defects, a PGL of the neck should be included in the differential diagnosis. Careful evaluation of family history of tumors is important. Diagnosis is made on both clinical and imaging criteria. Our patient presented with a 3 cm asymptomatic right sided neck mass, highly suggestive for a carotid paraganglioma.

Imaging procedures, which include a mandatory ultrasound of soft tissue and vessels of the neck and CT or magnetic resonance angiogram (MRA) are recommended preoperatively. On ultrasound the tumor appears as a homogeneously hypoechogenic mass, while on Doppler scan a multidirectional hypervascularization is the main feature. Due to contrast medium enhancement the tumors give high signal intensity on MRA. The Shamblin groups can be predicted preoperatively on MRI.

CT can be useful to reveal relevant bony erosions or punctuate calcification in the tumor (20). To reduce radiation exposure and improve soft-tissue visualization, MRA is preferred to CT (20). DUS confirmed the clinical suspicion of a CBT for our patient and contrast-enhanced CT helped plan the surgical resection.

The clinical value of embolization is discussed controversially in the literature (10, 18, 21, 22). It can be considered in large tumors, because hypervascularization can be challenging during surgery. The risk for neurologic complications such as transient ischemic attacks (TIAs) or stroke caused by angiography or embolization is considerable. In large tumors surgical resection can be facilitated by preoperative embolization. Our patient had a Shamblin

group II CBT, and therefore we decided the risks of embolization outweighed the benefits.

Complete surgical excision is the aim of any operative procedure for PGL and is the only curative option (22). Tumor doubling time is very slow and lies between 4 and 14 years; hence conservative treatment, such as a wait-and-see strategy, can also be discussed in elderly patients, who are free of PGL symptoms but do have other comorbidities. Ipsilateral lymphadenectomy should be performed simultaneously to identify malignant tumors. In these tumors malignancy is defined by metastases in non-neuroendocrine tissue (21). Complete resection of the tumor and excision of an adjacent lymph node was carried out in the case presented.

To reduce intra-operative bleeding, temporary clamping or ligation of the external carotid artery (ECA) may be required. Clamping but not ligation of the ECA was necessary during our surgical resection procedure, to minimize blood loss and improve cranial nerve detection. The larger the tumor the bigger the risk for inevitable nerve lesions or additional vascular procedures increases during surgery (4). Treatment in specialized vascular surgery centers is advisable because the incidence of vascular reconstructions is more than 25%.

Good blood pressure control is recommended. Baroreceptor failure syndrome (BFS) was described by Toma and Netterville and found in 16% of cases (23, 24). It might occur in patients after bilateral resection of the carotid glomus with postoperative acute hypertension and tachycardia. No complications were noted at 1 and 6 months postoperative follow-ups for our CBT patient.

Urinary vanillylmandelic acid and urinary or plasma metanephrine preoperative determination is important if an extra-adrenal tumor is suspected. Early diagnosis and surgical treatment of tumors before the tumor reaches 3 cm is recommended in order to avoid intra-operative nerve damage.

The patient must be referred to a surgeon who is familiar with the head and neck region and has the technical options for vascular reconstruction.

After histopathological confirmation of the paraganglioma, genetic testing and family examination is recommended.

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CLINICAL, EPIDEMIOLOGICAL AND PROPHYLACTIC ASPECTS OF DYSPEPSIA

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CLINICAL, EPIDEMIOLOGICAL AND PROPHYLACTIC ASPECTS OF DYSPEPSIA (Abstract): Dyspepsia or indigestion is a term that refers to a multitude of symptoms and disorders of the digestive system which cause suffering in many patients. Dyspepsia means pain or discomfort that is centrally localized in the upper abdomen. The following categories of symptoms may also be present: pain localized in the right or left hypochondrium, dysphagia, upper gastrointestinal bleeding, acute abdomen, symptoms of reflux, transit disorders. On the other hand, the term "discomfort" was defined in order to designate a condition characterized by or associated with early satiety, upper abdominal bloating, nausea, epigastric fullness. As dyspeptic syndrome is often caused by excessive food intake or by consumption of certain nutrients, the disease may have a severe prognosis. Hearty meals, the nature of foods, the preparation of meals, possible special conditions of eating may often contribute to dyspepsia.
Keywords: HEREDITY, INTESTINAL TRANSIT, EXCESSIVE FOOD INTAKE, DISCOMFORT, NAUSEA, BLOATING.

Dyspepsia is a condition without systemic involvement, characterized by symptoms localized in the upper abdomen and manifesting itself in epigastric pain, fullness, bloating or discomfort. Dyspepsia is characterized mostly by pain that varies from day to day, without any obvious relationship to meals or certain foods. Dyspepsia occurs as burning and cramp after an emotion or a certain mental state (1).

Dyspepsia, often called indigestion, is a common disease, often associated with the ingestion of certain foods. The dyspeptic syndrome is most commonly caused by excessive food intake or by consumption of certain foods and has a possible severe prognosis. Dyspepsia was widely considered a psychosomatic disorder, as it is due to a functional disturbance in the nervous system or in the muscular activity of the stomach or of the small intestine.

Dyspepsia has an important *epidemiological* significance, representing about 2-5% of all clinical examinations of the family doctor and 20-45% of the gastroenterologist consultations. 70-80% of patients seeking medical care for upper abdominal symptoms have no organ injuries, but functional dyspepsia, and the remaining 20-30% show systemic manifestations related to gastro duodenal ulcer, gastric neoplasm and chronic pancreatitis. Dyspepsia is thought to affect up to 46% of the population (2).

Dyspepsia can be designated as an "iceberg symptom" with a major impact on health systems, requiring special attention from both family physician and gastroenterologist, but also from decision makers of the community health system (3).

The human body is anatomically built not to consciously perceive the function of its organs. Metabolic and digestive processes are genetically programmed to develop within certain limits and to show self-regulating variations that do not reflect themselves in the consciousness. They become conscious when disturbances occur in the normal evolution of phenomena and when functional imbalances or abnormal states are present (3).

On an organic and functional level, dyspepsia is expressed by how the individual and his digestive organs react to food. Their susceptibility to food and the complex process of digestion are influenced by many factors.

Although dyspepsia affects women and men of all ages, it is more common in women aged 16 to 60 years. Women have often dyspepsia during menstruation or pregnancy. Smoking is another risk factor for dyspepsia (4).

Gastrointestinal diseases inducing dyspeptic phenomena are as follows: air intake (aerophagia); gastric juice or regurgitated gas caused by gastro esophageal reflux disease or by hiatal hernia; peptic gastric or duodenal ulcer; gastric cancer; the inability to digest milk and milk products (lactose intolerance); irritable bowel syndrome, a disorder that affects the transit of food through the intestines. The significant overlap of irritable bowel syndrome and dyspepsia may be explained by their common visceral hypersensitivity. Dyspepsia and gastro esophageal reflux overlap is caused by high somatisation, especially insomnia and medication use (e.g. proton pump inhibitors) (5).

Many non-gastrointestinal diseases are also associated with dyspepsia: diabetes, thyroid disease, hyperparathyroidism, severe renal disease, cholecystitis, anxiety or depression, side effects of caffeine, alcohol or medication.

The symptoms of dyspepsia originate in the upper gastrointestinal tract, particularly in the stomach and in the first section of the small intestine. In most cases, symptoms occur after the ingestion of food in the form of abdominal pain, allergic manifestations, eructation, bloating, burning sensation after eating, chronic irritable bowel, chronic fatigue, constipation, diarrhea, bloating, insomnia, joint and muscle pain, nausea, intestinal gurgling, vomiting.

Dyspeptic symptoms may also accompany gastritis, gastroenteritis, gastric ulcer, vesicular lithiasis, pancreatic disease. Pregnancy and advanced diabetes may also be associated with dyspepsia that however may occur without accompanying other digestive diseases.

Many studies suggest that the overlap of dyspepsia and other conditions, such as gastro esophageal reflux or *Helicobacter pylori* infection, may represent a distinct syndrome that needs different management strategies (5, 6).

Factors that may affect digestive process

Individual characters governed by heredity (presence or absence of certain enzymes, abnormal conformation of some digestive organs or segments) and the presence of a certain neuro vegetative tone, a certain combination of neuro humoral and endocrine factors are clearly involved in the process of digestion. A medical history with acute digestive diseases during child-

hood, chronic digestive diseases or digestive diseases that have required surgery, infectious diseases with digestive involvement etc, chronic abusive use of certain medications (aspirin, phenylbutazone) or coffee and alcohol abuse can lead to dyspeptic disorders (7).

There has been shown that nonsteroidal anti-inflammatory drugs, antibiotics and estrogens are commonly associated with dyspepsia.

The digestive process, especially in the modern times, when food is excessively consumed or when nervous tension is frequent, digestive disturbances occur immediately and last for days.

Abusive consumption of certain foods (meat, pasta, sweets, vegetables with high cellulose content) and other factors involved in digestion may lead to functional disorders localized in different segments of the digestive tract and manifesting as various signs and symptoms that define dyspepsia (8, 9).

Other factors that may cause or contribute to dyspepsia are intestinal obstruction, malabsorption or liver and pancreatic diseases, allergies and food intolerances.

Food that is difficult to digest and gut bacteria can produce toxins that affect the mucosal tissue and may lead to leaky gut syndrome. This condition occurs when undigested food particles which are normally eliminated in the feces pass through the structures of the intestinal lining and are absorbed by the system, causing digestive disturbances.

The factors contributing to this phenomenon are abnormal intestinal flora, food allergies, alcohol consumption, parasites, chemicals or drugs that irritate the small bowel.

Psychological factors, such as anxiety, stress or anger, may contribute to disturbance of the nervous mechanism controlling bowel and stomach muscles.

If the reason that caused indigestion remains unclear, there may be functional dyspepsia. This is a type of indigestion caused by the alteration of the capacity of the stomach to digest food, before it passes into the small bowel.

Theories on the causes of dyspepsia suggest abnormal perception at the level of the intestinal sensory nerves, followed by impaired information processing and disturbed bowel stimulation by motor neurons.

Clinical aspects

Dyspepsia may persist for years, if not the whole life time, and it manifests itself periodically and the symptoms may be more common over some days, weeks or months. The Rome II definition of functional dyspepsia describes it as the presence of abdominal pain or discomfort in the epigastria for at least 12 weeks over the last 12 months that cannot be explained by upper gastrointestinal investigation. The more recent Rome III definition requires symptoms to be present for the last 3 months, with symptoms onset at least 6 months before diagnosis (10).

When digestion is complete, the stomach empties into the upper part of the small bowel through mechanisms regulated by the brain and by a nerve network located in the muscles of the digestive tract wall. The coordination between these nerve endings that secrete neurotransmitters ensures that hormones and muscle fibers in the wall of the digestive tract regulate the movement of the digestive tract and consequently favor digestion, absorption and elimination of the ingested food.

There are three clinical forms of dyspeptic syndrome, depending on the prevalence of some of the symptoms: *reflux type* (regurgitation, eructation and retrosternal

burning are common), *ulcerative type* (characterized by epigastric pain and feeling of hunger in the stomach), *dysmotility type* (mostly colicky pains, bloating).

However, the most important symptom is *the pain*. It is generally perceived as abdominal discomfort or real pain in the whole abdomen or on the flank, under the right or left costal margin or in the epigastrium. Sometimes the pain is even perceived as cramps. The pain may have an esophageal, gastric, pancreatic, biliary or intestinal origin. The pain may be continuous, intermittent, at a certain time after meals or at night. Another group of dyspeptic symptoms is caused by excessive production of gas in the digestive tract and is manifested by eructation, flatulence, gurgling, and abdominal bloating. Patients experience sometimes loss of appetite, may lose weight and have discomfort, especially after meals. Although these problems occur, the general state of the patient is good most of the times. Of course, not all of these symptoms are found together in the same dyspeptic patient (11).

Sometimes dyspeptic phenomena are better grouped and systematized. In the intestine, carbohydrates undergo a fermentation process and proteins undergo a putrefaction process under the influence of the microbial population at this level. When meals with a lot of vegetables high in cellulose or with a lot of milk or meat are preferred and when gastric, pancreatic or biliary secretion disorders are present, associated with an increased sensitivity of the intestinal mucosa, there is a risk of *fermentation* or *putrefaction* dyspepsia with excitation of intestinal peristalsis and diarrhea. Therefore, one-sided food patterns may change the balance of intestinal bacterial flora, leading to disturbances in the activity of the small bowel and especially of the large intestine (12).

Due to the disturbance of carbohydrate digestion, *fermentation* dyspepsia is accompanied by excessive production of intestinal gases and is manifested by bloating, gurgling and flatulence. These gases are eliminated with the feces and have a pungent sour smell. Stools are light colored and with acidic reaction.

Putrefaction dyspepsia is caused by excessive growth of putrefaction proteolytic flora, due to the disturbance of protein digestion. It is manifested by pasty smelly stools with alkaline reaction. The disease is more common in persons with excessive consumption of meat and meat products, in which case the digestion and absorption of proteins is not carried out completely. They are poorly digested in the small bowel. Afterwards, they reach the colon, where they will be subjected to the action of the flora of putrefaction, irritating the wall of the colon and accelerating the transit.

In disorders of fat digestion caused by biliary pancreatic or intestinal deficiency, soap dyspepsia may occur. The patients complain of loss of appetite, abdominal fullness, eructation, bloating, abdominal cramps at about an hour after the meal. The stools contain large amounts of crystallized soap, fatty acids and fat bubbles. Malaise, various intestinal absorption disorders and intolerance or allergy to certain foods may also occur, with consequences on the general health status (13).

Aerophagia and excessive intestinal fermentation cause pain in the anterior and left thoracic region, resembling sometimes pain of heart and vessel diseases, due to the distention of the upper portion of the stomach and of the large intestine.

Clinical diagnosis of dyspepsia

Dyspepsia is diagnosed based on typical

symptoms and in the absence of other gastrointestinal diseases, in particular those related to hyperacidity and non-gastrointestinal diseases.

The dyspeptic syndrome may occur as the result of a temporary eating disorder, but when symptoms occur in a young person or they persist, worsen and disturb the daily activities of the individual, an investigation of the upper digestive tract should be made.

Indigestion is a discomfort rather than a serious problem. However, it may be a sign of a major underlying disease and it is important to be carefully investigated, especially when the onset is recent.

Laboratory investigations are needed in order to rule out other diseases, rather than to diagnose dyspepsia. They are mainly used in order to exclude the presence of other gastrointestinal and non-gastrointestinal diseases.

Additional tests include stool tests that determine whether the stomach inflammation had *Helicobacter pylori*. Upper digestive endoscopy, radiography of the esophagus, stomach and small intestine, abdominal ultrasound may also be performed. Some studies warn that inadequate work up in primary care leads to unnecessary endoscopic investigation, when the guidelines are not considered and the age of the patients might hardly be associated with the risk of malignancy (2).

For the diagnosis of functional dyspepsia, tests are necessary in order to rule out severe gastrointestinal diseases, but they are rather ineffective in identifying the cause of dyspepsia, which is caused by a malfunction of the nervous system or of the muscular activity of the digestive tract.

Initial diagnosis can be made by the family doctor through physical examination and medical history review.

Differential diagnosis is made in case of systemic disorders, irritable bowel syndrome, and lower abdomen distress, designated as a single entity called "irritable digestive tract disorder". *Evolution* and *prognosis* are favorable.

Treatment of dyspepsia

The treatment includes dietary and hygienic measures. If the symptoms persist, a pharmacological treatment to control symptoms is required (14). Therapies for dyspepsia can be subdivided depending on the underlying disease that is being treated: gastro esophageal reflux disease, peptic ulcer disease, functional dyspepsia (15).

The treatment of dyspepsia is mainly educational and prokinetic drugs and muscle relaxants are used. Antidepressants and the change of the hygienic and dietary regimen may also have a beneficial role.

If the indigestion has a specific cause (ulcer or gastritis, etc.), treating these disorders relieves the symptoms. When the cause is not identified, diet changes and administration of drugs is the most effective treatment.

The treatment of dyspepsia begins with a proper diet, adapted to gastric chemistry, intestinal motility and subjective symptoms, and with the treatment of the systemic disease that has caused dyspepsia (parasitosis, gallstones or gallbladder dyskinesia, colopathy, appendicitis, adnexitis).

Drug therapy includes antisecretory medication such as proton pump inhibitors (omeprazole, esomeprazole, lansoprazole, pantoprazole), H2 blockers (cimetidine, ranitidine, famotidine), prokinetics (metoclopramide) and antibiotics. Acotiamide is a novel prokinetic that has been shown to be effective in functional dyspepsia in a phase III randomized trial (15).

When peptic ulcer caused by *Helicobac-*

ter pylori is diagnosed, an antibiotic is also prescribed. When even a thorough investigation did not reveal a cause of the symptoms, antidepressant medication may be used, in order to alleviate the discomfort of indigestion by reducing the sensation of pain and eliminating symptoms such as headache, nausea, night sweats, restlessness and constipation.

CONCLUSIONS

Any disturbance of the normal function of the nervous system or of the muscular activity of the digestive tract can cause dyspepsia. Dyspepsia is diagnosed based on evaluation of typical symptoms and on the elimination of non-functional gastro-intestinal diseases, non-gastro-intestinal disorders and mental illness.

Excessive food intake that stresses and disturbs digestion, the disproportion between food intake and digestion capacity, hereditary or acquired individual characteristics of the digestive structures, special psychological conditions of eating may cause dyspepsia, which may be seen as an epiphenomenon of esophageal, gastric, biliary, pancreatic and intestinal diseases.

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AGE AT INITIATION OF SEXUAL LIFE, PROTECTION AT FIRST INTERCOURSE AND SOURCES OF INFORMATION REGARDING SEXUAL AND REPRODUCTIVE HEALTH

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AGE AT INITIATION OF SEXUAL LIFE, PROTECTION AT FIRST INTERCOURSE AND SOURCES OF INFORMATION REGARDING SEXUAL AND REPRODUCTIVE HEALTH **(**Abstract): **Objectives:** this study is intended to determine: age at initiation of sexual life, protection at first intercourse and sources of information regarding sexual and reproductive health. The trends identified by this study can form the basis of public health programmes. **Material and methods:** between 2011 and 2012, 564 subjects, 18-35 years old, from urban and rural environment, have responded to a questionnaire with 96 items on family-related topics. In the present study we focused on three items. The Pearson chi-square tests were employed using the statistical programs *SPSS*. **Results and discussion:** most of the subjects had engaged in their first intercourse at 17–18 years old (48.58%). The number of individuals who had started their sexual life earlier than at 17 years of age was higher in males and in young subjects ($p<0.001$). The protection at first intercourse was very low (38.4%) and the condom was the main method (34%). The protection was significantly higher in urban environment and in case of people with average and high educational level ($p<0.001$). As for the information on the subject of sexual behavior, the smallest contribution came from doctors and sanitary staff (12.6%), from the school (15.2%), from parents, relatives (17%). Friends and acquaintances contributed to the largest extent - 45.7%, and the contribution of Internet was 41%. **Conclusions:** Diminishing the pregnancy rate in teenagers and the unwanted pregnancy rate, as well as the rate of sexually transmitted infections, requires a multidisciplinary approach in the prevention programmes. The school and parents are crucial factors within the education programmes. **Keywords:** FIRST INTERCOURSE, CONDOM USE, SEXUAL AND REPRODUCTIVE HEALTH, SEX EDUCATION

Early initiation of sexual activity can have multiple risks such as: cervical cancer, breast cancer, early aging, infertility, infection with Human Immunodeficiency Virus (HIV) and other sexually transmitted infections **(**STIs). The youth who start their sexual life too early are in danger of having more sexual partners, of using condoms to a lesser extent and of engaging in other health risk behaviors, such as: alcohol consumption, smoking, drug consumption, delinquency, violence (1, 2).

The age of beginning sexual life is determined by biological aspects such as menarche and pollution, but especially by culture, environment, society, and the

world in which the individual lives. Religious education, religious belonging, their history and the constitutional provisions or the supplementary legislation regarding Church have a more or less important influence on the sexual and reproductive behavior (3, 4, 5).

The sources of information have an important role in the formation of the concepts of sex, sexuality, gender and in the development of sexual behavior.

In 1990, after the collapse of Communism and the establishment of a democratic regime, the health of women and children was very precarious, which imposed since 1993 the launch of public health programmes and strategies for family planning by modern means of contraception, the improvement of neonatology services, for the control of STIs and HIV, for the screening for genital and breast cancer, for sexual education etc. Unfortunately, since 2006, Romania has not had any national strategy in the field of health, sexual rights and reproduction. As a result of the precarious presence of national programmes of prevention and education on SRH, as compared with western European countries, Romania reports one of the highest incidence of Cervical Cancer and one of the highest rates of diagnoses of syphilis (6, 7).

Taking into consideration the facts presented above, this study aims at the determination of: age at initiation of sexual life, protection at first intercourse (FI), sources of information regarding sexual and reproductive health (SRH), and impact of social-demographic variables on these items in Romania. The trends identified by this study regarding sexual activity and the sources of information in the field of sex can form the basis of public health programmes which are targeting the SRH.

MATERIAL AND METHODS

Design and sampling, setting of the study. This research was conducted using a cross-sectional study with quantitative data. The sample, which is not representative for the whole country, includes N=564 subjects. The distribution of the subjects was relatively homogenous with respect to environment, gender, educational level, and age (tab. I).

TABLE I

Distribution of subjects according to environment, gender, educational level and age group (socio-demographic characteristics)

Social-demographic characteristics	Number	%
Environment		
Urban	317	56.2
Rural	247	43.8
Gender		
Male	265	47.0
Female	299	53.0
Educational level		
Low (primary education <10 years)	158	28.0
Medium (secondary education 10–12 years)	229	40.6
High [a]	177	31.4
Age groups (years)		
18–26	317	56.2
27–35	247	43.8

Legend: [a]·Tertiary education >12 years, completed by obtaining a diploma. University diploma. Post-university diploma

The study was conducted between 2011–2012 in urban areas within Bucharest, Craiova and Satu Mare; it was also carried out in the villages of Cioroiaşi and Stolnici and in villages in the county of Satu Mare.

Informed written consent was obtained from each participant at recruitment. The subjects were informed that they could withdraw from the study at any stage and they were assured of confidentiality. The study was approved by the Ethics Commission of the "Francisc I. Rainer" Anthropology Institute of the Romanian Academy.

Questionnaire design, data management and statistical analysis. We used an *omnibus* questionnaire of 96 items that focused on topics related to family functioning: domestic economics, education, cohesion & solidarity and sexual reproduction (8). In the present study we focused on three items: age at FI, protection at FI, sources of information regarding SRH. The Pearson chi-square tests were employed using the statistical programs *SPSS (SPSS* Inc., Chicago, IL). Demographic variables used in the statistical analyses were: environment, gender, educational level and age group.

RESULTS

***First intercourse*.** At the moment of investigation, a number of 15 subjects (2.7%), of which 13 women, had not started their sexual life. Among the 549 subjects who had started their sexual life, most of them, 48.58% (N=266), had engaged in the first intercourse at 17–18 years old. The ratio of those who had started their sexual life between 15–16 years old was 21.1%, and those who had had FI between 13–14 years old amounted to 5%. In the whole sample, the average age at the initiation of sexual life was 17.67 years, median 17 years with a minimum of 13 years, a maximum of 30 years. The average age at FI was higher in the urban environment (17.84 years in urban vs. 17.46 years in rural area) was higher for women (18.08 years females vs. 17.23 years males), for individuals with a higher educational level (18.19 years in high-level education subjects vs. 17.44 years in subjects with low level of education). The average age of virginity loss decreases with the age of the respondent; the average age at the start of sexual life in the lot born between 1976 and 1984 was 18.41, and in the lot born between 1985 and 1993 it was 17.07 years. The ratio of the individuals who started their sexual life at an age earlier than 17 years was higher in men ($p<0.001$). Those with medium and high levels of education tend to begin sexual life at an age higher than 17 years ($p=0.015$). The ratio of the individuals who started their sexual life earlier than at the age of 17 years is higher in young subjects, ranging between 18-26 years old ($p<0.001$) (tab. II).

Protection at first intercourse. The protection at FI was very low, only 38.4% (N=211) of the 549 subjects who had started their sexual life used protection. Condoms were the main protection method at FI, namely 34% (N=192). The protection was significantly higher in individuals from urban environment ($p<0.001$) and in those with average and high educational level ($p<0.001$) (tab. III). Both men and women, in similar proportions, used no protection at all against STIs or pregnancy at their FI.

TABLE II

Distribution of subjects by groups of age at FI, according to gender, educational level and age groups

Age groups at FI	Gender*		Educational level**			Age groups (years)*	
	Male	Female	Low	Medium	High	18-26	27-35
<=15	74.6	25.4	32.2	39.0	28.8	61.0	39,0
16–17	51.7	48.3	27.8	46.1	26.1	71.7	28,3
18–19	39.0	61.0	28.6	39.6	31.9	46.7	53,3
>= 20	37.2	62.8	24.4	26.9	48.7	20.5	79,5
Total	47.9	52.1	28.1	40.4	31.5	55.0	45

*p<0.001; **p<0.05

TABLE III

Distribution of subjects regarding protection at FI, depending on environment and educational level

Protection at FI	Environment *		Educational level*		
	Urban	Rural	Low	Medium	High
No	47.6	52.4	34.3	37.3	28.4
Yes	68.7	31.3	18.0	45.5	36.5
Total	55.7	44.3	28.1	40.4	31.5

* p<0.001

Sources of information on sex. To the information on the subject of sexual behavior, the smallest contribution came from doctors and sanitary staff (12.6 %), from the school (15.2), from parents, relatives (17%). Friends and acquaintances contributed to the largest extent – 45.7% - to supplying information on the topic of sexual behavior; then followed the mass-media and the Internet with a contribution of 41%, and specialized books with 24.3%. In most cases, subjects from urban environment, women, and people with average and high educational level received to a greater extent information from all sources (tab. IV).

TABLE IV

Distribution of subjects regarding the sources of information on sex, depending on socio-demographic characteristics

Sources of information on sex		Environment		Gender		Educational level		
		Urban	Rural	Male	Female	Low	Medium	High
Doctors, sanitary staff	A little[a]	54.2**	45.8**	49.1**	50.9**	30.2**	39.1**	30.6**
	A lot	70.4**	29.6**	32.4**	67.6**	12.7**	50.7**	36.6**
School	A little[a]	56.1***	43.9***	47.7***	52.3***	30.8*	35.8*	33.5*
	A lot	57.0***	43.0***	43.0***	57.0***	12.8*	67.4*	19.8*
Parents, relatives	A little[a]	52.6*	47.4*	50.4*	49.6*	30.8**	37.6**	31.6**
	A lot	74.0*	26.0*	30.2*	69.8*	14.6**	55.2**	30.2**

Sources of information on sex		Environment		Gender		Educational level		
		Urban	Rural	Male	Female	Low	Medium	High
Specialized books	A little[a]	49.6*	50.4*	51.3*	48.7*	35.1*	39.6*	25.3*
	A lot	76.6*	23.4*	33.6*	66.4*	5.8*	43.8*	50.4*
Newspapers, radio, TV, internet	A little[a]	42.9*	57.1*	48.0***	52.0***	41.4*	36.0*	22.5*
	A lot	75.3*	24.7*	45.5***	54.5***	8.7*	47.2*	44.2*
Friends, acquaintances	A little[a]	51.0**	49.0**	41.2**	58.8**	31.4**	36.3**	32.4**
	A lot	62.4**	37.6**	53.9**	46.1**	24.0**	45.7**	30.2**

[a] Little, almost none; *p<0.001; **p<0.01; ***p>0.5

DISCUSSION

First intercourse. Many teenagers who started their sexual life earlier, without being ready for it, had regrets and did not really enjoy that unique experience. Most of them acted under the psychological pressure of peer group norms, to be accepted and valued (9).

When comparing the data of an American study conducted on teenagers which the prevalence of early sex, defined as having one's first sexual intercourse before age 14, a much lower prevalence is obvious for Romania (more than one third in the American study vs. 0.4 % in the present Romanian study) (10). Just like other studies, this study shows that women have waited longer before having their first sexual experience (11). This could be explained by the following aspects: Young men are encouraged to lose their virginity and to have as many sexual partners as possible as a sign of virility and manhood. On the contrary, girls are encouraged to postpone as much as possible the moment of their first sexual act, to have a small number of partners as a proof of sobriety and obedience.

Just like other studies, we identified a change of patterns of sexual activity by lots of age (12, 13). The lot born in the interval 1985-1993 started sexual life at an average age lower by almost one and a half years as compared to the lot born between 1976 and 1984.

Protection at first sexual intercourse. In Romania after 1990 it was possible to legally use modern means of contraception. So, those who were born in 1976, being 35 years old in 2011, when the data were collected, possibly starting their sexual life at the age of 13, had every possibility to practice protected sex. The ratios of subjects who did not use any protection at FI (61.6%) are alarmingly high. When comparing the studies conducted in Romania: Reproductive Health Survey Romania, 1999 (RHSR 1999), Reproductive Health Survey Romania, 2004, and the Study Rada–Tarcea (2005–2006) (14, 15, 16), the present study shows that the prevalence of using the condom in the population between 18-35 years old has slightly increased.

There are studies which identified that potential condom users raised the question that the condom could reduce the pleasure of sexual intercourse (17, 18). Therefore, one of the methods of encouraging protection could be the stronger highlighting of advantages and positive consequences in the long run of using the condom with occasional partners, as against the risks of unsafe sex. At the same time, it is necessary to make men responsible with respect to SRH and to educate women to negotiate the use of condom.

Sources of information on sex. Just like other studies (19), in this study it has

been found that friends and acquaintances contributed to the largest extent to the information on the subject of sexual behavior. Pertinent sources such as school, doctors, sanitary staff, are very poorly represented. Almost half had mass-media as a source of information. In comparison with the Romanian study RHSR 1999, we have identified an increase of the contribution of doctors and mass media and a decrease of the contribution of friends and acquaintances. E-health (healthcare, offering health-related information by Internet) must be regarded as an opportunity of improving the healthcare services. The greatest challenge for e-health is the accuracy and reliability of information. Internet is teeming with inaccurate, incomplete information which can lead to making wrong decisions (20).

In Romania, after 1990, volunteers were trained by international agencies and non-governmental organizations which taught in high schools lessons about the health of reproduction, family planning and STIs. Starting with the school year 2004-2005, the optional subject Education for Health was introduced in the curriculum for grades I–XII (21). Unfortunately, in the school year 2011-2013, only a few of the students attended classes for this subject matter.

Parents can play an important role in protecting youth against risky sexual behaviors, especially if they are well informed. The studies show that when parents discuss with their children about SRH and when they monitor their behavior, young people are less likely to exhibit a risky behavior, both in general circumstances and in sexual matters in particular (22).

In this study, communication with parents on questions relating to sexual and reproductive health was very scarce.

CONCLUSIONS

The results of the study show that Romania, just like other ex-Communist East-European countries, has caught up with the western countries as to the issues of sexual behavior. The main issues are a poorer implementation rate of the education for SRH, and insufficient dealing in education and teaching with the psychological and emotional elements of a relationship.

As for behaviors with risk for SRH, such as FI with no protection, and age of FI 17 years or less, it has been ascertained in the rural environment, in men and in those with low and average educational level. As compared to the oldest studies conducted in Romania, the present study shows only a slight improvement of obtaining information on sex from specialists in the field, from school and parents. For diminishing the pregnancy rate in teenagers and the rate of unwanted pregnancies, as well as the STIs rate, a multidisciplinary approach is required in the prevention programmes. Both school and parents are crucial factors in this extensive undertaking within the education programmes for SRH.

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NEWS

LYMPHOPROLIFERATIVE CONDITIONS OF THE SEROSA

A diverse and complex variety of lymphoproliferative diseases may involve the serosa, with widely differing clinical outcomes encompassing a spectrum of benign and malignant conditions. The World Health Organization classification of hematologic malignancies recognizes each disease by distinct clinical, morphologic, immunohistochemical, and genetic features. Accurate diagnosis commences with the morphologic interpretation but also requires numerous sophisticated tests such as immunohistochemistry, flow cytometry, molecular genetic clonality studies, and molecular cytogenetics (eg, fluorescence in situ hybridization). All forms of hematologic malignancy may involve the various serosal sites, although this is usually observed as secondary involvement in persons with known lymph nodal, marrow-based, or extranodal disease. Primary pericardial, pleural, and peritoneal lymphomas are rare. Many nonneoplastic conditions may mimic lymphoma and a variety of nonhematolymphoid tumors may simulate hematologic malignancies. An understanding of the role of ancillary tests, together with an appreciation of their limitations, will prevent misdiagnosis (Richard Attanoos. Lymphoproliferative Conditions of the Serosa. *Arch Pathol Lab Med*. 2012;136:268–276).

Doina Butcovan

ROMANIAN BLOOD DONORS SCREENING: IS IT REALLY NECESSARY AND/OR MANDATORY?

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ROMANIAN BLOOD DONORS SCREENING: IS IT REALLY NECESSARY AND/OR MANDATORY? (Abstract): Blood services are required to provide the safest possible products, but no transfusion can ever be totally free of the risk of transfusion transmissible infections (TTI). Over the past decade, the risk of TTI through transfusion has been reduced (e.g. 1 in 300 000 for HBV to 1 in 2 million for HIV). With the introduction in 1999 of sensitive and expensive nucleic acid testing (NAT) technology in some countries, the disease transmission rate and the window period have been significantly reduced, but a remaining concern is the chance that a blood donor will be infected and not detected by such tests. To obtain safe blood and blood components it is important to ensure that the donors are healthy and free from TTI by using a donor selection procedure meticulously made, using a donor questionnaire to assess donor health and safety and for reducing the risk of transmission of infection, in particular for infections for which no suitable screening tests are available. In Romania the prevalence of TTI among donor population is high in comparison with other European Union (EU) countries. This may require significant improvements in the screening process of both donors and donations to minimize the infectious risk. **Key words**: BLOOD DONATION, TRANSFUSION TRANSMISSIBLE INFECTIONS, SCREENING

Worldwide, more than 92 million blood donations are collected annually. The demand for blood and its components is increasing and there is a need for more donors to be recruited and retained. An estimated 1.6 million units are discarded annually due to the presence of infectious markers for transfusion transmissible infections (TTI).

In developed countries, blood transfusion or plasma-derived products have been the main route of HCV transmission. Zero risk, which has been the goal, has yet to be achieved. False negatives still persist and transmissions of transfusion transmissible viruses still occur, although rarely (1).

In all south-eastern Europe countries, including Romania, the prevalence of TTI among donor population is high in comparison with others European Union (EU) countries (tab. I) (2).

A study performed by Brumboiu *et al* in 2003-2010 (submitted), in blood donors from Cluj and Satu Mare, two counties of the North West region of Romania, describes a higher seroprevalence for HCV in comparison with other studies (3).

Donors are our essential partners in ensuring a safe and adequate blood supply (4).

The difficult question is how to balance the burdening of the donor, faced with an

increasing number of very private questions, with the voluntary character of donating blood (5).

TABLE I
Prevalence of TTI in donor population from SEE countries

COUNTRY	HIV%	HBV%	HCV%	SYPH%	HTLV
Albania	0.01	3.02	0.13	0	-
Bosnia and Herzegovina*	0.038	0.504	0.389	0.179	-
Serbia	0.030	0.973	0.316	0.145	-
Federation BH	0	1.85	0.950	0.037	-
Brcko district of FBH					
Bulgaria**	0.0039	2.12	0.38	0.06	-
Croatia***	0.003	0.022	0.021	0.012	-
Republic of Moldova	0.025	5.8	3.0	2.6	-
Romania	0.010	0.32	0.045	0.40	0.001
Serbia	0.0095	0.25	0.21	NA	-
Montenegro	0.001	0.30	0.30	0.13	-
The former Yugoslav Republic of Macedonia	0.00	1.32	0.53	0.39	-

* No confirmation tests performed
** Data for first time donors
*** Confirmed positive
(source: national reports) Albania, Bosnia and Herzegovina, Bulgaria, Croatia, the Republic of Moldova, Romania, Serbia and Montenegro and The former Yugoslav Republic of Macedonia

WHO. Blood services in south-eastern Europe:Current status and challenges. 2007

This paper aims to summarise the necessity and possibility of minimizing the infectious risk in order to assure safe blood products in Romania.

Transfusion transmissible infections (TTI)

While in the past, the risk of transfusion-transmitted infections (TTI) was accepted by patients and physicians as unavoidable, a low-risk blood supply is expected today. Since the early nineteen sixties, blood banks, as well as plasma manufacturing industries, have aggressively pursued strategies to reduce the risks of TTI (6).

The magnitude of the TTI varies from country to country, depending on TTI's load in that particular population from where blood units are sourced. 7 There is a long list of viruses, parasites and bacteria which can be transmitted through blood transfusions. Among them, from far the most important TTI are human immunodeficiency virus (HIV-I/II), hepatitis B virus (HBV), hepatitis C virus (HCV), syphilis and transfusion-associated malaria infection.

Given the potential transmission of viruses during the 'immunological window period' [i.e. the period of early infectivity when an immunologic test is non-reactive], novel non-serology based approaches such as viral nucleic acid testing (NAT) have been established (6).

Today, the residual risk of TT HBV infection varies between 0.75 per million blood donations in Australia, 3.6 – 8.5 in the USA and Canada, 0.91 – 8.7 in Northern Europe, 7.5 – 13.9 in Southern Europe up to 200 per million donations in Hong Kong, largely reflecting the global epidemiology of HBV. The estimated risk for HIV transmission to date is between 0.14 – 1.1 and for HCV between 0.10 – 2.33 per million units transfused (6)

Table II describes the trends and prevalence of markers for HBV, HCV, HIV and HTLV I in Romanian blood donors from 2000 to 2004 (2).

TABLE II
Trends and prevalence of TTI markers in Romanian blood donors, 2000 to 2004.

	TTI MARKERS							
	ANTI-HIV-1+2(*)		ANTI-HTLV-I/II(**)		ANTI-HCV		AgHBs	
	FTBD (%)	RBD (%)	FTBD (%)	RBD (%)	FTBD (%)	RBD (%)	FTBD (%)	RBD (%)
2000	0.040	0.0026	0.073	0.0111	1.33	0.030	5.30	0.067
2001	0.045	0.0026	0.089	0.0019	1.30	0.029	4.95	0.083
2002	0.020	0.0007	0.061	0.0001	1.27	0.027	5.11	0.075
2003	0.032	0.0040	0.043	0.0002	1.17	0.010	5.70	0.071
2004	0.038	0.0022	0.046	0.0002	1.21	0.010	5.40	0.073

Abbreviations:
FTBD: First Time Blood Donors
RBD: Repeat Blood Donations
Observations:
(*) - Only one case of HIV-2 has been confirmed in a FTBD.
(**) - Only HTLV-I but no HTLV-II has been confirmed.

WHO. Blood services in south-eastern Europe:Current status and challenges. 2007

Although the risk of transfusing a blood unit collected during the window period may be decreased using NAT, the actual benefit in most populations has first to be determined and should be balanced against

the complexity and high cost of performing NAT, including the infrastructure required. The potential benefit of detecting early infections and preventing possible transmissions of infection should be assessed in relation with the incidence and prevalence of infection in the blood donor population, the effectiveness of the blood donor selection process, the sensitivity of the serological screening currently undertaken and the ability to enhance this through (8).

Donor Selection

The purpose of donor selection is to assess the suitability of an individual to be a blood donor and that the blood products derived from this donation are safe for the recipients. Donor selection is designed to identify high risk volunteers and defer them from donating blood. 9It became clear that non-remuneration of donors is an important criterion to avoid high risk donors; this is the main reason why efforts have been made to promote a volunteer unpaid donor system in various countries in the world (5).

The promotion of voluntary non-remunerated blood donation in Romania is included in the current legislation of the blood service, but voluntary paid donors are still present in the country, 79% remunerated vs. 21%-non-remunerated donors (2).

By law, voluntary ”remunerated” donors receive one day of holiday, free admission to health care services and food tickets (2). An inadequate stock of blood usually forces transfusion service officials to turn to replacement or possibly ‘‘hidden’’ paid donors which might ultimately increase safety risks attributed to these types of donations (10)

It is known that individuals cannot objectively assess their own level of risk. Extensive questioning of all donors on their sexual behaviour could lead to a loss of existing donors who may find the process intrusive. 11 A study realized in 2012 in Cluj Blood Transfusion Centre highlights that the existing routine screening methods used in the selection process of blood donors cannot detect all risk factors for blood borne infections, particularly the sensitive behavioural risk factors (drug use, sexual promiscuity) (12).

One of the most debated issues is the definite deferral of men who had sexual contacts with other men (MSM) (even if it was once in their life), whereas heterosexual individuals are excluded for only one year after a one time risk for HIV infection. 11,13Van der Bij *et al* described that donors infected with HIV often reported male-to-male sex and a high proportion reported other sexual risk behaviours, such as anonymous sexual contacts.

Although some of this risky behaviour was associated with MSM, which is a permanent deferral for donating, more attention could be given to sexual risk behaviours in donor screening (14).

Ramezani and co-workers describe that among rejected unemployed donors the most common risk factor found was unsafe sexual conduct, which confirms that acquiring answers to standard questions from blood donors can prevent the transmission of blood-borne infectious diseases (15).

Another issue is that many donors either do not understand transfusion-related risks or they do have knowledge on transmission routes but believe it doesn’t apply to them. For example, a large proportion of HCV infected donors has a history of injecting drug use but deny such use at the time of screening for blood donation. People who have used and stopped using drugs in the past might not consider themselves as “addicts” or might even believe that their past injecting drug use is not harmful for the safety of the blood they now wish to donate

(14).

Hepatitis C was found mostly among intravenous drug users and their spouses, probable virus carriers and donors with a history of unsafe sexual contact (15). In Cluj blood donors population no intravenous illicit drug use was reported (12).

The key to minimize the infectious risk is the screening of both donors and donations. Screening is thus a two-stage approach: the donor selection process is the first stage of the process and laboratory testing represents the second stage (14, 16). This strategy is used to protect the supply excluding the high-risk and potentially infected donors by selection and laboratory screening the donated blood. After the systematic selection and screening of donors, the residual risk of transfusion-transmitted HCV is very low nowadays.

A study carried out in the Netherlands in 2006 found a significant correlation between blood-borne infectious diseases and high risk behaviour (9,15).

Donor selection appears to be an efficient and cost-effective way to reduce the risk of TTI to an acceptable level because high risk donors mirror a high incidence rate of TTI (17).

Donors Questionnaire

The donor questionnaire is the key tool in donor selection, being designed to protect potential donors who may be at risk for an adverse consequence of blood donation and to enhance recipient safety (13).

The screening of blood donors in Romania includes a donor self-deferral questionnaire, an interview with the responsible physician, investigating each donor for possible risk behaviour for TTI and laboratory testing used to ensure the safety of the blood supply. If a screening test sample is reactive, the donation will be discarded and more specific confirmatory testing will be asked. In most cases, even if the result of confirmatory testing is negative, donors are permanently deferred; however, in some countries, re-entry algorithms are permitted with certain donor retesting protocols (13).

Along with data collected during this screening process, each individual donor confirms by signature, that he or she has fully understood all the risks that would defer him or her from blood donation. The physician examining the donor qualifies him or her as eligible to donate blood, respecting the established criteria for donor selection, or defers the donor, thus protecting the safety of the blood stock and its potential users. In addition, all the collected blood units undergo laboratory testing for human immunodeficiency virus, hepatitis B virus, hepatitis C virus and syphilis.

The questionnaire is the only line of protection against certain infections for which no testing is performed, such as malaria, babesiosis, leishmaniasis, and Chagas disease (13).

Certain questions aim to identify recently infected individuals who may be undetectable by currently used screening tests (window-period donors). For example, donors are asked if they have had a tattoo or a needle stick injury in the last 12 months. Other questions aim to reduce the number of prevalent infections, in part to offset testing and inventory release errors or lack of test sensitivity. For example, intravenous drug users are permanently excluded from blood donation. (13).

It is unclear if individuals currently deferred for MSM behaviour would consider additional questions about behaviours specifically addressed to them as being discriminatory or as being a positive development in identification of a low-risk group that would then be allowed to donate (4).

The use of a donor questionnaire prompts donor selection staff to ask im-

portant questions and carefully assess the donor's health. By presenting all relevant information in a standard format, a donor questionnaire facilitates decisions on the acceptance or deferral of the donor.9 However, individuals who do not respond accurately or truthfully to the questionnaires about infectious disease risk factors at the time of screening represent a potential threat to the safety of the blood supply due to enhanced risk for window-period infections. There are donors, who had donated primarily to receive the results of an HIV test and nearly 25% thought it was reasonable to donate to be tested for HIV, and that 12% believed that donating with known risk factors is acceptable (5).

The decrease in HCV seroprevalence in blood donors observed in other studies was not present in the study performed by Brumboiu *et al.*, where the prevalence evolution trend was stable. Such trend may reflect the HCV prevalence in the general population, the need for maintaining the same methods for donors' selection 3 and at the same time the necessity of educating donors on transfusion-related risks and transmission routes for TTI.

CONCLUSIONS

In most countries - Romania included - it seems difficult to keep a balance between the increasing demand for blood and the need to recruit and retain more donors. What stays the most important is to ensure safe blood supply by excluding high risk donors. The assessment of the donor's health and TTI risks requires a sensitive, non-judgemental approach to ensure confidentiality (9).

Most blood donors perceive themselves to be healthy, but some are unsuitable to donate blood. Additional time and effort should be spent by the trained personnel who interview the donors to explain why all very private questions are necessary.

The process of selecting blood donors based on their potential exposure to transmissible diseases is not always simple and straightforward, nor is it always strictly and / or exclusively based on scientific and epidemiologic data (4).

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NEWS

PERINATAL CHARACTERISTICS AND BREAST CANCER RISK IN DAUGHTERS

Authors from Divisions of Cancer Epidemiology and Genetics, National Cancer Institute, in a paper published in Journal of Developmental Origins of Health and Disease, highlighted that the *in utero* origins of breast cancer are an increasing focus of research. However, the long time period between exposure and disease diagnosis, and the lack of standardized perinatal data collection makes this research challenging. Authors assessed perinatal factors, as proxies for *in utero* exposures, and breast cancer risk using pooled, population-based birth and cancer registry data. Birth registries provided information on perinatal exposures. Cases were females born in Norway, Sweden or Denmark who were subsequently diagnosed with primary, invasive breast cancer (n=1419). Ten controls for each case were selected from the birth registries matched on country and birth year (n=14,190). Relative risks (RRs) and 95% confidence intervals (CIs) were estimated using unconditional regression models. Breast cancer risk rose 7% (95% CI 2-13%) with every 500 g (roughly 1 S.D.) increase in birth weight and 7% for every 1 S.D. increase in birth length (95% CI 1-14%). The association with birth length was attenuated after adjustment for birth weight, while the increase in risk with birth weight remained with adjustment for birth length. Ponderal index and small- and large-for-gestational-age status were not better predictors of risk than either weight or length alone. Risk was not associated with maternal education or age, gestational duration, delivery type or birth order, or with several pregnancy complications, including preeclampsia. These data confirm the positive association between birth weight and breast cancer risk. Other pregnancy characteristics, including complications such as preeclampsia, do not appear to be involved in later breast carcinogenesis in young women. (Troisi R, Grotmol T, Jacobsen J, et al. Perinatal characteristics and breast cancer risk in daughters: a Scandinavian population-based study. *J Dev Orig Health Dis.* 2013; 4(1):35-41)

Alina Manole

NECROTIZING ENTEROCOLITIS, A RARE BUT SEVERE CONDITION WITH INSIDIOUS POSTOPERATIVE COMPLICATIONS

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Necrotizing enterocolitis, a rare but severe condition with insidious postoperative complications(Abstract): Necrotizing enterocolitis (NEC) is one of the most frequent causes of gastrointestinal perforation in premature neonates, only few case series and reports being described in adult patients. Early in the course of the disease, superficial mucosal ulceration, sub mucosal edema and hemorrhage occur. Further progression leads to transmural necrosis leading sometimes to bowel perforation. Six cases encountered in our clinic in recent years led us to resume discussions on necrotizing enteritis, not because it is a rare disease, but due to the severe postoperative complications. **Material and methods:** Our lot consisted of four stage 1 patients and two with Bell stage III NEC and severe intestinal injury, necrosis, and perforation. All of the patients were diagnosed preoperatory with other surgical conditions, like appendicitis with peritonitis, perforated duodenal ulcer or acute cholecystitis. **Results**: We present to review two cases. For patients undergoing laparotomy, resection of the involved intestine mandates either enterostomy formation or primary anastomosis. An intermediate option is laparotomy with intestinal resection and delayed anastomosis 48 to 72 hours later. Because of the small number of patients in our lot, we cannot advise a certain surgical treatment, but a strategy involving bienterostomy*per primam* should be further analyzed. The choice of operative intervention reflects multiple variables, including age, physiologic status, institutional resources and surgeon preference based on experience. Primary peritoneal drainage for perforated NEC may help to resuscitate and treat a critically ill patient initially, and in some instances, may be definitive operative intervention. **Conclusions**: Relatively rare disease, of unknown etiology and elusive pathogenesis, NEC has initial non-specific symptoms and clinical features that mimic more common surgical diseases. There is considerable controversy regarding which procedure is preferable. Currently, in the absence of rigorous evidence supporting the superiority of one approach over the other, surgical intervention depends mostly on the treating institution or the individual surgeon. **Keywords:** ENTEROCOLITIS, LAPAROTOMY, PERFORATION.

Necrotizing enterocolitis (NEC) is one of the most frequent causes of gastrointestinal perforation in premature neonates, only few case series and reports being described in adult patients. Relatively rare disease, of unknown etiology and elusive pathogenesis, has initial nonspecific symptoms and clinical features that mimic more common surgical diseases.

Due to the multifactorial nature of the

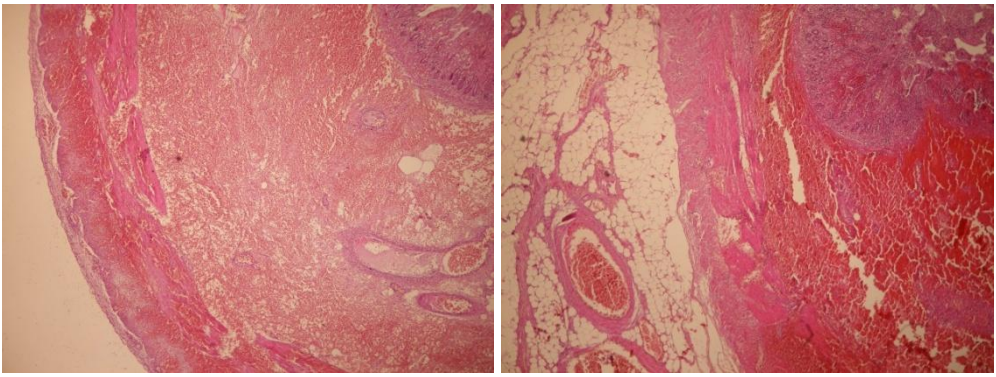
disease and limitations in disease models, NEC remains, above all, a problem of diagnosis and treatment, being accompanied by severe complications in evolution.

It is characterized by diffuse ulceration and necrosis of the distal small bowel and the colon, leading to perforation of the bowel, followed by high morbidity and mortality.

Although the exact etiology and pathogenesis of NEC remains elusive, it is well established that several bacterial and viral species have been associated with outbreaks of NEC (*Clostridium spp.*, *Klebsiella spp.*, *Staphylococcus epidermidis*, *Escherichia coli*, Rotavirus) (1). No single pathogen has been identified as causative and the ability of the microflora to colonize the epithelium and to ferment unabsorbed nutrients may be more important than the strain itself (2, 3).

Perforation seems to be caused by enteral acute ischemia due to quickly installed obstruction of the parietal microcirculation, strictly limited to the intestinal wall, without mesenteric involvement (4).

The ileum and proximal colon are the most commonly affected sites in NEC although any segment of the gastrointestinal tract can be involved including the stomach. Severity of bowel wall necrosis ranges from a small localized mucosal necrosis of a segment to transmural necrosis of the entire small intestine and colon in most severe cases (5).



a. **b.**

Fig. 1 a, b. Hemorrhage and transmural necrosis ("Sf. Pantelimon" Anatomopathological Laboratory)

In more advanced stages of NEC, pathological findings include gastrointestinal bleeding, inflammation, bacterial overgrowth, intestinal distension with multiple dilated loops of small bowel, pneumatosis-intestinalis, intestinal perforation, necrosis, hypotension, septic shock, pneumoperitoneum, and intra-abdominal fluid (6).

Six cases encountered in our clinic in recent years led us to resume discussions on necrotizing enteritis, not because it is a rare disease, but due to the severe postoperative complications (fig. 1 a, b).

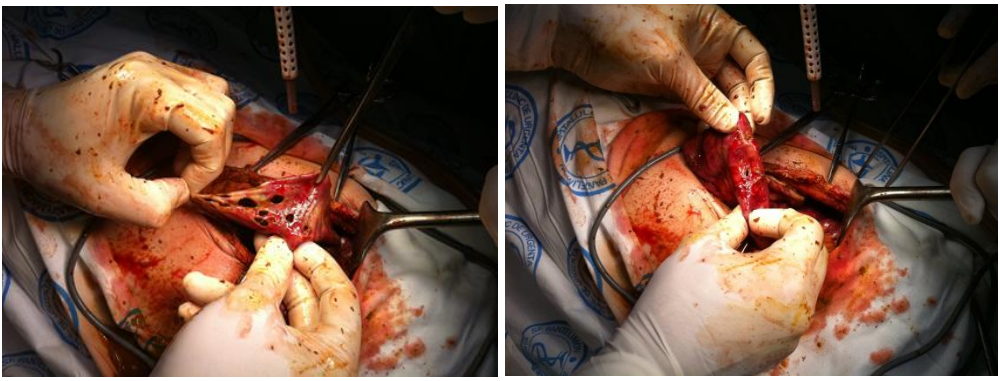
CASE REPORTS

First case, a man (MI aged 39 years) was admitted to the emergency room with the diagnosis of duodenal penetrating ulcer and pancreatitis. Admission status is marked by pallor and hypotension, severe

abdominal pain in a patient with grade II-III obesity.

First 12 hours of evolution are followed by shock, upper abdominal pain exacerbation and signs of peritoneal irritation requiring intervention (cholecystectomy for acute cholecystitis).

Due to multiple hydroaeric levels on Rx and bloating, reintervention was required for early postoperative ileus.



a. **b.**

Fig. 2 a, b. Enterectomy with termino-terminal anastomosis

Intraoperative: ecchymotic, thin-walled intestinal loops and multiple mesenteric lymph nodes. A jejunal segment of about 30 cm of necrosis and multiple perforations, blocked by the great omentum were discovered (fig. 2 a, b).

Starting at 6 days after reoperation follows a series of other 4 reinterventions imposed by anastomotic fistulas or perforations distant to the anastomosis.

The sixth intervention unveils both anastomotic failure and necrosis of afferent intestinal loop, requiring bienterostomy with Pezzer and Folley catheterization of intestinal ends. Enteral nutrition is used for the next 40 days, until complete extinction of peritonitis phenomena is achieved. A new reintervention with reintegration and anastomosis results in slow, favorable outcome with complete healing.

Second case also has special clinical features. Patient D.L. aged 15 years, was admitted for symptoms of acute appendicitis. Surgical intervention reveals acute catarrhal appendicitis for which appendectomy and drainage is performed. From the 5th postoperative day symptoms worsen and diffuse abdominal pain, bloating, lack of intestinal transit and fever require reintervention under the suspicion of peritonitis by cecal fistula. Laparotomy unveils generalized peritonitis with micro perforations of ileum in a 40 cm area, for which enterectomy and subsequent termino-terminal anastomosis are performed.

At the 11-day postoperative relaparotomy uncovers anastomosis failure and, in addition, an area of necrosis on jejunal loop, compelling right hemicolectomy and cuneiform resection of jejunal necrosis. After a couple of days, a new jejunal fistula required enterectomy and reanastomosis.

DISCUSSION

The main factors responsible for the development of NEC are intestinal ischemia

and bacterial infection. Although the etiology still remains unclear, the common organisms implicated are bacteria like *Klebsiella spp., E. coli, Enterobacter spp., Pseudomonas, Clostridium spp.,* and *Staphylococcus epidermidis*. It occurs most often in the areas of the bowel, where the blood flow from the major mesenteric arteries overlaps (7).

Early in the course of the disease, superficial mucosal ulceration, sub mucosal edema and hemorrhage occur. Further progression leads to transmural necrosis leading sometimes to bowel perforation. The clinical presentation include; abdominal distension, bilious vomiting and bloody diarrhea (5).

NEC can be classified clinical and radiological into 3 stages, as proposed by Bell and later modified by Walsh and Kliegman (5).

Stage 1 consists of non-specific clinical and radiological signs (fever, abdominal distension, dilated loops on X-ray and bowel thickening). Despite the presence of intestinal necrosis and perforation, pneumoperitoneum may not be observed. Stage 2 is characterized by blood in the stools, metabolic acidosis and classical signs of intestinal pneumatosis on X-ray. In stage 3, patients present with hypotension and shock, disseminated intravascular coagulation, occasionally peritonitis and sepsis.

Our lot consisted of four stage 1 patients and two with Bell stage III NEC and severe intestinal injury, necrosis, and perforation. All of the patients were diagnosed preoperatory with other surgical conditions, like appendicitis with peritonitis, perforated duodenal ulcer or acute cholecystitis.

Traditional surgical management of intestinal necrosis relies on the principles of controlling the source of peritoneal contamination by laparotomy, exploration of the intra-abdominal viscera, and excision of the involved, necrotic intestine.

Management options include: aggressive perioperative resuscitation, often including inotropic support; expedient and minimal operative behavior with attempts at limiting physiologic insult; evaluation of the entire intestine with resection of only necrotic or perforated bowel; enterostomy proximal to intestine of questionable viability; and preservation of the ileocecal valve if possible (8).

The choice of operative intervention reflects multiple variables, including age, physiologic status, institutional resources and surgeon preference based on experience.

For patients undergoing laparotomy, resection of the involved intestine mandates either enterostomy formation or primary anastomosis. An intermediate option is laparotomy with intestinal resection and delayed anastomosis 48 to 72 hours later.

Finally, primary peritoneal drainage for perforated NEC may help to resuscitate and treat a critically ill patient initially, and in some instances, may be definitive operative intervention.

There is considerable controversy regarding which procedure is preferable. Currently, in the absence of rigorous evidence supporting the superiority of one approach over the other, surgical intervention depends mostly on the treating institution or the individual surgeon.

These are studies that suggest that the type of operation performed for perforated necrotizing enterocolitis does not influence survival or other clinically important early outcomes in preterm infants (8).

Because of the small number of patients in our lot, we cannot advise a certain surgical treatment, but a strategy involving bien-

terostomy*per primam* should be further analyzed.

Necrotizing enterocolitis is a severe inflammatory disorder of the intestine and a major cause of death and morbidity. In contrast to medicine improvement during the past 30 years, the mortality rate of 30 to 50 percent for patients with intestinal perforation due to necrotizing enterocolitis remains essentially unchanged (9).

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RADICAL RETROPUBIC PROSTATECTOMY – INITIAL EXPERIENCE

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RADICAL RETROPUBIC PROSTATECTOMY - INITIAL EXPERIENCE (Abstract): **Aim:** To evaluate our experience with the first cases of radical retropubic prostatectomy and to use the results for patient counseling and optimizing therapeutic decision. **Material and Methods**: In the interval January 2011 - December 2012, 23 patients aged 56-69 years (mean age 62.6 years) were treated by radical retropubic prostatectomy. The retrospective study included an analysis of the significant data in the case records, surgical protocols and outpatient postoperative check-ups, special attention being given to indications, intra- and early postoperative complications (within 30 days) and hospital stay. **Results:** Preoperatively, prostate specific antigen (PSA) ranged between 4.5 and 27.2 ng/ml (mean 9.5 ng/ml), and Gleason score was 5 in one patient, 6 in 20, and 7 in 2 patients. Clinical stage was T1 in 5 patients (21.7%), T2 in 16 (69.6%) and T3 in 2 (8.7%). Mean intraoperative blood loss was 1214 ml (range 400-2500 ml), and 17 patients received blood transfusions. Postoperative complications included acute renal failure and extravasation of contrast medium during retrograde cystography in 3 cases each, urinary infection in 4 patients and wound dehiscence in 1 case. The average postoperative hospital stay was 19.57 days (range 13-43). **Conclusions:** Radical retropubic prostatectomy is a safe technique, involving a reduced risk of complications. For most patients with localized prostate cancer, radical prostatectomy is the best treatment option. **Keywords**: PROSTATE CANCER, RADICAL PROSTATECTOMY, COMPLICATIONS.

Of the therapeutic options in localized prostate cancer (PC), radical retropubic prostatectomy gives patients the best chance of survival. However, despite improvement in surgical technique (the so-called anatomic variation or with neurovascular bandelet preservation), radical retropubic prostatectomy is associated with the risk of severe complications (bleeding, urinary incontinence and erectile dysfunction). The aim of this study was to evaluate the experience with these first cases of radical retropubic prostatectomy and to use it in patient counseling and optimizing therapeutic decision.

MATERIAL AND METHODS

In the interval January 2011 - December 2012 at the Iasi Clinic of Urology and Kidney Transplant, radical retropubic prostatectomy was performed in 23 patients. The retrospective study included the analysis of the significant data in the medical records, protocols, outpatient postoperative check-ups, with special emphasis on indications, intra- and early postoperative complica-

tions (within 30 days), and hospital stay.

Preoperatively, prostate cancer was generally diagnosed by at least six prostate biopsies. Routine staging included digital rectal examination, PSA determination, and magnetic resonance imaging or abdominopelvic computed tomography. Bone scintigraphy was performed in only 5 patients (21.7%) in which PSA values were higher than 10 ng/ml (tab. I) (1).

TABLE I

TNM staging (2009) adapted, with localized prostate cancer highlighted

T – Primary tumor
T1 Clinically inapparent tumor, neither palpable nor visible by imaging **T1a** Tumor incidental histologic finding in ≤5% of tissue resected **T1b** Tumor incidental histologic finding in >5% of tissue resected **T1c** Tumor identified by needle biopsy because of elevated PSA
T2 Palpable tumor localized intraprostatic **T2a** it involves less than half of a prostatic lobe **T2b** it involves more than half of a prostatic lobe **T2c** it involves both prostatic lobes
T3 Tumor extends through the prostate capsule **T3a** extracapsular extension, including microscopic invasion of vesical neck **T3b** invades seminal vesicle
T4 Tumor is fixed or invades adjacent structures, other than seminal vesicles: external sphincter, rectum, levator muscles and/or pelvic wall
N – Regional lymph nodes
N0 no regional lymph node metastasis
N1 metastases in the regional lymph
M – Metastasis
M0 no metastasis
M1 metastasis **M1a** nonregional lymph nodes **M1b** bone **M1c** other sites

Postoperatively, urethral catheter was removed 10-14 days after surgery if retrograde cystography showed no contrast extravasation at the anastomotic site. Patients were discharged the next day if the surgical wound was healed and they urinated spontaneously.

The urethral catheter was removed 10-14 days after surgery if retrograde cystography showed no contrast extravasation at the anastomotic site. Patients were discharged one day after catheter removal if the surgical wound had healed, and they urinated spontaneously.

RESULTS

Patient age ranged between 56 and 69 years with an average of 62.6 years, and preoperative PSA level was 4.5 to 27.2 ng/ml (mean 9.5 ng/ml). Gleason score was 5 in one patient (4.3%), 6 in 20 patients (87%) and 7 in 2 patients (8.7%). Three patients (13.1%) received neoadjuvant hormone therapy (LH-RH analogues/antiandrogens) (tab. II).

The neurovascular bandelets were preserved in 19 patients (82.6%), bilateral in 13 (56.5%) and unilateral in 6 (26.1%). Pelvic lymphadenectomy was performed in one patient (4.3%).

Intraoperative blood loss was 400-2500 ml, with an average of 1214 ml, 17 patients (73.9%) requiring blood transfusion.

TABLE II

Clinical stage distribution

Clinical stage	Patients	
	No.	%
T1c	5	21.7
T2a	7	30.4
T2b	3	13.1
T2c	6	26.1
T3a	2	8.7

No death was recorded in the study group. In 14 patients (60.1%), postoperative course was simple, without complications. The complications, most of them minor, recorded in 9 patients (tab. III).

TABLE III

Postoperative complications

Complications	Cases	
	No.	%
Acute renal failure	3	13.1
Extravasation of contrast media (retrograde cystography)	3	13.1
Urinary infection	4	17.4
Surgical wound dehiscence	1	4.3

Urine loss following urethral catheter removal ranged between 30 and 1510 ml/day, with an average of 396 ml/day, but decreased 30 days postoperatively to an average of 204 ml/day (0 - 650 ml / day). Mean postoperative hospital stay was 19.57 days (range 13-43 days).

DISCUSSION

To determine the prognosis of the patients diagnosed with PC we applied the Partin tables (James Buchanan Brady Urological Institute, Johns Hopkins Medical Institutions, Baltimore, USA) and MSKCC nomogram (Memorial Sloan-Kettering Cancer Center, New York, USA), which are commonly used worldwide prior to initiating treatment.

The Partin tables use the clinical features of PC (Gleason score, serum PSA and clinical stage) to determine the likelihood of localized disease and the risk of extracapsular extension, seminal vesicle and regional lymph node invasion (2).

Pre-treatment MSKCC nomogram uses the following data: PSA value, Gleason score, clinical stage, number of positive and negative biopsies and possible neoadjuvant treatments (hormonal, radiation). The results obtained after processing the above data are

used to calculate the likelihood of malignant disease extension (localized disease, extracapsular extension, seminal vesicle and regional lymph node invasion) and the evolution after curative-intent therapy (5 and 10-year progression-free and 10 and 15-year cancer-specific survival). Progression-free survival means that PSA levels remain undetectable and PC will not progress after radical prostatectomy (3); cancer-specific survival estimates the likelihood of surviving prostate cancer within 10-15 years of radical prostatectomy (4).

The mean probability of localized disease (61.1%) and the risk of extracapsular extension (31.6%), seminal vesicle invasion (5.3%) and regional lymph node invasion (1.9%), as it results from the use of the Partin tables and the mean probability of localized disease (63.2%), extracapsular extension (30.6%), seminal vesicle invasion (8.8%) and regional lymph node invasion (3.7%) as it results from the use of MSKCC nomogram. Note the similitude in values and graphical presentations between the two techniques for assessing prognosis in prostate cancer patients (fig.1, fig.2).

Prognosis after radical prostatectomy calculated using MSKCC nomogram was encouraging; thus progression-free survival at 5 and 10 years was likely in 92.2% and 88.7% of the patients, respectively, while cancer-specific survival at 10 and 15 years was 99% (fig.3).

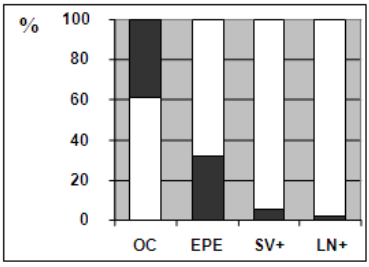

Fig. 1. Mean probability of organ-confined disease (OCD), extracapsular extension (EPE), seminal vesicle invasion (SV+) and regional lymph nodes (LN+), Partin tables

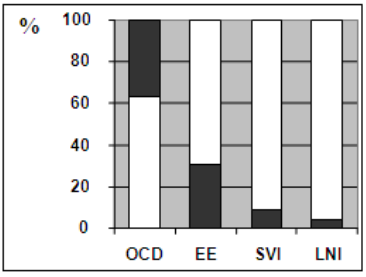

Fig. 2. Mean probability of organ-confined disease (OCD), extracapsulare extension (EE), seminal vesicle invasion (SV1) and reginal lymph node invasion (LNI), MSKCC nomogram

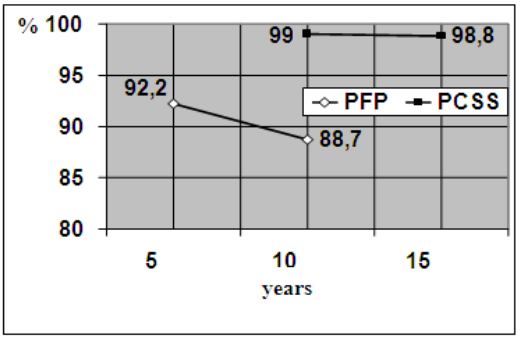

Fig. 3. Progression-free period (PFP) and cancer-specific survival Prognosis after radical prostatectomy, calculated by MSKCC nomogram)

Three patients (13.1%) developed acute renal failure in the early postoperative period, but it was rapidly resolved after hydroelectrolytic rebalancing.

Retrograde cystography revealed extravasation of contrast medium in 3 patients, requiring prolonged bladder catheterization.

Pelvic lymphadenectomy was performed in but one patient, the remaining patients being at low risk for dissemination according to the prognostic algorithm.

Besides the curative role of radical retropubic prostatectomy, patient quality of life, depending on the incidence of intra- and postoperative complications, especially of those with long-term impact (urinary incontinence, impotence) is a major aspect of this surgical technique (5).

Despite the small number of cases, our results are similar to those in the literature (5). This can be explained by the strict patient selection in terms of therapeutic indication and low level of co morbidities.

Three (13.1%) patients developed acute renal failure in the early postoperative period, rapidly resolved after electrolyte rebalancing. Extravasation of contrast material, demonstrated by retrograde cystography in 3 patients, required extended bladder drainage by urethral catheter. The 4 patients (17.4%) with postoperative urinary infection received specific antibiotic therapy and the patient with surgical wound dehiscence had secondary suture.

Pelvic lymphadenectomy was performed only in one patient because the remaining patients were at low risk for dissemination according to the prognostic algorithm.

CONCLUSIONS

Radical prostatectomy is considered the best therapeutic option in organ-confined PC for patients whose life expectancy is at least 10 years (6). The initial experience of the Iasi Urology and Kidney Transplant Clinic confirms this, also underlying the relatively low rate of intra-and postoperative complications.

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SURGICAL-ORTHODONTIC TREATMENT OF MALOCCLUSIONS THROUGH BEGG TECHNIQUE

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SURGICAL-ORTHODONTIC TREATMENT OF MALOCCLUSIONS THROUGH BEGG TECHNIQUE (Abstract): **Aim**: To assess the effectiveness and quality of surgical-orthodontic treatment outcomes through Begg technique in patients with malocclusions. **Material and methods**: Of a total of 117 patients referred to the Orthodontics Clinic Iasi, 16 patients with malocclusions (7 boys and 9 girls) were selected according to certain criteria and received surgical and orthodontic treatment through Begg technique. The outcomes were assessed clinically and radiological. Statistical analyzes were performed in *SPSS 16.0* for Windows. **Results**: 56.2% of the patients presented Angle Class I malocclusion, 12.5% Class II/1, and 31.3% Class II/2. Orthodontic therapeutic extractions were performed symmetrically on maxillary and/or mandible in 12 patients: first premolars in 18.8%, second premolars in 37.5%, or permanent first molars in 18.7%. Following extraction all patients were treated with Begg appliances. The duration of active treatment stages was variable, depending on stage results and some individual inconveniences. After five years, the therapeutic outcomes were stable, there were no post-treatment relapses, and the degree of root resorption of upper incisors that occurred in 60% of the patients was low. **Conclusions:** The indications of the orthodontic treatment through Begg technique have some limitations, the dental and periodontal response varied from patient to patient, major post-treatment root resorptions did not occur, and the assigned time was on average 6 month longer. **Keywords:** BEGG TECHNIQUE, MALOCCLUSIONS, PERMANENT TEETH EXTRACTIONS, ROOT RESORPTION.

Begg technique (TB) (1, 2, 3) is an original method that uses an arch-bracket system and a completely different treatment philosophy than Tweed system.

The basic principles used by Begg were the concept of attritional occlusion, differential force system, and the use of light orthodontic forces, and in collaboration with A. Wilcock (cit.1) the best alloy for orthodontic springs was developed. By the use of light orthodontic forces, this technique does not require extraoral anchorage, but it may be associated with minor oral surgery (extractions of permanent teeth for orthodontic treatment) and removable appliances.

Begg technique had been evolving over two decades, when many experts have adopted and developed system of brackets, archwires and auxiliary devices (3). In America and Europe it received wide popularity in the '70s, and had a considerable decline due to poor dental dimensional control (4). Currently this technique is practiced sporadically worldwide despite

its low cost, largely due to time-consuming archwire adjustment by the orthodontist.

We have assessed the surgical-orthodontic treatment by Begg technique in a series of patients with malocclusion in view of testing its effectiveness and the quality of treatment outcomes and compared them with results reported by other authors.

MATERIAL AND METHODS

The study was conducted on 16 patients (7 boys and 9 girls) with malocclusion, aged 12 to 19 years. They were selected from 117 patients according to the following criteria: age over 12 years, complete eruption of permanent molars, no dysfunction or vicious habits, and absence of Angle class III malocclusion. After obtaining the informed consent, all patients were subjected to clinical and laboratory examinations (model, photostatic, orthopantomography and lateral cephalometry studies) and surgical-orthodontic treatment by Begg technique.

Surgical extraction for orthodontic purposes or non-extraction was decided depending on space requirements and odontal status. Dental extractions were performed in the Clinic of Oral and Maxillofacial Surgery of the Iasi "St. Spiridon" University Emergency Hospital.

Patients were examined at each intermediate stage of treatment, at the end of retention period, and then annually for five years. The final results were assessed clinically. STRATO-X orthopantomograph with magnification factor of 11.8% was used for radiographic evaluation of the degree of root resorption of upper incisors, measuring directly on pre- and post-treatment retroalveolar radiographs the root height (distance from anatomic neck to apex).

Database and statistical analyzes were performed in SPSS 16.0 for Windows and descriptive statistics was used. Results were displayed in MS Excel.

RESULTS

Of the 16 randomized patients 56.2% (n = 9) presented Angle Class I malocclusions and 43.8% (n = 7) Angle Class II, of which 12.5% Class II/1 (n = 2) and 31.3% class II / 2 (n = 5) (fig. 1).

Minor oral surgery was initiated three weeks before the start of orthodontic treatment and extractions for orthodontic purpose were performed in 12 patients. To make space on arcades, tooth extractions were symmetrical on maxillary and/or mandible. To select the appropriate sites of orthodontic tooth extraction, facial esthetic criteria, odontal and periodontal status, facial growth pattern, and type of malocclusion were considered. Teeth selected for extraction were the first premolars (3 patients), second premolars (6 patients) or permanent first molars (3 patients) (fig. 2).

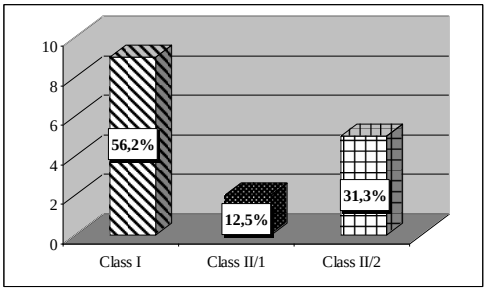

Fig. 1. Frequency of malocclusion types

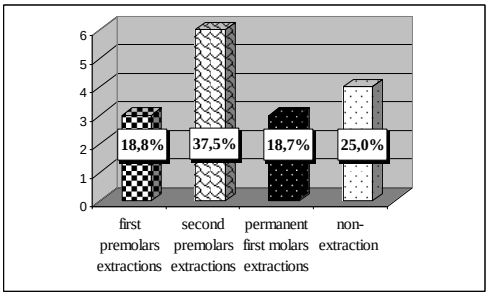

Fig. 2. Frequency of minor oral surgical treatment types

Orthodontic treatment was initiated simultaneously in the 16 patients. We used Begg-type fixed poly-aggregate appliances: orthodontic bands made with cylindrical and oval vestibular tubules and hooks on the molars, prefabricated brackets with vertical, round slots and pins for archwire fixation into brackets; main archwire, plain or looped, made by the orthodontist of two types of round cross section Australian wire (Regular and Special^{+}); second archwire for all types of root movements (derotation and uprighting), placed in bracket slot and attached to the main archwire; class I, II or III elastics. Begg appliance was placed monomaxillary (10 patients) and bimaxillary (6 patients), depending on the severity of malocclusions (fig. 3).

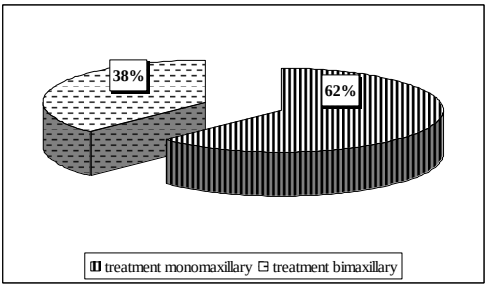

Fig. 3. Frequency of orthodontic treatment types

Orthodontic treatment was carried out in three active stages (alignment of teeth, spaces closure, uprighthing and torque), followed by retention period. The first stage of treatment had a mean duration of 9 months, during which crowding of teeth, frontal tooth rotations, overbite to an edge to edge relationship, open bite, crossbite, and axial relations of anchor permanent molars were corrected and spaces closed. The second stage of treatment lasted on average 7 months and was aimed at extraction space closure, distal tipping of the incisor crowns, over-correction of derotations, and completion of tooth movement. The third stage of treatment had a mean duration of 14 months and consisted in corrections of the mesiodistal, axial and vestibulo-oral relations, completion of over-corrections of derotations, and completion of tooth movement. Retention was achieved with such passive appliances as Hawley retainer and had a mean duration of 12 months (fig. 4).

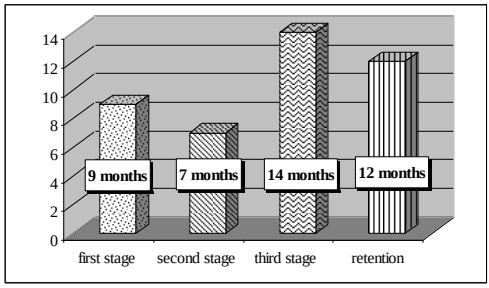

Fig. 4. Duration of orthodontic treatment stages

During treatment some subjective and objective problems have occurred, which prolonged the individual duration of orthodontic treatment. Some patients did not follow the monthly activation appointments, proper nutrition, good oral hygiene, frequently resulting in loosened brackets, ruptured rigid ligatures, arches deformed by chewing, band decementation, and gingival inflammation. In patients with rotation of inferior canine teeth and extractions of permanent lower first molars, rotation of lower second molars and early eruption of lower third molars required the simultaneous use of some removable appliances to increase control over second molars mezialization. In other patients there were excess space and periodontal reactions. Of the 16 patients, one abandoned treatment for aesthetic reasons.

Post-treatment results were stable over the 5 follow-up years in all patients and

there were no recurrences. Radiographically, the degree of root resorption of upper incisors was between 2-4 mm in 4 patients, between 1–2 mm in 5 patients, and undetectable or below 1 mm in 6 patients (fig. 5).

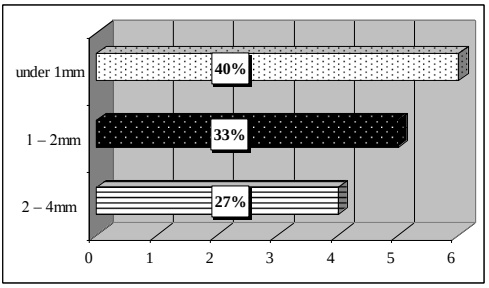

Fig. 5. Post-treatment degree of root resorption of upper incisors

DISCUSSION

In orthodontic practice, Begg technique, together with Edgewise technique, is a basic technique of fixed polyaggregate treatment. Begg technique is simpler and less expensive than Edgewise technique (1, 2, 3).

For a successful orthodontic treatment, Begg said that essential are a complete and correct diagnosis and individualized treatment protocol for each clinical case.

Our study patients presented dental and/or skeletal abnormalities specific to our population, in agreement with Begg's initial indications. Angle class III skeletal abnormalities were excluded from the study, because these are one of the limitations for application of Begg technique.

Controversy over extraction prior to orthodontic treatment and non-extraction has not yet been elucidated. Begg was in favor of extraction of four first premolars, application of the polyaggregate system three weeks later, and use of Class I, II and III elastic traction. Tooth extractions solve the need of space in frontal area in Angle class I or Angle class II incisive protrusion by applying in the first stages of treatment of elastic tractions that induce distoversion of canine teeth, and in the third stage of treatment of secondary archewires that causes root movements in the opposite direction and closure of extraction spaces (2).

Some of our study patients presented severe dental clinical situations, complicating the treatment plan by the absence of a second premolar or permanent first molar, which required the selection of other teeth for symmetrical extractions on two or four dental quadrants. For these reasons, in some patients treatment stages were longer and there were some unwanted dental movements of the permanent second molars, solved by the use of movable devices.

Begg appliance has the advantage of light orthodontic forces, minimal brackets-main archwire friction, rapid teeth alignment, dental occlusion opening, minimal oral anchorage, and low cost. Disadvantages are related to complex archwire modeling, difficult positioning of secondary arches, imprecise three-dimensional teeth control, absence of rectangular arches, monthly activation appointments, and inestethic components.

In our study patients, the duration of intermediate stages of treatment was short, but the finishing stage required a longer period of time due to the poor control of teeth positions by single-point brackets, as mentioned by Swain (5).

Some problems occurring during treatment were mentioned in the literature and determined changes in Begg appliance. Thus, the following were introduced: segmental arches, used in Edgewise technique for canine distalization (6), separate arch system for extraction and non-extraction cases (7), and Edgewise brackets on the front group and rectangular wire (8), which were beneficial to control tooth movement.

Dermaut and De Munck (9) have

demonstrated that there is an obvious correlation between the degree of tooth intrusion and root resorption. Scott and Green (10) have studied the phenomenon of root resorption after orthodontic appliance removal and concluded that it does not correlate with tooth movements during active treatment, but with other factors (traumatic occlusion, wearing retention devices). McFadden et al. (11) found that root resorption was present in some teeth before treatment due to increased individual susceptibility, and that after treatment with Begg appliance, root resorption was 95% in lower incisors, 90% in upper central incisors, 87% in upper lateral incisors, and between 79 and 72% in canine teeth.

Due to the use of light forces, the degree of root resorption in the incisive group was low and affected a small percentage of patients. The obtained results were stable over time, without significant changes five years after treatment. Forty percent of the patients experienced varying degrees of resorption (1, 2), similar with the data reported in the literature (12).

CONCLUSIONS

Researches have shown that not all malocclusions can be treated by Begg technique, and when associated with extractions, the dental and periodontal response varies from patient to patient. This technique does not cause large amount of root resorption, but time for treatment is on average 6 months longer than with other fixed techniques.

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NEUROPEPTIDE Y STIMULATION AS PRIMARY TARGET FOR PREVENTIVE MEASURES OF MALADAPTATIVE CARDIOVASCULAR REACTIONS IN OCCUPATIONAL CHRONIC STRESS EXPOSURE

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NEUROPEPTIDE Y STIMULATION AS PRIMARY TARGET FOR PREVENTIVE MEASURES OF MALADAPTATIVE CARDIOVASCULAR REACTIONS IN OCCUPATIONAL CHRONIC STRESS EXPOSURE (Abstract): Chronic stress may produce a decrease in central NPY expression and subjects exposed to it may prove hypersensitivity to a novel stressor with dysfunctions in the NPY system and cardiovascular maladaptation to stress, even hypertension. Upregulation of NPY expression may contribute to successful behavioral adaptation to stress by reducing cardiovascular tone and suppressing anxious behaviors. Adaptogens, a new class of metabolic regulators stimulate NPY expression and release. **The aim** of this study is to increase tolerance and adaptation to stress of hypersensitive to novel stressor, occupational chronic stress exposed subjects with cardiovascular maladaptation to mild new stressor using adaptogens as part of prevention protocol. **Material and methods:** 40 military personnel with known cardiostressor reactional mode and occupational chronic stress exposure were exposed to mild novel stressor: occupational medicine routine evaluation and clinically assessed for maladaptative cardiovascular response prior and before application of 30 day prevention protocol. Employees were randomly split in two groups, one receiving standard prevention protocol (lifestyle counseling) plus adaptogens in multiple dose administration, twice daily and the other receiving only standard prevention protocol. **Results:** We found significant statistic differences in all cardiovascular parameters in adaptogen group and only in diastolic blood pressure in control group. **Conclusions:** Adaptogens could be an important factor in successful prevention protocols of chronic occupational stress dysfunctions involving NPY systems. **Key words**: NPY, ADAPTOGENS, CHRONIC OCCUPATIONAL STRESS, CARDIOVASCULAR MALADAPTATION, NOVEL STRESSOR

Neuropeptide Y (NPY) is a central neuromodulator and peripheral sympathetic neurotrans mitter with significant regulatory roles in cardiovascular, neuroendocrine, immune and metabolic adaptation to stress and its release has been linked to a variety of stress-induced changes whose actions are dependent on the activation of multiple G-protein-coupled receptors,Y1-Y5 (1). In the brain the concentrations of NPY are significantly higher than other neuropeptides , being found mainly in the limbic system, including amygdala and hypothalamus, areas known for regulation of emo-

tional behaviours and stress response (2). Central, NPY acts as an anxiolytic and inhibits sympathetic activity with lowering blood pressure and heart rate resultants (3). Plasma NPY levels are positively related with behavioral performance under stress (4). Low plasma levels of NPY are associated with nervous autonomic system hyperactivity to stressors, hyperarousal, exaggerated alarm reactions and anxiety reactions (5). Chronic stress may produce a decrease in NPY expression in several central nervous system regions (6) and chronic stress exposed subjects may prove hypersensitivity to a novel stressor (7) with dysfunctions in the NPY system that might lead to cardiovascular maladaptation to stress and even hypertension (8). Heilig and Thorsel proposed that an upregulation of NPY expression may contribute to successful behavioral adaptation to stress through its buffering of stress promoting signals (9) and later it was demonstrated that activation of NPY systems reduces cardiovascular tone and suppresses anxious behaviours (8). Adaptogens, a new class of metabolic regulators which increase the ability of an organism to adapt to environmental factors and to avoid damage from such factors, raising the state of non-specific resistance (10) were recently proved to stimulate the expression of NPY and its release from neuroglia cells as primary upstream target (11). Panossian et al. study was done using ADAPT 232, a combination of *Eleutherococcus senticosus* root extract, *Schisandra chinensis* berry extract and *Rhodiola rosea* root extract and they demonstrated that increased tolerance and adaptation to stress obtained through this formula might be due to stimulation and release of NPY into systemic circulation as an innate defense response (12). Standard occupational prevention programs of maladaptation to chronic stress concentrate on lifestyle changes and relaxation methods promotion.

MATERIAL AND METHODS

Our study is open label randomized type of study done on 40 military personnel, all males, recruited during routine occupational medical evaluations between February and March 2012, in occupational medical office from Emergency Military Hospital, Iasi. Research group was randomly split in two, 20 employees being the study group (SG) and 20 employess being the control group (CG). All signed informed consent and the selection was based on inclusion and exclusion criteria. The inclusion criteria were age between 25 and 50 years, being healthy, having no significant history, BMI- 18.50-24.99 kg/m2, stress reactive type- cardiostressor- arterial blood pressure $\geq$ 140/80 mmHg and rest heart beat >80 beats/minut. The exclusion criteria were cardiovascular disease, endocrine disease, neurologic disease, obesity, arterial blood pressure <140/80 mmHg and rest heart beat <80 beats/minute.

Military employees are prone to more maladaptative cardiovascular stress responses than general population (5) and percentage of such dysfunctions is quite significant as we found in previous studies done in our occupational medicine office. Recent studies on stress protective on stress protective effect of a combination of adaptogens- ADAPT 232- through NPY expression stimulation and release from neuroglia cells (11) incited us to determine if this combination of standardized adaptogens might be useful as preventive measure in maladaptation to occupational stress. We considered the occupational medicine routine evaluation a mild novel stressor and

cardiovascular dysfunctions finded(arterial blood pressure ≥ 140/80 mmHg and rest heart beat >80 beats/ minute) an exaggerated alarm reaction and anxious behavior of a chronic occupational stressed hypersensitive subject with low central levels of NPY (5). We selected apparently healthy male military employees with known cardiostressor reactional mode, aged 25 to 50 years and as told earlier in the text, they were randomly split in 2 groups. Girst group,SG group received a standardized combination of adaptogens – Adaptonic- *Eleutherococcus senticosus* root extract, *Schisandra chinensis* berry extract and *Rhodiola rosea* root extract from *Alevia*, *Falticeni*, multiple dose administration, twice daily and lifestyle counselling and CG group received only standard protocol that is lifestyle counselling . We hypothesized that stimulation of NPY through Adaptonic will improve tolerance to novel stressors in a chronic occupational stress exposed subject. Subjects were reevaluated 30 days after, being exposed to same mild stressor but with 30 days interference and we studied alarm reaction through cardiovascular parameters.

RESULTS AND DISCUSSION

We used descriptive statistic studies and TTEST from Microsoft Excel 2003. There were no significant statistic differences between the two groups on age parameter (35.60±7.38 years for SG compared with 35.95±8.17 years for CG. We found significant statistic differences ($p<0.05$) in all cardiovascular parameters (tab.I). Thus the stimulation of NPY through the combination of adaptogens- Adaptonic- improved tolerance to novel stressor in the chronic stress exposed subjects.

TABLE I

Cardiovascular parameters in study and control groups before (t0) and after 30 days of standard protocol and adaptogens (t1)

Group	N	Age(years)	Systolic blood pressure in t0 (mmHg)	Systolic blood pressure in t1 (mmHg)	Diastolic blood pressure in t0 (mmHg)	Diastolic blood pressure in t1 (mmHg	Rest heart rate (beats/min) in t0	Rest heart rate (beats/min) in t1
Study group (SG)	20	35,60±7.38	156.20±11.63	138.50±6.3	91.50±7.27	73.25±7.38	96.50±7.96	74.85±6.01
Control group (CG)	20	35.95±8.16	154.80±9.75	154±9.4	90.25±7.34	86.50±6.97	96.15±10.70	94.80±8.91

In group 2 we found significant statistic differences only in parameter: diastolic blood pressure ($p<0.05$). This finding could be due to prior experience of NPY systems to the mild stressor we used that is medical evaluation, taking in account that NPY influence vascular tonus but adaptation to the stressor is incomplete as cardiovascular parameters: systolic blood pressure and rest heart rate had no significant statistic differences ($p>0.05$) (tab. I).

Testing plasma levels of NPY as routine measure of occupational medical evaluation in chronic military stress exposed subjects, with cardiovascular maladaptation to mild novel stressor might bring important, adia-

cent information about their alarm reaction capacities and gives us the possibility to correct any dysfunction of the stress response system before the units are involved in high-stress, high-risk operations.

CONCLUSIONS

We found out that adaptogens could be an important factor in a successful preventive measures protocol of chronic stress dysfunctions involving NPY systems. Early diagnosis of these type of dysfunctions and their treatment with a combination of adaptogens might prove organizational and individual health saving. Extensive studies on larger populations and with different occupations are neede for further development of the treatment protocol.

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EXTRAHEPATIC COMPLICATIONS OF CHRONIC CHOLESTASIS: CURRENT DIAGNOSIS AND TREATMENT

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EXTRAHEPATIC COMPLICATIONS OF CHRONIC CHOLESTASIS: CURRENT DIAGNOSIS AND TREATMENT (Abstract). Pruritus, fatigue and osteoporosis are the main symptoms of the extra hepatic manifestations of chronic cholestasis that affect patients' quality of life. Pruritus affects more often female patients, varies as intensity during a day and for longer period of time, typically can be localized on the palms of hands and soles of feet or can be generalized. Pruritus can be treated with anions resines exchange – cholestiramine, the pregnanne X receptor agonist Rifampicine, Naltrexone. Liver transplantation can be considered if severe pruritus remains refractory to all medical treatments. Fatigue is the most disabling complain in chronic colestasis. No specific therapies are available for fatigue and liver transplantation doesn't improve it. Osteoporosis and the risk of fractures are more severe with the duration and severity of hepatic disease. For treatment are recommended regular physical exercise, vitamin D and Ca supplimentation and bisphosphonates (Alendronate 70 mg/week) in severe cases. Only patients with atherosclerotic risk and hyperlipemia can be treated with statines. Fat soluble vitamin supplementation can be administrated only in symptomatic and proved vitamin deficiency. **Key words**: CHOLESTASIS, PRURITUS, FATIGUE, OSTEOPOROSIS

Intrahepatic chronic cholestasis is not just an isolated disease but a complex process resulting from a number of hepatic conditions, such as primary biliary cirrhosis (PBC), primary sclerosing cholangitis (PSC), drug-induced cholestasis, idiopathic biliary ductopeny in adults, cholestasis of pregnancy, cystic fibrosis, sarcoidosis, granulomatous hepatitis and HIV-associated cholestasis.

Complications of chronic cholestasis can be classified as hepatic (portal hypertension, ascites, encephalopathy, liver failure) and extrahepatic - frequent (pruritus, fatigue, osteoporosis, hyperlipidaemia, malabsorption of fat-soluble vitamins and steatorhhea) and rare (peptic ulcer, hyperpigmentation). Usually, extrahepatic complications of cholestasis are not fully appreciated. Their identification, early diagnosis and prophylactic or curative treatment leads to improvement in quality of life in patients with chronic cholestatic liver disease.

PRURITUS

Pruritus and lethargy are the commonest

extra hepatic manifestations of chronic cholestasis. In patients with cholestasis pruritus can represent either a minor problem, or on the contrary an extremely significant one, leading to loss of quality of life, attention deficit, social inconvenience, insomnia, poor intellectual function, depression or even suicide. Disabling chronic pruritus is an indication for liver transplant if all other therapeutic measures have failed (1, 2).

Pruritus in chronic cholestasis has a variable intensity throughout the day, as well as over longer periods of time. It is usually worst at night. It may also demonstrate a circadian variation, being worse in the afternoon (3). It usually affects the palms and feet and is not associated by characteristic skin lesions (4). Scratch marks secondary to pruritus can lead to eczema, skin changes, secondary bacterial infections and even sepsis (5).

Pruritus in chronic cholestasis diminishes in the final stages of chronic liver disease and appears to be inversely proportional to severity of cholestasis. It is more common in women compared to men. Its prevalence varies according to the chronic conditions that evolve with cholestasis. In PBC and PSC it is found in 25 – 80% of cases and in 5 – 15% of hepatitis C sufferers. In chronic hepatitis B and steathohepatitis pruritus is not usually manifested, despite the presence of cholestasis (6).

Etiology

The etiology of pruritus in chronic cholestasis is yet to be identified. The cholestatic liver appears to synthesize pruritus-inducing substances either directly or as cofactors (7). Pruritus has been attributed to a number of agents in the absence of any solid evidence: bile salts, steroid hormones, endogenous opioids, histamine and serotonin (8). Current research is focusing on autotaxine and its product lysophospatidic acid.

Bile salts were first associated with pruritus in cholestasis; however, the following were demonstrated:

- The level of serum, urine and skin bile salts does not correlate with the severity of pruritus;
- Low levels of bile salts are found in cases of severe pruritus.

A good example is that of pruritus in PBC. This can be severe in moderate forms of the disease (where bile salt levels are not significantly elevated) and minor or absent in severe forms (in the presence of high levels of bile salts). In conclusion, bile salts and their metabolites could have an indirect role in the etiology of pruritus in cholestasis (9).

Steroid hormones and their metabolites could be cofactors in cholestatic pruritus. This may be suggested by a higher prevalence of cholestasis in women and its presence in pregnancy, where pruritus is most intense in the third trimester where steroid hormones and their metabolites reach the highest concentrations. After delivery, the level of steroid hormones decreases together with pruritus, as well as the reduction in cholestasis parameters (10). It has been hypothesized that steroid hormones might modulate neuronal excitability of pruritus-mediating and/or nociceptive fibers in patients with cholestasis (6).

Endogenous Opioids. Although the administration of opioids can lead to pruritus, no relationship has been established between opioid concentration and intensity of pruritus. This is exemplified by a similar level of opioids present in PBC patients with and without pruritus (10). At present it is unclear how endogenous opioids lead to pruritus.

Histamine levels are raised in chronic cholestasis – however sufferers do not

respond to antihistamine therapy. Furthermore, skin changes associated with histamine release (erythema and urticaria) are missing in patients with cholestasis and pruritus. It is therefore unlikely that histamine is involved in the etiology of cholestatic pruritus (6).

Serotonin is not considered a mediator of pruritus, although cholestasis alters serotonin homeostasis, and consequently the perception of pruritus.

Lysophospatidic acid (LPA), being a potent neuronal activator, has the potential to cause pruritus in cholestasis. Studies have demonstrated that the serum level of ***autotaxine*** (LPA forming enzyme) is raised in patients with cholestasis, and is directly proportional with the intensity of pruritus (1).

Therapy

The Etiology of pruritus remains unknown. Despite this, many agents have demonstrated total or partial therapeutic benefit:

1. Drugs that bind or increase faecal excretion of pruritus-mediating agents: cholestyramine, colestipol and colesevelam. *Cholestyramine, colestipol and colesevelam* are anion exchange resins which bind pruritus-mediating substances and aid their faecal excretion. They bind bile salts in the bowel lumen, interrupting the enterohepatic circulation and leading to increased synthesis of bile salts from cholesterol in hepatocytes. Cholestyramine was the first drug used for the effective treatment of pruritus and remains a first line therapeutic agent, leading a reduction or even resolution of symptoms in 2 – 4 days. Recommended doses are 4 – 16 g / day (1, 6). Any other therapeutic agent can be bound by anion exchange resins (thryoxine, digoxin, oral contraceptives, ursodeoxycholic acid, etc) and these must be taken at least 2 – 4 hours prior to cholestyramine. Adverse reactions of cholestyramine include metallic taste, bloating, constipation, worsening of fat malabsorption and fat-soluble vitamins (2,10,12). Colestipol and colesevelam have not been investigated in clinical studies, and cannot therefore be recommended as first line therapy (13).

2. Enzyme inducers. *Rifampicin* is an enzymatic inducer and agonist of pregnane X receptor (PXR). It is used as second line therapy of cholestatic pruritus; it can be administered as first line therapy in those who cannot tolerate cholestyramine (1). Mechanism of action include (14): competing with bile acids for hepatocyte uptake; decreasing the intracellular concentration of bile acids, as well as their detergent activity on cell membranes; induce microsomale enzymes facilitating urinary excretion; modify gut flora leading to disturbance of the bile salt metabolism and that of pruritus-mediating substances. Pruritus subsides in less than a week from commencement of therapy. Doses administered are 150 mg/day if bilirubin is under 3mg/dl and 150 mg twice daily if bilirubin levels are greater than 3 mg/dl (2, 6); doses can reach up to 600 mg/day (1). Many adverse reactions have been described, affecting 12% of cases 2-3 months following treatment (12). Severe and frequent reactions include: drug-induced hepatitis, hemolytic anemia, renal failure and thrombocytopenia (15, 16). Due to these side effects initial doses must be low and carefully monitored once increased.

3. Therapy with agents which interfere with opioid neurotransmission represents third line therapy, according to European and US guideline. Cholestasis related pruritus is associated with a rise in opioidergic tonus, which interacts with opioid-mediated neurotransmission in order to reduce pruri-

tus. *Naltrexane* is an oral opioid antagonist which, according to literature meta-analyses, leads to effective reduction in pruritus (17). It is administered in increasing doses, starting with 12.5 mg. The dose is increased by 12.5 mg at 3-7 days until the pruritus is controlled. The maximum dose is 50 mg/day. Inpatients may be given IV *naloxone* 4 mg twice or three times per day, followed by *naltrexane* (1,6).

The use of opioid antagonists is limited by withdrawal-like reactions, which occur spontaneously and unpredictably. Some data suggest that these reactions may relate to the severity of the pruritus (18), although there is a lack of consensus. These reactions manifest as abdominal pains, a rise in arterial blood pressure, tachycardia, nightmares and depersonalization. Prolonged use may be associated with chronic pain syndrome (19). Patients must be monitored both clinically for the above mentioned adverse reactions, as well as biochemically due to potential liver toxicity.

4. Anti-depressants. According to the EASL guide, *sertraline* is a fourth line therapeutic agent, belonging to the antidepressesant class of drugs. It is a selective serotonin receptor inhibitor. Doses of 75 – 100 mg/day lead to almost complete remission of pruritus, independent of its antidepressant side-effects. The mechanism of action is not fully understood (20). Antipruritic results in patients with chronic cholestasis obtained with opioid antagonists and sertraline are inferior compared to those seen in patients treated with cholestyramine and rifampicin – these remain first and second line therapeutic modalities, respectively.

5. Extra-corporeal dialysis with albumin and plasmaphoresis are recommended in cases of disabling pruritus, resistant to therapies mentioned previously. Initial therapy involves 3 sessions per week and gradually is decreased to one session per week. This acts predominantly on unidentified plasma pruritus-mediating substances (12, 21, 22).

6. Liver transplant is indicated in pruritus caused by chronic cholestasis resistant to all other therapeutic measures mentioned above, having an increased risk of depression and suicide (23).

7. Other therapies used include anabolic hormones, dexamethasone, phototherapy, serotonin antagonists (ondansetron). Currently their value is only historical. *Androgenic steroids* (*norbetalon, methyltestosteron*) were used for a short duration. They act at a hepatocellular level. Their adverse reactions (worsening of cholestasis, virilisation, growth disturbances, and increased risk of hepatocellular carcinoma) and lack of benefit in improving choelstasis and pruritus lead to their removal from current therapeutic strategies (24). *Dexamethasone* was used in large doses (12 mg/day) in pregnancy-induced cholestasis. *Gabapentin* and *cimetidine*, similar to dexamethasone, have no proven effect in improving pruritus associated with chronic cholestasis. The presence of adverse reactions led to discontinued use. *Phototherapy*: ultraviolet light led to improvements in pruritus secondary to cholestasis. Starting from this observation, progressive exposure to ultraviolet light can decrease pruritus, but increases the risk of skin cancer. *Antihistamine therapy* is less effective (chronic cholestasis-associated pruritus is not associated with histamine release). Similar to *phenobarbital*, these have a sedative effect, and both address the insomnia associated with pruritus (1, 6). The use of phenobarbital can be associated with gingival hyperplasia. Antihistamine therapy can lead to dry oral mucosa, limiting their use in

chronic cholestasis associated with *sicca syndrome* (12). *Serotonin antagonists*: sfklmerotonin is involved in nociceptive neurotransmission. Ondansetron 4-8 mg three times per day, a serotonin type 3 receptor antagonist, has been used to treat chronic cholestatic pruritus without obvious improvements (25).

FATIGUE

Fatigue, together with pruritus, is a common symptom in chronic cholestasis. It has been predominantly studied in PBC (26). Tiredness in chronic cholestasis is not related to the severity of chronic liver disease and is not improved by liver transplantation (1). In order to attribute fatigue to chronic cholestasis, other concomitant pathologies need to be excluded, such as anemia, hypothyroidism, electrolyte abnormalities, diabetes, and depression.

Similar to pruritus, fatigue can be explained by changes in central serotonergic neurotransmitters (1). Different mechanisms have been suggested to explain fatigue, such as aberrant release of corticotrophin releasing factors (CRH) and accumulation of magnesium in the globus pallidus (26). In PBC fatigue is associated with changes in the autonomic nervous system (postural hypotension) and sleep disturbance, especially during the day (27).

Currently there is no available therapy specific for the fatigue associated with chronic cholestasis. In PBC studies have shown a reduction in daytime somnolence and objective tiredness (quantified through various scales and questionnaires) by using *Modefenil* 100 - 200 mg/day (28, 29). Psychological support and antioxidant therapy are required, as well as avoiding activities which exacerbate dysfunction of the autonomic nervous system (careful monitoring anti-hypertensive medication) and avoiding of sleep disturbance (avoiding coffee consumption in the afternoon) (1, 30).

BONE PATHOLOGY IN CHOLESTASIS

Osteodystrophy in cholestasis is mainly a consequence of osteoporosis and less one of osteomalacia (31). *Osteoporosis* represents a decrease in bone volume as a consequence of bone thinning with changes in micro architecture and a rise in the risk of spontaneous fracture. *Osteomalacia* is a defect in bone demineralization, secondary to a lack of vitamin D.

The majority of studies indicate that osteoporosis in chronic cholestasis has a moderate prevalence of 35%, with a wide range (9 – 90%), according to diagnostic criteria used and the degree of liver disease (32). Fractures secondary to osteoporosis have a prevalence of 2- 20% (33). The significance of osteoporosis and the risk of fracture increases with the severity and duration of hepatic cholestatic disease (1, 34). Other risk factors for osteoporosis independent of liver disease are: history of bone fractures, early menopause, and prolonged steroid therapy or with ion exchange resins, and not least alcohol consumption and smoking (34).

Disease mechanisms involved in the development of osteoporosis in cholestasis are not entirely clear. Some studies suggest impaired mechanisms of bone resorption, whilst others advocate changes in bone formation (35). High levels of osteoprotegerine in PBC seem to counterbalance the osteoclastic activity mediated by RANKL (Receptor activator of nuclear factor kappa-B ligand) (36).

The most accepted form of identifying osteoporosis involves the measurement of bone density through the biophotonic absorption of x-rays (DXA) in the vertebral

spine (L2 – L3) or in the superior segment of the femur. This method is quick, precise, reproducible and minimally invasive. Results are expressed though the Z score (number of standard deviations - SD compared to normal values in a person of similar age and sex) or T score (number of standard deviations from a absolute value in a young individual). According to the T score, the World Health Organization (WHO) classifies bone density in 4 levels:

Normal bone density - T score < -1 SD

Osteopenia - T score between -1 and -2.5 SD

Osteoporosis - T score < -2.5 SD

Severe osteoporosis - T score < -2.5 SD and at least one osteoporotic fracture

In addition to DXA assessment, a number of other parameters need to be quantified when monitoring of osteoporosis: calcium and phosphate metabolism, 25 OH – Vitamin D and parathyroid hormone. These need to be determined at the beginning of the assessment and at subsequent intervals (6 months to 3 years) according to the severity of cholestasis (1,12,32,37).

Therapy can be classified as being is prophylactic or curative.

Prophylactic treatment includes identifying and treating extra-hepatic risk factors previously described. Physical exercise, calcium and vitamin D intake and dietary supplements (Calcium: 1000 – 1500 mg/day; Vitamin D: 260 μg 25- OH vitamin D every 2 weeks or 400-800 IU/day) (32).

Currative tratement is commenced if DXA values are under -1.5 in the presence of previously mentioned risk factors or if the DXA score is ≤ 2.5 (1,12). *Bisphosphonate* non-hormonal therapies can also be used. Alendronate (70 mg/week for 2 years has an anti-resorptive effect, causing a rise in bone density and a decrease in the number of fractures (38). *Oestrogen based hormones* are no longer used due to hepatotoxic effects, whilst *calcitonin* therapy has been discontinued due to lack of treatment effect. Current evidence does not support the use of *Zolendronic acid* and *strontium* in the management of osteoporosis in cholestasis (12).

HYPERLIPIDAEMIA AND RISK OF ATHEROSCLEROSIS IN CHRONIC CHOLESTASIS

Studies investigating the risk of hyperlipidaemia in chronic cholestasis were predominantly performed on patients with PBC (39). These studies have shown that hypercholesterolemia in chronic cholestasis is not associated with clinical or subclinical atherosclerosis in a manner different from that in the general population (1, 2). Usually, in patients without cholestasis, the risk of atheroma formation is raised if the level of HDL cholesterol is low and LDL cholesterol levels are raised. In chronic cholestasis in PBC there are three factors with raised values and protective effects regarding the risk of atheroma formation in hyperlipidaemia: LpX originated from the oxidation of LDL and maintains it at a low level, HDL cholesterol and adiponectine. Consequently, only patients with chronic cholestasis with risk factors for atheroma formation benefit from therapy (39 – 41). Treatment consists of lifestyle changes (weight loss, physical activity, a good diet regarding cholesterol levels, etc) and medical therapy. *Statins* reduce LDL cholesterol, with minimal hepatic toxicity. They can be used in this group of patients in order to improve cholesterol levels. *Fibrates* (normally reduce the absorption of cholesterol, reduce inflammation and the production of antibodies) have not been shown to be efficacious in dyslipidaemias seen in chronic cholestasis (42). Current research

is investigating the role of *Ezetimibe*, which inhibits the intestinal absorption of cholesterol (Niemann Pick C1 like protein). This alone or in combination with statin reduces LDL cholesterol in cholestasis (43, 44). A promising therapy in dyslipidaemia in cholestasis is the *nuclear receptor inhibitors* which impact on cholesterol metabolism, the synthesis of bile acids and hepatic mechanisms of detoxification in chronic cholestasis (45).

MALABSORPTION OF FAT SOLUBLE VITAMINS (A, D, E, K)

A healthy adult stores 500,000 units of **vitamin A** in the liver as retinol – this reserve lasts 12 months. Symptomatic deficits of vitamin A are unusual in adults with cholestasis (46).

The assessment of vitamin A status in cholestasis is difficult and requires measurement of serum retinol levels, serum retinol/retinol binding protein, as well as ophthalmological assessment of adaptation to night vision. Asymptomatic deficit of vitamin A can be corrected using 15,000 U / day, followed by 45,000 U twice weekly. In symptomatic deficits, 100,000 U of retynil palmitate are administered IM every 2 months for 6 months, followed by oral vitamin A supplementation of 45 – 50,000 U twice weekly (47). Vitamin E aids the intestinal absorption of other fat-soluble vitamins and is recommended as an adjuvant in vitamin A deficiency (11).

Vitamin D. Vitamin D requires bile acids in order to be intestinally absorbed. Furthermore, vitamin D is transported in blood bound to proteins synthesized in the liver – patients with liver disease and biliary stasis do not produce sufficient transport proteins. Vitamin D levels are determined by serum measurement of 25-OH vitamin D levels (normal levels 35 – 55 μg/ml). Osteomalacia is the clinical manifestations of vitamin D deficit. Treatment consists of exposure to sunlight, physical exercise, monthly IM administration of 100,000 U vitamin D2 or D3 or daily oral administration of 50 – 100 μg 25-OH vitamin D. Vitamin D must not be given without adequate monitoring (47, 48).

Vitamin E. To determine vitamin E status in cholestasis it is necessary to determine serum concentration of vitamin E (normal levels 5-20 μg/ml). In hyperlipidaemia, vitamin E is bound to lipoproteins; consequently the vitamin E / total lipid level ratio (normal adult level 0.8 tocopherol / g total lipids) reflects vitamin E levels better (11). Vitamin E deposits provide sufficient reserve for 1 – 2 years, with degenerative changes in the motor neurological system occurring only after 5 – 10 years of vitamin deficiency. Whilst progressive muscular degeneration in children with cholestasis is frequent, in adults neuropathy is unusual. Neurological changes affect primarily the posterior columns and are characterised by the absence of reflexes or loss of proprioception and ataxia. Correcting Vitamin E deficiency in children is achieved with α tocopherol, α tocopherol acetate or α tocopherol succinate. The initial dose 10 – 25 IU /Kg/day is increased progressively to 100 – 200 IU/Kg/day in order to obtain a normal vitamin E / total lipid ratio. Adults must be monitored for neurological signs. In moderate cholestasis parenteral vitamin E is administered: 200 mg IM twice per month or 400 mg PO once daily. Therapy continues for the duration of cholestasis, aiming to maintain a vitamin E / total lipid ratio of > 0.8 (47).

Vitamin K. Vitamin K deficiency occurs in severe cholestasis and leads to coagulopathies: spontaneous bleeding, severe hemorrhage in response to minimal trauma, etc.

The liver uses vitamin K in the synthesis of clotting factors: II, V, VII, VIII, X, XI, XII and XIII. Vitamin K status is determined through prothrombin time (PT), serum vitamin K levels and clotting factors II, VII, IX and X. Vitamin K deficit can pe corrected by oral administration of soluble vitamin K (2.5 – 5 mg twice weekly) which can be increased up to 5mg daily until the prothrombin time is corrected. Patients with severe hemorrhage must be treated with IV, IM or SC vitamin K: 10 mg/day for 2 – 3 days, then 10 mg IM monthly (48).

In conclusion, clinical manifestations of fat-soluble vitamin deficiencies occur only in advanced cholestatic disease. The degree of vitamin deficit is proportional to the severity and evolution of the disease. Vitamin levels must be assessed twice per year. Vitamin supplements are administered indefinitely only in symptomatic cases or if biochemical analysis reveals vitamin deficiencies (49).

STEATORRHEA

Bile salts are needed for the luminal absorption of fat-soluble vitamins. Steatorrhea is a normal manifestation of severe cholestatic conditions that evolve over long periods of time. It occurs as a result of changed absorption, solubilisation and lipolysis of triglycerides in the intestinal lumen, which can be associated with exocrine failure of the pancreas (especially in PBC), celiac disease and *sicca syndrome* (50). Therapeutic intervention include: decreased fat intake: 40 – 50g / day, intake of short chain triglycerides (which do not require bile salts for absorption), a reduction in the dose of cholestyramine, pancreatic enzymes, a gluten free diet in the presence of celiac disease.

CONCLUSIONS

Often ignored in clinical practice, the assessment of extra-hepatic complications in patients with hepatic cholestatic conditions allows a more efficient management of this patient group. Pruritus, fatigue and osteoporosis are often overshadowed by other manifestations of liver disease which dominate the clinical presentation. In the last decades, significant progress has been made in understanding the molecular path physiology of cholestasis, although not many findings can be clinically applied. In an age of research targeting nuclear receptors in cholestasis, the pathogenesis of pruritus and fatigue remains as yet uncertain. Furthermore, current guidelines recommend the same first line therapeutic interventions – cholestyramine and rifampicin – as were reported 20 years ago. New studies are needed to determine more effective methods of diagnosis, prophylaxis and therapy of the extra-hepatic manifestations of cholestasis, in order to improve the quality of life in this patient group.

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CUTANEOUS MYIASIS CAUSED BY CORDYLOBIA ANTHROPOPHAGA. CASE REPORT

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CUTANEOUS MYIASIS CAUSED BY CORDYLOBIA ANTHROPOPHAGA. CASE REPORT (Abstract): Cutaneous myiasis due to infestation by the larva of *Cordylobia anthropophaga* (*C. anthropophaga*) is rarely seen in Europe. It is important to correctly identify such cases to prevent a delay in treatment in geographical regions where *C. anthropophaga* (tumbu fly) is endemic or in the case of patients who have travelled to these areas, because the clinical findings can be attributed to other furuncular lesions (such as furunculosis, cutaneous abscesses, tuberculosis, or mycosis). We report the case of a patient with furuncular myiasis due to the larvae of *C. anthropophaga* earlier misdiagnosed as furunculosis in a patient returning from tropical Africa (Senegal), part of the habitat of *C. anthropophaga*. The patient was treated in Luxembourg, Europe, country that does not belong to tumbu fly endemic areas. Our case was successfully resolved by minimally invasive surgery followed by antiseptic treatment. Antiparasitic medication was not required. **Keywords:** MYIASIS, CORDYLOBIA ANTHROPOPHAGA, CUTANEOUS FURUNCLE.

Cordylobia anthropophaga, also known as tumbu fly, putzi fly, tumba fly, mango fly, or skin maggot fly, is a species of *Calliphoridae* (blow-flies) with a habitat restricted to Central and East Africa. During the larval stage of development, it is a parasite of large mammals, sometimes affecting humans (1). The name anthropophaga stems from anthropophagos, "human eater" in Greek (2). Myiasis represents the infestation of a live host, large vertebrate mammals or humans, with maggots (larvae) belonging to flies of Order Diptera (two winged). The lesions appear predominantly on the skin but may affect even body orifices (nose, ears, eyes, genital area) or even intestines (3). A distinction can be made between post traumatic lesions (when the larvae are deposited in wounds) and furuncular lesions (4).

Similar lesions can be made by other parasites such as *Cordylobia rwandae* and *Cordylobia rodhaini*, (5) otherwise known as the Lund fly, an organism endemic to the tropical forests of Africa, and *Dermatobia hominis*, or the human botfly, which can only be found in Central and South America (8). However, the transmission of *Dermatobia hominis* differs significantly from that of *Cordylobia* species. *D. hominis* usually requires an intermediate vector, such a blood-sucking insect (ticks, mosquitoes).

Adult flies deposit their eggs onto the intermediate host (6). Once the host encounters a worm-blooded host larva begin to hatch and invade exposed skin areas of the limbs, trunk or head. Females from the *Cordylobia* species tend to deposit between 100 and 300 eggs directly in sandy soil, often contaminated with feces or onto dirty or wet clothing that has been let outside to dry (7). The hatched larvae can survive in the ground for about two weeks, afterwards requiring a host to continue developing (7) Movement of the contaminated soil determines the larvae to surface and attach to the skin. If a larva finds a host, it penetrates the skin and in 8 to 12 days it develops through the three larval stages before reaching the prepupal stage, after which it can leave the host to burry itself into the ground where it pupates, finally becoming an adult fly that is capable of reproduction to continue the life cycle again (7). Thus, the larvae tend to hatch and invade either covered skin areas that were in contact with clothes such as the genital area, buttocks, trunk or areas that were in contact with contaminated soil such as the soles of the feet (4).

CASE REPORT

A 33-year-old woman living in Luxembourg was referred to the surgery clinic of the Centre Hospitalier de Luxembourg by the Department of Infectious Diseases with a week-old history of several boils (furuncles) on the thighs. The patient was right-handed, smoked 12 cigarettes a day and occasionally consumed alcohol. She worked as a schoolteacher and was allergic to pollen. Her past medical history revealed malaria and surgical interventions, a left adnexectomy in 2016, tonsillectomy and adenoidectomy in her infancy. At the time of her consultation she was under treatment with Malarone, a drug combination of atovaquone/proguanil, an antimalarial medication used in both the treatment and prevention of malaria.

Her symptoms gradually increased in severity over the past week, with itching, inflammation and pain that motivated the patient to present herself to the emergency department of the hospital where a diagnosis of furunculosis was initially made. The patient was examined by our colleagues from the Department of Infectious Diseases and referred to the Plastic Surgery Department for surgical excision and drainage of the furuncles. Routine history taking revealed that she had recently returned from a trip to Dakar, Senegal, one of the countries where *Cordylobia anthropophaga* is endemic. The locals usually hang their clothes to dry outside in the sun and do not iron them. Upon examination several discharging orifices were identified on the dorsal aspect of both thighs, each surrounded by mild inflammation. Closer inspection revealed movement in the center of the sinuses (fig. 1).

Fig. 1. Furuncular lesion with sinus containing *Cordylobia anthropophaga* larva

We diagnosed five furuncular myiasis lesions on the dorsal aspect of the thighs. The larvae were easily removed using pressure and small forceps. One larva was dif-

ficult to extract from the affected area, requiring surgery. After injecting local anesthesia, we performed a small incision following the Langer lines of skin tension to facilitate forceps extraction followed by a careful debridement of the purulent content. The larvae were subsequently identified by the hospital pathologist as belonging to *Cordylobia anthropophaga*. The patient was placed on a 7-day course of amoxicillin trihydrate equivalent to 875 mg amoxicillin and potassium clavulanate equivalent to 125 mg of clavulanic acid t.i.d. and tetanus toxoid prophylaxis, analgesia and daily dressings with Chlorhexidine were prescribed. A routine follow-up one week later showed that all the lesions had healed, and the patient did not present any complications or recurrence.

Fig. 2. The extracted *Cordylobia anthropophaga* larva

DISCUSSION

Mammals such as dogs and small rodents can also be a reservoir for this parasite with occasional human infection (8). Many cases of myiasis affecting different human organs have been described since then (3, 9-11) In most cases the diagnosis can be made solely relying on the clinical findings. The patient either lives in an endemic area or has recently travelled to one. Clinically upon inspection we can find several non-healing cutaneous lesions, itching or movement under the skin, inflammation or pain, sometimes serous discharge or even a worm-like structure protruding from the central punctum (4). The clinical appearance can be attributed to other furuncular lesions such as cellulitis, furunculosis, cutaneous abscesses, tuberculosis, mycosis, actinomycosis, fungating carcinoma, tuberculosis and chronic ulcerating abscess (4). In most cases the diagnosis is confirmed by the extraction and examination of larvae morphology, however sequencing of the 28S rRNA gene can also be performed (12, 13). Radiological investigations such as ultrasonography and mammography may be helpful, having already been used in the identification of *Dermatobia hominis* by hyperechoic nodule representing the larvae surrounded by a hypoechoic halo corresponding to the cavity (14).

The therapeutic objective is to remove the larvae and prescribe a course of antibiotics. Very few *C. anthrophaga* infestations get complicated by secondary bacterial infection, in most cases the secretions from the larva tend to inhibit bacterial growth, a common property identified in several fly types (15). In most cases the larvae can be removed without incising the skin, by covering the punctum (also serving as the air supply for the maggot) with oils, Vaseline petroleum jelly or other ointments, even butter or liquid paraffin (8). The same effect can be obtained by using a clear film dressing such as Opsite or Tegaderm, causing the maggot to begin struggling for air, forcing it to come out of the skin (16). Another treatment suggested by Dybbro (17) is to inject a combination of anesthetic and epinephrine into the insect chamber to obtain the same suffocating effect. In some cases, the use of a forceps may be required, also after injecting local anesthesia, such as lidocaine, an incision can be made over the lesion to facilitate

extraction. Complete removal of the larva is essential, as any leftover fragments could cause an inflammatory response or a granuloma (10). This must be done carefully, and the entire larva extracted as any remnant may provoke an inflammatory response (15). Sometimes simply applying pressure to the sides of the furuncle is enough to mobilize the larva, *C. anthropophaga* having smaller hooked bristles on the cuticle than those of *Dermatobia hominis* (18).

Boggild (19) described a method for extraction of *D. hominis* larvae using a snake venom extractor However, snake venom extractors are not commonplace in European Emergency Departments, thus we propose a more affordable and accessible method. Using two Luer Lock type Syringes and a Luer Lock type Syringe Connector, we can create a vacuum chamber by removing the piston from one of the syringes and attaching it to the skin over the myiasis lesion, creating enough suction to pull out the larva, after careful expansion of the punctum using small forceps. Morselli (20) described this type of installation, used in fat grafting procedures for processing and producing the adipose cells. After removal the lesions tend to heal quickly. Rare complications have been cited in the literature such as cellulitis, abscess formation, osteomyelitis and tetanus (21).

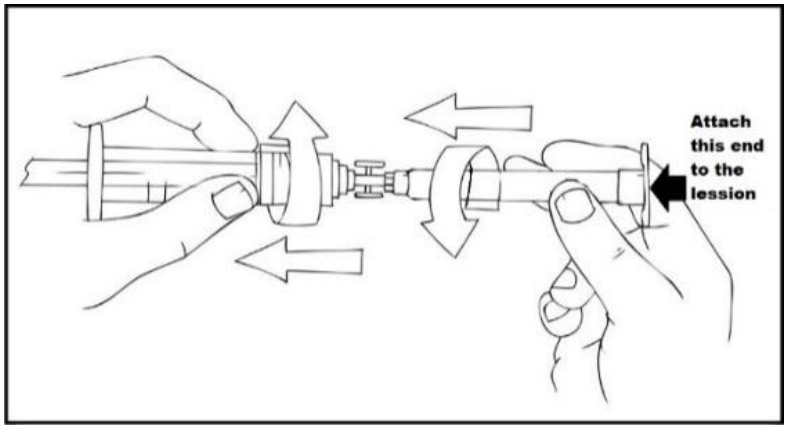

Fig. 3. Extraction method using a vacuum chamber created with the help of two syringes and a syringe connector. (Image adapted by the author from https://www.pfizer.de/medikamente-produkte/rezeptpflichtige-medikamente/pfizer-produkte/detailansicht/refacto-afR/)

CONCLUSIONS

Our paper presents the case of a European patient who had travelled to an endemic country and presented several furuncular lesions that had been initially misdiagnosed. Considering recent developments with an increasing number of people traveling to endemic countries or coming to European countries from these destinations, as well as the effects of climate change, cases of human myiasis could become even more frequent in countries in which it is rarely encountered. Although it remains a relatively uncommon condition in Europe, an increased awareness of its clinical aspects is more and more important to rapidly provide proper treatment.

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TRATAMENTUL CARCINOMATOZEI PERITONEALE DE ETIOLOGIE DIGESTIVĂ - STUDIU RETROSPECTIV PE UN LOT DE 203 PACIENȚI

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TREATMENT OF PERITONEAL CARCINOMATOSIS OF GASTRO-INTESTINAL ORIGIN - A RETROSPECTIVE STUDY ON 203 CASES (Abstract) Peritoneal carcinomatosis of gastrointestinal origin (PC-GI) is an advanced digestive tumor and is found in 10-30% of patients (P) with primary surgery for cancer (C) and up to 50% of C recurrences. **Aim**: to evaluate the main characteristics, ethio-pathogenesis, prognosis and imaging to track of P with PC-GI admitted to the Third Surgical Clinic, „St. Spiridon" Hospital, Iaşi. **Material and methods**: A retrospective study was carried out on series of 203 patients admitted in the period June 2006 - March 2011. The patients were aged between 27-80 years (average 62), with a women / men ratio of 95/108. The duration of hospitalization was between 1 and 61 days, with an average of 13.5 days for emergency cases and 15 days for elective cases. The data from observation files, the operating protocols, pathology reports and follow-up files were collected and analyzed. **Results**: 136 patients were hospitalized with synchronous PC (the most common gastric N = 60) and 67 with metachronous PC (the most common colon N = 29). Imaging investigations consisted of ultrasound and computer tomography that showed a sensibility and specificity of 80% and 73 % respectively, mainly in regard to ascites but less in assessing the presence of peritoneal deposits. The most common complication was septic shock and mortality was 9.5% (17 patients). Average survival was 5.7 months. **Conclusion**: PC-GI is a disease with a poor prognosis, posing difficulties in early diagnosis, establishing the surgical indication and protocol. Consistent advances in systemic and locoregional chemotherapy, surgical techniques, intraoperative radiotherapy, as well as immunotherapy are expected to improve prognosis. **Key words:** PERITONEAL CARCINOMATOSIS, CHEMOTHERAPY, PALLIATION, ASCITIS.

Carcinomatoza peritoneală cu punct de plecare digestiv (CP-D) a fost considerată mult timp o boală incurabilă. Incidența CP-D este dificil de apreciat. În 2002 (Global Cancer Statistics - 2002) dintr-un total de 1.023.152 pacienți cu cancer colorectal, 15% aveau carcinomatoză peritoneală (CP), din 933.937 pacienți cu cancer gastric, 40 % aveau CP, din 232.306 pacienți cu cancer pancreatic 25% aveau CP (1).

Diagnosticul şi stadializarea în CP-D sunt dificile: dozarea serică a markerilor tumorali, examenul ecografic şi examenul tomografic computerizat (CT) sunt metode

frecvent utilizate. Markerii tumorali au o specificitate şi o sensibilitate crescute atunci când sunt determinaţi în lichidul de ascită (2). Sensibilitatea examinării CT variază funcţie de dimensiuni: 9-24% pentru nodulii subcentimetrici şi până la 65% pentru cei de peste 5 cm (3). Studiile recente (4) recomandă FDG-PET ca fiind cea mai utilă explorare în CP.

În studiul EVOCAPE din 370 de pacienţi cu CP cu chimioterapie sistemică speranţa de viaţă a fost cuprinsă între 3 luni (noduli de CP peste 2 cm) şi 9 luni (noduli sub 5 mm).

Mai multe studii multicentrice comparative nerandomizate au evidenţiat beneficiul în ce priveşte supravieţuirea pacienţilor şi durata fără semne de boală la P la care administrarea chimioterapicului s-a efectuat intraperitoneal intraoperator şi apoi sistemic. În singurul studiu randomizat referitor la CP cu punct de plecare colorectal, Wervaal şi col.(5) au constatat o supravieţuire medie de 22 de luni în lotul de P la care s-a practicat chimioterapia intraperitoneală hipertermică asociată citoreducţiei peritoneale faţă de o supravieţuire medie de 12 luni la lotul de P la care la tratamentul chirurgical s-a asociat chimioterapia sistemică, diferenţa a fost una semnificativă statistic.

MATERIAL ŞI METODĂ

În perioada iunie 2006 - martie 2011 în Clinica a III - a Chirurgie au fost internaţi 203 pacienţi cu diagnosticul de CP-D stabilit prin intervenţie chirurgicală cu confirmare bioptică şi/sau descriere macroscopică a leziunilor peritoneale sau în baza unor explorări imagistice cu suspiciune ridicată pentru CP la pacienţii cu istoric de tumoră digestivă operată.

Din foile de observaţie, din protocoalele operatorii, buletinele anatomo-patologice, registrele de control, protocoalele oncologice şi din datele furnizate de Serviciul pentru Evidenţa Populaţiei au fost extrase principalele caracteristici clinico-epidemiologice, de diagnostic, tratament, evoluţie şi prognostic. Culegerea datelor şi statistica descriptivă a fost efectuată cu ajutorul programului *Microsoft Excel 2007*.

REZULTATE

Din analiza principalelor caracteristici clinico-epidemiologice ale P (tab. I) se observă o uşoară predominanţă a sexului masculin, o durată de spitalizare în medie egală între P cu CP sincronă (CP-S) şi CP metacronă (CP-M).

TABELUL I

Caracteristici clinico-epidemiologice ale pacienţilor cu carcinomatoză peritoneală internaţi în Clinica III Chirurgie în perioada iunie 2006-martie 2011

Pacienţi 203	Femei → 95
	Bărbaţi → 108
Vârsta 20-87 ani	Media → 62 (38-86)
Durata de spitalizare 1-61 zile	Media → 14 (1-27)
	Urgenţe → 13,5 (1-28)
	Electiv → 14,3 (3-27)
CP Sincronă 136	Electiv → 92
	Urgenţe → 44
	Ocluzii intestinale → 28
CP Metacronă 67	Electiv → 37
	Urgenţe → 30
	Ocluzii intestinale → 23

Cea mai frecventă cauză de CP-S a fost cancerul gastric (60 de cazuri – 44%) urmată de cancerul de colon; cea mai frecventă cauză de CP-M a fost cancerul de colon (29 cazuri – 39%) urmată de cancerul gastric (tab. II)

TABELUL II

Etiologia carcinomatozei peritoneale digestive la cei 203 pacienți

136 pacienți cu carcinomatoză sincronă				**67 pacienți cu carcinomatoză metacronă**		
Stomac	60			13		
Colon	31	cecoascendent	14	29	cecoascendent	10
		transvers	4		transvers	5
		transvers	13		transvers	14
Pancreas	19	cefaloistmic	5	7	cefaloistmic	5
		corporeocaudal	14		corporeocaudal	2
Rect	8			8		
Esofag	6			-		
Duoden	2			3		
Căi biliare	7			-		
Altele	7			7		

Au fost operați 109 pacienți cu CP-S (80%). În 25 cazuri (fig.1) s-au practicat rezecții cu viză paleativă, la 2 pacienții cu neoplasm colon drept cu CP s-a practicat hemicolectomie dreaptă cu peritonectomie parțială şi chimioterapie intraperitoneală normotermică pre- şi postoperatorie.

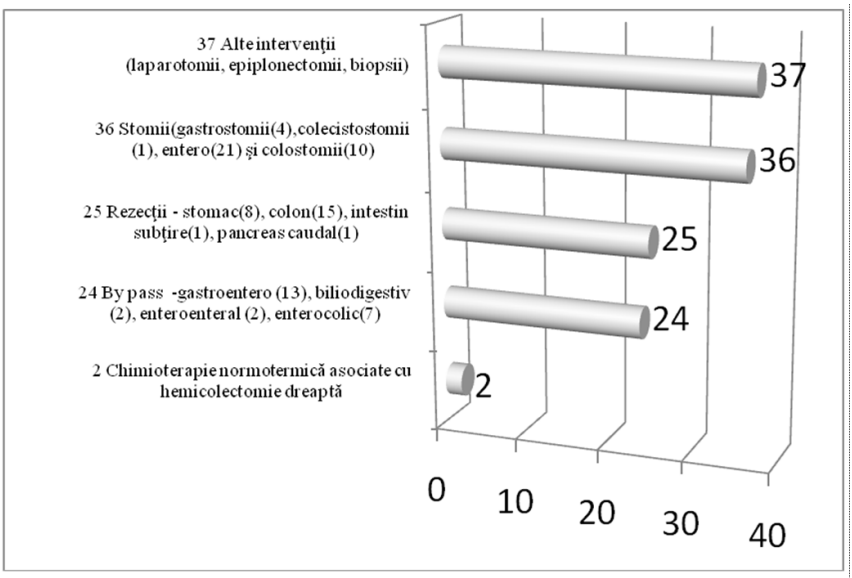

Fig. 1. Intervențiile efectuate în carcinomatoza peritoneală sincronă

Din 67 de pacienți cu CP metacronă au fost operați 53 de pacienți(79%); cele mai frecvente intervenții (fig.2.)au fost cele de *by-pass*. La 3 pacienți cu CP-M în antecedente cu cancere colon operate s-a practicat chimioterapie intraperitoneală pre- şi post-operatorie.

Examenul ecografic a fost a fost efectuat la 198 pacienți. 177 dintre aceştia au fost operați. Sensibilitatea şi specificitatea examinării ecografice în evidențierea ascitei a fost de 82% şi respectiv 71%.

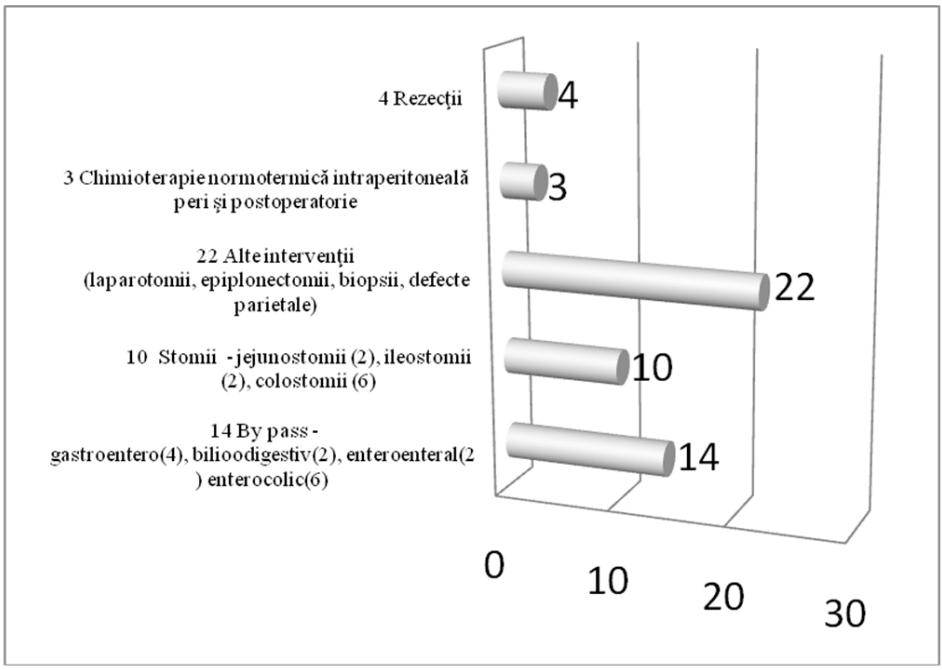

Fig. 2. Intervențiile efectuate în carcinomatoza peritoneală metacronă

Examinarea CT a fost efectuată la 40 din cei 203 pacienți (20%), aceasta s-a efectuat la 29 din 136 pacienți (21%) cu CP sincronă şi la 11 pacienți (16%) cu CP metacronă. În funcție de modul de prezentare a pacienților în 34 de cazuri (85%) examenul CT a fost efectuat la P electivi şi în 6 cazuri (15%) în urgență. Ascita a fost evidențiată la 12 P din cei operați şi care au efectuat şi examen CT, cu o sensibilitate şi o specificitate de 81% şi respectiv 78%. Îngroşări ale seroasei peritoneale au fost evidențiate la 23 P din care 17 au fost operați, sensibilitatea şi specificitatea au fost de 79% şi respectiv 73%).

Biopsia intraoperatorie asociată sau nu cu alte intervenții a fost efectuată la 131 pacienți din cei 179 operați (73%), a fost efectuată la 99 din cei 128 de pacienți cu CP sincronă (76%). În 29 de cazuri (24%) biopsia intraoperatorie nu a fost efectuată – 13 cazuri cu cancer gastric. La 32 din cei 51 de pacienți (62%) cu CP metacronă operați biopsia a fost efectuată.

Laparoscopia diagnostică a fost utilizată la 15 pacienți (7,5%). Laparoscopia a fost folosită la 12 pacienți (esofag-2, stomac-1, ficat-3, corp pancreas-5, necunoscute-2),

din cei 136 pacienți cu CP-S (9%) şi în 11 cazuri s-a prelevat şi biopsie. S-a efectuat ca singură intervenție laparoscopia diagnostică asociată cu biopsie şi la 3 cazuri de CP-M.

Din totalul de 136 de pacienți cu CP sincronă la 49 dintre aceştia s-a efectuat o sutură digestivă (25 rezecții, 24 operații de tip *by-pass*). Au fost 2 fistule (4%) digestive tratate conservator. Dintre cei 18 pacienți cu CP metacronă la care s-a practicat o sutură digestivă (4 rezecții, 14 operații *by-pass*) 2 P au dezvoltat postoperator fistulă digestivă, aceştia au decedat prin sepsis.

Mortalitatea postoperatorie la cei 177 pacienți operați a fost de 9,5% (17 decese postoperatorii). 15 P (88%) au fost operați în urgență iar 2 (12%) electiv. Principala cauză de deces a fost şocul septic - 8 pacienți (47%). Alte cauze de deces au fost hemoragia digestivă superioară cu soc hemoragic – 2 pacienți, insuficiența hepatorenală – un pacient, alte cauze – 6 pacienți.

Din 203 pacienți internați în Clinica III Chirurgie în perioada iunie 2006-martie 2011 la 1 martie 2011, 178 pacienți erau decedați. Supraviețuirea medie la pacienții cu CP de origine gastrică a fost de 4,2 luni. Supraviețuirea medie la pacienții cu CP de origine colorectală a fost de 6,5 luni. Un singur pacient diagnosticat în 2008 cu CP de la o tumoră de colon transvers metacronă (cu un neoplasm de colon drept operat în antecedente) la care s-a practicat totalizarea colectomiei şi apoi chimioterapie sistemică era în viața la 1 martie 2011. Supraviețuirea pacienții cu CP cu punct de plecare pancreas, ficat, căi biliare a fost sub 3 luni.

DISCUȚII

CP-D a fost considerată mult timp o afecțiune incurabilă iar tratamentul chirurgical propus unul paleativ. În prezent a crescut interesul tratamentul agresiv al CP cu scop creştere a duratei şi calității vieții şi chiar vindecare. Au fost definite noțiunile de citoreducție optimală şi suboptimală, stadializări în funcție de distribuția carcinomatozei scorul de completitudine al citoreducției. Deoarece tratamentul asociază mai multe manevre agresive (peritonectomii întinse, rezecții viscerale largi, hipertermia) intervențiile nu sunt lipsite de riscuri. Într-un studiu retrospectiv Sugarbaker şi col. (6) pe un lot de 356 pacienți operați pentru CP au constat o mortalitate intraspitalicească de 2%, complicații medii şi severe la 19 % din pacienți şi 11 % din pacienți au necesitat reintervenții chirurgicale.

Pe lângă morbiditatea şi o mortalitatea crescută un alt impediment în folosirea acesteia este prețul. Bonastre şi col.(7) pe un lot de 73 de pacienți operați între 2002-2003 în Institutul Gustave-Roussy au evidențiat un cost pe pacient de 39350 Euro cu o durată medie de spitalizare de 27 zile, în condițiile în care spitalului i-au fost rambursate o medie de 20500 Euro a rezultat un deficit de 1,4 milioane. Într-un alt studiu Deraco şi col.(8) pe un lot de 382 de pacienți pe în perioada 1995-2008 remarcă un cost pe pacient de 36.000 Euro cu o medie de spitalizare de 24,3 zile şi un deficit între valoarea valoare serviciilor şi valoarea rambursată de aproximativ 1.860.000 euro pe ultimii doi ani.

În prezent sunt în desfăşurare mai multe studii clinice de fază I/II cu noi clase chimioterapice iar rezultatele preliminare sunt încurajatoare. Catumaxomab este un anticorp trifuncțional recent introdus în tratamentul CP-D. Un studiu recent (9) pe un lot de pacienți cu CP-D tratați cu catumaxomab arată o supraviețuire medie de 502 zile cu absența evoluției la 11 pacienți

din 17.

În statistica Clinicii a III - a Chirurgie în perioada iunie 2006 - martie 2011 au fost tratați 203 pacienți. Cancerul gastric a reprezentat cauza cea mai frecventă de CP-S iar cancerul de colon cauza cea mai frecventă de CP-M. Datorită istoriei naturale mai mulți pacienți cu cancere de colon local avansate operate ajung în faza complicațiilor ocluzive ce necesită reintervenții chirurgicale.

Principala investigație imagistică la pacienții internați în clinică a fost examinarea ecografică. Examenul CT în ciuda faptului că are o sensibilitate şi o specificitate mai mare a fost efectuat doar la 40 pacienți din cei 203, fapt explicabil atât prin accesul mai greu la explorare cât si printr-un procent relativ ridicat de pacienți internați în urgență (31%).

În ceea ce privește intervențiile chirurgicale, acestea au fost în marea majoritate paleative, ablația tumorilor a reprezentat 20% din intervențiile practicate pentru CP-S. La 5 pacienți (2 cu CP-S pentru neoplasm colon drept şi 3 CP-M pentru neoplasm colon operat) s-a efectuat chimioterapie preoperatorie normotermică urmată de chimioterapie intraperitoneală postoperatorie pe o perioadă cuprinsă între 2 şi 4 zile. Administrarea chimioterapicului (CIS-PLATIN) intraoperator şi apoi postoperator nu a influențat evoluția postoperatorie imediată a pacienților, un singur pacient a prezentat insuficiență renala acută reversibilă. După externare pacienților li s-a administrat chimioterapie sistemică. Supraviețuirea pacienților a fost de 6,8 şi 9 luni, 2 pacienți au fost pierduți din evidență.

Rata relativ ridicată de complicații şi mortalitatea crescută (9,5%) sunt explicate prin numărul mare de pacienți internați cu complicații, de obicei ocluzive. Raportările centrelor cu experiență în tratamentul CP, o mortalitate de 0-6% după intervenții agresive (10-12) pot fi explicate şi prin faptul că aceste centre de obicei tratează pacienți atent selecționați şi care nu sunt urgențe chirurgicale.

CONCLUZII

Cu o incidență în creştere, o calitate a vieții afectată, supraviețuire scurtă, morbiditate şi mortalitate operatorie crescute, carcinomatoza peritoneală de etiologie digestivă rămâne o afecțiune dificil de tratat. În absența studiilor prospective multicentrice randomizate terapia acestei afecțiuni cu tablou clinic polimorf rămâne un subiect controversat si dificil de apreciat ca eficiență reală. Introducerea în arsenalul terapeutic a unor noi clase de chimioterapice, a unor intervenții chirurgicale agresive, şi eventual a terapiilor genice ar putea îmbunătăți prognosticul acestor pacienți.

MULȚUMIRI

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DIAGNOSTIC APPROACH OF PNEUMONIA IN AN ELDERLY PATIENT WITH CO MORBIDITIES - CASE REPORT

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DIAGNOSTIC APPROACH OF PNEUMONIA IN AN ELDERLY PATIENT WITH COMORBIDITIES **(**Abstract): Dyspnea is one of the symptoms that has a major impact on patients' health, with a negative influence on the quality of life. The main causes of dyspnea are cardiac, pulmonary and mixed (cardiac or pulmonary). There are several other causes such as metabolic conditions (acidosis), pain, neuromuscular disorders, otorhinolaryngeal disorders, anxiety, panic disorders and hyperventilation. Acute pneumonia in the elderly is a common occurrence and its incidence grows as the elderly population increases. We report the case of a 76 years old patient with a known cardiovascular condition, recently hospitalized for right pulmonary infarction. He presented to our clinic for influenced general state, rest dyspnea, fever, shiver, and productive cough in the last two days. Current medication included oral anticoagulants and triple antihypertensive treatment (responsible for his low blood pressure). Laboratory results showed a nonspecific inflammatory syndrome with leukocytosis and neutrophilia and mild normochromic normocytic anemia; D-dimers were within normal range, fibrin degradation products 1+; myocardial enzyme testing showed no alteration, and BPN (beta natriuretic peptide) was 790 pg/ml. Chest X-ray showed diffuse bilateral reticular shadows (more pronounced on the right side) and left costodiafragmatic opacification. It appears that age-related increase in morbidity and mortality in community-acquired pneumonia is not due to age per se, but to interactions between the immune system, systemic diseases and nutritional factors. Community-acquired pneumonia in the elderly is known to have a high mortality. Although the diagnosis can be easy, the physician must also investigate for less obvious causes of dyspnea such as the presence of comorbidities and fragility of the elderly patient. **Key words**: PNEUMONIA, ELDERLY, DYSPNEA.

Dyspnea is one of the symptoms that have a major impact on patients' health, with a negative influence on the quality of life. There are many causes of dyspnea in the elderly, most commonly cardiac (1) and pulmonary.

A list of causes of dyspnea should include: cardiac pathology (congestive heart failure, coronary artery disease, myocardial infarction, cardiomyopathy, valvular dysfunction, left ventricular hypertrophy, asymmetric septal hypertrophy, pericarditis, arrhythmias), pulmonary pathology (COPD, asthma, restrictive lung disorders, hereditary lung disorders, pneumothorax), mixed cardiac or pulmonary diseases (COPD with pulmonary hypertension and cor pulmonale, chronic pulmonary emboli, trauma), and

other causes (metabolic conditions such as acidosis, pain, neuromuscular disorders, otorhinolaryngeal disorders, anxiety, panic disorders, hyper-ventilation). Among the respiratory causes, pneumonia is of special importance due to its high frequency and its difficult management in the exponentially growing elderly population (2, 3). The diagnostic and therapeutic approach of this illness for patients aged over 65 requires extensive knowledge in the field of geriatric medicine because, on the one hand, it frequently presents with atypical symptoms and, on the other hand, because it often has a negative impact on the quality of life. It is also important to note that acute pneumonia in the elderly is a common occurrence and its incidence is growing as the elderly population increases (4).

Pneumonia is considered to be the sixth among the leading causes of death in the United States, with approximately 600.000 admissions per year (5, 6). Often, it can lead to serious complications and/or death.

The common symptoms of all viral pneumonias are fever, chills, nonproductive cough, rhinitis, myalgias, headaches, and fatigue. These symptoms are similar to those encountered in pneumonia, although studies have shown a lower probability of experiencing chest pain in viral pneumonia (7). Although most patients present with cough, in the elderly it may be only scant.

Viruses account for the largest proportion of childhood pneumonias. Viral pneumonia decreases in frequency in healthy young and middle-aged adults, but then increases substantially among the elderly. Studies on community-acquired pneumonias in patients over 65 years have consistently proved viruses to be the second most common etiologic cause (with the first one being *Streptococcus pneumoniae*), ranging from 13-50% of the diagnosed cases (7, 8, 9, 10).

CASE REPORT

We present the case of a 76 years old man with former tobacco and alcohol addiction, previously diagnosed with multiple cardiovascular co morbidities: high blood pressure (systolic pressure =230 mmHg), heart failure, atrial fibrillation, IIIrd degree heart block (treated with permanent VVI pacing), stage III peripheral ischemia (treated with thrombendarterectomy on left superficial femoral artery and left ilio-femoral by-pass), ischemic stroke in left carotid territory (that occurred 10 years ago and resulted in right hemiparesis). Two months before admission he underwent abdominal surgery (cholecystectomy); few days after the intervention he presented dyspnea; investigations revealed a right pulmonary infarction for which he received oral anticoagulation therapy at home.

His current reasons for admittance were rest dyspnea, productive cough with mucopurulent sputum, fever and shiver that had started 2 days before admission. Clinical examination showed an influenced general state and dyspnea with tachypnea; pulmonary auscultation revealed bronchial crackles on both pulmonary areas, and bilateral rales, more pronounced in the right hemithorax; his blood pressure was 85/60 mmHg under triple antihypertensive medication, heart rate 72/min. He also had presacral pressure sores 4/6 cm and right hemiparesis.

Laboratory analysis showed a nonspecific inflammatory syndrome with leukocytosis and neutrophilia - white cells = 16370/mm^3 (Normal value: 4000-9000/ mm^3), neutrophyles = 80% (Normal value: 55-70%), erythrocyte sedimentation rate = 34 mm/1h (Normal Value: 2-10 mm/1h) and mild normochromic normocytic anemia

(hemoglobin = 11.7 mg/dl and hematocrit = 36.2%).

D-dimer blood levels were within normal range, fibrin degradation products were 1+; myocardial necrosis enzymes were at normal value, BPN (beta natriuretic peptide) was 790 pg/ml (Normal value: <100), INR was 2,16 and oxygen saturation was 93% (Normal value: 95-100%) without the aid of oxygen mask.

Chest X-ray showed diffuse bilateral reticular shadows (more pronounced on the right side) and left costodiafragmatic opacification with an elongated lower left arc. Echocardiography revealed a mild dilatation of the left atrium (AS=41/51/58 mm), paradoxic movement of the interventricular septum and stage I mitral insufficiency; no vegetations were identified.

The Mini Mental State Examination (MMSE) showed mild cognitive dysfunction, with an average score of 24 (25 during the first examination and 23 the next day).

The patient was afebrile during his hospitalization, with preserved diuresis and heart rate between 70 and 76/min.

After a 10 - day therapy with broad spectrum antibiotics, i.v. corticotherapy and expectorant, symptoms decreased in intensity and dyspnea improved.

The dosage for antihypertensive medication was adjusted in order to obtain a good control of blood pressure and oral anticoagulant therapy was monitored according to INR values.

DISCUSSION

Although dyspnea in the elderly can be caused by both respiratory and cardiovascular diseases, the first diagnostic suppositions were pulmonary embolism and community-acquired pneumonia (CAP).

Dyspnea can be assessed by several tests and procedures in order to establish a definitive positive diagnosis. Those used on a regular basis are chest X-ray, spirometry, arterial blood gas measurement, EKG, exercise treadmill and echocardiography

Chest radiography is usually included among the first diagnostic tests. It can identify parenchymal or interstial lung abnormalities, opacities, nodules, etc. It can also evaluate the cardiac shape and size (relevant for conditions such as pericarditis and ventricular hypertrophy) and skeletal abnormalities relevant to the differential diagnosis.

Spirometry can help differentiate between obstructive lung disease (COPD, asthma) and restrictive lung disease (obesity, kyphosis or scoliosis, pleural effusion, neuromuscular disorders, systemic lupus erythematosus, sarcoidosis).

Arterial blood gas measurement can provide information about altered pH, hypercapnia, hypocapnia or hypoxemia. This measurement is more commonly used for the evaluation of acute dyspnea but it can also be used in the evaluation of chronic patients.

EKG can reveal arrhythmias, ischemia and/or other cardiac conditions that can cause dyspnea.

Exercise treadmill testing can identify ischemia as a cause of dyspnea. This test can be performed when symptoms are atypical for angina or when silent ischemia is suspected.

Echocardiography can detect a valvular abnormality and may be diagnostically helpful in patients with questionable murmurs in the context of dyspnea. It may also exclude sepsis and endocarditis (11) as possible dyspnea causes.

Pulmonary embolism and pneumonia are common complications that occur espe-

cially after stroke (12, 13, 14, 15), and due to the fact that they are clinically resemblant they can often be confounded. It is also true that the two pathologies can coexist, although many practitioners would rarely consider the possibility of pulmonary embolism if CAP is proved (16). These complications are frequently seen in the second to fourth week after stroke (16).

Among patients with symptomatic pulmonary embolism or deep venous thrombosis (DVT) that receive anticoagulant treatment for 3 months, fatal pulmonary embolism is rare during and following anticoagulant therapy. Patients presenting with pulmonary embolism are more likely to die of recurrent pulmonary embolism or DVT than patients presenting with DVT (17).

Riquelme et al.'s study underlined that the clinical picture of CAP in the elderly is often incomplete; they may have a large variety of signs and symptoms, with no association between them. Pleuritic chest pain was considered an independent risk factor for pneumonia caused by common microorganisms. Malnutrition was more common in patients with pneumonia than in patients without this pathology (18).

The quality of the diet in the aging population is influenced by physiological factors such as poor dentition, altered taste acuity, limited mobility, and polymedication. Elderly aged 74–80 years have less than 100 taste buds, whereas young adults have 250 (19). Psychological factors in this group that can contribute to poor oral intake include depression, dementia, and lack of motivation. Social factors that further compromise dietary intake include institutionalization, living alone, isolation during meals, and poor education. Similarly, in an aging population, economic factors such as reduced income, inadequate cooking, and health care costs can affect the quality of the diet (20).

In an elderly patient with pneumonia, the management plan is developed according to the clinical and instrumental assessment results. Compensatory strategies for increasing eating and swallowing efficiency and for reducing the risk of aspiration may involve modifying food and liquid consistencies and volumes. Dietary modification is a common management approach. Patients vary in their ability to swallow thin and thick liquids, semi-solids, and solids. The consistency of the patients' food should be individualized according to clinical testing findings. Dehydration in the elderly is one of the leading problems in nursing homes and long-term care facilities (21). Caution should therefore be taken with regard to the modification of fluids, as compliance with thickened liquids is often reduced. Small sips of cold water in patients with good oral hygiene may bring relief to thirsty patients and may reduce the resultant dehydration (22).

While the presence of dysphagia and the volume of the aspirate are key factors that predispose elderly patients to aspiration pneumonia, a number of other factors play an important role (23). Colonization of the oropharynx is an important step in the pathogenesis of aspiration pneumonia. The elderly have increased oropharyngeal colonization with pathogens such as *Staphylococcus aureus* and aerobic Gram-negative bacilli (*eg, Klebsiella pneumoniae* and *Escherichia coli*) (24, 25, 26).

Elderly patients with CAP represent a special group due to comorbidities, neurological impairment, and immunological and/or nutritional deficiency (18).

The incidence of fever in elderly with CAP is between 33% and 60% (4, 27).

Elderly patients with pneumonia commonly present with neurological symptoms. Delirium prevalence in hospitalized elderly with pneumonia varies from 50% (20), to values between 15% and 47% (4, 29).

In one study, chest radiography was compared to high resolution CT for the diagnosis of CAP (30). Chest radiographs detected an opacity in 18 of 47 patients (38.3%) suspected of having pneumonia, whereas high-resolution CT detected opacities in these 18 patients and in an additional 8 (55.3%) (30).

COPD, heart disease, malignancy, malnutrition, congestive heart failure, and diabetes mellitus have been implicated as risk factors leading to CAP in the elderly (18, 31, 32, 33, and 34).

It appears that age related morbidity and mortality in CAP is not due to age *per se* (29), but due to interactions between the immune system, systemic diseases and nutritional factors.

Mortality related to CAP is high. In his study, Riquelme showed that mortality was 26% (18) and it was in accordance with the results of other studies (4). Venkatesan and colab. reported that apyrexia is a prognostic indicator of mortality (4).

Optimal duration of antibiotic therapy for CAP has not yet been defined in prospective studies. Studies should be carried out to determine if a longer duration of therapy is necessary for elderly patients. Since about half the patients with CAP have respiratory symptoms at a 30-day follow-up, it is possible that longer therapy than that given at present may be beneficial. By convention, 10–14 days is the usual duration of therapy. Legionnaires disease should be treated for 21 days, as should pneumonia due to *Pneumocystis carinii* (31).

All elderly patients with CAP should undergo follow-up chest radiography to make sure that the pneumonia has resolved (31).

Optimum hospitalization in elderly with CAP is 15-16 days (4, 18).

Elderly patients who are functionally impaired (the frail elderly (35)) should be referred for geriatric assessment. Some of these patients may require admission to a geriatric rehabilitation center after the pneumonia has resolved. Studies have shown that geriatric assessment teams do improve the care of the elderly, resulting in a reduction in the number of patients who need discharge to long-term-care institutions (36). However, such assessment with only limited follow-up has not been effective (37).

Although the diagnose can be obvious, the differential diagnosis, clinical and paraclinical investigations must evaluate all etiological causes of dyspnea due to the presence of co morbidities and the fragile status of the patient.

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NOUTĂȚI

EVALUAREA FUNCȚIEI RENALE REZIDUALE UTILIZÂND CYSTATINUL C

Su Jin Kim şi colaboratorii săi din Korea, au publicat un studiu important privind relaţia dintre funcţia renală reziduală (RRF) şi cystatinul la copiii cu dializă peritoneală (PD), atât la pacienţii anurici cât şi la cei cu RRF. Pornind de la faptul ca cystatinul C nu are corelaţie cu parametrii de baza pe care se evaluează eficienţa dializei, cum ar fi Kt/V pentru uree dar are o corelaţie semnificativă cu RRF, autorii conchid ca cystatinul C ar putea fi un bun indicator pentru RRF. Editorialul trece în revistă diferitele legături între cystatinul C şi uree, prezentate în literatura de specialitate privind adulţii, precum şi factorii determinanţi ai clearance – ului cystatinului C la pacienţii dializaţi. La adulţi, nivelurile cystatinului C sunt predominant determinate de RRF dar nu în exclusivitate. La hemodializa la pacienţi anefrici şi pacienţii cu PD există o corelaţie cu Kt/V săptămânal pentru uree. Nivelurile de cystatin C sunt, de asemenea, dependente de ultrafiltrare. În pofida acestor factori care influenţează cystatinul C după RRF, cystatinul C este un parametru util de monitorizare a pacienţilor cu PD care poate fi mai strâns corelat cu prognosticul pe termen lung decât o fac parametrii clasici de eficienţă ai dializei. (Filler G, Huang S-H S, Lindsay RM, Residual renal function assessment with cystatin C. *Ped Nephrol* 2011; 3(26): 333-335).

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DRENAJUL ȚESUTULUI GRAS SUBCUTANAT LA GRAVIDELE OBEZE SUPUSE OPERAȚIEI CEZARIANĂ

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SUBCUTANEOUS FAT TISSUE DRAINAGE IN OBESE PREGNANT WOMEN AFTER CAESAREAN SECTION (Abstract): The authors have analyzed the outcome of cesarean section celiotomy in obese pregnant women. The occurrence of postoperative wound infections, eventually dehiscence, in 2015 pregnant women who underwent caesarean section in a 3-year interval at the Craiova Ist Clinic of Obstetrics was studied. The analysis revealed the relation between postoperative wound infection and its topography, the type of fat tissue drainage (if done), and skin suture. The authors present a very effective method of subcutaneous drainage in obese pregnant women after cesarean section. **Key words:** OBESITY, DRAINAGE, INFECTION, CESAREAN SECTION

În ultimii ani se constată o creştere a procentului femeilor obeze în lume. In SUA aproximativ jumătate din populația feminină prezintă un grad de obezitate. În România procentul variază între 15-30%. Obezitatea creşte riscul de HTA, diabetului, infertilității, iar la gravide creşte prematuritatea, malformațiile congenitale, apare macrosomia fetală. În mod deosebit creşte riscul infecției puerperale (uter, anexe, cavitate buco-dentară, sâni, plagă postoperatorie).

În obstetrică plăgile în țesut gras se infectează cel mai frecvent. Infecția plăgii de celiotomie în țesut grăsos la femeile obeze este de 15 ori mai fracventă fată de femeile cu greutate normală.

În operațiile pe cale abdominală cavitatea peritoneală se deschide prin două feluri de incizii la peretele abdominal, respectiv incizie mediană şi incizie transversală (de obicei Pfannenstiel).

Alegerea inciziei depinde de cauza pentru care se intervine. De obicei incizia mediană se face în laparotomiile iterative, în abdomenul acut chirurgical, în urgențele mari obstetricale. Incizia transversală Pfannenstiel este preferată mai ales la femeile obeze. Avantajul inciziei mediene este că se execută rapid, permite orice fel de drenaj. Dezavantajul constă în faptul că expune la eventrații, la infecția plăgii, este mai puțin preferată din motive estetice. Incizia transversală Pfannenstiel este estetică, nu expune la eventrații şi infecția este mai rară.

Pentru a reduce o infecție locală este nevoie de o hemostază ingrijită, respectarea planurilor anatomice la sutură, evitarea spațiilor moarte, drenajul țesutului gras, asepsie riguroasă, material de sutură corespunzător. Majoritatea operațiilor obstetricale se fac prin incizie transversală suprapubiană. Cea mai frecventă este incizia

Pfannenstiel. Incizia la femeile obeze se execută în pliul de revărsare a şorțului gras abdominal, la 2-3 laturi de deget deasupra simfizei. Atenție la eventuala secționare a arterelor epigastrice la extremitatile secțiunii transversale. Închidrerea se face cu un eventual drenaj supraaponevrotic.

Mai există şi alte feluri de incizii transversale: incizia Rapin-Kusther în care se secționează tegumentele transversal şi țesutul subcutanat gras care se împinge spre ombilic, după care se deschide aponevroza şi peritoneul longitudinal; incizia Bordenheuer-Bastien în care incizia pielii se face de la o spină iliaca la cealaltă, la un lat de palma deasupra simfizei, se incizează transversal şi aponevroza cu fibrele muşchilor si peritoneul, la închidere capetele muschilor se suturează.

MATERIAL ŞI METODĂ

Definirea obezității în practică diferă de la autor la autor deoarece greutatea ideală este o noțiune abstractă. Astfel se consideră obez orice individ care depăşeşte cu 10-20% normele MTI (Metropolitan life insurance company statist. Bull 1959, 41 SUA). Noi am considerat obeze gravidele care au depăşit cu 15% normele MTI, dar am luat în calcul şi greutatea impusă de vârsta sarcinii – la termen 9-12 kg. Am exclus greutatea dată de patologia sarcinii sau altă patologie.

S-a urmărit pe o perioada de trei ani (2008-2010) prezența infectiei plăgilor abdominale şi eventual dehiscenței acestora în intervențiile chirurgicale obstetricale la garvidele obeze operate. Cu predilecție s-a urmărit infecția plăgii abdominale în relație cu topografia plăgii, cu drenajul supraaponevrotic, cu tratamentul instituit, cu germenii existenți în supurație.

Se descrie un procedeu, mai nou folosit în operațiile obstetricale, de drenaj al țesutului gras cu rezultate foarte bune.

REZULTATE ŞI DISCUȚII

Se constată o frecvență mare a infecției plăgii abdominale la femeile obeze, majoritatea cu incizie transversală. Pliul abdominal determinat de şorțul de țesut gras ce se revarsă este de fapt zona în care se face incizia transversală Pfannenstiel si zona unde există un grad mare de umezeală continuă şi greu aerisită.

Plăgile supurate au fost populate cel mai frecvent de germeni gram pozitiv (*Stafilococul Aureu şi Escherichia Coli*). Tratamentul a presupus în primul rând o toaletă riguroasă, administrare de spray cu aloe, sutura persecundum la nevoie, antibiotice, antiinflamatorii nespecifice.

Pentru a evita infecția țesutului gras în plăgile cu țesut gras intens reprezentat am procedat folosind un sistem de drenaj mai adecvat. Eficiența a fost foarte mare, nici o plagă neinfectandu-se.

Din 4704 de naşteri în Clinica I a Spitalului Județean Craiova 2015 (42%) au născut prin operație cezariană, din care 1800 (89%) prin incizie transversală Pfannenstiel. Din cele 2015 de operatii cezariană am contatat prezența obezitații la 350 (17,36%). Dintre acestea 7% au prezentat infecția plăgii care uneori a devenit dehiscentă. La nici un caz drenat supraaponevrotic prin metoda descrisă mai jos nu s-a produs infecția plăgii abdominale.

Descriem mai jos procedeul folosit:

Astfel am efectuat incizia transversal un pic mai sus de pliul şorțului de grăsime abdominal cu respectarea timpilor inciziei Pfannenstiel (fig. 1).

La deschiderea peretelui abdominal drenăm supraaponevrotic cu două tuburi de polietilenă scoase la comisurile plăgii, dacă

este posibil prin contraincizie. Tuburilor le adaptăm seringi la capetele exterioare cu care creăm vid (fig. 2).

Fig. 1. Drenajul țesutului grăsos în incizia Pfannenstiel

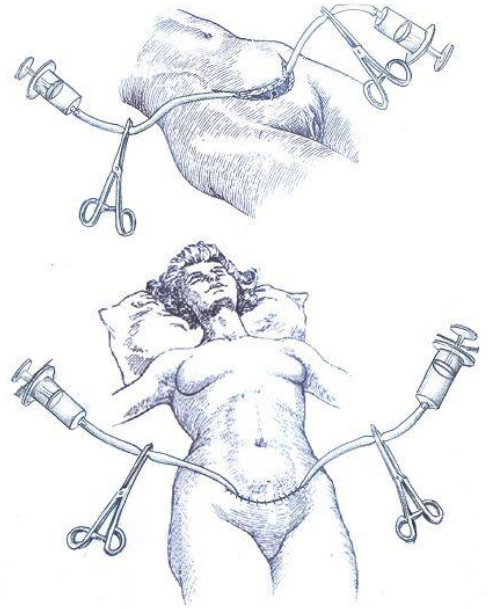

Fig. 2. Drenajul supraaponevrotic cu vid

Țesutul gras îl suturăm cu fire rare, iar tegumentul cu fire foarte dese. Prin aspirație cu seringa de o parte, apoi de cealaltă, creăm vid. Pensăm tubul, aruncăm conținutul seringii, apoi atașăm seringa, desfacem pensa, iar sucțiune, iar pensăm, aruncăm conținutul, atașăm seringa, desfacem pensa și așa mai departe pană eliminăm tot ce se absoarbe din plagă. La sfarșit atașăm seringa, desfacem pensa, tragem pistonul pentru a crea vid și îl fixăm cu un ac de seringă trecut transversal prin mânerul pistonului, pentru a se menține în pozitie de vid. Astfel secrețiile se adună în seringă și apoi procedăm din nou a doua zi la sucțiune. Drenajul durează 5-6 zile.

Procedeul este eficient și în inciziile mediene (fig. 3) și în suturile persecundum după plăgile dehiscente.

Fig. 3. Drenajul țesutului grăsos în incizia mediană

CONCLUZII

Infecția plăgii operatorii abdominale este mai frecventă la femeile obeze cu predominență la femeile gravide obeze.

Incizia Pfannenstiel făcută la nivelul plicii de revărsare a șorțului gras abdominal este des însotită de supurație datorită umezelii continue și spațiului neaerat.

Incizia transversală deasupra pliului este mai lipsită de risc supurativ.

Drenajul supraaponevrotic (după procedeul nostru) sub vid are o eficiență maximă, el poate fi aplicat şi în suturile persecundum.

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CLINICOPATHOLOGICAL FEATURES OF JUVENILE GRANULOSA CELL TUMOR WITH AN EXTENSIVE MUCINOUS COMPONENT. CASE REPORT

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CLINICOPATHOLOGICAL FEATURES OF JUVENILE GRANULOSA CELL TUMOR WITH AN EXTENSIVE MUCINOUS COMPONENT. CASE REPORT (Abstract): Granulosa cell tumors (GCTs) are a rare type of ovarian neoplasm accounting for only 2-3% and their mix with mucinous elements is uncommon. Despite their favorable natural course, correct diagnosis is imperative for proper management to prevent metastatic spread and local recurrences. We herein report a case of a 34-year-old female patient presenting to our clinic with a pelvic mass which required surgical removal. Her past medical history was peculiar: she was diagnosed with a right ovarian tumor for which she underwent total hysterectomy with bilateral salpingo-oophorectomy. Histological features of the specimen suggested the diagnosis of mucinous ovarian carcinoma. Seven years later, she underwent a right hemicolectomy for a tumor recurrence involving the ascending colon, followed by the same histological diagnosis. After another eight years, a computed tomography scan showed a pelvic tumor which was removed and after histological evaluation with extensive immunohistochemical staining the diagnosis was clarified: juvenile granulosa cell tumor with extensive mucinous component. Our case report emphasizes the necessity of immunohistochemical staining for accurate diagnosis, especially when clinical features do not correlate with histology findings. Moreover, due to the rarity of these types of tumors, further reports and studies are encouraged to standardize their diagnosis and treatment. **Keywords:** JUVENILE GRANULOSA CELL TUMOR; IMMUNOHISTOCHEMISTRY; OVARIAN CARCINOMA.

Granulosa Cell Tumors (GCTs) are a type of malignant sex cord-stromal tumors and account for only 2-3% of all ovarian neoplasms (1). They present as slow-growing masses with an indolent natural course. Two types of GCTs are described based on their histologic appearance: adult and juvenile GCTs. Adult type is the most common occurring in perimenopausal women, while juvenile GCTs are rare, accounting for roughly 5%, and arise in young females (2). Imaging findings vary

from well-defined solid masses with internal cysts to pure cystic lesions (3). Macroscopically they appear as encapsulated lesions with a smooth surface resembling cystadenoma. The classic microscopic features found in GCTs are Call-Exner bodies which are amorphous, eosinophilic, fluid-filled spaces formed by secretions from neighboring granulosa cells; however, Call-Exner bodies are found only in the adult-type GCT thus their absence cannot exclude a juvenile GCT. Immunohistochemistry (IHC) is the only tool to provide a specific diagnosis. Inhibin, calretinin, FOXL2 and SF-1 are specific positive markers, while epithelial markers such as CK7, EMA are negative markers (4, 5). Due to their rarity and favorable prognosis, standard treatment protocols are yet to be implemented. However, current management is multimodal. Surgery is the first step. Stage I tumors are candidates for fertility-sparing surgery, while advanced stages require total hysterectomy with bilateral salpingo-oophorectomy and, if needed, cytoreductive procedures. Adjuvant therapy consists of chemotherapy, radiation therapy and hormone therapy. Follow-up regimens are hardly adopted, however GCTs are prone to late recurrence therefore, imaging follow-up is needed to early depict tumor relapse (6, 7). Our aim is to present the clinical course of a rare recurrent GCT with mucinous infiltration initially misdiagnosed as mucinous ovarian carcinoma. GCTs can present in many different facets and have a tendency to behave unpredictably with late relapses demanding a high degree of expertise for accurate clinical and pathological diagnosis. Our target is to include this entity in the differential diagnosis of ovarian masses, to support immunohistochemical staining in inconclusive cases and by reporting them, prospective studies may be conducted to standardize GCT diagnosis, optimal treatment and follow-up, considering their recurrence rate.

CASE REPORT

A 34-year-old female patient was admitted to our clinic after being diagnosed on a follow-up computed tomography (CT) scan with a pelvic tumor. Physical examination revealed an abdominal mass in the hypogastrium, painful on palpation. The abdomen showed signs of collateral venous circulation, while lower extremities showed signs of bilateral deep vein thrombosis (DVT). Laboratory results were insignificant except for an iron-deficiency anemia and slightly elevated CA125 (37.8 U/mL).

In her past medical history, we found some prominent features. She was diagnosed with mucinous ovarian carcinoma, based on the immunohistochemical findings (tab. I), for which she underwent total abdominal hysterectomy with bilateral salpingo-oophorectomy. Despite the confirmed results, the patient refused chemotherapy.

TABLE I

Immunohistochemical analysis of specimen suggestive for mucinous ovarian carcinoma

Marker	CK7	ER	CEA	S100	Vimentin	CK20	P53	PCNA
Result	Focally +	60% +	-	-	+	-	-	30%

Seven years later the patient was diagnosed with a tumor recurrence involving the ascending colon. A right colectomy was performed as the tumor was adherent

to the cecum. Specimen analysis revealed the diagnosis of mucinous ovarian carcinoma. Noteworthy is that IHC was not performed. The patient received six cycles of chemotherapy with carboplatin and paclitaxel.

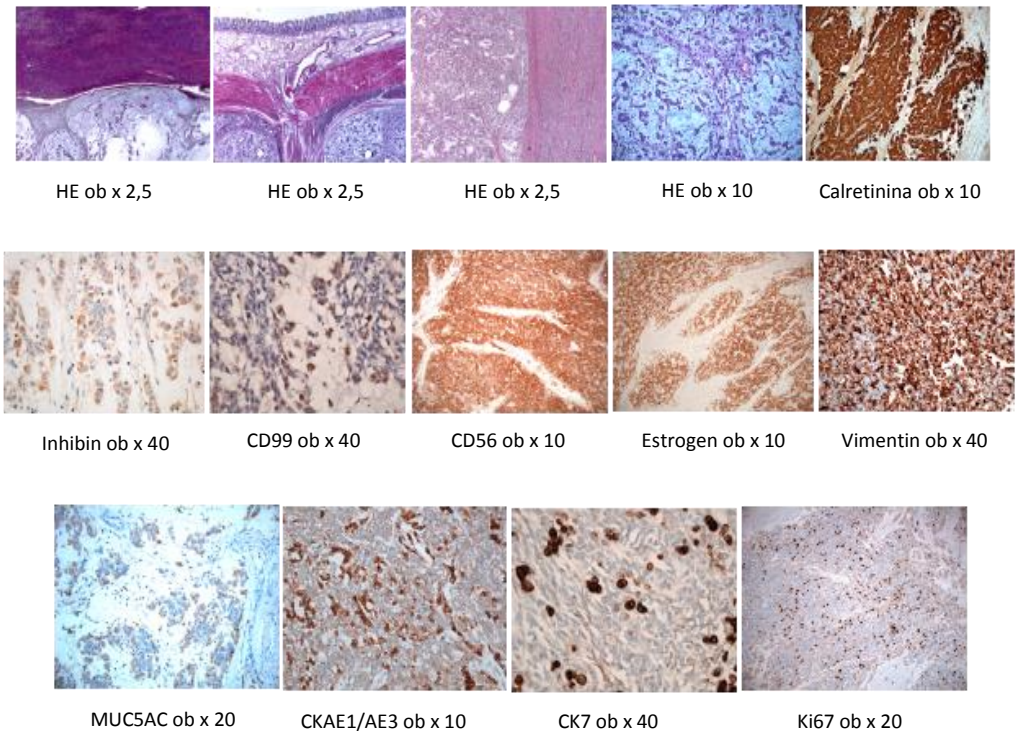

Fig. 1. IHC panel. Hematoxylin Eosin (HE) staining can be observed in the first four images. The next ten images show the presence of different IHC markers

Eight years later she was diagnosed in our clinic with an abdominopelvic tumor, the CT scan revealing a well-defined interhepatorenal tumor measuring 162/143/160 mm with direct invasion of the liver segment 6, right kidney, adrenal gland, and right diaphragmatic pillar. The mass had a compressive effect on the psoas muscle, caudate lobe, hepatic hilum, gallbladder and inferior vena cava (fig.1). Beside this mass, two other tumors were identified with the same features. One at the mesenteric root measuring 89/127/90 mm and another in the left pelvic area in direct contact with the sigmoid colon and bladder. In addition, multiple enlarged lymph nodes suggestive of metastatic spread were found in the paraaortic and external iliac regions, obturator fossa and mesentery. Intraoperatively, three tumors were identified. One measuring 120/15 mm located on the greater omentum, another measuring 80/100 mm attached to the mezosigmoid, and an infiltrative multilobulated mass in the right iliac fossa extending to the right upper quadrant and retroperitoneum. The masses were

removed along with the resection of the greater omentum, marginal hepatectomy, partial diaphragmatic peritonectomy, right nephrectomy and adrenalectomy, sigmoid colectomy and aortocaval lymph node dissection.

On receipt of the specimens, multiloculated, encapsulated masses were found. Cut section of the lesions showed a grey to white appearance with areas of hemorrhage, large cystic degeneration and mucinous content. Histopathological examination revealed round and fusiform cells with rich eosinophilic cytoplasm arranged in a lax architecture surrounded by mucus and rare Call-Exner bodies. Mitotic figures were 10/10 high power field. IHC testing was conducted and based on the expanded panel of markers (tab. II) the diagnosis was clarified: juvenile granulosa cell tumor with extensive mucinous component. Postoperative chemotherapy was initiated as soon as possible with cisplatin and etoposide.

TABLE II

IHC panel for the retrieved specimens

Marker	Result
CD 10	negative
EMA	negative
WT1	negative
CDx2	negative
CK20	negative
Podoplanin	negative
Inhibin A	focal positive
Calretinin	diffuse positive
ER	intense positive
CD 99	focal positive
CD 56	positive
MUC 2 and MUC 5 AC	low positive
Vimentin	positive
Cytokeratin AE1/AE3	positive and negative alternation
CK 7	focal positive
Ki 67	18% positive

DISCUSSION

Firstly, described by Rokitansky in 1885 (8), GCTs are sex cord tumors which can originate not only from granulosa cells, but also from interstitial cells, fibroblasts or techa cells explaining their occurrence in men. Morphologically, they are divided into two groups: adult GCT and juvenile GCT. This classification is based on their histological features, not on their epidemiology, thus adult GCT is indeed more frequent in postmenopausal women, but not exclusively. Adult granulosa cells show a solid organoid-like architectural pattern, with frequent pathognomonic Call-Exner bodies. In contrast, juvenile granulosa cells organize in nodular patterns, have a high mitotic index (and a worse prognostic), wide cytoplasm and rare Call-Exner bodies. Both types are usually unilateral, and both can differentiate into hormone-producing tumors. Juvenile GCTs are more frequent in younger patients, 90% of them being diagnosed at less than 30 years of age (3, 4, 5, 9, 10, 11).

Typically, GCTs present as slow-growing masses with a low malignant potential and favorable prognosis in comparison to epithelial ovarian cancers; however, they are at risk of early and late local recurrence, so a follow-up scheme is recommended including CT scans and tumor marker determinations. In these tumors the prognostic value of CA-125 is poor. Serum inhibin determination is a good choice for monitoring GCTs. Surgical removal followed by chemotherapy in recurrent cases is the mainstay of treatment. Radiation therapy has limited efficacy and hormone therapy is an option for hormone-producing tumors (12, 13, 14). Long-term follow-up including pelvic and abdominal examination, CT scan and tumor marker determina-

tion is recommended as delayed recurrences are characteristic for this disease.

Our case had a clinical presentation as a mucinous ovarian tumor with late relapses which required a high degree of suspicion for pathological diagnosis. We emphasize the importance of a complete immunohistochemical panel in differentiating ovarian masses. GCT cells usually stain positive for inhibin, calretinin, CD99, CD56, vimentin, estrogen and progesterone receptors. They are negative for CK7 and epithelial membrane antigen (EMA). After the first surgical excision of the ovarian tumor, the condition was mistaken for mucinous ovarian carcinoma. This confusion was possible precisely because the whole immunohistochemistry panel was not performed, in our case missing the determinations of inhibin, calretinin, CD 99, CD 56 and EMA. Next, after the removal of the right iliac fossa mass, immunohistochemical staining was not performed, supported by the previous histological exam, and a chemotherapy regimen for mucinous ovarian carcinoma was initiated. The recurrences were diagnosed each time in a locally advanced stage with adjacent visceral invasion because a follow-up sequence was not maintained since the patient did not stick to monitoring. Once the panel of immunohistochemistry markers is accomplished, a correct diagnosis can be made. The direct implications are connected to the appropriate adjuvant therapy and the necessity to track it in the long term. All these highlight the importance of identifying the whole immunohistochemistry panel in the case of patients with ovarian neoplasm, especially in the case of the young patients and when the tumor relapses over long periods of time.

CONCLUSIONS

We here presented a rare case of juvenile granulosa cell tumor misdiagnosed as mucinous ovarian carcinoma, emphasizing the necessity of performing a complete panel of immunochemistry markers. The rarity of these tumors as well as the lack of guidelines for the diagnosis and monitoring requires more insight into studying this disease.

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NEWS

FOLIC ACID AND RISK OF POSTPARTUM DEPRESSION

Postpartum depression, as a common complication of childbearing, is defined as an obvious depressive symptom or a typical depressive episode within 1 to 12 months after delivery. A cohort study aimed to assess the association between duration of folic acid supplementation during pregnancy and the onset of PPD in Chinese women. A total of 1592 participants were recruited, and data collected between July 2015 and March 2017 in Tianjin, China. Participants' baseline data were collected regarding socio-demographic and lifestyle characteristics, obstetric history, and folic acid supplementation during pregnancy. The Self-Rating Depression Scale, Chinese version was used to assess depressive symptoms at 6-12 weeks postpartum, and the prevalence of PPD in participants was 29.4%. Pregnant women who took folic acid supplements for more than 6 months had to lower prevalence of PPD, compared to those who took folic acid for less than 6 months. (Jing Yang, Yuyan Liu, Lujia Cao, Yuzhi Zheng, Wen Li and Guowei Huang. Association between Duration of Folic Acid Supplementation during Pregnancy and Risk of Postpartum Depression. *Nutrients* 2017; 9: 1206)

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EVALUĂRI CLINICO-EPIDEMIOLOGICE ASUPRA UNUI LOT DE 3521 DE PACIENŢI, CARE AU SUFERIT ACCIDENTE DE CIRCULAŢIE ÎN JUDEŢUL IAŞI, ÎN RAPORT CU LOCALIZAREA ŞI SEVERITATEA TRAUMATISMELOR, ASISTAŢI ÎN U.P.U. "SF. IOAN", ÎN PERIOADA 2002-2009

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CLINICAL EPIDEMIOLOGICAL ASSESSMENTS ON 3521 PATIENTS SUFFERING FROM ROAD TRAFFIC INJURIES, IN RELATION WITH TRAUMA LOCALISATION AND SEVERITY, ASSISTED IN "SF. IOAN" EMERGENCY UNIT, DURING 2002 - 2009 (Abstract): **The aim** of study was to assess road traffic injuries in relation with their localisation and severity. **Material and methods**: A sample of 3521 patients suffering from road traffic injuries and assisted in "Sf. Ioan" Emergency Unit, Iaşi, Romania was assess regarding age group, sex and residence area, type of lesions and ther localisation and severity, between 2002-2009. Data were collected using a special epidemiological inquiry and processed using SPSS and MS Excel statistical softs. **Results**: The incidence of road traffic injuries increased during the last decade, with a report men/women of 1.5, urban and 21-30 age group predominance. The most frequent were leg fractures (16.7%) and thoracal contusions (19.1%), cranial and facial trauma (32.4%), with open injuries (10.5%). **Conclusions**: Prevention programmes with a high efficiency at the national level, as well as a concret identification of risk factors with a multidisciplinar approach of road traffic accidents, are needed. **Key words**: ROAD TRAFFIC INJURY, INCIDENCE, FRACTURE, CONTUSION

Accidentele de trafic rutier reprezintă o problemă majoră de sănătate publică, care afectează toate sectoarele societăţii. În anul 2009, datele statistice arată că 1.260.000 de oameni au decedat în accidente de trafic rutier, reprezentând 2,2% din totalitatea deceselor şi 25% din decesele provocate de accidente în general (cauza principală a deceselor pentru persoanele cu vârsta între 15 şi 44 ani şi pe locul 9 pentru toate categoriile de vârstă) (1,2,3,4).

Prognoza OMS pentru 2020 indică trecerea accidentelor de trafic rutier, de pe locul 9, pe locul 3 la cauza deceselor, după sinucideri şi afecţiuni cardiovasculare (şi acestea din urmă provocate de stres, sărăcie şi suprasolicitare) (2,5,6,7). Faptul că 90% din accidentele de trafic rutier, la nivel mondial, au loc în ţările sărace şi mediu dezvoltate şi numai 10% în ţările dezvoltate (care au reuşit să reducă consecinţele accidentelor de circulaţie cu 50% în ultimul deceniu), indică necesitatea unei mai mari implicaţii în realizarea unor programe de prevenţie, cu eficienţă sporită şi aplicabilitate la nivel comunitar (8,9,10,11,12).

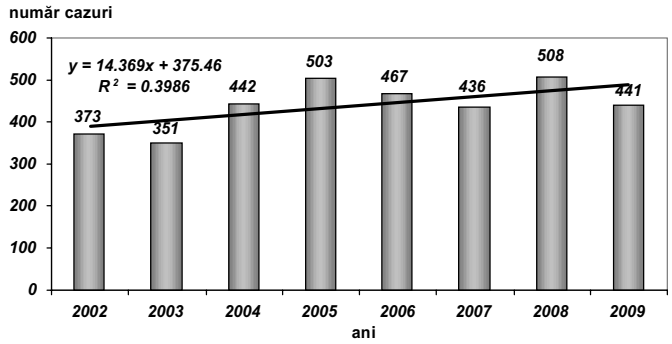

Fig. 1. Repartiţia numărului de cazuri de accidente de trafic rutier, pe ani, în judeţul Iaşi, în perioada 2002 - 2009

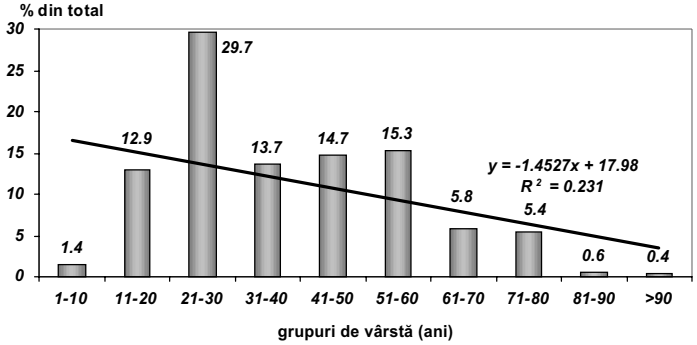

Fig. 2. Repartiţia numărului de cazuri de accidente de trafic rutier, pe grupuri de vârstă

MATERIAL ŞI METODE

Studiul a fost efectuat pe un lot de 3521 pacienţi, internaţi în secţia U.P.U. a Spitalului de Urgenţe „Sf. Ioan" Iaşi, în perioada 2002-2009. Culegerea datelor a fost realizată retroprospectiv, utilizându-se registrele de internare şi externare ale spitalului şi foile de observaţie ale pacienţilor. Prelucrarea datelor s-a efectuat cu ajutorul programului de statistică S.P.S.S. şi programului MS EXCEL 2007.

REZULTATE ŞI DISCUŢII

1. Incidenţa cazurilor de accidente de trafic rutier, pe ani, în judeţul Iaşi în perioada 2002-2009, arată o tendinţă crescătoare a numărului de cazuri, deşi în ani precum 2003, 2007 şi 2009 au fost înregistrate mai puţine cazuri, comparativ cu ceilalţi ani (2003 – 351 cazuri; 2007 – 436; 2009 - 441). Cele mai multe cazuri de accidente de trafic rutier au fost semnalate în anul 2008 (508 cazuri) (fig. 1).

Această tendinţă crescătoare a numărului de cazuri de accidente de trafic rutier poate fi pusă pe seama creşterii, mai ales în ultimii ani, a numărului de autovehicule, în situaţia în care condiţiile de carosabil au rămas aceleaşi sau chiar s-au deteriorat, aspecte observate şi de alţi autori (5,6,8,10).

2. Repartiţia pe sex şi medii a cazurilor de accidente de trafic rutier, în judeţul Iaşi, în intervalul de timp studiat, arată că sexul masculin a fost mai frecvent implicat (60,2%), faţă de feminin (39,8%), cu un raport bărbaţi / femei de aproximativ 1,5. Acest fapt are două posibile explicaţii: prima, ţinând cont de faptul că numărul de conducători auto de sex masculin este mai mare şi, deci, riscul este mai crescut; a doua explicaţie, de ordin psihologic, femeile conduc mai

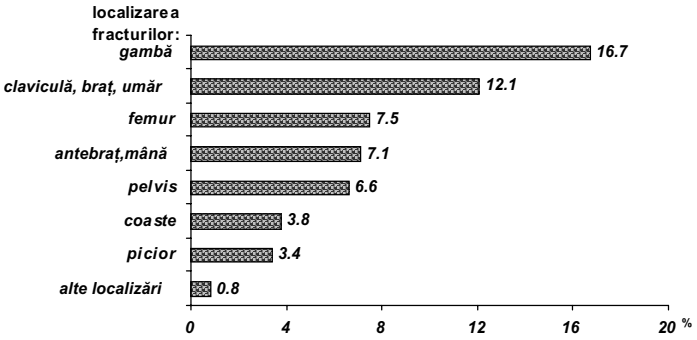

Fig. 3. Distribuţia fracturilor în funcţie de localizare

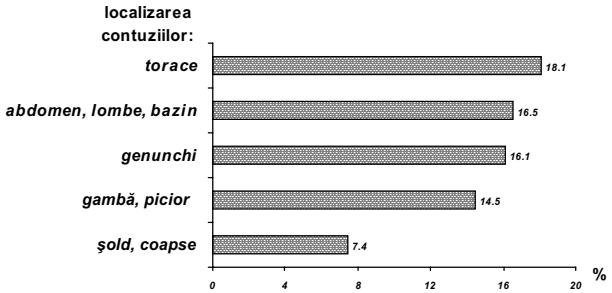

Fig. 4. Distribuţia contuziilor în funcţie de localizare

prudent, evită viteza excesivă, acordă o atenţie mai mare la semnele şi restricţiile de circulaţie. Repartiţia pe medii de reşedinţă a fost în favoarea celui urban, cu diferenţe statistic semnificative, în evaluarea cumulativă, pentru întreaga perioadă, cu explicaţia că în mediul urban, traficul este mai aglomerat, atât pietonal, cât şi pe carosabil.

Şi alte lucrări, publicate în ţară şi străinătate, evidenţiază faptul cert că bărbaţii sunt mai adesea implicaţi în accidente de trafic rutier decât femeile, cele mai multe accidente având loc în urban (2,4,6,9).

3. Repartiţia pe grupuri de vârstă a cazurilor de accidente de trafic rutier, luate în studiu, a înregistrat, pentru fiecare an, cât şi pentru întreaga perioadă, valori semnificativ mai crescute pentru grupul de vârstă 21 – 30 de ani (29,7% din total cazuri), urmat de 51-60 ani (15,3%) şi 41-50 ani (14,7%) (fig. 2). Această distribuţie a cazurilor poate fi explicată prin faptul că tinerii sunt mai frecvent implicaţi în trafic, atât ca pietoni, cât şi în conducerea autovehiculelor, în cadrul unor situaţii profesionale, familiale, relaţionale.

Date similare au fost menţionate şi în alte cercetări efectuate în diferite regiuni ale globului, atât în ţările dezvoltate, cât şi în cele cu un nivel socio-economic mai scăzut (3,5,10,11,12).

4. Repartiţia cazurilor de accidente de trafic rutier în funcţie de gravitatea leziuni arată faptul că procentul persoanelor care au suferit plăgi deschise a fost de 10,5%, comparativ cu plăgile închise, în procent de 89,5%. Numărul cazurilor de plăgi deschise, deşi mic, în comparaţie cu plăgile închise, este totuşi de luat în consideraţie, dată fiind gravitatea, de multe ori, extremă, a acestor leziuni, însoţite adesea de şoc hemoragic, comă, fiind responsabile de decesul rapid al acestor pacienţi accidentaţi în traficul rutier, înainte de a ajunge ambulanţa, aşa cum este precizat şi în lucrările altor

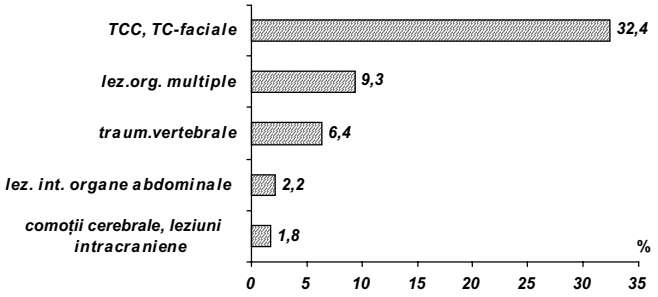

Fig. 5. Distribuţia leziunilor în funcţie de gravitate

cercetători (7,8,9).

5. Repartiţia fracturilor şi contuziilor în funcţie de localizare a arătat faptul că cele mai frecvente *fracturi* s-au situat la nivelul gambei (16,7%), urmate de localizarea la nivelul membrului superior (claviculă, braţ, umăr – 12,1%) (fig. 3). Comparativ, la nivelul toracelui s-au înregistrat cele mai multe *contuzii* (19,1%), urmând apoi localizarea acestora la nivelul abdomenului, lombelor şi bazinului (16,5%) (fig. 4).

Aceste rezultate denotă gravitatea ridicată şi riscul leziunilor organelor vitale toracale (cord, plămâni) şi abdominale (contuzii şi rupturi ale splinei, în special, care pot fi „în doi timp", ducând la şoc prin hemoragie internă şi deces, chiar după o perioadă de timp de la accidentul de trafic rutier) (3, 9, 10, 11).

6. Distribuţia leziunilor în funcţie de gravitate, pentru cazurile de accidente de trafic rutier, luate în studiu a evidenţiat un procent ridicat al traumatismelor cranio-cerebrale şi cranio-faciale (32,4%), urmate de leziuni multiple ale organelor interne (9,3%), iar comoţiile cerebrale şi leziunile intracraniene, de gravitate crescută, fiind întâlnite în 1,8% din situaţii (fig. 5).

Şi alte studii din ţară şi de la nivel mondial pun pe primul loc traumatismele cranio-cerebrale, atât prin frecvenţa lor, cât şi prin gravitatea şi riscurile vitale, care implică o supraveghere şi un tratament imediat şi prompt, în cadrul spitalizării în secţii de neurochirurgie (7,8,9,11).

CONCLUZII

Din punct de vedere al localizării leziunilor, aproape jumătate afectează extremitatea cefalică, un sfert nivelul membrelor şi o cincime torace. Un calcul economic exact al consecinţelor accidentelor de trafic este practic imposibil de realizat, singura certitudine pe care o avem este că tributul plătit de societate, atât medical, cât şi economic, este mult prea mare.

Devine, astfel, necesară instituirea unor programe de prevenţie eficiente la nivel naţional, precum şi adoptarea unor norme care să implice domenii variate ale activităţii umane, identificarea verigilor fragile ale factorilor implicaţi şi eliminarea sau minimalizarea acestora. Considerăm că abordarea multidisciplinară a prevenţiei accidentelor de circulaţie presupune adoptarea unor măsuri *legislative* adecvate şi verificarea respectării lor, a unor măsuri *administrativ - economice* care să vizeze spaţiul rutier, a unor măsuri de *educaţie pentru sănătate,* şi măsuri cu caracter *medical şi psihologic*.

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CANCER GENETIC COUNSELING IN EUROPE. PART II: CENTRAL AND EASTERN EUROPEAN COUNTRIES

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CANCER GENETIC COUNSELING IN EUROPE (PART II) CENTRAL AND EASTERN EUROPEAN COUNTRIES (Abstract): Hereditary Breast and /or Ovarian Cancer (HBOC) is the leading cancer among women worldwide. Since the discovery of the *BRCA* mutation in the ´90s, several healthcare programs have been established in Western European countries. These programs provide nationwide access to individualized care for families at high risk for HBOC. The genetic counseling starts with mutations screening programs with a guideline for further management of the high-risk group for breast cancer. Unlike Western European in the case of Central and East European countries, genetic testing for mutations in *BRCA1* and *BRCA2* genes is limited due to the high costs not covered by the insurance funds. As an exception between Eastern European countries in the Czech Republic genetic counseling and testing for hereditary breast and/or ovarian cancer is available to all women who are at risk of developing an early onset disease. Because of limited resources the rest of Eastern European countries try to identify some specific solutions for overcome this problem. One solution implemented with success in Poland is to identify founder recurrent mutations in order to offer testing for these recurring mutations to a large population. The high frequency of 3 founder mutations in Polish women supports the implementation of a national program that provides genetic counseling and testing with significantly reduced costs. The same panel of *BRCA1* founder mutations like for Poland, seems to be found in countries from Baltic sea region so the recommendation for national screening for these mutations in HBOC women exist since 2003. The other solution is to offer sequencing of the entire coding region of the gene, which is more precise and covers all mutations, to a highly selected and less numerous populations. For instance, in Romania, free genetic *BRCA1* and *BRCA2* genes testing and counseling is available only in some particular programs conducted in few Medical Centers, but Slovenia implemented a national program for genetic testing and counseling but restricted to EOC patients that were diagnosed with early onset high grade-serous EOC. **Keywords:** HEREDITARY BREAST AND OVARIAN CANCER, BRCA, GENETIC COUNSELING.

Hereditary Breast and/or Ovarian Cancer (HBOC) is the leading cancer among women worldwide. This form is mostly caused by mutations in such genes as *BRCA1* and *BRCA2* and is thought to account for 5 to 10% of all cases of breast cancer (1). Female *BRCA* mutation carriers face an elevated lifetime cancer risk of up to 60% for BC and ovarian cancer (OC) and an age-dependent contralateral BC risk of up to 55% (2, 3). Since the discovery of the *BRCA* mutation in the ´90s, several

health care programs have been established in Europe such as the German Consortium for hereditary BC/OC established by 17 specialized academic centers; The clinical guideline (CG) 41 developed by National Institute for Health and Clinical Excellence (NICE), in UK; or "The Cancer Plan" developed by the French Ministry of Health in coordination with the French National Cancer Institute (Institut National du Cancer / INCa) (4). These programs provide nationwide access to professional, individualized structured care for families at high risk for HBOC including interdisciplinary genetic, clinical and psychological counseling that establish individual risk assessment on the basis of a detailed pedigree and molecular genetic testing for those who fulfill the risk criteria. An intensified BC surveillance program is recommended depending on patients' age, morbidity, and personal preferences, even when individuals test negative for *BRCA1* or *2* mutations if the family history indicates a "high-risk" for BC through pedigree analysis This surveillance is well accepted and includes regular breast MRI, mammography, ultrasound, and a physical exam, all financed by the health insurances systems (5). Prophylactic surgery is also a topic of discussion for *BRCA* mutation carriers (6).

However, experiences from other countries show that not everywhere in the Europe women with HBOC get genetic testing and counseling. In Central and Southeastern European countries, incidence and mortality trends show an increment, due to recent changes in risk factors (smoking, age at menarche, age at first pregnancy, estrogen intake, etc.) (7). The delay in transition and European integration of these countries has reflected also on the status of the investigation of hereditary disorders, including hereditary breast and ovarian cancer (HBOC), resulting in the fact the most of eastern and central European countries are currently without such a national cancer genetic testing and counseling programs (7). The genetic counseling, starting with mutations screening programs is available at some oncological centers, with a guideline for further management of the high-risk group for breast cancer specific for each institution. Unlike Western European in the case of Central and East European countries genetic testing for mutations in *BRCA1* and *BRCA2* genes related to breast cancer, as well as for any other cancer type of cancer, is limited due to the high costs (2,000 €) that is not covered by the insurance funds (8). Moreover, the group of genetic counseling (geneticist or molecular biologist, psychologist, oncologist), is not always available at the Cancer Centers. So, free genetic testing is available only in some particular programs (8).

In this article we aim to describe the current status and trends of cancer genetic testing and counseling programs in several Central and Eastern European countries to raise awareness among physicians and payers, and to propose the most important changes needed to improve oncology care in CEE countries.

Cancer genetic counseling in Eastern European countries

It is difficult to estimate the model of genetic counseling applied in Central and Eastern European countries, particularly because, to our knowledge, data on adherence to the various models are lacking. As in all countries in transition with very limited resources, the introduction of highly sophisticated and very expensive laboratory techniques into clinical practice is always questionable. Due to the lack of hot spots or recurrent mutations in the majority of

geographical areas, direct sequencing of the entire coding region of both *BRCA* genes is strongly recommended for *BRCA* analysis, which would make population-based screening expensive. Because of limited recourses Eastern European countries try to identify some specific solution for overcome this problem. The first solution is to conduct some HBOC genetic screening study in order to identify the recurrent mutations, even less frequent than Israel`s or Iceland`s, in order to offer testing for these recurring mutations to a large population. The second solution is to offer sequencing of the entire coding region of the gene, which is more precise and covers all mutations, to a highly selected and less numerous populations. Identification of BRCA mutation carriers, establishment of spectra and frequency of BRCA mutations, especially in breast cancer patients, together with long-term follow-up of mutation carriers, should allow research on the factors affecting penetrance of BRCA gene mutations. Further, systemic BRCA analysis will enable introduction of clinical management for mutation carriers into the clinical practice, resulting in real benefit for this highly vulnerable part of the population (9).

One of the countries that successfully implemented the first strategy is **Poland**. Three mutations in *BRCA1* (*5382insC*, *C61G* and *4153delA*) are common in Poland and account for the majority of mutations identified to date in Polish breast and breast-ovarian cancer families. It is not known, however, to what extent these 3 founder mutations account for all of the *BRCA* mutations distributed throughout the country (10). This question has important implications for health policy and the design of epidemiologic studies. The high frequency of this mutations in Polish women supports the recommendation to testing for genetic ovarian cancer susceptibility with significantly reduced costs. A national program has been established in Poland that provides genetic counseling and testing services for these 3 mutations to the entire population at no cost to the participants (11). At this moment several Genetic Counseling Units like Warsaw Cancer Center Institute of Oncology offers risk counseling based on the occurrence of cancer in the pedigree. In the families identified with an increased cancer risk, genetic testing is critical for preventive healthcare, including specialized surveillance programs and prophylactic mastectomy and/or oophorectomy, which reduce the risk of breast/ovarian cancer by over 90%. However, according to Polish guidelines and recommendations, the government sponsored surveillance program, which includes annual mammography and breast magnetic resonance imaging, pelvic ultrasonography, and cancer antigen 125 levels, is provided only to women with known pathogenic mutations (12).

The same panel of *BRCA1* founder mutations like for Poland, seems to be found in countries from Baltic sea region. The most frequently detected mutation in this Baltic Sea region is the *c.5266dupC*, found from the Baltic Sea to the Pacific Ocean and from Europe to Siberia (13). The *c.4035delA* (*4154delA*) mutation is the second most prevalent mutation in the Baltic states (13). Despite these founding, what is assuring extremely high cost-effectiveness in identification of individuals with high risk of breast and/or ovarian cancers, was recommended for practical genetic testing purposes since 2003 (13), there is no national program available in these countries at this moment.

As an exception between Eastern European countries genetic counseling and test-

ing for hereditary breast and/or ovarian cancer is available to all women in the **Czech Republic** who are at risk of developing early onset disease. There is access to genetic counselling in all regions of the country and two major molecular genetic laboratories provide *BRCA1* and *BRCA2* testing for eligible women. Specialized preventive care is organized by 2 centers, within the Complex Oncology Treatment Clinic of MMCI in Brno, and the Department of Oncology of the General Faculty Hospital in Prague, however, some patients and their family members are under the care of other oncology departments and clinics (14). Oncologists, gynecologists and geneticists from both centers draw up and publish guidelines for the specialized preventive care of *BRCA1* and *BRCA2* carriers in the Czech journal Clinical Oncology. Since 1999 almost three thousand probands (patients) and two thousands of family members were tested at Masaryk Memorial Cancer Institute (MMCI). The protocol for the *BRCA1* and *BRCA2* carrier's follow-up and for other individuals with high risk of breast cancer was published in 2001 and 2009. The main purpose of these publications was to standardize the prevention within oncology centers and provide to the carriers the most updated preventive procedures.

The first study of 20 **Bulgarian** familial BC patients was performed in 1998 aiming to develop a screening approach for the *BRCA1* gene (15). No mutations but only benign polymorphisms were identified, most likely due to the small sample size, the low stringency of the selection criteria and the lack of complete analysis of *BRCA2*. Up to 2015 no other surveys on *BRCA1/2* mutations in Bulgarian patients with familial BC/OC have been published. The lack of sufficient genetic studies for inherited mutations in *BRCA1/2* genes in the Bulgarian population impedes the introduction of an effective mutation screening that would identify the individuals at high risk in BC/OC families. In 2015, team from Departments of Surgery and General and Clinical Pathology, University Hospital, "Alexandrovska", Medical University of Sofia, and the Clinic of Medical Oncology, Specialized Hospital for Active Treatment in Oncology, Sofia, conduct a genetic analysis for *BRCA1/2* germline mutations in a cohort of 200 Bulgarian women with BC, fulfilling the recognized international criteria. This is the first comprehensive study in Bulgaria aiming to ascertain the contribution of *BRCA1/2* germline mutations to familial BC in the Bulgarian population. The project has been supported by Ministry of Education and Science, Republic of Bulgaria. No other data about genetic counseling programs is available (16).

In **Romania** the first molecular investigation of the role of the *BRCA* genes in breast and ovarian cancer was made in 2010 at "Grigore T. Popa" University of Medicine and Pharmacy, Iasi (17). In 2014, after 10 years of collaborative research with *Jean Perrin Center* from Clermont-Ferrand, France, at "Grigore T. Popa" University of Medicine and Pharmacy Iasi was founded the Department of Oncogenetics. A founded by University program for implementation of efficient and coherent system of HBOC molecular diagnostic, rick evaluation and further ontogenetic counseling and management, using the French model, was conducted in collaboration with *Jean Perrin Center*. The program offers free genetic testing d counseling for the first 100 HBOC families. Another project of *BRCA1* /*BRCA2* testing of 200 women with beast/ovarian cancer was started in

2015 at "Prof. Dr. I. Chiricuţă" Institute of Oncology in collaboration with "Iuliu Haţieganu" University of Medicine and Pharmacy, Cluj-Napoca. This prospective study will enhance the result of germline *BRCA1* /*BRCA2* mutations in Romanian high-risk breast cancer patients (18).

Genetic screening for mutations on inherited breast/ovarian cancer in **Slovenia** started at the Institute of Oncology Ljubljana, in 1999. The first step in implementing a *BRCA1/2* genetic counseling was the approval of the Commission for Medical Ethics at the Ministry of Health, Republic of Slovenia for a study that aim to include into the process of genetic counselling all living patients with epithelial ovarian cancer (EOC) younger than 45 years at the time of diagnosis in the period 1999-2008 according to the data of the Slovenian National Cancer Registry made at the Institute of Oncology Ljubljana (19). The process of genetic counselling included the possibility of genetic *BRCA1/2* testing, with all possible clinical implications offered afterwards. All germinal *BRCA1/2* mutation (*gBRCA1/2m*) positive patients were offered inclusion into the screening and prophylactic program for the high-risk group for breast cancer. In addition, the PARP inhibitor therapy was offered to relapsed *BRCA* positive serous ovarian cancer patients. Following new guidelines of SGO and National Comprehensive Cancer Network (NCCN), genetic counselling is offered now to all EOC patients in Slovenia. However, genetic testing is still restricted to EOC patients that were diagnosed with high grade-serous EOC (20).

CONCLUSIONS

Large differences in cancer epidemiology, in the majority of oncology care parameters and in treatment outcomes, exist between CEE countries and neighboring Western European countries. Since the European Union (EU) is built on the concept of free movement of goods, services, people and capital, the EU aims to safeguard the quality of health care for consumer protection reasons. Delivery of health care is still the responsibility of the individual countries within the EU. Because of the growing mobility of professionals and patients, the EU's mission is to encourage and support progress to ensure high quality and comparable care across Europe. In this condition to accelerate the development of cancer genetic testing and counseling in CEE countries and to reduce the apparent disparities across Europe, in CEE countries a shared set of prioritization criteria would be desirable. Prioritization should be based on considerations of medical benefit; health need and costs and all decisional factors should be included in further discussion and better evidence is needed before clearly defined common recommendations and strategy for improving patient management in all European Union countries. Screening programs for genetic testing of HBOC patients should be implemented, and the results of the research study on local founder mutations should be centrally evaluated and used for increase cost/benefit efficiency of genetic testing programs. Improved education for all involved in oncology care is needed. Special attention should be on training specialists in cancer genetic counseling and management. Also, very important and needed throughout the region is development of clinical guidelines and training for counseling and cancer care management and follow-up of cancer patients and survivors is needed. Countries in the CEE region urgently need to increase investments and make higher allocation of health care budg-

ets for oncology in order to diminish gaps between them and neighboring Western European countries. Implementation of all these measures needs additional EU funds to decrease inequalities in cancer control in Europe.

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CANCERUL MAMAR LA FEMEILE CU VÂRSTA PESTE 60 DE ANI

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BREAST CANCER IN WOMEN OVER 60 YEARS OF AGE (Abstract) : The breast cancer is the most frequent malignancy in women over 60 years of age in the developed countries and it represents the second frequent cause of death through malignancies in women. **Material and method** : The study was performed on 63 women over 60 years old diagnosed with breast cancer in an one year period (2006) in our service. The aim of the study is to establish the clinical particularities and the optimal therapeutically management of this disease on elderly women. **Results** : The incidence of the malignancy is high (78% of all cases) in women of 60 to 69 years of age. The most common risk factor revealed in this study is the obesity (33%). The cardiovascular diseases (70%) and the atrophic cervico-vaginitis (43%) are the most frequent associated conditions. The incidence of the low stages of the malignancy is low (14%) and usually the tumors are well differentiated ductal invasive carcinomas (63%). The modified Madden radical mastectomy was performed in most of the cases (75%). **Conclusions** : The breast cancer in elderly women has an incidence peak at the age of 60 to 69 years . Despite the numerous associated conditions the surgical treatment remains the most effective therapy. **Key words** : BREAST CANCER, ELDERLY WOMEN, RISK FACTORS, THERAPEUTICALLY MANAGEMENT

Cancerul mamar reprezintă cea mai frecventă afecţiune malignă a femeilor din ţările dezvoltate şi constituie a doua cauză de mortalitate prin cancer în rândul femeilor cu vârsta de peste 60 de ani (1).

Cancerele ginecologice la persoanele vârstnice sunt diagnosticate, de obicei, în stadii avansate, deoarece acestea nu beneficiază, de cele mai multe ori, de consultaţii ginecologice de rutină în scopul realizării unui screening oncologic genital.

Incidenţa mai crescută a malignităţilor la pacientele vârstnice se explică prin mai multe ipoteze :

- mecanismele de reparare a ADN-ului sunt afectate în mod negativ odată cu avansarea în vârstă ; în consecinţă celulele îmbătrânite nu mai controlează replicarea ADN-ului, fapt ce duce la aneuploidie şi proliferare necontrolată. În cazul persoanelor tinere aceste anomalii declanşează mecanisme ce duc la moartea celulei afectate (apoptoză) (2) ;
- scăderea funcţiilor sistemului imun, în mod special a imunităţii mediate celular (3) ;
- patologia malignă diagnosticată la persoanele vârstnice apare , deobicei , după o lungă perioadă în care factorii de risc au acţionat favorizând apariţia bolii. Acest fapt coroborat cu ipotezele mai sus menţionate determină apariţia cu o frecvenţă mai crescută a maladiei neoplazice la persoanele vârstnice (2,3).

Agresivitatea relativ mai scăzută a neoplasmelor la pacientele vârstnice se poate explica prin teoria neoangiogenezei mali-

gne, aceasta fiind mai redusă la pacienta vârstnică, limitând rata de creştere tumorală.

Femeile cu vârsta de peste 60 de ani deţin o treime din totalul cazurilor de cancer mamar şi jumătate din decesele datorate acestei afecţiuni. Studiile efectuate relevă faptul că femeile în vârstă au frecvent tipuri histologice mai favorabile, nivele mai mari de receptori estrogenici si progesteronici, rată de creştere tumorală mai scăzută şi mai puţine metastaze (3).

Factorul vârstă este unul dintre cei mai importanţi factori de risc pentru cancerul mamar. Riscul ca o femeie cu vârsta sub 40 ani să dezvolte cancer de sân este mai mic de 0,5%, acest risc crescând de peste 20 de ori la aceeaşi femeie(10%) la vârsta de 90 ani (4).

Depistarea precoce a neoplaziei mamare prin efectuarea mamografiei ca metodă de screening ar reduce considerabil stadiul neoplaziei mamare în momentul diagnosticării acesteia. Mortalitatea prin cancer mamar la 10 ani s-ar reduce cu 25-30%. Din păcate, chiar şi în ţările dezvoltate, screeningul prin mamografie al cancerului mamar la persoanele vârstnice este deficitar. În USA peste 50% din femeile cu vârsta de peste 60 de ani nu au efectuat vreodată o mamografie (4,5).

Mamografia, ca singură metodă exploratorie de screening, nu diagnostichează aproximativ 10-15% din cancerele mamare (6).

Asociaţia Americană de Geriatrie recomandă ca şi metode de screening mamografia şi examenul clinic al sânului , care trebuiesc efectuate anual până la vârsta de 75 de ani şi după această vârstă se recomandă ca mamografia să fie efectuată la 2 ani iar examenul clinic al glandei mamare anual (7,8).

MATERIAL ŞI METODĂ

Lotul de studiu este constituit din 63 de cazuri reprezentând pacientele cu vârsta peste 60 de ani diagnosticate în perioada 1 ianuarie-31 decembrie 2006 cu neoplazie mamară în Clinica a II-a Obstetrică-Ginecologie, Spitalul de Obstetrică-Ginecologie "Cuza-Vodă" Iaşi.

Lotul principal, în funcţie de criteriul vărstă, s-a subdivizat în 3 subloturi - cu vârsta cuprinsă între 60-69 ani, 70-79 ani şi peste 80 de ani.

Factorii urmăriţi în studiu sunt: vârsta, mediul de provenienţă, factorii de risc, stadiul bolii, forma anatomo-patologică, prima secvenţă terapeutică, tipul intervenţiei chirurgicale, durata medie de spitalizare, patologii frecvent asociate.

REZULTATE

Incidenţa maximă a neoplaziei mamare se află în subgrupul pacientelor cu vârsta între 60-69 ani – 49 cazuri, restul cazurilor fiind distribuite astfel: 13 cazuri - subgrupul 70-79 ani, 1 caz – subgrupul pacientelor cu vârsta peste 80 de ani. Mediul rural prezintă o incidenţă uşor mai crescută comparativ cu cel urban (53,96% vs 46,04%).

Dintre factorii de risc obezitatea a fost cea mai frecventă manifestare întâlnită (33,3%), fiind urmată în ordinea frecvenţei de menopauza tardivă, lipsa alăptatului la sân, antecedente familiale de neoplazie mamară, menarha precoce

Patologiile asociate, mai ales cele cardiovasculare, pot reprezenta la acest grup de vârstă un obstacol în instituirea imediată a terapiei bolii canceroase, necesitând în primul rând echilibrarea acestora.

Cervico-vaginita atrofică a fost întâlnită în

TABELUL I
Factori de risc

Factor de risc	Număr cazuri
Obezitatea	**21**
Menopauza tardivă	**8**
Lipsa alăptatului la sân	**6**
Antecedenta familiale de neoplazie mamară	**5**
Menarha precoce	**5**
Nuliparitatea	**4**

TABELUL II
Patologie asociată

Patologie asociată	Sublot I 60-69 ani	Sublot II 70-79 ani	Sublot III >80 ani
Cervico-vaginita atrofică	18	8	1
H.T.A.	14	7	1
Cardiopatie ischemică	10	6	1
D.Z.	5	2	0
Prolaps genital	3	2	0
Varice membre inferioare	3	1	0

cele mai multe cazuri (42,85%), fiind urmată de bolile cardio-vasculare (cardiopatia ischemică - 27%, HTA - 35%), diabet zaharat (11,1%), prolaps genital (7,93%), varice la nivelul membrelor inferioare (6,34%).

Din punct de vedre anatomo-patologic carcinomul invaziv ductal domină ca frecvenţă –(63,49%), fiind urmat în ordine de carcinomul invaziv lobular (25,41%), mastita carcinomatoasă (6,34%) şi de carcinomul microinvaziv ductal (4,76%).

Stadiul neoplaziei în momentul diagnosticului a fost avansat în cele mai multe cazuri, stadiul III a fost întâlnit cel mai frecvent (34,9%), excepţie făcând sublotul pacientelor cu vârsta cuprinsă între 70-79 ani în care stadiul IV a fost întâlnit cu aceeaşi frecvenţă ca şi stadiul II (4 cazuri). Stadiile incipiente ale neoplaziei mamare (0/I) au fost diagnosticate în 9 cazuri (stadiul 0 – 3 cazuri, stadiul I – 6 cazuri) .

Conduita : tratamentul chirurgical a fost instituit de primă intenţie în cele mai multe cazuri (54,14%), factorul vârstă, în absenţa unei patologii grave asociate, neinfluenţând intervenţia chirurgicală, în restul cazurilor s-a optat în 39,69% din cazuri ca primă intenţie terapeutică polichimioterapia, iar în 3,79% din cazuri radioterapia.

Durata medie de spitalizare a pacientelor, în special a celor operate, a crescut uşor odată cu vârsta acestora (de la 11 zile în sublotul celor cu vârsta între 60-69 ani la 14 zile în sublotul pacientelor cu vârsta cuprinsă între 70-79 ani).

DISCUŢII

Variantele tratamentului chirurgical sunt multiple, mulţi practicieni optând în cazul pacientelor în vârstă cu cancere mamare în stadii incipiente pentru excizia sectorială în limite de siguranţă oncologică urmată de evidare ganglionară axilară şi radioterapie postoperatorie. O altă variantă menţionată în literatură constă în hormonoterapie (Tamoxifen, Raloxifen) ca unic tratament pentru pacientele vârstnice cu cancere mamare cu receptori estrogenici prezenţi şi cu patologii asociate ce prezintă un risc anestezic şi chirurgical crescut (7).

Conduita noastră a constat într-o abordare mai radicală a bolii canceroase în cazul pacientelor la care starea generală a permis acest lucru. Pornind de la premiza că neoplazia mamară prezintă un grad crescut de multicentritate (până la 70% în cancerele lobulare şi până la 40% în cancerele ductale) (3) mastectomia radicală tip Madden cu limfadenectomie axilară a fost metoda de elecţie în tratamentul cu scop curativ al cancerului mamar, constituind 75% din intervenţiile chirurgicale practicate. La mastectomie tip Pattey cu limfadenectomie axilară s-a recurs în 5,5% din cazurile operate, iar mastectomia simplă s-a practicat în 19,5% din intervenţii.

Bolnavele în vârstă cu cancer mamar beneficiază de tratament multimodal. Pentru stadiile I-II secvenţa chirurgicală este prima metodă terapeutică. Pentru stadiile III-IV radioterapia este prima metodă terapeutică urmată de mastectomia “de curăţire”. Mas-

tectomia simplă este indicată numai cazurilor la care terenul nu permite o operaţie de durată.

Complicaţiile tratamentului chirurgical sunt relativ rare. Complicaţiile intraoperatorii constau din leziuni neurovasculare şi ale pleurei. Leziunile nervului Charles Bell şi subscapular nu sunt urmate întotdeauna de o atitudine vicioasă a omoplatului sau sechele respiratorii. Leziunile pachetului vascular axilar sunt rare şi obligă la sutură vasculară şi nu ligatură. Complicaţiile postpoperatorii imediate pot fi reprezentate de: hematoame, colecţii limfatice în regiune axilară, supuraţii, dezunirea suturilor, tromboflebita venei axilare (9). Lotul studiat nu a prezentat complicaţii intraoperatorii sau postpoperatorii imediate.

Complicaţiile postoperatorii tardive constau în: edemul important al membrului superior de partea sânului operat –cea mai redutabilă complicaţie a intervenţiilor radicale, având cauze multiple: obstrucţie venoasă sau limfatică, scleroză cicatricială (9). În lotul studiat s-au semnalat 2 cazuri care au prezentat un edem moderat al braţului datorat unei tromboflebite de venă axilară. Sub tratament anticoagulant evoluţia a fost favorabilă în ambele cazuri.

Complicaţiile postoperatorii datorate decompensării patologiei preexistente au constat în 3 cazuri în dezechilibrarea diabetului zaharat, 2 cazuri - agravarea simptomatologiei datorate cardiopatiei ischemice, 4 cazuri - agravarea hipertensiunii arteriale, 2 cazuri - tromboflebită profundă membre inferioare. Evoluţia acestor cazuri a fost favorabilă sub tratament, nu s-a înregistrat niciun deces în perioada postoperatorie.

CONCLUZII

Cancerul mamar prezintă o creştere a incidenţei odată cu avansarea în vârstă. Pe lotul studiat, paciente cu vârsta peste 60 de ani, incidenţa maximă a neoplaziei regăsindu-se în subgrupul pacientelor cu vârsta între 60 şi 69 de ani.

Stadiul bolii în momentul diagnosticului este, de cele mai multe ori, avansat şi creşte odată cu înaintarea în vârstă.

Formele anatomo-patologice întâlnite la aceste vârste nu diferă semnificativ ca frecvenţă şi tip în comparaţie cu cele întâlnite la vârste mai tinere, agresivitatea bolii canceroase neprezentând o creştere la persoanele vârstnice.

Bolile cardiovasculare (H.T.A., cardiopatia ischemică) domină ca frecvenţă şi severitate patologia asociată bolii canceroase la acest grup de vârstă.

Obezitatea, factor de risc al majorităţii neoplaziilor ginecologice, este o condiţie patologică frecventă.

Tratamentul chirurgical, în lipsa comorbidităţilor severe şi coroborat cu stadiul bolii, rămâne secvenţa terapeutică principală, factorul vârstă necontraindicând aplicarea acestuia.

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THE ROLE OF INTERLEUKIN 10 (rs1800872) AND HEAT SHOCK PROTEIN 70-2 (rs1061581) GENES IN THE MECHANISMS OF COLIENTERITIS DEVELOPMENT

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THE ROLE OF INTERLEUKIN 10 (rs1800872) AND HEAT SHOCK PROTEIN 70-2 (rs1061581) GENES IN THE MECHANISMS OF COLIENTERITIS DEVELOPMENT (Abstract): *E. coli* is one of the commonest causes of diarrhea worldwide responsible for 20-50% of all infectious diarrheas. Genetic background of the condition, including the possible role of IL-10 (rs1800872) and thermal shock protein 70-2 (rs1061581) genes remains unclear. **Aim:** To find the possible role of IL-10 (rs1800872) and Heat Shock Protein (HSP) 70-2 (rs1061581) genes in the mechanisms of colienteritis pathogenesis. **Material and methods:** 95 patients with diarrheal escherichiosis and 30 practically healthy individuals (control) participated in the study. Gender composition presented with 62 women (65.26%) and 33 men (34.74%), mean age – 38.66±3.11 yrs. The single nucleotide polymorphisms (SNP) of HSP70-2 (A1267G) and IL-10 (C-592A) genes were studied in PCR. **Results**: Among patients, IL-10 (rs1800872) and HSP70-2 (rs1061581) genetic mutations were observed with frequencies of 9.47% and 3.16%, respectively, compared to absence in control. Allelic distribution of HSP70-2 gene's 1267G>A SNP showed 2.62 times G-allele domination over A-allele ($p<0.001$): among patients by 43.16% ($\chi^2=70.78$; $p<0.001$), and in control by 50.0% ($\chi^2=30.0$; $p<0.001$), with insignificant excessive heterozygosity, not disturbing expected Hardy-Weinberg's equilibrium. Allelic distribution of IL-10 gene's C-592A SNP shows C-allele 2.9 times dominated ($p<0.001$) in general population: among patients by 43.16% ($\chi^2=70.78$; $p<0.001$), in control – by 66.66% ($\chi^2=53.33$; $p<0.001$), with preserved population equilibrium. **Conclusions**: Mutant A-allele of the IL-10 (rs1800872) gene associates with almost twice increased colienteritis risk in observed population [OR=1.98; 95%CI OR=1.0-4.20; p=0.046], with protective role of wild C-allele [OR=0.50; 95%CI OR=0.24-1.01; p=0.058]. HSP70-2 gene's SNP (rs1061581) does not associate with colienteritis risk. **Keywords:** COLIENTERITIS, DIARRHEA**,** PATHOPHYSIOLOGY, INFLAMMATION, SINGLE NUCLEOTIDE POLYMORPHISM, HSP70-2 (RS1061581) GENE, IL-10 (RS1800872) GENE, ALLELE, RISK.

According to WHO's Diarrheal disease Fact sheet and Global Burden of Diseases, about 1.7 to 5 billion cases of diarrhea occur per year worldwide. Total deaths

from diarrhea are estimated at 1.26 million in 2013, down from 2.58 million in 1990. In 2010, it is the second most common cause of deaths (0.76 million or 11%) in children younger than five years (1, 2).

Colienteritis is one of the commonest causes of diarrhea with incidence of 20-30 cases per 100,000 individuals in developed countries and is caused by pathogenic strains of *Escherichia coli* (3). The pathophysiology of colienteritis clinical manifestations to some extend depends on *E. coli* variant (enterotoxigenic, enteroinvasive, enteropathogenic, enterohemorrhagic, and enteroaggressive). However, major pathogenetic mechanisms of colienteritis development are universal, involving pro- and anti-inflammatory cytokines (4). Genetic background for intestinal disorders underwent explosive development in recent years, mainly focusing on microbiome and its relationship with metabolome and proteome. Genetic background for digestive system diseases as well as other inflammatory conditions is well established, proving associations of several genes' single nucleotide polymorphisms (SNPs) with hepatitis, metabolic syndrome (5, 6) . However, lesser attention was paid to genetically determined susceptibility to *E. coli* emphasizing development of intestinal and systemic inflammation. Our attention attracted genes regulating IL-10 and Heat Shock Protein (HSP) synthesis. Both IL-10 and HSP play an important role in regulating systemic inflammation, though the role of respective genetic mechanisms in colienteritis requires clarification. Therefore, *the aim of this study* was to find the possible role of IL-10 (rs1800872) and Heat Shock Protein (HSP) 70-2 (rs1061581) genes in the mechanisms of colienteritis pathogenesis.

MATERIAL AND METHODS

The study involved 95 individuals with escherichiosis (mean age 38.66±3.11 yrs. ranging from 25-52 yrs.), among them 62 (65.26%) female and 33 (34.74%) male patients. Colienteritis diagnosis was based on clinical symptoms, history, laboratory findings, including microbiology and coded as A04.8-A.04.9 in IDC-10. Enteropathogenic, enteroinvasive, enterohemorrhagic *E. coli* , hemolytic (*E. coli* Hly+), and lactose-negative *E. coli* were isolated and identified. Identification of other possible intestinal pathogens led to exclusion from the study group. Control group formed by 30 practically healthy individuals of respective age and gender without any acute or chronic inflammatory processes and no antibacterial drugs intake in any form for at least 6 months prior to the study.

This study *conforms to international bioethical standards* (European Convention on Human Rights and Biomedicine, the Declaration of Helsinki of the World Medical Association on ethical principles of scientific medical research involving human subjects, GCP, EUC directive #609, etc.) and approved by Commission for Bioethics in Research of the Bukovinian State Medical University, Ukraine. All patients signed written permissions and obtained full information about the study prior to participation.

Polymerase chain reaction (PCR) was used to study the polymorphism of HSP70-2 (A1267G, rs1061581) and IL-10 (C-592A, rs1800872) genes. DNA was isolated from peripheral venous blood lymphocytes using a set of DNA-sorb-V reagents. PCR was performed using Taq-DNA-polymerase and following specific primers for HSP70-2 (5′-CATCGACT TCTACACGTCCA-3′ – *forward* and 5′-CAAAGTCCTTGAGTCCC AAC-3′ – *reverse*) gene (7), and IL-10 (5′-

CCTAGGTCACAG TGACGTGG-3′ – *forward* i 5′-GGTGAG CACTACCTGA-CTAGC-3′ – *reverse*) gene (8).

Allele discrimination of the HSP70-2 gene was performed using restriction endonuclease PstI (Fermentas, Lithuania). The products of DNA amplification (amplicons) of the C-592A SNP of the IL-10 gene and those of the restriction of HSP70-2 gene A1267G SNP were separated in horizontal electrophoresis in a 3% agarose gel concentrated with 4 μL of ethidium bromide for 45-60 minutes. Visualization using transluminator with addition of GeneRuler 50 bp DNA Ladder (Thermo Scientific, USA) molecular weights marker. Visualized images analyzed in *SnapGene* and *Vitran* software packages.

Statistical processing was carried out using the *MS Excel, Primer of Biostatistics 6.05* and *Statistica 7.0* software. Logic regression was used for establishing genetic dependencies.

RESULTS

No statistically reliable differences in genotypes and alleles of both HSP70-2 (A1267G) and IL-10 (C-592A) genes' SNPs relative frequencies (tab. I-II) between study and control groups were observed. In general population, from 190 identified alleles, in both control and study groups HSP70-2 (rs1061581) gene's G-allele dominated 2.52 and 3 times ($p<0.001$), and IL-10 (rs1800872) gene's C-allele dominated 2.52 and 5 times ($p<0.001$), respectively. Distribution of HSP70-2 gene's 1267A→G and IL-10 gene's C-592A SNPs in studied cohort meets the Hardy-Weinberg equilibrium ($p>0.05$) with a slight excess of heterozygosity, which generally does not disturb the population balance.

Colienteritis risk emphasizing HSP70-2 (rs1061581) SNP is presented in table III depicting the fact that polymorphic variants of HSP70-2 gene may not be additional risk or protection factors for colienteritis in general observed population.

Epidemiologic analysis of coli enteritis risk associations with IL-10 gene's (rs1800872) genotypes is presented in table IV. Minor A-allele associates with almost double increased colienteritis risk in general population [OR=1.98; 95%CI OR=1.0-4.20; p=0.046] with marginal protection role of IL-10 gene's C-allele [OR=0.50; 95%CI OR=0.24-1.01; p=0.058].

TABLE I

Genotypes and alleles frequencies of HSP70-2 gene's A1267G (rs1061581) SNP

Studied gene, n (%)		Study group, n=95 (%)	Control, n=30 (%)	OR [95% CI]	χ^2 p
HSP70-2 (A1267G), n (%)	GG	44 (46.31)	15 (50.0)	0.86 [0.38-1.96]	$\chi^2<1.0$ p>.05
	AG	48 (50.53)	15 (50.0)	1.02 [0.45-2.32]	$\chi^2<1.0$ p>0.05
	AA	3 (3.16)	0	-	-
χ^2 p		$\chi^2=58.77$ p<0.001	p>0.05	-	-
HSP70-2 (A1267G), n (%)	G-allele	136 (71.58)	45 (75.0)	0.84 [0.43-1.63]	$\chi^2<1.0$ p>0.05
	A-allele	54 (28.42)	15 (25.0)		
χ^2 p		$\chi^2=70.78$ p<0.001	$\chi^2=30.0$ p<0.001	-	-

TABLE II

Genotypes and alleles frequencies of IL-10 gene's C-592A (rs1800872) SNP

Studied gene, n (%)		Study group. n=95 (%)	Control, n=30 (%)	OR [95% CI]	χ^2 p
IL-10 (C-592A), n (%)	CC	50 (52.63)	20 (66.67)	0,56 [0.23-1.31]	χ^2=1.82 p>0.05
	CA	36 (37.89)	10 (33.33)	1.22 [0.51-2.90]	χ^2<1.0 p>0.05
	AA	9 (9.47)	0	-	-
χ^2 p		χ^2 =41.15 p<0.001	χ^2 =6,67 p=0.01	-	-
IL-10 (C-592A), n (%)	*C*-allele	136 (71,58)	50 (83.33)	0.50 [0.24-1.06]	χ^2=3.31 p>0.05
	A-allele	54 (28,42)	10 (16.67)		
χ^2 p		χ^2 =70,78 p<0.001	χ^2 =53,33 p<0.001	-	-

TABLE III

Polymorphic variants of HSP70-2 gene as risk factors for coli enteritis in general studied population

Potential risk factor	Indices				
	RR	95% CI RR	OR	95% CI OR	p
AA-genotype (hypothetically)	0.93	0.10-8.59	0.92	0.09-9.24	>0.05
AG-genotype	0.99	0.66-1.49	0.98	0.43-2.25	>0.05
GG- genotype	1.08	0.71-1.64	1.16	0.51-2.64	>0.05
A-allele	0.88	0.54-1.44	0.84	0.43-1.63	>0.05
G-allele	1.05	0.88-1.24	1.19	0.61-2.31	>0.05

TABLE IV

Polymorphic variants of IL-10 gene as risk factors for coli enteritis in studied population

Potential risk factor	Indices				
	RR	95% CI RR	OR	95% CI OR	p
AA-genotype (hypothetically)	2.82	0.38-21.53	3.03	0.37-24.99	>0.05
AG-genotype	0.88	0.50-1.55	0.82	0.35-1.95	>0.05
GG- genotype	0.79	0.57-1.08	0.56	0.23-1.31	>0.05
A-allele	1.71	0.93-3.14	1.98	1.0-4.20	0.046
G-allele	0.86	0.74-0.99	0.50	0.24-1.01	0.058

DISCUSSION

Naturally, colienteritis pathophysiologic mechanisms studies are focused primarily on microbiologic factors, which definitely have a meaning. Following contamination by similar pathogen, presence of similar concomitant environmental, age and gender associated risk factors, the response of the immune system differs, as well as the severity of the disease, the appearance of complications. It is likely that an important role in the immunological response to the

pathogen belongs to non-specific immune resistance provided by neutrophils and is subject to mRNA expression of protein activity encoded by the corresponding genes. One of the molecular systems for protecting cells from cytotoxic reactions is HSP. Previous studies proved the protective role of HSP70 on intestinal cells (9). Tahara *et al.* found that HSP70-2 gene associated with a reduced risk of gastric cancer in older persons infected with H. pylori (10) but increases the risk of duodenal ulcer (11). J.A. Lin et al n showed that A1267G SNP of this gene (rs1061581) does not associate with the development of spondylitis in the Chinese population (12), vulvaginitis in adolescent girls of Northern Bukovina (8), but increases the risk of intestinal perforations, formation of fistulas and abscesses in patients with Crohn's disease in Japanese and European populations (13). This is the first available study of the HSP70-2 gene's A1267G SNP associations with the colienteritis. However, it shows that this SNP of the HSP70-2 gene is not an additional risk factor for colienteritis.

The IL-10 gene in humans is highly polymorphic and at the promoter region several single nucleotide polymorphisms have been described. In individual studies, an association was established between the C592A polymorphism of the IL-10 gene and the onset of coronary heart disease in people younger than 45 years old (14); with higher serum IgE levels in schistosomiasis (15) among the Brazilian population (in mutation A-allele carriers); osteoarthritis severity with diabetes mellitus and obesity (7). In this study we found almost double increase in the risk of colienteritis only in the carriers of the mutational A-allele of the IL-10 gene (rs1800872). Other researchers did not establish links between the studied IL-10 gene SNP with the appearance and severity of several infections including leprosy (16) and viral hepatitis B (17). Valença *et al.* observed no association between the polymorphisms of the IL-10 gene (G1082A / -C819T / -C592A) and the regression of periportal fibrosis after specific treatment for schistosomiasis (18).

CONCLUSIONS

In the observed colienteritis patients, mutations of IL-10 (rs1800872) and HSP70-2 (rs1061581) genes in the homozygous state occur at frequencies of 9.47% and 3.16%, respectively, compared to the absence in control. IL-10 (rs1800872) and HSP70-2 (rs1061581) genes' relative frequencies of alleles did not differ significantly between patients and control.

Mutant A-allele of the IL-10 (rs1800872) gene associates with almost twice-increased colienteritis risk in observed population, with protective role of wild C-allele. Polymorphic variants and alleles of the HSP70-2 (rs1061581) gene are not an additional risk factors for colienteritis in the examined population.

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THE ALTERNATIVE METHOD FOR DETECTING DENTAL CARIES USING AN OPEN SOURCE SYSTEM ARDUINO

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THE ALTERNATIVE METHOD FOR DETECTING DENTAL CARIES USING AN OPEN SOURCE SYSTEM ARDUINO (Abstract): **Aims:** Developing an instrument based on the electrical impedance measurement for dental caries detection and transmission of the result on a fast access device (laptop, tablet and smart phone). **Material and methods:** The system is based on a microcontroller as an Open Source Hardware and an easy to use Open Source Software. The hardware architecture contains signal collection electrodes for resistance measurement of tooth, a microcontroller with Bluetooth data communication and a human machine interface (HMI) with Bluetooth interface and software for data visualization. **Results:** The measured impedance value is used to classify caries types. The extracted features results are showed as a text that suggests the diagnosis. **Conclusions:** The instrument is very useful for fast evaluation of dental health. The system has low power consumption and is very easy to operate with it. **Keywords:** INSTRUMENTATION, DENTAL DECAY, OPEN SOURCE, IMPEDANCE MEASUREMENT.

Every biological material possesses its own electrical signature. When a current pass through this biological material, it will produce an effect on the impedance (1, 2).

The technique of electrical impedance measurements is a promising alternative method for occlusal caries diagnosis. It was described as an original method in 1951, and then used in 1980: Vanguard Electronic caries detector (Massachusetts Manufacturing Corporation, Cambridge) and Caries Meter L (GC International Corporation Interleuvenlaan, Leuven). In 2001 there was made a device for the measurement of the electrical impedance of the hard-dental tissues which was verified in the Cariology laboratory from the "Grigore T. Popa" University of Medicine and Pharmacy Iasi, with the purpose of detecting occlusal caries in incipient phases (3). Then in 2011 it has been created a virtual instrument used to diagnose dental caries using NI data acquisition device and LabVIEW software (4).

Especially in children and adolescents, crack cavities represent 80% of the untreated cases. These are often hidden by an intact enamel layer. Conventional methods of detecting hidden caries are not safe enough in these situations.

There are three directions that influence

the development of medical instruments and establish an early position in a very competitive market: producing safe, high-quality devices for patient care, reduced development time and low-cost system.

It is very difficult to design a medical product that has features and capabilities necessary to be used by a particular customer. To overcome this challenge, we need a new approach that allows increased modularity of design complexity, while permitting the implementation of innovative ideas from non-technical experts. Also, the new trend in Open Source encourages the researchers to develop instruments using open hardware at low cost and free open software (5). Open source systems are widely used in various domains. Some authors are using it for ECG measurements, smart sensors or intelligent home (6), (7).

In recent years, several research groups started to investigate alternative systems for monitoring dental diseases. In this paper, we present an instrument for early detecting dental decay, based on open-source electronics prototyping platform, flexible, easy-to-use hardware and software.

MATERIAL AND METHODS

In our days, the modern microcomputer technologies allow us to simple install various data acquisition systems by using friendly open hardware and open software.

The developed instrument is working with low frequency while out-of-phase component is negligible, and we can use the term resistance. We chose to use a sine wave signal with a frequency of 400 Hz and effective value of 1 μA generated on an analogical output channel of the microcontroller (8, 9). On the output analog channel, we used an electronic passive low-pass RC filter of first order. One simple low-pass filter circuit consists of a resistor in series with tooth impedance and a capacitor in parallel with tooth impedance. We use a combination of resistance (R) and capacitance (C) in order to give the value of 400 Hz for generated frequency (C=100 nF and R=3.98 kΩ). The break frequency, also called cutoff frequency (f_c) is determined by the time constant τ:

$$t = RC \quad (1)$$

$$f_c = \frac{1}{2\pi\tau} = \frac{1}{2\pi RC} \quad (2)$$

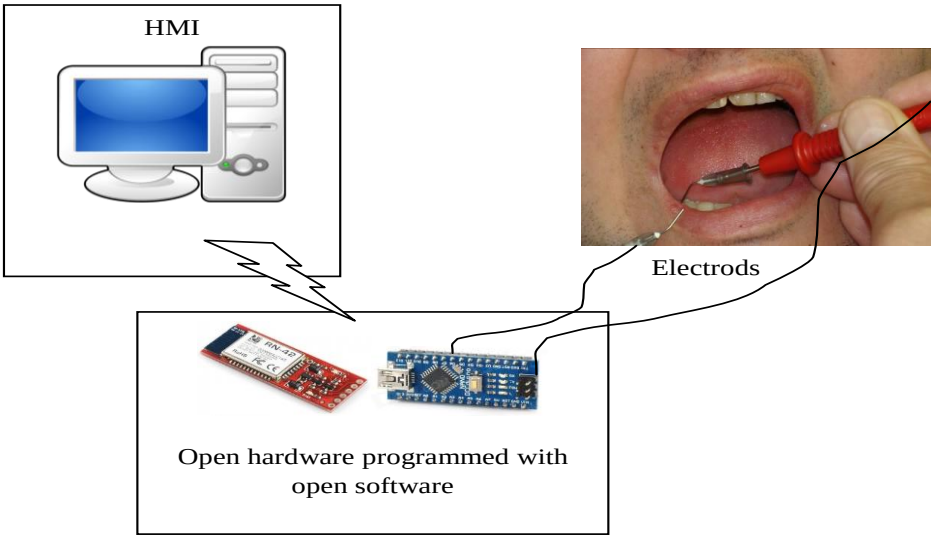

Fig. 1. The hardware architecture of detecting dental decay instrument

The developed system architecture consists of the following elements: signal collection electrodes, the microcontroller with Bluetooth data communication and human machine interface (fig. 1).

Signal collection electrodes for resistance measurement of tooth, active electrode, are applied in the mouth: the active electrode on the tooth and the neutral one on the lip, using single use sterile probes with equal and negligible resistance (R=0.914 Ω). The instrument would not produce pain due to the extremely low generated current.

The microcontroller with Bluetooth data communication was designed to be small so that it fits into a mobile device like a pen which would give the dentist more freedom of handling. We use Arduino micro and RN42 Bluetooth module.

The third element is the human machine interface (HMI) with Bluetooth interface and software for data visualization, represented by a laptop with Java technology-based software.

RESULTS

We use open software like Arduino IDE to program the microcontroller and Java for desktop applications. The implemented firmware is made for the low and high level implemented software. In Arduino IDE we set the functionality of the microcontroller:

1) setup serial communication and pins communication;

2) wait for serial port configuration from desktop application;

3) generate sinusoidal signal over PWM (Pulse Width Modulation) output;

4) acquire data from electrodes throughout analogue input channel;

5) voltage calculus;

6) resistance calculus starting from read voltages.

The instrument was calibrated against standard resistors and the linearity is relatively constant over the full scale (fig. 2).

$R = a \cdot U^3 + b \cdot U^2 + c \cdot U + d$ (3) where the constants a, b, c, d take the values: $a = -3 \cdot 10^{-5}$, $b = 1{,}54 \cdot 10^{-2}$, $c = 5{,}55 \cdot 10^{-1}$, $d = -8{,}062 \cdot 10^{-1}$.

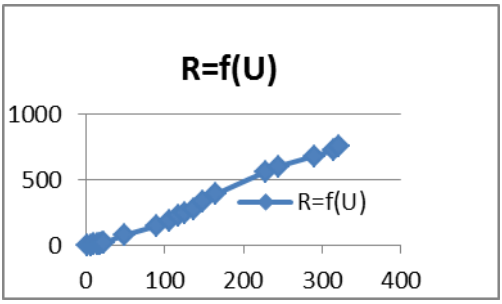

Fig. 2. Calibration graph

7) classify caries types using calculated impedance as shown in the paper;

8) send data over Bluetooth.

The desktop application made in Java software as a human interface, that provides setup communication parameters and has main function to show and save data, which is after sent to the instrument (fig. 3).

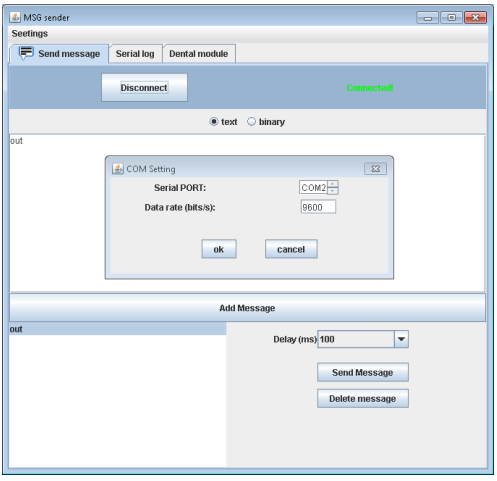

Fig. 3. Java application software - Settings

Measured impedance value is used to classify caries types. The extracted features results

are shown as a text that suggests the diagnosis. Depending on the impedance value Z, the displayed text will be different. An example is showed in the figure 4.

By using "Serial log", the dentist can check and view the history measurement (fig. 5).

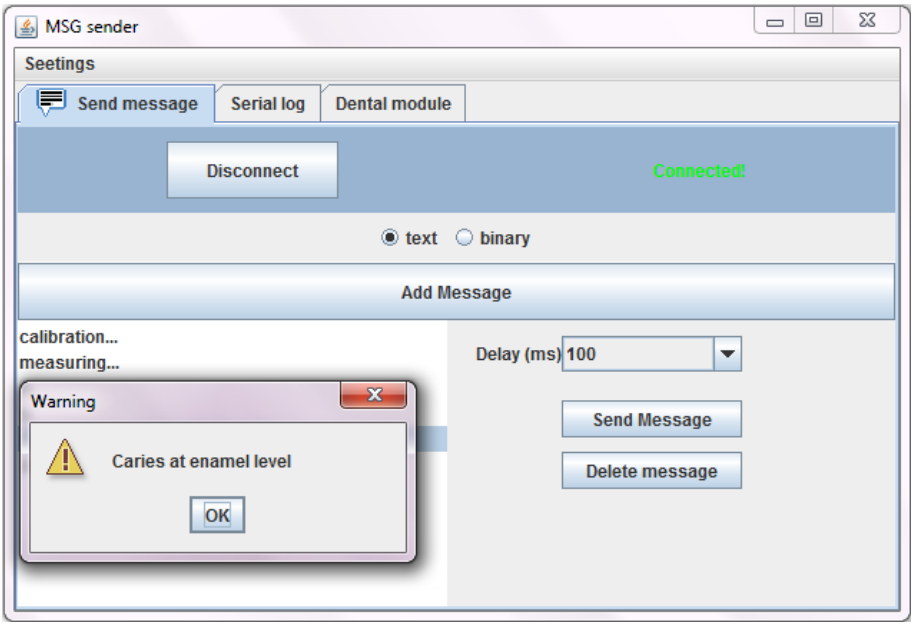

Fig. 4. Java application software – Warning message

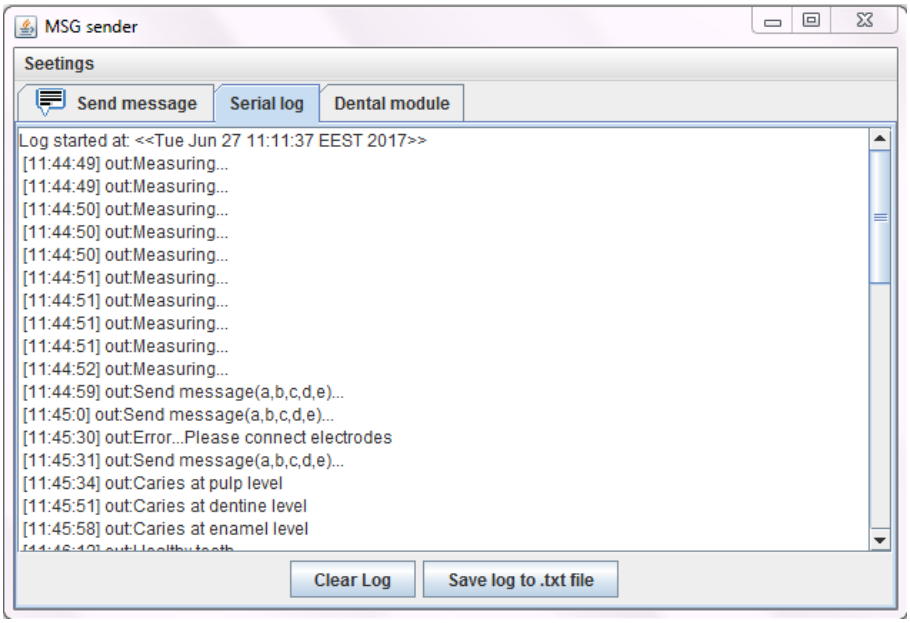

Fig. 5. "Serial log" - the history measurement

The device has been tested in a Dental Office, on a group of 18 subjects in order to put the right diagnosis. The measurements were taken not only on decayed teeth, but also on healthy ones. The results were also validated by a dentist, who diagnosed the subjects through classical methods.

DISCUSSION

Starting from the research of Mayuzumi and Suzuki (10), our instrument software is based on the measurement of electrical impedance of dental tissues. When a sinusoidal current cross the dental tissue with 400 Hz frequency, the impedance is more than 600 KΩ. It has been observed that the electrical impedance decreases as the dentin caries progresses, so the impedance of enamel caries presents values between 250 and 600 KΩ and in dentin caries, the impedance varies between 15 and 250 KΩ, depending on the depth of decay (fig. 6).

The term impedance is used to describe the resistance to an alternating current at a certain frequency. The total impedance of the circuit is the sum of the components in phase and out of phase. Due to the fact that, the frequency in the device is small, the out of phase component is negligible, and the term resistance can be used.

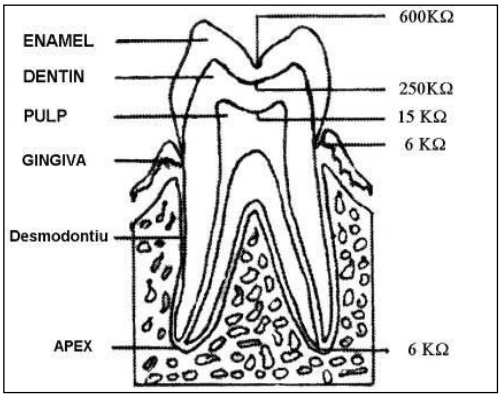

Fig. 6. Impedance values representation depending on the decay type

The classification of caries types using calculated impedance:

- Z = 0÷15 kΩ - "Caries at pulp level"
- Z = 15÷250 kΩ - "Caries at dentine level"
- Z = 250÷600 kΩ - "Caries at enamel level"
- Z > 600 kΩ - "Healthy tooth"
- Z < 0 – "Error" (10).

By tightly integrating hardware, software, validation, and reporting tools, the Java technologies provide the best solution for rapidly developing and testing medical devices and allow an expert in designing integrated systems, to accelerate the development of medical devices.

CONCLUSIONS

Starting from the theory underlying the use of electrical resistance in the diagnosis of caries (that enamel is a good insulator, but during the caries process, porosity formed in tissue which will be filled with water and ions saliva, acting like networks conductive, causing reduced electrical resistance), we formed an instrument which measures the resistance and that can provide us to give a diagnosis.

The device is easy to use by the dental practitioner and has technical advantages in comparison with other techniques used so far because the results are transmitted via Bluetooth to a device much easier to follow (laptop, tablet and smartphone).

The work presented is also a part of the open source systems trend, in a sense of reducing the costs, flexibility and biological signal processing systems modularization. Using an HMI and a low-cost hardware to process and measured signals, our solution allows a rapid development of the product, resulting in quicker deployment. The software that we developed represents an important tool, which helps dentists to speed up diagnosis.

Comparing with other diagnosis methods, our measurement method for the electrical resistance of the tooth seems to offer the highest hope for achieving reliable, accurate detection of the earliest stages of enamel demineralization. The time for taking measurement data is shorter comparative to other methods.

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NEWS

CHARACTERIZATION OF LARYNGOTRACHEAL MICROBIOTA IN PATIENTS WITH LARYNGOTRACHEAL STENOSIS

Knowing that microbial flora may play a role in mucosal inflammation, Hillel AT *et al*. studied the microbiota associated with laryngotracheal stenosis of various etiologies, in adults. The study showed significant differences in microbial community composition between scar and nonscar samples, as well as depending on etiology and sample site. Thus, scar samples and samples taken from the glottis region had decreased microbiota diversity. Members of the family *Moraxellaceae*, including *Moraxella and Acinetobacter*, were associated with idiopathic scar samples. There was an inverse correlation between Prevotella and Streptococcus among samples. The study concluded that these differences may influence the management of the disease (Hillel AT, Tang SS, Carlos C, Skarlupka JH, Gowda M, Yin LX, Motz K, *et al.* Laryngotracheal Microbiota in Adult Laryngotracheal Stenosis. *mSphere*. 2019; 4(3). pii: e00211- 19. doi: 10.1128/mSphereDirect.00211-19).

MECHANICAL VIBRATION APPLICATION AND LOW-LEVEL LASER THERAPY TO ACCELERATE ORTHODONTIC TOOTH MOVEMENT, AN OVERVIEW

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MECHANICAL VIBRATION APPLICATION AND LOW-LEVEL LASER THERAPY TO ACCELERATE ORTHO-DONTIC TOOTH MOVEMENT, AN OVERVIEW (Abstract): It is well-known that in the last decades, orthodontic treatments are successful not only for children and young people, but also for adults. One of the drawbacks of classical orthodontic treatments, reported by patients, is the prolonged period of the orthodontic treatment. In this context, specialty research focuses on the improvement and successful application of devices for accelerating orthodontic treatments, which would allow a considerable reduction in treatment time. From the available devices that can accelerate the rate of orthodontic tooth movement, two completely non-invasive devices, that need no individualization, have a wide addressability and have been discussed more and more frequently in the literature are, Low Level Laser Therapy (LLLT) and mechanical vibration application. Being relatively new techniques in orthodontics a need is felt to pay more attention to the understanding of the basic principles of using these methods and to identify and summarize the main characteristics of these two devices. The aim of this overview study is to lead to a better understanding of the possibilities of using these devices in day to day practice. This paper presents some of the characteristics of these methods that we found through the literature searches conducted for reviews and research articles related to vibrational devices and LLLT that accelerate orthodontic tooth movement. **Keywords***:* ORTHODONTICS, TOOTH MOVEMENT, LOW LEVEL LASER THERAPY, VIBRATIONAL DEVICES.

In orthodontics, in order to achieve tooth movement a mechanical stimulus is introduced by placing fixed appliances on the teeth (1). The fixed orthodontic appliances are bonded to the tooth surfaces that were previously conditioned in order to facilitate adhesion (2) using various materials (3). Then certain forces are applied to the dentoalveolar complex in order to realize these movements (1). Applying external physical force will lead to the inflammation or ischemia of the periodontal ligaments and bone remodeling, this phenomenon with inflammatory reactions can sometimes be painful for the patient. The time needed for a classic, fixed orthodontic treatment is around 2-3 years and a prolonged treatment can also lead to complications. Reducing the duration of orthodontic treatment can decrease the incidence of side-effects, there-

fore create more comfort for patients (4). Knowing the importance of the correlations between dental, skeletal and chronological age in orthodontics (5), the initial situation, treatment evolution and outcome need to be evaluated and stored using the currently available digital technologies (6, 7).

In an effort to reduce treatment time and increase effectiveness, new methods have been introduced to accelerate orthodontic tooth movement. Four non-invasive techniques, that are used to accelerate orthodontic movements, are described in the literature: application of electric currents (8) or electromagnetic fields (9), LLLT (10, 11, 12) and mechanical vibration application. Studies have shown that cellular response may be modified by application of mechanical forces and this response may be controlled using these non-invasive devices during the treatment period.

Intrusive application of these techniques can lead to unwanted and very harmful side effects. For instance, early signs of orthodontic overload are manifested in the vascularization of the dental pulp and the marginal gingiva, which can be assessed objectively by using a Laser Doppler Flowmetry (LDF) with a proper technique (13, 14, 15, 16). LDF is a non-invasive diagnostic tool that allows the early, objective, continuous and real-time evaluation of the vascular dynamics in the dental pulp (17, 18, 19, 20) and marginal periodontium (21, 22, 23).

Low-Level Laser Therapy (LLLT) has been shown to have biological effects on hard and soft tissues in dentistry. LLLT stimulates bone remodeling by induction of osteoblasts proliferation in the tension site and osteoclasts in the pressure site (24).

As another non-invasive method vibrational stimulus, used as a method to increase the rate of bone remodeling have been shown as a promising and convenient solution.

In this overview the earlier mentioned techniques will be discussed in order to better understand their work mechanisms and their effectiveness during orthodontic treatment.

MATERIAL AND METHODS

An electronic search for relevant articles regarding accelerated orthodontic tooth movements was conducted using *Medline-PubMed, ScienceDirect, Springer Link* and *Web of Science* via *e-information.ro* electronic platform. From the studied literature, the articles that mentioned LLLT and vibrational devices that may accelerate tooth movement and shorten the orthodontic treatment period were selected.

Low Level Laser Therapy

Low-Level Laser Therapy (LLLT) or photo biomodulation is successfully applied to orthodontics and is known as Light Accelerated Orthodontics (LAO) (25). In the specialty literature are mentioned multiple effects of the light radiation in the tissues such as stimulation of the dentoalveolar complex with a potential impact on ATP production by mitochondrial cells. During the tooth movement phase, higher ATP availability helps cells 'turnover' more efficiently leading to an increased remodeling process due to an elevated metabolic activity and accelerated tooth movement. Moreover, LLLT could increase vascular activity, which would also contribute to the rapid turnover of the bone and is amenable to light.

Many researches support the claim of increased osteoblastic activity following LLLT *in vivo* (26) and *in vitro* (27, 28). On the other hand, recent studies have indicated enhanced osteoclastic activity after Low-Level Laser Therapy *in vivo* and *in vitro* (29).

A number of clinical studies (30, 31, 32,

33) have suggested that LAO may also lead to increased velocity of canine movement and decreased pain, and a significantly higher acceleration of retraction of treated canines. In addition, LLLT stimulates vascularization, epithelization and collagen synthesis (34) and has an analgesic, biomodulatory and anti-inflammatory effect on dental tissues (35, 36, 37) that may influence the outcome of orthodontic treatment.

Vibrational devices

Cyclic vibration (38) also known as low-level oscillatory signals application Low Intensity Pulsed Ultrasound (LIPUS) (39), Low-Intensity Vibration (LIV) (40, 41) or Low-Frequency Mechanical Vibration (LFMV) (42) can have various positive effects. There is compelling evidence that bone remodeling can be significantly accelerated through the application of pulsating force of various frequency and magnitude (43) and also depends on general health status (44).

Studies support the idea of two main theories that explain the bone remodeling process: piezoelectricity generated in the alveolar bone and pressure-tension sites within the periodontal ligaments. (45). Mechanical forces have a great influence on bone remodeling, mechanical cyclical stretching, fluid shear stress, compression, and microgravity play different roles in cell differentiation and proliferation by affecting intracellular interactions (46).

It has been shown that cyclic forces transmitted multiple times to biological structures lead to a faster response at cellular level (43). Clinical studies have shown that factors increasing the rate of bone remodeling also increase the rate of tooth movement (10, 11).

DISCUSSION

Low-Level Laser Therapy or **photo biomodulation** is a non-invasive technique which uses specific wavelength range, intensity and light penetration.

We emphasize that the impact of the LLLT on the tissues will be dependent on the wavelength and light intensity and other specific working parameters of laser radiation.

According with Elkatan *et al.* (47) acceleration of orthodontic tooth movement using LLLT is dose-dependent, very low dose or very high dose will not cause acceleration**.** LLLT with the optimum dose can be used during retention to decrease the retention period, but a retainer should be used. Otherwise the relapse tendency would increase.

Fromont-Colson *et al.* (12) stated that LLLT can be effective for accelerating orthodontic movement but it may be necessary to concentrate research efforts on finding the optimal dose for LLLT.

Kau *et al.* (25), had determined that LAO leads to increase in the rate of orthodontic tooth movement and also demonstrate its efficacy in humans in a large cohort. An important observation to note is that the impact of LAO was independent of the center and investigator effect, which makes it even more viable in clinical settings in general practice.

It is well known that LLLT has a biostimulation effect on cell proliferation and wound healing (10) and also could be used on soft tissues as a photodynamic therapy (48). In certain, *in vivo* animal and human experiments LLLT enhanced the velocity of orthodontic tooth movement and improved the quality of bone remodeling (4, 10, 11).

Several clinical trials based on animal models and human subjects have investigated the effect of LLLT on accelerating orthodontic tooth movement (47, 49, 50).

Also reviews conducted on the subject suggested that LLLT may be effective but given the limited amount of information

available and relatively small number of studies further investigation may be required (12, 51).

Ge M.K. *et al.* (52) throughout their research concluded that LLLT might accelerate orthodontic tooth movement and they found that there is no adverse effect associated to this treatment. Also, they related that relatively low energy densities (2.5, 5, 8 J/cm^2) were more effective, however an optimal dose remained yet undetermined.

Samara S.A. *et al.* (53) found that photo biomodulation therapy applied for 3 min/ day accelerates orthodontic space closure, however in their opinion to establish a standardized protocol further clinical studies are needed.

Sedky Y. (54) *et al.* concluded that both low-level laser therapy and cortectomy-facilitated orthodontics can reduce the treatment period, but LLLT has the advantage of being non-invasive.

In the study conducted by Yassaei S. *et al.* (55), applying a 980 nm diode laser would not significantly increase the rate of orthodontic tooth movements, although changes at cellular level were noticeable.

The limited number of clinical studies, different protocols and potential publication bias is the reason that LLLT effectiveness may be questioned, idea sustained by Long H. *et al.* (51) in their meta-analysis and by Sonesson M. *et al.* (56) in their systematic review.

The results of our literature search regarding LLLT was summarized in tab. I.

TABLE I.

Literature search for LLLT articles

No.	Author names and year	Study Type	Results
1.	Elkattan A.E. *et al.*, 2012	Clinical study	Increases the rate of orthodontic tooth movement
2.	Isola G. *et al.*, 2019	Clinical study	Increases the rate of orthodontic tooth movement
3.	Alazzawi M. *et al.*, 2018	Clinical study	Increases the rate of orthodontic tooth movement.
4.	Fromont-Colson C. *et al.*, 2017	Review	Can be effective. Further investigation is necessary.
5.	Ge M.K. *et al.*, 2015	Meta-analysis	Effective in accelerating the rate of orthodontic tooth movement.
6.	Ghizlane G. *et al.*, 2013	Clinical study	Increases the rate of orthodontic tooth movement.
7.	Mohammed M. *et al.*, 2014	Review	May increase the rate of tooth movement, useful tool in clinical orthodontics.
8.	Kau C. *et al.*, 2013	Clinical study	Increases the rate of orthodontic tooth movement.
9.	Sachin K. *et al.*, 2019	Review	Increase in bone density, as well as increased expression of osteogenesis markers.
10.	Samara S.A. *et al.*, 2018	Clinical study	Effective in orthodontic active space closure.
11.	Sedky Y. *et al.*, 2019	Clinical study	Increases the rate of orthodontic tooth movement and has the advantage of being non-invasive compared to cortectomy-facilitated orthodontics
12.	Long H. *et al.*, 2015	Meta-analysis	Weak evidence, although may accelerate orthodontic tooth movement. Insufficient data, more studies are needed.
13.	Sonesson M. *et al.*, 2017	Review	Low quality of evidence, need for consistency in study design and conformity of laser method.
14.	Üretürk S.E. *et al.*, 2017	Clinical study	Increases the rate of orthodontic tooth movement.
15.	Yassaei S. *et al.*, 2016	Clinical study	No significant changes in the rate of orthodontic tooth movement.

Low-level mechanical oscillatory signals (vibrations) have been proved to increase the rate of bone remodeling (38).

Goyal V. *et al.* (43) in their review concluded that mechanical vibrations have positive effect on the bone architecture and promote osteogenic activity.

We accentuate that the effectiveness of vibrational devices to accelerate the rate of orthodontic tooth movement is influenced by vibration parameters. The results will mainly be dependent on duration of use and the frequency of the cyclic loading.

In recent years, several clinical studies have investigated the effects of vibrations on the rate of orthodontic tooth movement (38, 57, 58, 59).

Pavlin D. *et al.* (38) in their clinical trial have found that applying cyclic loading (vibrations) of 0.25 N at a frequency of 30 Hz in association with a fixed orthodontic appliance will significantly accelerate the rate of orthodontic tooth movement.

Jing D. *et al.* (60) have presented in their review that there is a need for well-designed high-quality clinical trials in order to obtain reliable results. They have also concluded that vibrational stimulus is effective for accelerating tooth movement in canine retraction but not in the alignment phase.

Lobre W. *et al.* (1) used vibrational devices to lower the pain perception that is associated with the use of fixed orthodontic appliances. During the 4-month test period the micro pulsed vibration device group felt significantly more comfortable compared to the control group. These findings are challenged by Miles P. *et al.* (45) clinical trial results, where the vibrational devices had no effect on pain alleviation.

Orton-Gibbs *et al.* (58) demonstrate that the use of vibrational devices in an orthodontic practice can significantly reduce treatment time, but they also consider that further testing is necessary. These findings correspond with Bowman S.J. *et al.* (57) results, where a 30% increase in the rate of tooth movement during orthodontic levelling phase was noticeable.

Given that root resorption is a potential side effect of any orthodontic treatment, the effects of these devices had to be evaluated. There is no clear evidence that using a vibrational device as an adjuvant during treatment may cause root resorption. Chung H.K. (61) after radiological evaluation, found that there is no significant difference after using these devices and states that further testing and a longer follow-up period is needed.

The results of the literature search conducted on vibrational devices applied in orthodontics was introduced in table II.

TABLE II

Literature search for articles about vibrational devices

No.	Author names and year	Study Type	Results
1.	Pavlin D. *et al.*, 2015	Clinical study	Increases the rate of orthodontic tooth movement.
2.	Lobre W. *et al.*, 2016	Clinical study	Reduces the perception of pain. Further studies are needed.
3.	Orton-Gibbs S. et al., 2015	Clinical study	Effective in accelerating the rate of orthodontic tooth movement.
4.	Jing D. *et al.*, 2017	Review	Increases the rate of orthodontic tooth movement. There is a need for well-designed clinical trials in order to obtain more reliable information.
5.	Bowman S.J. *et al.*, 2015	Clinical study	Increases the rate of orthodontic tooth movement.
6.	Miles P. *et al.*, 2012	Clinical study	No advantage for the early resolution of crowding or the alleviation of pain during orthodontic treatment.
7.	Goyal V. *et al.*, 2018	Review	Increases the rate of orthodontic tooth movement.
8.	Chung H.K., 2011	Clinical study	There is no clear evidence that using a vibrational device may cause root resorption.

CONCLUSIONS

From the articles about LLLT that were included in our study 12 out of 15 stated that its use increases the rate of orthodontic tooth movement and 3 out of 14 concluded that there is no, or weak evidence that it would reduce treatment time. From the studies conducted on vibrational devices, that we analyzed, 7 out of 8 confirmed that its use during orthodontic treatment has positive effects and only one study described the device as having no effect.

Given the limitations of our overview, it can be concluded that LLLT and vibrational devices can be a genuine way to reduce classic orthodontic treatment duration and they have close to no side-effects. However, larger and longer clinical trials are required to assess the long-term stability of the treatment outcomes as well as the biological mechanisms in order to determine future clinical guidelines so that these devices could be routinely applied.

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NEWS

POSITIVE EFFECTS OF NATURAL SUBSTANCES IN CHEMOTHERAPY-INDUCED TOXICITY – A STUDY ON MICE

A recent study conducted on mice, shows the positive effects of the combination of chemotherapy with natural products. three such natural products, 1,8-cineols - terpenes present in many aromatic plants such as *Artemisia herba alba*, eucalyptus, sage, rosemary, and cardamom, *Exopolysaccharide* (EPS - an extracellular polysaccharides isolated from marine microorganisms by microbial fermentation, and Ellagic Acid (EA) - a natural phenol, which is found in various vegetables and fruits, have a positive effect on chemotherapy-induced toxicity. Supplementary substance-based studies on the human subjects are required to fully demonstrate the positive outcome of the combination. (Abdallah HMI, Abdel-Rahman RF, El Awdan SA, Allam RM, El-Mosallamy AEMK, Selim MS, Mohamed SS, Arbid MS, Farrag ARH. Protective effect of some natural products against chemotherapy-induced toxicity in rats. *Heliyon* 2019; 5(5): e01590).

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ASPECTE CLINICO-EVOLUTIVE ALE MENINGITEI TUBERCULOASE LA COPIL

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CLINIC AND EVOLUTIVE ASPECTS OF CHILDREN TUBERCULOUS MENINGITIS (Abstract) : The aim of the study was to investigate the particularities of the clinical manifestation and evolution of tuberculous meningitis at children. **Material and method** : The study was made between January 2000 and December 2008 in Clinic of Infectious Diseases Iaşi and Emergency County Hospital „Sf. Ioan cel Nou" Suceava on a group of 169 children with tuberculous meningitis. **Results** : The majority (78.1%) of patients had poor socio-economic conditions and 22.4$ had a family TB contact. The onset of the symptoms was atypical in infants and small children with fever associated with digestive, neurological or pulmonary manifestations. The admission in hospital was delayed in 56.8% of patients and 39.05% had a severe general status with coma. The positive diagnosis was based on cytological and biochemical features of CSF, results of QuantilFERON. TB Gold, pulmonary images, family TB contact and evolution under anti-tuberculous therapy. We observed a high rate of complications represented by hydrocephaly (28.9%). 18 patients died (4 infants), the cause of dead being meningeal coma or complications. **Conclusion** : The diagnosis of tuberculous meningitis at children remains a problem because of the atypical clinical manifestation, the delay of initiating the therapy causing high mortality and frequent complications. **Key words** : CLINICAL TUBERCULOSIS, MENINGITIS, CHILDREN

Tuberculoza este una din problemele majore de sănătate populaţională la nivel mondial. În România sunt raportate, anual peste 30.000 de cazuri noi sau recidive, cu dezvoltarea unor forme grave de boală la copilul mic şi sugar (1, 2). Concomitent cu tuberculoza pulmonară, o recrudescenţă deosebită o au în prezent şi formele extrapulmonare, printre care şi meningita tuberculoasă (2, 3).

Studiul nostru a urmărit evidenţierea particularităţilor clinice şi evolutive ale meningitei tuberculoase la copil, într-o zonă din nord-estul României.

MATERIAL ŞI METODE

În studiu au fost incluse 169 de cazuri de meningită tuberculoasă la copil, internate în Clinica de Boli Infecţioase Iaşi şi Spitalul Judeţean de Urgenţă „Sf. Ioan cel Nou", Suceava, în perioada 2000-2008. Au fost analizate, retroprospectiv, datele demografice (vârsta, sexul, mediul de provenienţă), factorii favorizanţi, manifestările clinice, importanţa examenelor paraclinice, evoluţia clinică şi programele terapeutice.

REZULTATE

1. Date demografice. Incidenţa anuală a

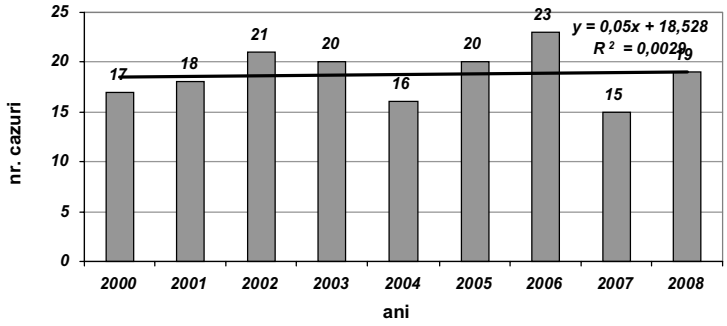

Fig. 1. Incidenţa anuală a meningitei TB la copil

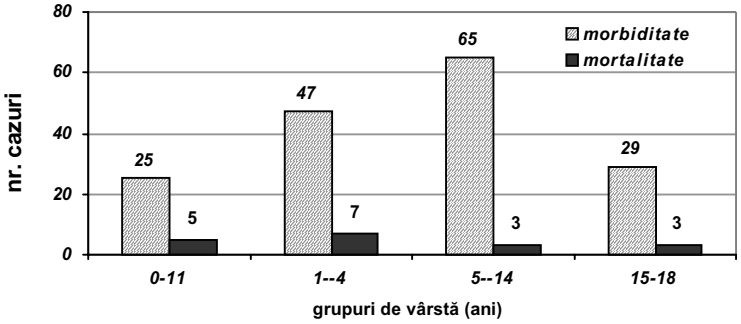

Fig. 2. Distribuţia cazurilor de meningită TB pe grupuri de vârstă

cazurilor de meningită tuberculoasă (TB) în lotul studiat a fost influenţată de evoluţia epidemiei de tuberculoză cu valori ridicate, cu maximum în anul 2006, când s-au înregistrat 23 cazuri (13,6%) (fig. 1).

Repartiţia cazurilor pe grupuri de vârstă a evidenţiat o incidenţă maximă la grupul 5-14 ani (40,23%), urmată de 1-4 ani (27,81%) (fig. 2).

Incidenţa pe sex nu a indicat diferenţe semnificative ($p \geq 0,05$), fiind de M/F = 89/80, cu uşoară predominanţă a sexului masculin. Diferenţa pe medii a fost semnificativă ($p \leq 0,05$) înafara mediului rural (U/R = 21,8/78,1%). Nu s-a remarcat nici o diferenţă sezonieră.

2. Factori favorizanţi (exceptând infecţia HIV/SIDA), în apariţia meningitei TB, au fost astfel: condiţii socio-economice precare (78,1%); focar familial (22,48%); TB pulmonară netratată (4,73%); afecţiuni patologice preexistente care au condus la scăderea imunităţii (11,24%) (2,3).

3. Diagnosticul clinic al meningitei tuberculoase. Prodromul a fost frecvent insidios, cu durata între 1 şi 4 săptămâni marcat de scăderea apetitului (88%); agitaţie (72%); iritabilitate (68%); reducerea interesului pentru joacă (78%); senzaţie de greaţă şi vărsături (65%); dureri abdominal difuze (36%); febră rebelă la terapia cu antibiotic (18,3%). Simptomele de debut au fost polimorfe, atipice la sugar şi copilul mic, spre deosebire de şcolar la care s-a manifestat frecvent un tablou clinic sugestiv pentru meningită: febră + sindrom meningeal/sindrom encefalic (83,4%); febră + sindrom pseudotumoral (1,2%); sindrom febril prelungit peste 14 zile (5,3%); febră + tulburări gastrointestinal (8,9%); febră + imagine pulmonară de tip pneumonic persistent sub antibiotic (1,2%) (4,5,6).

Polimorfismul simptomelor de debut a stat la baza internării tardive pentru un număr de 96 (56,8%) cazuri (tab. I). Starea general la internare a fost gravă (39%) pa-

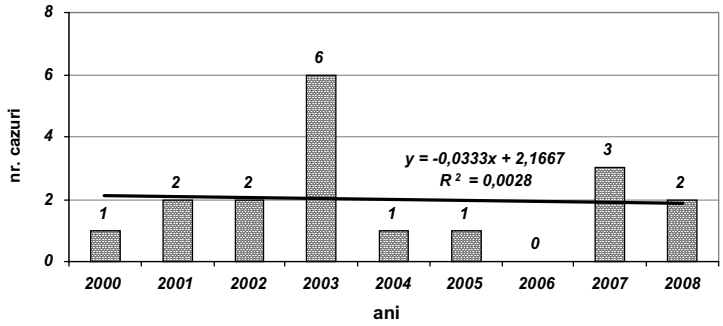

Fig. 3. Mortalitatea anuală prin meningita TB la copil

TABELUL I

Momentul internării în relaţie cu debutul bolii

	Intervalul: debut/internare (zile)			
	1-7	*8-14*	*15-21*	*>21*
Nr. cazuri	73	63	18	15
%	43,2	37,2	10,7	8,9

TABELUL II

Modificarea stării generale la internare

	Modificarea stării generale la internare		
	Gravă	*Uşoară*	*Medie*
Nr. cazuri	66	80	23
%	39	47,3	13,7

cienţi, dintre care 18,9% erau în comă gr. III (Scor Glasgow 5-8) (tab. II).

În perioada de stare s-au semnalat manifestări digestive (vărsături, dureri abdominale, constipaţie) şi fenomene de suferinţă encefalitică, acestea din urmă fiind prezente la majoritatea pacienţilor, şi s-au evidenţiat prin: convulsii tonico-clonice localizate şi generalizate – 34,9%; coma gr. II/III – 33,6%; pe parcursul internării coma cu sfârşit letal s-a aprofundat (gr. IV) la 8,3% dintre pacienţi; bradipsihie, somnolenţă – 28,9%; sindrom confuzional – 13,6%; tulburări cognitive şi de limbaj – 2,9%; paralizii de nervi cranieni – 32,5%; hemipareză/ hemiplegie – 19,5%; parapareză – 2,3%. Manifestările respiratorii au fost reprezentate prin detresă respiratorie şi apnee în 16,5%, iar manifestările cardiac prin bradicardie în 7,7% din cazuri.

4. Diagnosticul pozitiv. Elemente orientative: noţiunea de contact familial de TB la 34,3% sau de TB pulmonară în antecedente; simptome clinice, care de multe ori au fost polimorfe şi atipice, însă persistente sub terapia cu antibiotice (9,5%), în special la sugar şi copilul preşcolar au dus la suspiciunea de meningită TB. *Elemente de certitudine*: afectarea pulmonară caracteristică pentru TB a fost prezentă la 53,8% şi a constat în forma miliară – 20,7%; pneumonie cu pleurezie – 2,9%; pneumonie lobară – 9,5%; TB ulcero-cazeoasă – 1,8%; TB nodular-infiltrativă – 10%; adenopatii hilare – 8,8%; absenţa leziunilor pulmonare nu a infirmat etiologia bacilară a meningitei; modificări citobiochimice particulare ale LCR la 60,9%; examenul direct al LCR, în coloraţia Ziehl-Nielsen, a fost negativ în toate cazurile; cultura LCR, pe mediul Lowenstein-Jensen, a evidenţiat prezenţa *Mycobacterium tuberculosis* la 27,8%; în două cazuri s-a izolat o tulpină de *M. tuberculosis* rezistentă la izoniazidă; testul QuantilFERON TB Gold pozitiv în sânge la 14,8% pacienţi şi pozitiv în LCR la 60% din cele 40 de cazuri de meningită TB testate; examenul CT cranio-cerebral a fost practicat la 66,2% pacienţi îndeosebi cei cu complicaţii sau probleme de diagnostic; au fost evidenţiate modificări patologice de tip: hidrocefalie – 43,7%; tuberculoame – 20,5%; edem cerebral – 13,6%; infarcte multiple – 4,46%; hemoragii subrahnoidiene

TABELUL III

Evaluări asupra prevalenţei sechelelor, pe un lot de 169 cazuri de meningită TB

Sechele	Nr. cazuri	%
Hidrocefalie fără sunt VP	25	14,8
Hidrocefalie cu sunt VP	24	14,2
Retard psihomotor	15	8,9
Pareze ale membrelor	12	7,1
Cecitate	8	4,7
Hipoacuzie	7	4,14
Atrofie corticală	6	3,5
Paralizii ale nervilor cranieni	5	2,95
Encefalopatie gravă	5	2,95
Epilepsie GM	3	1,8
Higroma	2	1,2

– 2,67% ; atrofie corticală difuză – 6,25% ; encefalită presupurativă – 2,67% ; şi arahnoidită bazală la 3,57% pacienţi (7,8,9).

5. Evoluţia meningitei TB a depins de precocitatea diagnosticării şi introducerii tratamentului. Copiii care au primit tratament anti – TB în primele 10 zile de boală au fost recuperaţi în 86,4% din cazuri iar cei care au beneficiat de un tratament instituit după 10 zile de boală, s-au recuperat doar în 23,4% cazuri.

Urmărind relaţia dintre momentul introducerii terapiei anti – TB şi ameliorarea stării clinice au fost luaţi în considerare următorii parametri : ieşirea din comă a fost posibilă la 68,42% pacienţi din 57, după 5 şi 21 zile ; remiterea paraliziilor de nervi cranieni s-a constatat în 90% din cazuri la un interval de 14 – 30 de zile ; ameliorarea fenomenelor de focalizare a fost evidentă în 69,3% din 33 cazuri, după 6 săptămâni de tratament ; sindromul meningean îndeosebi la preşcolari şi şcolari s-a remis într-un interval de 4 – 21 zile ; apirexia s-a instalat, în medie, după 12,3 zile ; durata medie a spitalizării a fost de 63,5 zile, cu un minimum de 24 ore şi maximum 168 zile.

6. *Complicaţiile* care au apărut în decursul bolii au fost reprezentate de : hidrocefalie (28,9%) ; tuberculoame cerebrale unice sau multiple (12,4%) ; hemipareze / hemiplegie (19,5%) ; parapareză (2,3%) ; arahnoidita optochiasmatică (2,3%) ; paralizii ale nervilor cranieni II, VI, VII (32,5%) ; hipoacuzia (1,5%) ; hepatopatia medicamentoasă indusă, probabil de rifampicină (12,4%). Sechelele postmeningită TB au fost îndeosebi, de tipul hidrocefaliei (28,9%) (10,11) (tab. III).

7. Mortalitatea prin meningita TB. Din cei 169 pacienţi cuprinşi în studiu au decedat 18 (10,6%), dintre care 22,2% în primele 3 zile de spitalizare ; sexul feminin fiind cel mai afectat (7,1%). Incidenţa deceselor a fost mai mare la copilul mic şi sugar, înregistrându-se 4,1% decese între 1-4 ani. Mortalitatea a fost în scădere în ultimii ani, cele mai multe decese la copii înregistrându-se în anul 2003 (fig. 3).

Factorii care au agravat evoluţia bolii favorizând decesul au fost reprezentaţi de spitalizarea tardivă şi starea generală gravă la internare (5,9%), decesul survenind la un interval de 2,4 – 4 zile de la instituirea terapiei. Celelalte 7 (4,1%) cazuri de deces s-au produs după 16-35 de zile de tratament prin complicaţii : ramolisment cerebral, hidrocefalie tetraventriculară şi edem cerebral refractar la tratament patogenic.

DISCUŢII

Studiul a avut drept scop evidenţierea particularităţilor meningitei TB la copil, care în perioada 2000 – 2008 a reprezentat peste 30% din totalul cazurilor de neuro – infecţii. Anual, s-au înregistrat între 15 şi 23 îmbolnăviri.

Între factorii favorizanţi ai îmbolnăvirilor s-au semnalat : condiţiile socio-economice precare la 78,1% şi existenţa focarelor familiale de TB la 22,4% pacienţi. La sugar şi copilul mic au predominat simptome de debut polimorf, care au cauzat temporizarea internării în Clinica de Boli Infecţioase, în 65,2% din cazuri.

Suferinţa encefalitică a avut o frecvenţă semnificativă în lotul studiat, având aspecte clinice variate de la convulsii, paralizii de nervi cranieni, somnolenţă până la comă encefalitică de gradul III. Procentul de 91,7%, raportat de noi este, mult superior celui constatat în alte ţări (4,5,6). Simptomatologia predominant neurologică depistată la 39,4% dintre cazurile studiate este similară cu cea raportată în alte studii (5, 6, 7).

Diagnosticul de certitudine al meningitei TB s-a obţinut prin izolarea bacilului Koch din LCR în 27,8% din cazuri (4,89%). Dintre complicaţiile meningitei TB cea mai frecventă a fost hidrocefalia tetra– sau triventriculară, prezentă în 49 (28,9%) cazuri (10,11). Evoluţia a fost favorabilă pentru 24 (14,2) bolnavi cărora li s-a aplicat shunt ventriculo-peritoneal.

CONCLUZII

Simptomele de debut al meningitei TB au fost polimorfe, mimând diferite afecţiuni, ce au determinat adresabilitatea cu întârziere la medicul infecţionist. Starea generală la internare a fost gravă în 66 (39%) cazuri.

Cea mai frecventă complicaţie a fost hidrocefalia constatată la 28,9% din cazuri. S-au înregistrat 18 (10,6%) decese, dintre care 4 (2,3% cazuri la sugar şi 9 (5,3%) la copilul 1-4 ani. Cauza directă a morţii a fost coma meningitică sau complicaţiile de tipul hidrocefaliei tetraventriculară şi ramolismentul cerebral. Factorii majori ai prognosticului nefast au fost reprezentaţi de vârsta mică, gravitatea bolii la internare şi momentul tardiv al internării.

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NEW INSIGHTS INTO AORTITIS CLASSIFICATION: A MINIREVIEW

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NEW INSIGHTS INTO AORTITIS CLASSIFICATION: A MINIREVIEW **(**Abstract): Aortitis is a general term that refers to a broad category of infectious or noninfectious conditions in which an abnormal inflammation of the aortic wall occurs. These inflammatory conditions have different clinical and morphologic features and variable prognoses. The most common causes of noninfectious aortitis are the large vessel vasculitis, including giant cell arteritis and Takayasu arteritis, together with several rheumatic diseases. Infectious aortitis is a rare but potentially life-threatening disorder. In this paper, we reviewed the most common etiologic types of aortitis, noting some diagnostic issues, discussing some pathological classifications suggestive of a certain etiology and outlining the role of the pathologist. **Keywords**: AORTITIS, INFLAMMATION, VASCULITIS.

DEFINITION

Aortitis is an inflammatory process involving the aortic wall, regardless of the underlying cause. Most frequently the intima and media-intima junction, as well as the adventitia are involved. At the level of the media, the inflammatory infiltrate is associated with fragmentation of elastic fibers, smooth muscle cell (SMC) necrosis of various degrees and fibrosis (1).

AORTITIS ETIOLOGICAL CLASSIFICATION

Many criteria are used in aortitis classification. The most widely used classification of aortitis considers the etiology of the aortic inflammation (tab. I) (2).

In this classification, aortic wall inflammation may be either infectious or, more often, noninfectious. Noninfectious aortitis, a form of large-vessel vasculitis, can be grouped into: (a) giant cell aortitis, such as giant cell arteritis (GCA) and Takayasu arteritis (TA); (b) autoimmune aortitis, such as rheumatoid arthritis and ankylosing spondylitis; (c) idiopathic aortitis including inflammatory aortic aneurysm. Infectious aortitis may be secondary to fungal infection, tuberculosis, syphilis or infections with *Salmonella* or other bacterial agents.

CLINICAL MANIFESTATIONS AND DIAGNOSTIC APPROACH

There are various clinical presentations, including, for instance, hypertension in young patients suffering from TA; back or abdominal pain accompanying a thoracic or abdominal aortic aneurysm; vasculitis -

induces systemic inflammatory syndrome; aortic thrombosis with distal embolization; aortic dissection or rupture, etc. The recommended laboratory investigations are complete blood count, kidney and liver function tests, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), blood cultures, rheumatologic panel (antibodies to nuclear antigen-ANA, antineutrophil cytoplasmic antibodies - ANCA, rheumatoid factor - RF), skin test for tuberculosis, serological test for syphilis, etc. (3). Imaging is also useful for monitoring disease activity and guided biopsy in selected cases (e.g., in TA). Commonly, conventional angiography is the initial imaging study performed. Multidetector computed tomography (CT) helps with evaluating both arterial wall changes and abnormalities of the aortic lumen, whereas magnetic resonance imaging (MRI) is recommended for serial imaging in patient follow-up (4).

TABLE I
Etiologic classification of aortitis

1. Noninfectious	**2. Infectious**
○ Giant cell aortitis ○ Takayasu aortitis ○ Aortitis associated with autoimmune diseases • Rheumatoid arthritis • Ankylosing spondylitis • Behçet disease • Systemic lupus erythematous • Reiter syndrome • SystemicIgG4-related autoimmune disease ○ Lymphoplasmacytic aortitis	○ Bacterial • Salmonella • Staphylococcus species • *Streptococcus Pneumoniae* ○ Luetic (*Treponema pallidum*) ○ Mycobacterial (*Mycobacterium tuberculosis*) ○ Fungal
	3. Idiopathic aortic inflammatory aneurysm

The most widely used etiological classification, distinguishing between noninfectious and infectious causes, offers the advantage of better guiding the therapy of these disorders.

1. Non-Infectious Aortitis

GCA is a chronic granulomatous vasculitis that affects large and medium-sized vessels and usually the superficial cranial arteries are involved. Aortic injury occurs in 10-15% of GCA patients. The disease incidence increases with age (beyond the age of 50 years) and is also higher in women (two-fold to four-fold) (5). Aortic involvement usually manifests as aortic valve insufficiency and ascending aortic aneurysm in about 10% of aortitis (2). Aortic dissection rarely occurs (6).

GCA pathogenesis is secondary to a systemic granulomatous vasculitis and is closely related to polymyalgia rheumatica. Vascular inflammation may be widespread, most commonly involving the superior temporal artery, as well as the aorta and its branches (4, 5).

Histologically, the acute stage is characterized by inflammatory cellular infiltrate with multinucleated giant cells, lymphocytes, plasma cells and macrophages in the areas of destruction and lamellar fragmentation, while in the chronic stage there is progressive fibrosis of the vessel wall. Intimal fibrosis is present but not to a similar extent as in TA (5).

TA, also known as aortic arch syndrome, pulse less disease, or occlusive thromboaortopathy, is a necrotizing and obliterative segmental, large-vessel panar-

teritis of unknown cause. It is more common in young females (aged less than 50 years) and it is thought to be secondary to an autoimmune process. TA is associated with mycobacterial infection (tuberculosis) and with rheumatoid arthritis. Histology reveals degeneration of the media with a dense inflammatory infiltrate, including giant cells; there is a marked infiltration of mononuclear cells in the adventitia with perivascular cuffing of the vasa vasorum in the early stage, followed by fibrosis and calcification in the late stage, leading to occlusion and narrowing of the aorta and its branches (7).

TA is generally divided into two clinical stages. In the early stage, known as the systemic or prepulseless phase, clinical manifestations are mainly vague making diagnosis difficult during this interval. In the late stage, known as the occlusive or pulseless phase, aortic and branch vessel disease may manifest itself as stenosis or luminal narrowing or, less frequently, as aneurysmal dilatation that ensues after inflammation and destroys the media (8, 9).

In contrast to GCA, TA is more often associated with extensive intimal and adventitial fibrosis or scarring with resultant luminal narrowing. At the same time, in GCA "skip lesions" are more regular (10). Often, aortic wall thickness is greater among patients with TA than GCA.

Rheumatoid aortitis. Aortitis in rheumatoid arthritis (RA) is rare and less common than myocardial or valvular involvement. Aneurysms may occur in the thoracic or abdominal aorta, may rupture or cause aortic insufficiency. Histologically, inflammation consists of lymphocytes, plasma cells and macrophages, with or without the presence of rheumatoid nodules. Typical inflammation affects media and adventitia. In the destroyed media, SMCs and elastic lamellae are replaced by collagen fibers (11).

Ankylosing Spondylitis. Aortic root and aortic valve are the most commonly involved areas in patients with ankylosing spondylitis. In aortitis-dependent aneurysm formation, the aortic wall changes are common in affected patients, and aortic valve thickening is associated with valvular insufficiency (12).

Beçhet's Disease is an idiopathic vascular inflammatory disease characterized by mucocutaneous ulcers. Rarely, multiple aneurysms develop in large vessels such as the aorta. In the active stage of aortitis, there is a chronic inflammatory medial and adventitial infiltration with formation of a cuff around vasa vasorum with rare giant cells, while in the late stage there is fibrous thickening of the media and adventitia with endarteritis obliterans (13).

Systemic lupus erythematous. The aortic aneurysm is a rare complication in lupus erythematosus with involvement of ascending aorta that requires surgical aortic replacement (2, 14, 15).

Lymphoplasmacytic aortitis is characterized by a diffuse or multifocal medial inflammatory infiltrate consisting of lymphocytes and plasma cells, along with medial necrosis. As an autoimmune disease, it is characterized by the presence of IgG4-secreting plasma cells, hence the name of systemic IgG4-related autoimmune disease (16, 17).

2. Infectious Aortitis

Infectious aortitis is an inflammatory process within the aortic wall induced by microorganisms. Commonly, it is a complication of bacterial endocarditis secondary to *Streptococcus pyogenes*, *Streptococcus pneumoniae*, and *Staphylococcus*. Occasionally, specific infectious agents are involved, such as *Mycobacterium tubercu-*

losis, *Treponema pallidum*, and others (2).

Pyogenic aortitis was originally described and named "mycotic aneurysm" by Osler in 1885. The term of infected aneurysm is now preferred to mycotic aneurysm for avoiding confusion with fungal infection. The possible invasion route could be implantation on the intimal surface spread of infection through the vasa vasorum, direct extension from a contiguous extravascular focus, or traumatic inoculation of contaminated material. Histologically, it is characterized by medial destruction with the formation of micro abscesses. Morphologically, these aneurysms are mostly saccular rather than fusiform. The microbial agents generally involved are salmonella, streptococci, staphylococci and gonococci (1, 18).

Syphilitic aortitis. Syphilis is a sexually transmitted chronic systemic infection produced by the *Treponema pallidum* spirochetes. The clinical findings depend on the duration of infection and are divided into three stages: primary, secondary, and tertiary. Cardiovascular manifestations specific to tertiary syphilis are usually evident 5-30 years after the primary infection and are secondary to endarteritis obliterans of the vasa vasorum. Commonly, luetic aortitis involves the ascending thoracic aorta. Grossly, the intimal "tree bark" appearance is the result of the proximal aorta involvement. In the late stages, these characteristic lesion**s** may be masked by atherosclerotic (ATS) injuries. Histologically, there are perivascular lymphocytes and plasma cells around vasa vasorum affected by endarteritis obliterans. Inflammation spreads within the media, where it determines acute necrosis of the aortic wall with neutrophilic infiltration (microgome) which may contain treponemes, as demonstrated with Warthin-Starry staining (19, 20).

Tuberculous aortitis involves the thoracic and abdominal aorta with equal frequency. It occurs in patients with latent tuberculosis (TB) due to direct extension from contiguous mediastinal lymph nodes or by hematogenous or lymphatic spread. Pathologic samples display TB granulomas (caseous necrosis, multinucleated giant cells and epithelioid cells) in the adventitia and media. Complications include wall rupture or aneurysm formation (21, 22).

3. Idiopathic aortitis is typically asymptomatic and is diagnosed only by the pathologist after surgery for aortic aneurysms, because some patients with this form of idiopathic thoracic aortitis develop an aortic aneurysm also known as idiopathic inflammatory aortic aneurysm.

There are several hypotheses explaining this idiopathic aortic disease: immune response to various components of aortic fibro atheromatous plaques; viral infection (Herpes simplex virus, cytomegalovirus, Epstein Barr virus); periadventitial fibrosis secondary to focal wall ischemia; aortic localization of systemic vasculitis; primary autoimmune disease (23, 24).

A term often used as a synonym for this type of aortic inflammatory disease is "chronic peri aortitis" (CPA), which actually refers to a group of diseases, including retroperitoneal idiopathic fibrosis and perianeurysmal retroperitoneal fibrosis as well as inflammatory aneurysm of abdominal and possibly thoracic aorta, sharing the findings of marked periadventitial inflammatory infiltrate and fibrosis. The histopathological approach is based on the coexistence of intimal complicated fibro atheromatous plaques, significant adventitial inflammatory infiltrate with lymphoid follicles and germinal centers and periadventitial fibrosis (25).

Pathological classifications

In 2008, Burke *et al*. have proposed a strictly histological classification of aortitis considering the distinction between necrotizing and non-necrotizing forms, whereas the examining pathologists completely ignore the clinical findings (3). Considering the histological data analysis regarding the presence or absence of medial necrosis, the authors concluded that histology correlates with etiology: GCA is associated with non-necrotizing aortitis, while TA and other autoimmune diseases are more frequently associated with necrotizing aortitis.

Another pathological subclassification derives from the pattern of the inflammatory infiltrate that suggests possible etiologies, such as: (1) granulomatous/giant cell pattern with or without compact/well-formed granulomas (GCA, TA, and RA); (2) lymphoplasmacytic pattern (IgG4-RD, syphilitic aortitis); (3) mixed inflammatory pattern (Behçet's disease); and (4) suppurative pattern (Staphylococcus, Streptococcus, Salmonella, Pseudomonas, and fungal infections) (26).

According to Stone *et al*. (26) the subclassification of aortitis often requires a combination of clinical, radiologic, laboratory, and pathologic information, because there are different gray areas, including the existence of overlapping lesions (some forms of aortitis and inflammatory aneurysm) in the context of a varied etiopathogenetic, pathological and clinical-evolutive spectrum.

Thus, in the near future, a detailed diagnostic approach could equally provide useful elements for the identification of new therapeutic targets and methods, including newer biologic treatments such as target specific anti-inflammatory molecules (27, 28).

Recent acquisitions on aortic pathology, in the light of the complexity of histopathological, biomolecular and pathophysiological substrates, should result in diagnostic and therapeutic reconsideration of aortitis, with the widening of the "systemic" evaluation by improving clinical study efficiency and enriching trials with imaging techniques, tissue histopathology, biomolecular and genetic analysis.

CONCLUSIONS

So, in the future, more studies will be needed with larger numbers of patients and applying multifield researches including novel tissue marker identification. In the working team, the pathologist responsible for the pathology report is meant to guide clinical screening for previously unsuspected autoimmune disease, and suggest the etiology of the inflammatory process, as well. The pathologist should also provide information about the degree of activity of the inflammatory process, which can be useful in predicting possible aneurysm recurrence.

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MORPHOLOGICAL FEATURES OF ROMANIAN ENDEMIC *TEUCRIUM* L. SPECIES

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MORPHOLOGICAL FEATURES OF ROMANIAN ENDEMIC *TEUCRIUM* L. SPECIES (Abstract): *Teucrium* is a genus that belongs to *Lamiaceae* family, but the endemic plants from our country have not been studied before. Therefore, **the aim** of this study was to provide evidence and specific characteristics with taxonomic value. **Material and methods**: we collected the plants (*Teucrium chamaedrys* L., *T. montanum* L. and *T. polium* L.) from the wild flora in the Northern Romania and we prepared section and microscopic plates from various organs (stems, branches, leaves and bracts). **Results:** the observed features were both common and different (quantitatively and qualitatively) between the species. **Conclusions:** we obtained for each species several specific characteristics that allow the proper identification even when the environmental conditions are not optimal for the complete growth of the plants. **Keywords**: TEUCRIUM, MORPHOLOGY, STEMS, LEAVES, TAXONOMY

Most species of *Lamiaceae* are medicinal plants long used in folk medicine known and appreciated for their active principles (α-pinene, β-pinene, limonene and menthol isolated from volatile oils and diterpenes, flavonoids and iridoids isolated from polar fractions) (1). The Romanian flora comprises about 33 genera and 130 species and subspecies of the *Lamiaceae* family (2). At present, this is one of the most famous and studied families of plants, the available data is abundant in chemical composition with important biological actions, in the most diverse fields: medicine, gastronomy, cosmetology (1, 3, 4, 5).

Volatile oils are recognized for their powerful anti-antioxidant and antimicrobial properties, while polar compounds such as flavonoids, iridoids and diterpenes exert important anti-inflammatory, antioxidant, anticancer and gastroprotective action. All the justified aspects are the remedies for using such species not only in traditional and modern medicine but also in daily life, in the form of nutritious teas, spices or flavorings (6, 7, 8).

The genus *Teucrium* has a wide distribution in Europe, Asia, America, Australia

and especially in the Mediterranean area, the latter comprising approximately 96% of the species. In Romania, the genus *Teucrium* comprises 5 species and several subspecies that grow in spontaneous flora. Plants are usually perennial, in the form of shrubs or sub-shrubs (2, 9, 10). The representatives of this genus are appreciated for their medicinal properties, both in modern medicine, proof being the numerous studies performed in the last 40 years, but especially in traditional medicine. It seems that the first information on the beneficial actions of these species was recorded by a Trojan king, Teucrion, after which the plants were also named, and dates back to our era. In traditional medicine, *Teucrium* species have been used for hundreds of years for their antioxidant, tonic, diaphoretic, analgesic, antipyretic, antirheumatic, antiseptic and hypoglycemic (1, 4, 5). Recently, cytotoxic and anticancer action has also been discovered (6). The remedies obtained from the aerial parts are rich in aromatic compounds with a bitter taste, so that they can be used as flavorings or in the form of spices, with a beneficial effect on the digestive system.

Although the anatomical structure of the *Lamiaceae* species has been studied extensively in the 19th and 20th centuries, in works referring only to medicinal plants, the species of *Teucrium* are rarely mentioned; only Cole (1992), referring to the significance of terpenoids, deals extensively with species of this genus. Nevertheless, only exotic taxa from the *Teucrium* genus are cited in the available literature.

Therefore, the aim of our research was represented by the description of three species of *Teucrium* endemic in Romania (9, 10), trying to find several features of taxonomic importance that would allow the proper identification of these species.

MATERIAL AND METHODS

Plant material

For our study we selected three Romanian endemic species as follows: *Teucrium chamaedrys* L., *T. montanum* L. and *T. polium* L. The chosen species were very little anatomically researched, which is why we also addressed this aspect in our research. The plants were harvested from the spontaneous flora, of Horlesti (*Teucrium chamaedrys* L. and *T. polium*) and Bicaz (*T. montanum* L.), during 20th and 30th of June 2019.

Preparation of the microscopic plates

The fresh vegetative organs (leaf and stems - the main axis and a lateral branch), were fixed in 70% ethyl alcohol. Prior to analysis, each sample was cross-sectioned through and the sections were colored using the double staining method, with ruthenium red and iodine green (12, 13, 14). The permanent preparations were made in glycerol gelatin and evaluated using the Euromex microscope (iScope series with 4x10, 10x10, 20x10, 40x10 lens) and Image Focus Alpha software. Euromex sCMEX- 20 was used for photography of each sample.

RESULTS AND DISCUSSION

The morphological characteristics observed in our study revealed both common and uncommon elements, especially for *T. montanum* as compared to the other two species.

Stem shape and anatomy

The cross-section of the stem is round and somewhat similar for *T. montanum* and *T polium*. For both samples, the pericycle is made of bundles of sclerenchymatous and parenchyma cells. However, the stem from

T. chamaedrys is square, with the ribs slightly protruding (fig. 1).

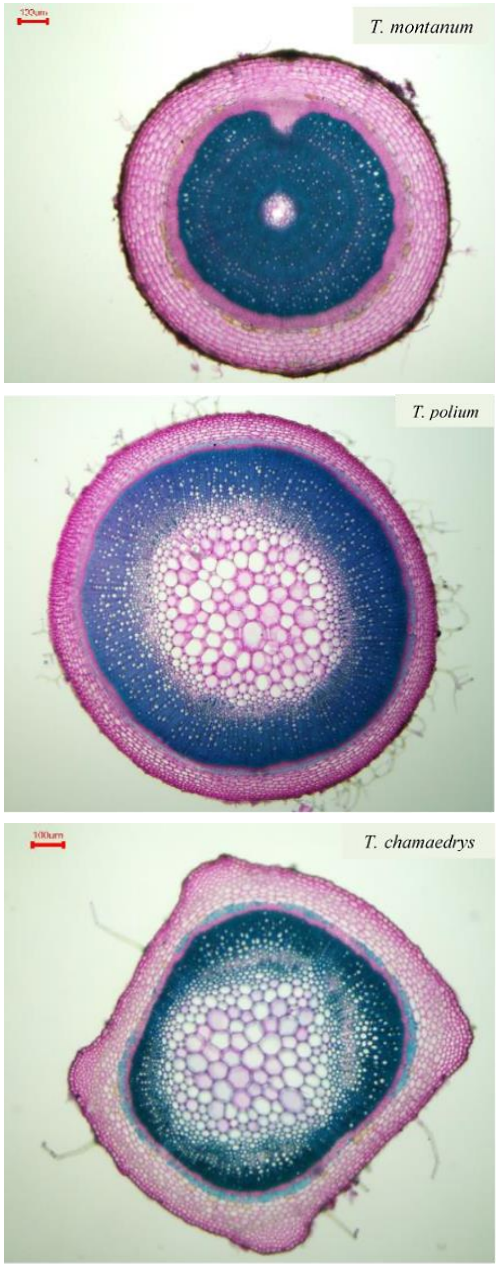

Fig. 1. Cross-sections through the stem of the investigated samples

Also, the bark of *T. montanum* is thicker (8 layers of cells) than the other two species and presents a thin collenchyma layer outward. The innermost layer of the cortex is primary endoderm, with cells strongly flattened radially and elongated tangentially. In the lateral walls, Caspary thickenings are also visible.

The epidermis of *T. montanum* is substantially exfoliated, which is why at the surface of the stem there are few trichomes, located only in the portions where this primary protective tissue persists. On the other hand, the epidermis of *Teucrium chamaedrys* has isodiametric cells of different size, with thicker internal and external walls, the latter being covered by a thin cuticle; just near the ribs the cuticle is very thick, the cutinization affecting all the thickness of the external wall. From place to place there are visible stomata, which project slightly above the epidermis. At the level of the epidermis, long, multicellular, uniseriate hairs are located, sometimes with arched tip and very short, pluricellular secretory beads (peltate hairs). Regarding the epidermis of *T. polium* contains isodiametric cells, with the outer wall thicker than the others and covered by the cuticle. Also, there are numerous multicellular trichomes, with the terminal cell much longer than the others and sometimes with a curved tip. The secretory trichomes are rare also multicellular, with a relatively short pedicel and a single or quadruple cell gland. Such elements have been previously noted in various *Teucrium* species from Serbia and Montenegro. Therefore, our results are in accordance with literature data (1, 8, 9).

Another visible difference between the stem sections is noted for *T. montanum* which presents a very large central cylinder

which does not start with a sclerenchymatous layer as in *T. polium* and *T. chamaedrys*. Moreover, the vascular bundles are almost entirely of secondary origin. The phloem fibers form a thin ring, made up of sieve tubes, attached cells and many parenchyma cells, whereas the xylem is thick and lignified and presents numerous vessels of different diameter, irregularly dispersed in the fundamental mass. Among the vessels and the cells of the xylem, there are few cells of lignified wood parenchyma, sometimes arranged in discontinuous radial rows.

For further confirmation, we also observed the features on the secondary branches of the same species. The branches contain similar cell layers, but they are thinner and differ from the main stem by the thickness of the sclerenchyma (maximum two layers) and the number of surface hairs. However, at the epidermis level of the secondary stems of *T. montanum* numerous long, multicellular and small, short secretory trichomes are visible. Moreover, in this case in the central pith, a large lacuna resulting from the disruption of the central parenchyma is observed. Sometimes this cavity is crossed by 1-2 bands of persistent medullary parenchyma (fig. 2).

With respect to *T. polium* main axis, the structure of the secondary branches differs by the following characteristics: the epidermis presents more numerous and longer surface hairs, the glandular trichomes are rarer and shorter, the collenchyma layer is thinner at the hypodermic level, the cells of the cortical parenchyma are often isodiametric; the sclerenchyma pericycle ring is thinner; the phloem and pith are thinner, but the primary xylem is slightly lignified

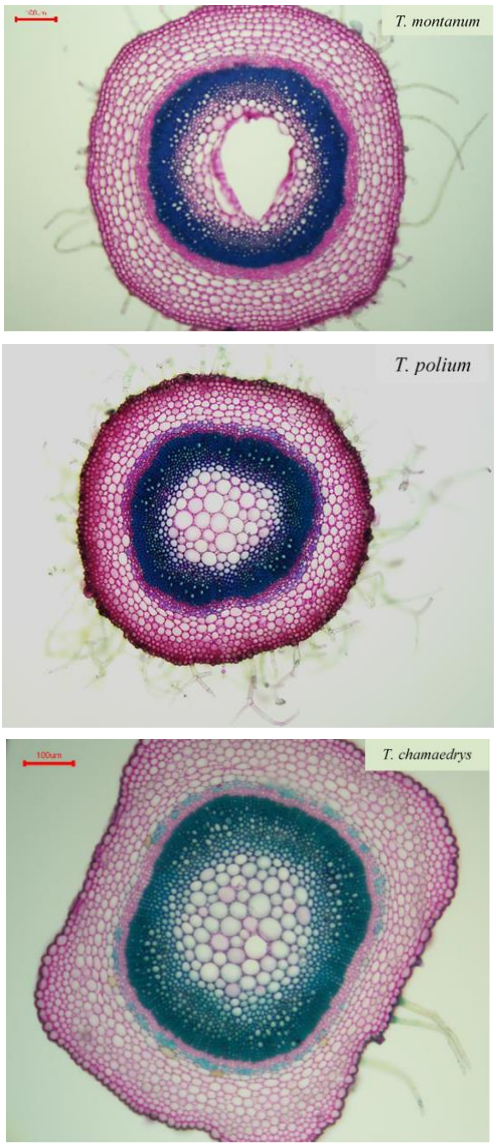

Fig. 2. The general aspect of the secondary branches obtained for the three *Teucrium* species

Leaf shape and anatomy

In general, all *Teucrium species* have the foliar lamina edges curved towards the abaxial face. Both the median vein and the lateral veins of the first order are protuber-

ant slightly to the lower face of the lamina. The lower epidermis has larger cells than the upper one, with the outer wall thicker and more intensely cutinized.

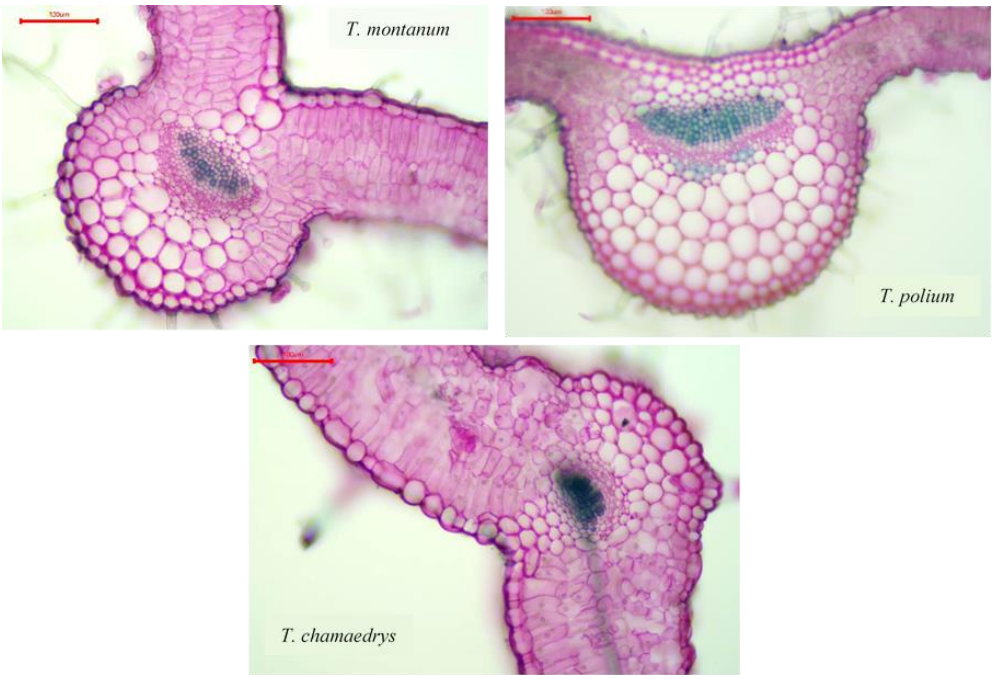

Fig. 3. The general aspect of the secondary branches obtained for the three *Teucrium* species

For *T. chamaedrys* there are surface hairs and secretory trichomes among the epidermal cells, but in smaller number on the surface unit than in the other two species, having the same structure as in the stem. Viewed from below, the lower epidermis has cells with sinuous side walls; from place to place numerous stomata are observed. The upper epidermis has cells with slightly wavy or straight walls, and the stoma is very rare. The mesophyll is differentiated into bistratified palisade tissue on the upper face and spongy multilayered tissue on the lower face, so the lamina has a hetero-facial bifacial structure. Regarding *montanum* sample, the epidermis has smaller cells on the lower face and larger cells on the upper face, the stomata are located in the lower epidermis, protruding slightly from the epidermis; so, the lamina is hypostomatic. The specific characteristics of *T. polium* leaf epidermis include isodiametric cells with the outer wall thicker and covered by a cuticular layer and the indumentum of both sides of the leaf is represented by numerous long, multicellular, uniseriate, often branched trichomes and fewer short secretory glands. Moreover, under both epidermis, there is a layer of collenchyma cells.

CONCLUSIONS

The structure plan of the analyzed organs is common to the three *Teucrium* species, with the same anatomical areas and component tissues. However, the investigated species differ in particular quan-

titative structure features such as cuticle thickness, frequency and size of trichomes, secondary xylem ring thickness, leaf mesophyll thickness, number of palisade structures and number of stomata on the surface unit lower epidermis.

Moreover, the species investigated by us differ in some qualitative traits, with significant taxonomic value, being able to distinguish the taxon when the plants are not flowering or to distinguish them when we only have fragments of the vegetative organs. The unique features that allow us to identify *Teucrium chamaedrys* plants are the bracts with homogeneous mesophyll and for *Teucrium polium* the high density of surface trichomes with branched tip. However, the stems are the main organ that are specific for *Teucrium montanum*, for which the presence of the aerial pith, the Caspary type endoderm on the bark and the lack of sclerenchyma are the characteristics necessary for identification. Nevertheless, further studies are undergoing to establish the main classes of active compounds and their pharmacologic potential.

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EVALUATION OF THE NEURO-AFFECTIVE STATUS IS A DECISION FACTOR IN THE THERAPEUTIC APPROACH OF SENIOR PATIENTS WITH HIP FRACTURES

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EVALUATION OF THE NEURO-AFFECTIVE STATUS IS A DECISION FACTOR IN THE THERAPEUTIC APPROACH OF SENIOR PATIENTS WITH HIP FRACTURES (Abstract): Both the cognitive function and the affective status are fundamental geriatric cues in the objective and comprehensive evaluation of a senior patient. **Aim:** To investigate the impact of cognitive and emotional disturbances of senior patients with hip fractures over the postoperative prognostic. And recovery period. **Material and methods:** We assessed the cognition and affective status as well as the degree of fragility in group of 44 senior patients admitted in the Trauma Clinic, Iasi for hip fracture. The patients underwent preoperatively a complete geriatric evaluation, focusing on the assessment of the cognitive function (MMSE - Mini-Mental State Exam), psycho-emotional status (GDS-Geriatric Depression Scale) and fragility (Groningen-IGF fragility index). **Results:** Most of the evaluated patients were female, and over 80 years old. Moderate cognitive impairment was approximately 40% in the studied group, 27% associating fragility. The mild depressed patients, with fragile status, accounted for more than a half of the percentage of those analyzed. The patients with cognitive and affective disorders registered a negative prognostic on the postoperative evolution and during the recovery period. **Conclusions:** The results of this study confirm the need for a complete geriatric evaluation in senior patients with hip fracture, or at least the evaluation of cognitive and emotional status associated with the degree of fragility as they are considered important prognostic factors in the post-surgical and recovery period. **Keywords**: COGNITIVE STATUS, NEURO-AFFECTIVE STATUS, FRAGILITY, HIP FRACTURE, SENIOR PATIENTS.

The assessment of the degree of impairment of the cognitive and emotional functions in the elderly patient is part of the basic geriatric evaluation. The need for cognitive evaluation, depressive status and degree of frailty resides in the optimal therapeutic approach and in the prognostic value of subsequent recovery in senior patients who have suffered a hip fracture. These parameters are not only markers of the state of health at the time of evaluation, but also important prognostic factors for

post-surgical and recovery periods (1).

The analysis of the neuro-emotional status of the elderly patients admitted with hip fractures through the use of standard geriatric instruments (MMSE, GDS) can determine the optimal subsequent behavior (2).The preoperative estimation of the patient's prognosis is of particular importance for healthcare providers, for patients and their relatives, not so much in terms of treatment decisions - surgical treatment represents the gold standard - but in terms of predicting the degree of subsequent recovery (3). In this context, the Groningen frailty index is also reported as a tool for anticipating post-hospitalization complications and the actual length of hospitalization in patients with hip fracture (4).

As senior individuals remain the fastest growing group in Western societies, a further increase in the number of traumatic events is expected. Due to this demographic change, hip fractures are also a growing economic problem, which demands the efficiency not only of the clinical care, but also of pre-intervention monitoring methods (5).

Preoperative depression, preoperative cognitive deficit and frailty were related to the postoperative delirium, medication management errors, postoperative complications, such as persistent pain, longer hospitalization, institutionalization frequency, increased costs, and increased mortality (6). There are several studies that focus on different factors that can influence afflicted groups of population (7, 8), and our preoccupations are focused on new factors that can affect the outcome of surgical orthopedic senior patients, such as the pre-operative detection of neuro-affective status and the degree of fragility in elderly patients with hip fracture.

MATERIAL AND METHODS

We present a prospective study that started in June 2019, from which we present the preliminary data collected in June-October 2019, on a group of 44 patients, aged between 62-93 years, hospitalized in the Trauma Clinic "Sf. Spiridon" County Clinical Emergency Hospital Iasi, for different types of hip fractures. A comprehensive evaluation was carried out preoperatively, with special attention to assessing the neuro-affective status and the degree of fragility with the help of MMSE (Mini Mental State Examination), GDS (Geriatric Depression Scale) and GFI (Groningen Fragility Index). Depending on the score obtained in the MMSE test, patients were considered to have normal cognitive status (>24 points), mild cognitive impairment (20-24 points) or medium-severe cognitive impairment (<20 points). The patients had an IGF≥4 and were all considered fragile. Depending on the score obtained in the GDS test, patients were considered to have normal affective status (GDS= 3-6), mild depressive status (GDS=7-10), severe depressed affective status (GDS= 10-15). The completion of the questionnaires was done after obtaining the informed consent of the patient, being made by the same trained medical team.

The data were collected by a geriatric team and were analyzed using SPSS 18.0 software. ANOVA Test, Chi-square Test and Paired T-test, Kruskall-Wallis and Pearson correlation for quantitative analyze where performed. The statistical significance was defined in 95% interval of confidence ($p < 0.05$).

RESULTS

Preoperative cognitive assessment

The percentage distribution of cases

according to the cognitive evaluation showed that severe cognitive impairment was found in 11.4% of the cases, moderate cognitive impairment was noticed in 34.0% of the total study group, and slight affectation was found in 27.3% of cases; only 27.3% of cases had no cognitive impairment (fig. 1).

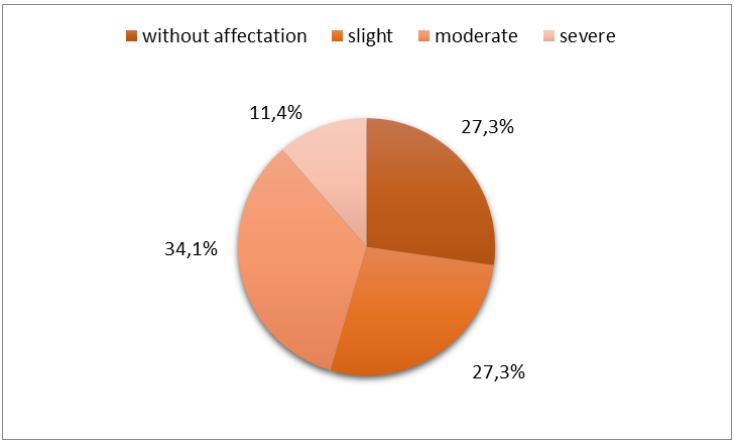

Fig. 1. The structure of the study group according to cognitive evaluation

MMSE score and age were in indirect correlation, moderate in intensity (r = -0.484; p = 0.001), noting that approximately 48% of patients had reduced MMSE scores at older ages (fig. 2).

The correlation of the cognitive impairment, as a percentage, with the marital status did not reveal statistically significant differences, 13.3% of the married persons and 20% of the divorced persons had severe neuro-cognitive impairment, and 41.7% of the patients had moderate neuro-cognitive impairment (p = 0.291) (fig. 3).

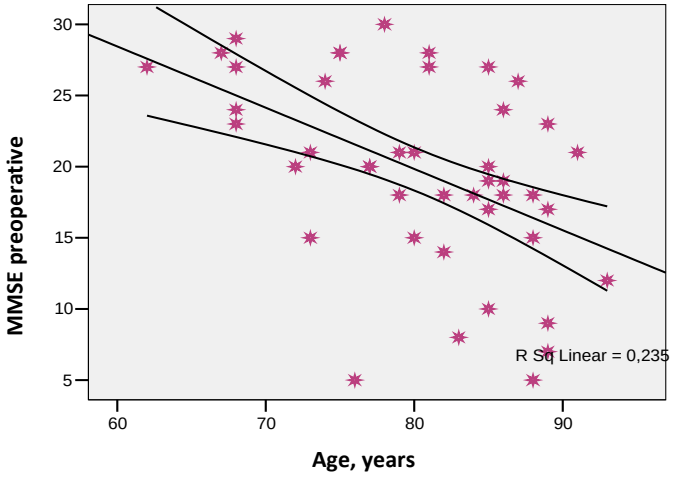

Fig. 2. Correlation of age with MMSE score

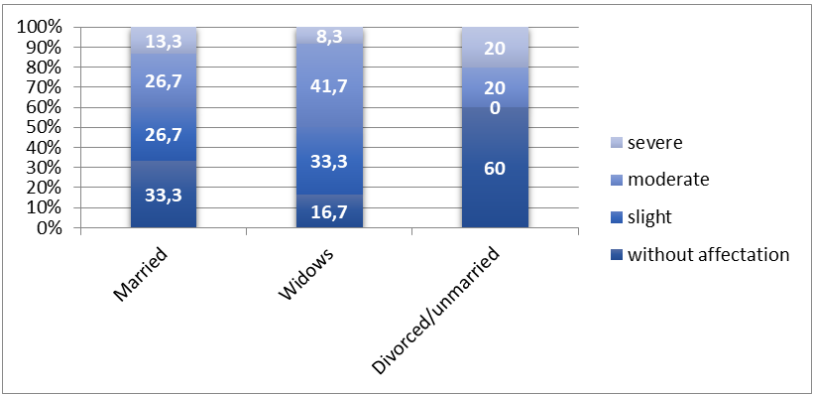

Fig. 3. Correlation of cognitive impairment with marital status

Preoperative emotional status

The percentage distribution of our cases according to emotional evaluation showed that 25% of the cases were very depressed, 54.5% of the total study group were mildly depressed and 20.5% of the cases were normal emotionally (fig. 4).

GDS score and age were apparently independent parameters (r = +0.074; p = 0.631) (fig. 5)

The correlation of emotional distress, as a percentage, with the marital status revealed statistically significant differences, 80% of unmarried or divorced people were very depressed, while 53.3% of married patients and 62.5% of widows were mildly depressed (p = 0.042) (fig. 6).

MMSE and IGF scores were in indirect correlation, reduced in intensity (r = -0.267; p = 0.08), noting that approximately 27% of the patients were associated with fragility with severe neuro-cognitive impairment, but the result cannot be extrapolated to the general population (fig. 7).

The GDS and IGF scores were in direct correlation, moderate in intensity (r = +0.590; p = 0.001), noting that 59% of the patients were associated with fragility with an extremely altered emotional status (fig. 8).

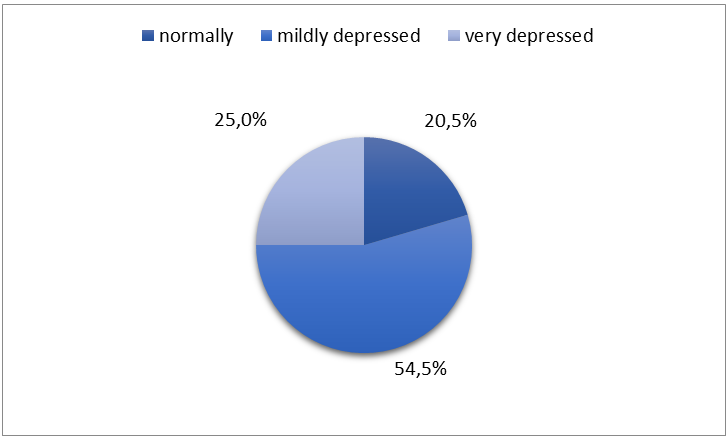

Fig. 4. The structure of the lot according to the emotional evaluation

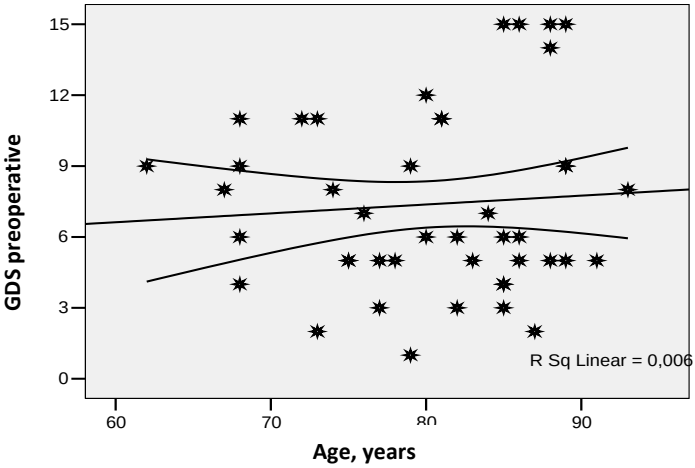

Fig. 5. Correlation of age with GDS score

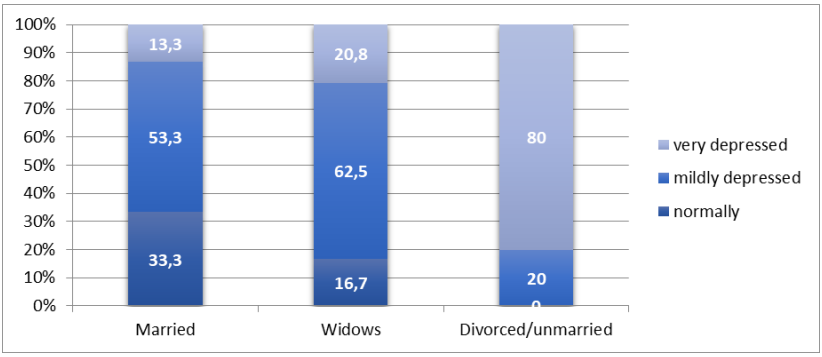

Fig. 6. Correlation of emotional affect with marital status

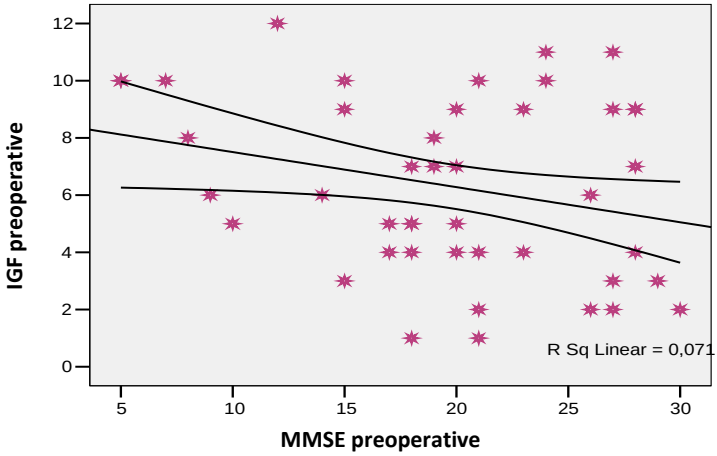

Fig. 7. Correlation of fragility with MMSE score

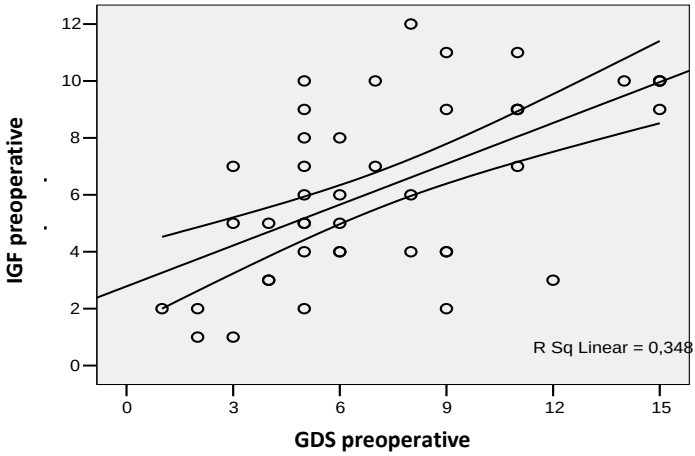

Fig. 8. Correlation of fragility with GDS score

DISCUSSION

Recent data shows the importance of performing MMSE and GDS scores in senior patients with hip fracture awaiting surgical treatment. There is an increased incidence of the alteration of functional (physical and mental) skills (9, 10) in the elderly persons exposed to such a traumatic event, despite previous functional independence (11).

It was estimated that approximately half of the seniors with hip fracture did not fully recover one year after the event, even with proper care during the recovery period (12). The one-year mortality rate for patients with hip fracture is about 25% and about one third of patients lose their independence during the same period.

There are several studies that report that there are strong connections between the onset of the traumatic event, the previous degree of depression and cognitive impairment. More than half (57.8%) of the seniors suffer from depression and cognitive impairment before the fracture (13), data confirmed by our study as well. It is important to know that trauma by itself aggravates the severity of these disorders (14, 15), which has a heavy negative impact on post-surgery mortality (16, 17), post-surgery complications and on the recovery period. A vicious circle is generated, that encompass age-associated comorbidities, the aggravation of the fragility and the prolongation of the hospitalization period (18).

Our study confirms the general opinion that the preoperative evidence of impairment of the emotional and cognitive status, in close relation with the degree of fragility, foretell a clear picture of the postoperative evolution and can advise an individualized therapeutic behavior (19), optimizing the chances of a full post-operative recovery.

CONCLUSIONS

The importance of the screening of affective and cognitive status in the senior patients that underwent a fall and a hip fracture was poorly understood and underestimated as prognostic factors in the post-surgical evolution. Our study confirms that the geriatric evaluation is very important in the preoperative assessment of these category of patients; if there is no geriatric team available, the completion of three simple questionnaires (MMSE, GDS and GFI) by any medi-

cal trainee can give valuable information about pre-surgical status of neuro-emotional and fragility status. Our data proved that there is a strong correlation between these three factors and the post-surgical evolution, complications and quality of recovery.

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METODOLOGIA DIAGNOSTICULUI HIPERPLAZIILOR ENDOMETRIALE EXAMEN CLINIC, PARACLINIC ŞI MANAGEMENT

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ENDOMETRIAL HYPERPLASIA DIAGNOSIS METHODOLOGY. CLINICAL, PARACLINICAL EXAM AND MANAGEMENT (Abstract): Factors associated with unopposed estrogenic stimulation such as obesity, exogenous hormone use endometrial hyperplasia are related to the development of the most common form of endometrial carcinoma, that is, the endometroid subtype. **Material and methods**: We selected a group of patients diagnosed with endometrial hyperplasia by endometrial biopsy and histopathological examination. The main complaint in all cases was abnormal uterine bleeding. All patients had gynecological examination, vaginal ultrasound, hysteroscopy endometrial biopsy or D&C. 32 patients had also immunohistochemical staining for Ki67, EGF, ER, PGR. **Results**: Cases with ages between 24-67 years were classified as: 100 simple hyperplasia, 10 complex hyperplasia, 43 atypical simple hyperplasia, 7 atypical complex hyperplasia. PR were 40-60% at all forms of hyperplasia, E2R were 30-40% in simple hyperplasia without atypia and 50-70% in complex hyperplasia without atypia. Correlation between immunohistochemical expression of E2R, PGR, Ki-67, EGF and body mass revealed an high immunohistochemical expression of E2R and Ki-67 in patients with hyperplasia without atypia and a low expression and high reactivity of EGF in cases with high body mass. **Conclusions**: Vaginal ultrasound and hysteroscopy are efficacious completion for histopathological diagnosis. We recommend an age/risk appropriate screening to detect risk factors and early disease in the asymptomatic patients. **Key words**: ENDOMETRIAL HYPERPLASIA, DIAGNOSTIC, MANAGEMENT

LITERATURE REVIEW OF ORAL TREATMENT OF TYPE 2 DIABETES MELLITUS

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LITERATURE REVIEW OF ORAL TREATMENT OF TYPE 2 DIABETES MELLITUS (Abstract): Type 2 non-insulin-dependent diabetes mellitus (T2DM), the most common form of diabetes that affects more than 90% of diabetic patients, is characterized by insulin resistance, β-pancreatic cells dysfunction, and increased hepatic glucose synthesis. The aim of the present paper was to review data on the efficacy of conventional oral dosage forms compared to controlled oral drug delivery systems for treating diabetes. Although the number of oral antidiabetic drugs has significantly increased in recent years, clinically there are still important limitations in terms of therapeutic efficacy, tolerability, and adverse effects, particularly regarding weight gain and medium and long-term glucose control. The last generation of oral pharmaceutical formulations such as modified-release tablets overcame some limitations, while injectable formulations with glucagon-like peptide-1 receptor agonists significantly increased the effectiveness of anti-diabetic medication and significantly reduced diabetes-specific comorbidities. Metformin extended-release formulation seems to be more effective than metformin conventional-release formulation in improving glyco-metabolic control, lipid profile, and levels of some adipocytokines in T2DM. Sustained-release glipizide formulation increases the compliance compared to immediate-release glipizide because it seems to achieve similar glucose control with decreased insulin secretion and to decrease the frequency of hypoglycemic episodes. Gliclazide modified release formulation seems to be effective and safe in T2DM with suboptimal glycemic control. Modified release formulations generate other advantages such as increasing treatment compliance, lowering the dose of drug substance, and even reducing side effects. **Keywords:** NON-INSULIN-DEPENDENT DIABETES MELLITUS, CONVENTIONAL RELEASE ORAL PHARMACEUTICAL DOSAGE FORMS, MODIFIED RELEASE ORAL PHARMACEUTICAL DOSAGE FORMS.

Diabetes mellitus is a metabolic genetically or inherited disorder, with chronic evolution, characterized initially by disruption of glucose metabolism, subsequently associated with suffering and disturbance of lipidic, protidic and mineral metabolism. The diagnostic criteria defined by the standard diagnostic criteria proposed by IDF and World Health Organization (WHO) for the patient with diabetes mellitus include a *venous blood glucose higher than 126 mg/dL or 7.0*

mmol/L at fasting (fasting for at least 8 hrs.) obtained for two separate tests or by *more than 200 mg/dL (11.1 mmol/L)* measured at any time of the day in patients with symptoms of hyperglycemia or hyperglycemic crisis. Another diagnostic criterion is *the glucose tolerance test*, which requires the administration of a defined amount of glucose (75 g) followed by blood glucose level test after two hours; in this case, a *glycemia higher than 200 mg/dL* confirms the diagnosis of diabetes mellitus. Last but not least, *glycosylated hemoglobin value above 6.5% (48 mmol/mol)* completes the biochemical status of the diabetic patient (1-3). Diabetes mellitus, according to its etiopathology, is classified as: *type 1 diabetes* (autoimmune β-pancreatic cells destruction), *type 2 diabetes* (progressive loss of insulin secretion), *gestational diabetes* (usually diagnosed in the second or third trimester of pregnancy in previously clinically healthy patients). There are also other types of diabetes such as *neonatal diabetes* due to exocrine pancreatic disorders (cystic fibrosis and pancreatitis) and *iatrogenic diabetes* as a result of chronic glucocorticoid administration in transplant patients and HIV/AIDS immunosuppressed patients (2, 3).

T2DM is the metabolic disorder with the highest incidence and is considered a worldwide public health issue. According to World Health Organization (WHO) statistics, the number of diabetes patients increased from 108 million in 1980 to 422 million in 2014 and it is estimated to increase to 642 million diabetic patients in 2040 (4). According to the 2019 edition of the International Diabetes Federation (IDF) Diabetes Atlas, 463 million adults are currently living with diabetes and 700 million adults are estimated with diabetes for 2045 (5). It is important to mention that the incidence of diabetes has significantly increased in developing countries compared to the developed ones. According to the results of the PREDATORR study finalized in 2014, which aimed to assess the prevalence of diabetes in the adult population of Romania, more than 11% of Romania's population is affected by diabetes. In 2014, there were registered over 875,000 diabetic patients and in 2015 there was an increase with 8.73% in the number of patients, reaching approximately one million diagnosed patients (6).

This fact has contributed to the approval of innovative antidiabetic molecules, thus ensuring effective monitoring of antidiabetic medication, especially in patients for whom conventional antidiabetic medication does not provide a rigorous and stable glycemic control. Nowadays, the pharmacological therapy of T2DM includes the following therapeutic classes: *insulin, biguanides, sulphonylureas (hypoglycemic sulfamides), methylglinides, thiazolidinediones (glitazones), α-glucosidase inhibitors, dipeptidyl peptidase 4 (DPP-4), sodium-glucose co-transporter 2 inhibitors (SGLT2 inhibitors)* and *glucagon-like peptide 1 (GLP-1) receptor agonists* (4, 7).

Most conventional pharmaceutical dosage forms of oral antidiabetic drugs are characterized by low bioavailability, short half-life, and the need to be taken more times a day, resulting in poor patient adherence. Therefore, more research has been made in developing new controlled oral drug delivery systems. The aim of the present paper was to review data on the efficacy of conventional oral dosage forms com-

pared to controlled oral drug delivery systems for treating diabetes.

The current dosage forms for oral antidiabetic drugs are presented in first table.

TABLE I

Dosage forms for oral antidiabetic drugs

Therapeutic class	Drug name	Strength (mg)	Brand name	Pharmaceutical form	Renal impairment	Hepatic impairment	Elderly	ANMDMR approval	EMA approval
Biguanides	Metformin	1000	SIOFOR®	film-coated tablet	SRI	NO	YES	✓	✓
		500; 750; 1000	SIOFOR®	prolonged-release tablet					
		1000	METFOGAMMA®	film-coated tablet					
		500	MEGUAN®	film-coated tablet					
		500; 850; 1000	GLUCOPHAGE®	film-coated tablet					
		500; 750; 1000	GLUCOPHAGE XR®	prolonged-release tablet					
Sulfonylureas derivatives	Glibenclamide	1,75; 3,5; 5	GLIBENCLAMID ARENA	tablet	SRI	SLD	YES	✓	✓
		3,5	MANINIL®	tablet					
	Gliclazide	60	DIAPREL MR®	modified release tablet	SRI	SLD	YES	✓	✓
		80	ESQUEL®	tablet					
		30	GLYCLADA®	prolonged-release tablet					
		60; 90	GLYCLADA®	modified release tablet					
	Glimepiride	1; 2; 3; 4; 6	AMARYL®	tablet	SRI	SLD	YES	✓	
	Gliquidone	30	GLURENORM®	tablet	YES	SLD	YES	✓	✓
Thiazolidine-diones (glitazones)	Lobeglitazone	0,5	DUVIE®	tablet				×	×
	Pioglitazone	15	PIOGLITAZONE TORRENT	tablet	YES	NO	YES	✓	✓
		15; 30; 45	ACTOS®	tablet					
Alpha-glucosidase inhibitors	Acarbose	50; 100	GLUCOBAY®	tablet	SRI	YES	YES	✓	✓
Dipeptidyl Peptidase-4 (DPP-4) Inhibitors	Alogliptin	6,25; 12,5; 25	VIPIDIA®	film-coated tablet	MRI=12,5 mg ESRD; SRI=6,25 mg	YES	YES	✓	✓
	Linagliptin	5	TRAJENTA®	film-coated tablet	YES	YES	YES	✓	✓

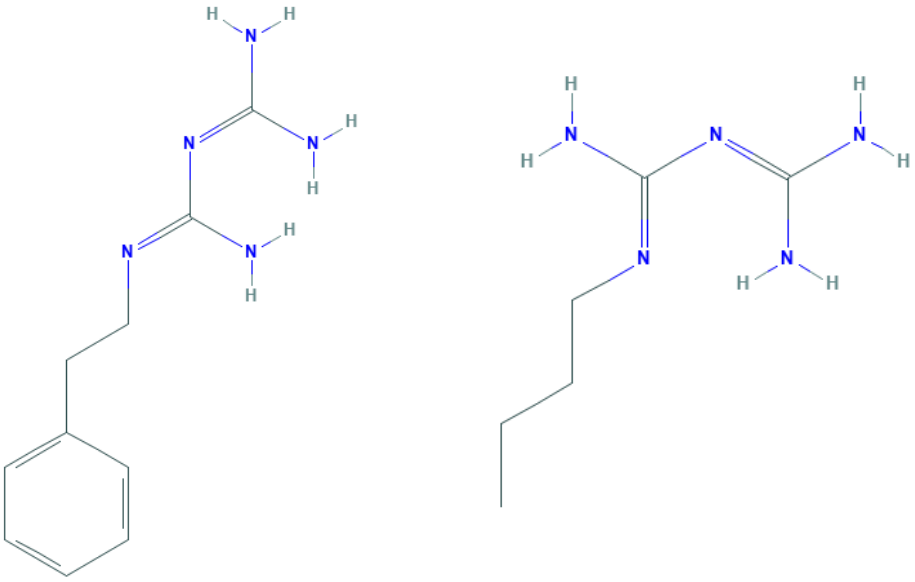
Therapeutic class	Drug name	Strength (mg)	Brand name	Pharmaceutical form	Renal impairment	Hepatic impairment	Elderly	ANMDMR approval	EMA approval
	Saxagliptin	2,5; 5	ONGLYZA®	film-coated tablet	ESRD	YES	YES	✓	✓
	Sitagliptin	25; 50; 100	JANUVIA®	film-coated tablet	ESRD	YES	YES	✓	✓
	Vildagliptin	50	GALVUS®	tablet	MRI; SRI; ESRD= 50 mg	NO	YES	✓	✓
Methylglinides	Mitiglinide	5; 10	GLUFAST®	tablet	-	-	-	×	×
		5; 10	GLUFAST OD®	orally disinte-grating tablet					
	Nateglinide	60; 120; 180	STARLIX®	film-coated tablet	YES	SLD	YES	✓	✓
	Repaglinide	0,5; 1; 2	RENEOS®	tablet	YES	YES	YES	✓	✓
SGLT-2 Inhibitors	Canagliflozin	100; 300	INVOKANA®	film-coated tablet	YES	YES	≥75 years	✓	✓
	Dapagliflozin	5; 10	FORXIGA®	film-coated tablet	YES	YES	YES	✓	✓
	Empagliflozin	10; 25	JARDIANCE®	film-coated tablet	YES	YES	YES	✓	✓
	Ertugliflozin	5; 15	STEGLATRO®	film-coated tablet	ESRD	YES	≥75 years	✓	✓

ANMDMR: National Agency for Medicines and Medical Devices of Romania; EMA: European Medicines Agency; ESRD: end-stage renal disease; MR: modified-release; MRI: moderate renal impairment; SRI: severe renal impairment; SLD: severe liver disease; XR: extended-release.

Biguanides - from conventional formulations to modified release

Since the active substance called phenformin (fig. 1a) was firstly discovered in the 1920s in the *Galega officinalis* plant extract (*French lilac*), the biguanides became the first therapeutic class of oral antidiabetic agents used in the treatment of diabetes. This compound was approved for therapeutic use only in October 1977 in the United States (US) but did not reach a major therapeutic success and was withdrawn a few years later due to the increased incidence of lactic acidosis as an adverse reaction (8). Researches in this field continued and in 1957 scientists Shapiro, Parrino, and Freedman synthesized 1-butylbiguanide (buformin) (fig. 1b), registered under the name of Silubin® formulated in delayed-release dragees/sugar-coated pills (100 mg buformin/dragee) by the German pharmaceutical company *Grünenthal*. Unfortunately, this active molecule did not offer maximum therapeutic results, being responsible for the same adverse effects as the phenformin molecule, which is the reason why it was withdrawn from the therapeutic use (9).

An important moment in the development of oral antidiabetics was the introduction of *metformin* (dimethyl biguanide HCl) molecule into therapy (fig. 2). It started to be used in European patient therapy in the mid-twentieth century, more precisely in 1957, and it was approved by the Food and Drug Administration (FDA) in the US in 1995 (3). Metformin is the first-choice oral antidiabetic drug in the management of T2DM across all age groups.



a **b**

Fig. 1. Chemical Structure of **(a)** phenformin **(b)** buformin (10)

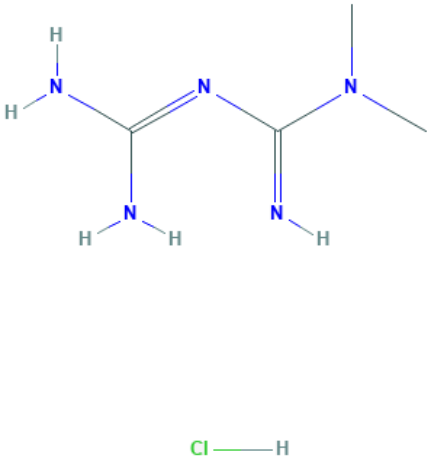


Fig. 2. Chemical Structure of metformin hydrochloride 3-(diaminomethylidene)-1,1-dimethylguanidine; hydrochloride (10)

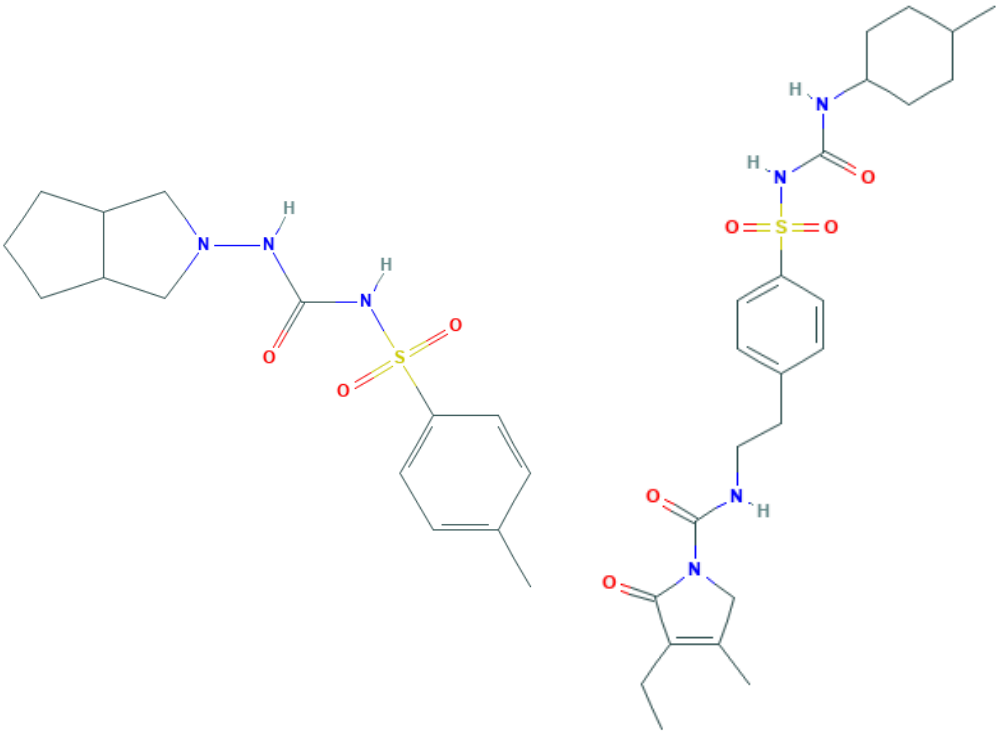
In the development of metformin clinical studies, French clinician Jean Sterne was the first diabetes specialist to introduce the molecule into therapy and gave the first commercial name of metformin conventional-release tablets, *Glucophage*® (8, 11, 12).

It has been shown that metformin alone reduces glycosylated hemoglobin by about 1.5% and is currently available as tablets with the immediate-release profile (IR) or extended-release profile (XR). The new studies of metformin pharmaceutical formulations revealed major benefits for lipid metabolism. Moreover, it was shown that prolonged-release tablets improved the glycemic control of diabetes patients. Reducing the frequency of administration at once per day in case of metformin XR formulations it was noticed an improved gastrointestinal tolerability through gradual and a controlled release of the active substance. Furthermore, by reducing the number of daily doses compliance to therapy increased significantly and the number and intensity of reported adverse reactions were also diminished (13-15).

In the Nomenclature of Medicinal Products for Human Use of the Romanian National Agency for Medicines and Medical Devices (ANMDMR), 50 pharmaceutical forms with metformin used in T2DM therapy are registered as single drug or as associations under different concentrations. Currently, metformin is the cheapest and most commonly used oral antidiabetic drug (16).

Sulfonylureas derivatives - from conventional formulations to modified release

Following the synthesis of hypoglycemic sulfonylureas derivatives, *tolbutamide* and *chlorpropamide*, which were also the first representatives, this class of oral antidiabetic drugs were intensively developed. Usually, this class of oral antidiabetic agents is recommended only in normal weight or lean patients with greater insulin deficiency, since β-pancreatic cells are still functional to secrete insulin and to lower plasma glucose levels. It is important to note that they may be associated with other oral antidiabetics, such as metformin or thiazolidinediones, but their use becomes more and more limited due to safety issues and emergence of new oral antidiabetics. The loss of β-pancreatic cell function does not allow the usage of antidiabetic sulfamides in the therapy of patients with type 1 or advanced stages of type 2 diabetes (17, 18).



a **b**

Fig. 3. Chemical Structure of **(a)** gliclazide and **(b)** glimepiride (10)

Sulfonylureas derivatives generically called sulfamides are classified into two major classes: first generation (chlorpropamide, tolazamide, tolbutamide), second-generation (glibenclamide, glibornuride, gliclazide (fig. 3 a) gliquidone, glisoxepide) and, according to some authors, third generation glimepiride (fig. 3 b) (tab. I). The second generation of sulphonylureas has a high potency and the therapeutic dose is expressed in units of milligrams of the active substance, but the maximum hypoglycemic effect is not superior to tolbutamide. Sulfamides and their metabolites have significant renal excretion, which is why caution is recommended in elderly patients and in patients with impaired renal function to avoid severe hypoglycemia (8, 19).

TABLE II

Sulfonylureas antidiabetic drugs - pharmacological characteristics

Oral antidiabetic drug	Usual daily dose (mg)	Duration of action (hrs.)	$T_{1/2}$ (hrs.)
Chlorpropamide	100-500	24-72	35
Tolazamide	100-1,000	12-18	7
Tolbutamide	500-3,000	6-10	7
Glibenclamide	2.5-15	12-16	5
Glibornuride	12.5-100	8-12	8
Gliclazide	80-240	6-12	10
Gliquidone	15-120	5-7	1.5-2
Glisoxepide	2-16	8-24	1.7
Glyburide	1.25-10	24	7-10
Glipizide	2.5-10	10-24	2-4
Glimepiride	3-6	12-12	7

Sustained-release glipizide formulation increases the compliance compared to immediate-release glipizide because it seems to achieve similar glucose control with decreased insulin secretion and to decrease the frequency of hypoglycemic episodes (21). Gliclazide modified release formulation seems to be effective and safe in T2DM with suboptimal glycemic control (22).

Oral antidiabetic drugs formulated only as conventional drug release forms

- **Thiazolidinediones (glitazones)**

The development of thiazolidinediones started from the observations of the clofibrate, ciglitazone and troglitazone derivatives (fig. 4) which were studied for their therapeutic effect on lipid profile; it was surprisingly found that these two molecules caused a decrease in blood glucose, but high hepatotoxicity though (20). In addition to the hypoglycemic effect, thiazolidinediones have a various number of pharmacological advantages, including decreasing the rate of albumin excretion, modulation of insulin resistance syndrome, lowering blood pressure, and improving the glucose tolerance threshold (7).

Based on the scientific observations of these two substances (ciglitazone and troglitazone), a new class of oral antidiabetics, called thiazolidinedione derivatives, has been developed. They are acting as agonists of peroxisome proliferator-activated receptors (PPAR). The main representatives of this therapeutic class are *pioglitazone* and *lobeglitazone* (19).

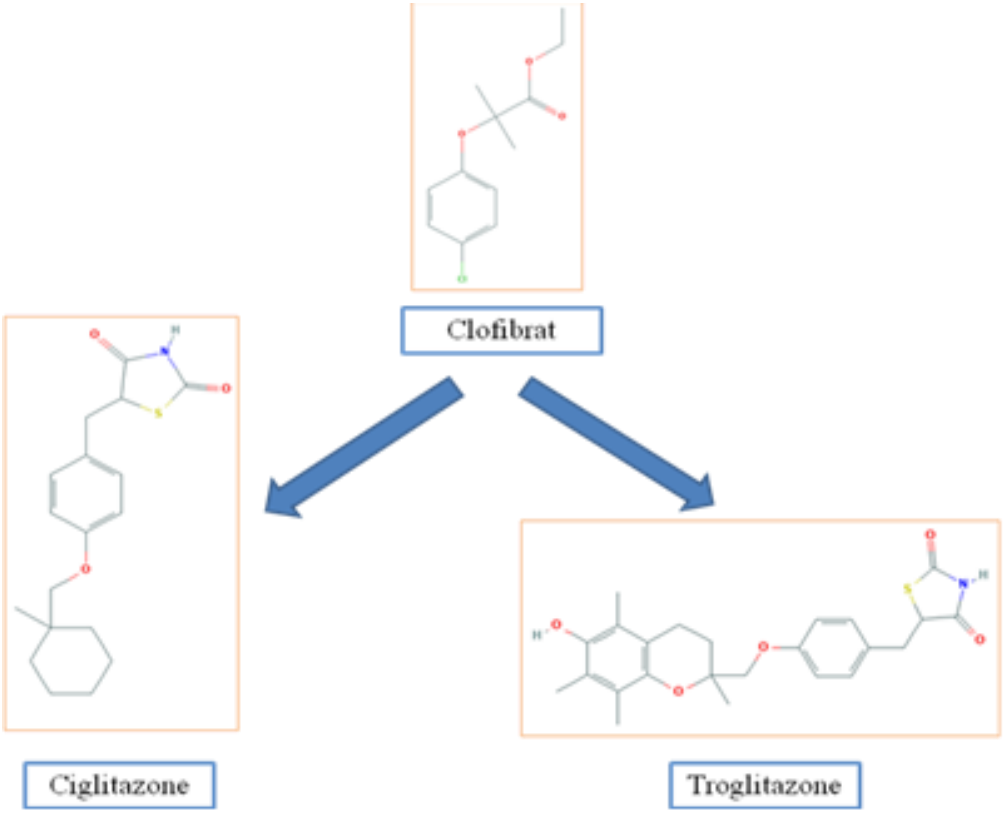


Fig. 4. Chemical structure of thiazolidinediones (10)

Pioglitazone (Actos®), the most used from this group, was authorized by the European Medicines Agency (EMEA, EMA) in October 2000 in T2DM in adult patients, particularly those who are overweight, and it is used as an adjunct to diet and exercise to improve glycemic control. Another derivative, Rosiglitazone (Avandia®) (fig. 5 a), authorized by EMEA in July 2000, was suspended in September 2010 because the benefits no longer outweighed the cardiovascular risks (23).

Lobeglitazone, a novel thiazolidinedione derivative, was approved in 2013, only in South Korea, and it is recommended in T2DM therapy. Even though the chemical structure of lobeglitazone is similar to pioglitazone and rosiglitazone, lobeglitazone was conceptually designed by modification of the rosiglitazone structure with a substituted pyrimidine. Unlike Pioglitazone, which is an agonist on both PPAR-alpha and PPAR-gamma, Lobeglitazone is a selective agonist only on PPAR-alpha. *Lobeglitazone* has a *p*-methoxy phenoxy group at the 4-position of the pyrimidine moiety leading to a more potent activity than the reference compounds (pioglitazone and rosiglitazone), with reduced side effects (fig. 5 b) (24).

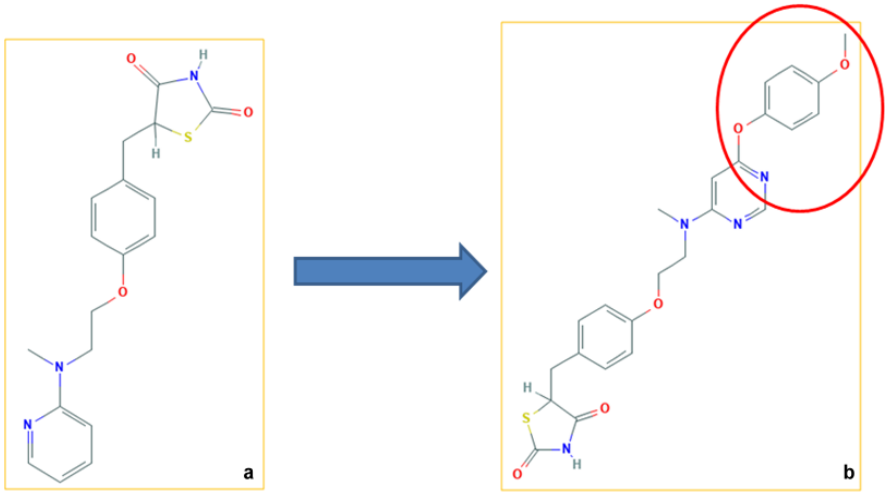


Fig. 5. Chemical Structure of **(a)** rosiglitazone **(b)** lobeglitazone (10)

➢ **Alpha-glucosidase inhibitors**

The beginning of the '90s is marked by the introduction of a new therapeutic class for patients with T2DM, α-glucosidase inhibitors, whose mechanism of action is delayed the absorption of dietary carbohydrates in the gastrointestinal tract, which generates the reduction in plasma glucose levels. Three drugs belong to this class, namely *miglitol*, *acarbose*, and *voglibose*, that were clinically approved in the US. In Romania, are used only the 50 and 100 mg film-coated tablets of *acarbose* (fig. 6), under the name Glucobay® (7, 17).

Voglibose, an unauthorized α-glucosidase inhibitor in Europe, is successfully used in Asia though. This compound was discovered in Japan in 1981, and since 1994 it has been used as an oral antidiabetic drug in T2DM therapy. *Voglibose* is used for lowering post-prandial blood glucose levels when either diet and/or lifestyle-related exercise, and other hypoglycemic agents (sulphonylureas) or insulin preparations are not able to provide adequate glycemic control.

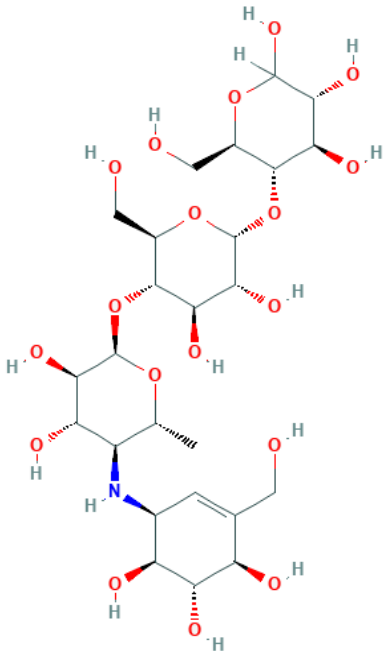


Fig. 6. Chemical Structure of acarbose (10)

This active substance produces an increased release of glucagon-like peptide 1 (GLP-1), which is an insulinotropic hormone, known to enhance insulin secretion and insulin sensitivity. Voglibose has a major advantage to acarbose, namely, it has no inhibitory activity against lactase and so, it does not cause lactose intolerance. In 2004, *voglibose orodispersible tablets* (*VODT - Voglibose oral disintegrated tablet*) were introduced into Japan, which allowed easier and faster administration of medication, in comparison with conventional oral tablets. The launch of VODT made a significant contribution to diabetic care by improving patient compliance. The development of VODT formulation brings important benefits to patients with swallowing problems, elderly patients as well as in emergencies when the patient has no access to water (25, 26).

- **Dipeptidyl Peptidase-4 (DPP-4) Inhibitors**

Dipeptidyl peptidase-4 inhibitors represent one of the newest T2DM therapy classes used as monotherapy. This therapeutic class includes drugs, such as *sitagliptin* (fig. 7), *saxagliptin*, *vildagliptin*, *alogliptin*, *linagliptin*, *gemigliptin*, *anagliptin*, *teneligliptin*, *trelagliptin*, *dutogliptin*, and *omarigliptin* (27-29). DPP-4 inhibitors have various mechanisms of action to control hyperglycemia, by stimulating the secretion of insulin from beta-cells, decreasing the secretion of glucagon from pancreatic alpha-cells, and at the same time reducing the production of glucose by the liver. The number of drugs doses administered per day is correlated with the half-life of the active substance. Thus, the sitagliptin, alogliptin, saxagliptin and linagliptin are administered in a single daily dose, while vildagliptin, with a lower half-life, is recommended for twice-daily administration. DPP-4 inhibitors may be used in combination with insulin with the great advantage of reducing the likelihood of developing hypoglycemia (3, 20).

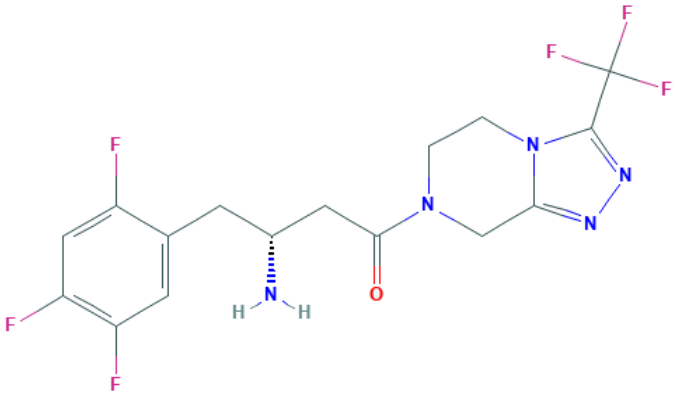


Fig. 7. Chemical Structure of sitagliptin (10)

DPP-4 inhibitors are safe, orally active, and highly tolerable, mainly in elderly patients, due to their minimal risk for hypoglycemic events.

- **Methylglinides**

Methylglinides form a class of drugs used to treat T2DM and include active substances, like *repaglinide* (fig. 8),

nateglinide and *mitiglinide*, with a mechanism of action to stimulate pancreatic secretion of insulin.

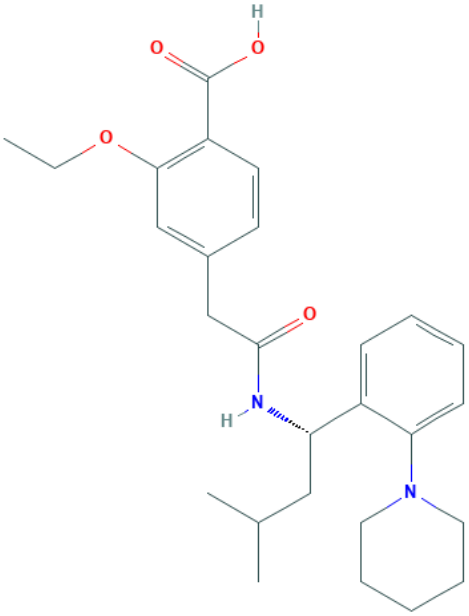


Fig. 8. Chemical Structure of repaglinide (10)

Methylglinides have a short half-life and are absorbed very rapidly from the gastrointestinal tract and therefore are recommended to be given 15 minutes before a meal. Methylglinides have the advantage that they can be used in patients who are allergic to sulphonylureas, and may reduce the risk of hypoglycemia, even if they induce similar weight gain to sulphonylureas. In case of inadequate glycemic control, they may be combined with metformin or thiazolidinediones. *Repaglinide* was introduced into therapy in 1997 as a benzoic acid derivative. Repaglinide can be administrated to patients with renal impairment, due to their excretion via feces. In 2001, EMEA approved the *nateglinide* molecule as 60, 120 and 180 mg film-coated tablets marketed under the brand name Starlix® (7, 19).

- **Sodium-glucose co-transporter 2 (SGLT-2) inhibitors**

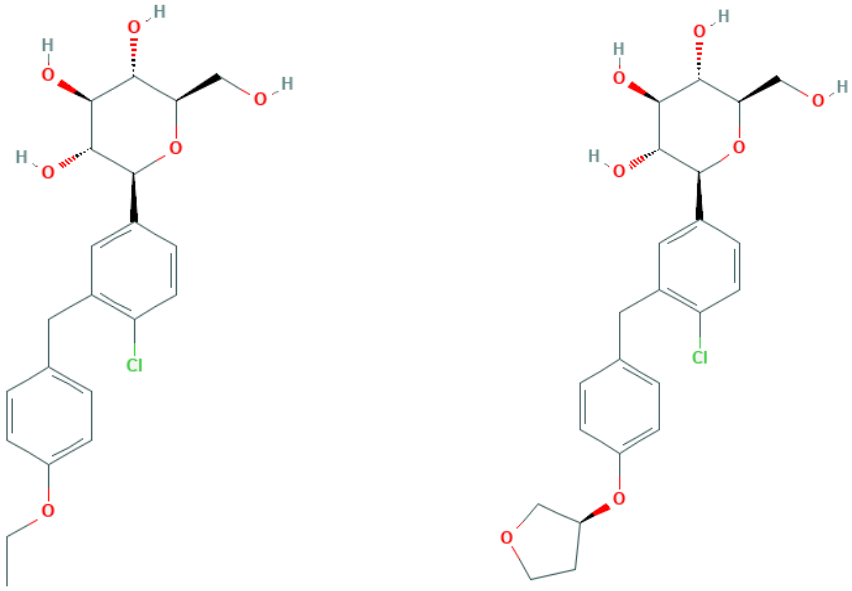
Phlorizin, a dihydroalcohol which was found primarily in fruits of the *Pyrus communis* (pear), but also in some closely related species such as cherries and apples, is the first model for synthetic analogs of SGLT-2 inhibitors.

This compound was studied as an antidiabetic therapeutic agent as a non-selective SGLT-1 and SGLT-2 inhibitor, but it was not an effective drug because when orally consumed, it was nearly entirely converted into phloretin by hydrolytic enzymes in the small intestine. So, all the attention of researchers has since focused on the synthesis of selective therapeutic compounds SGLT-2 for T2DM treatment (30).

In January 2019, the Committee for Medicinal Products for Human Use of EMEA approved a new therapeutic indication for the *dapagliflozin* molecule (fig. 9 a), thus becoming the first oral antidiabetic drug used in type 1 diabetes mellitus as an adjuvant to insulin in patients with the body mass ≥27 kg/m^2, when insulin monotherapy does not provide adequate glycemic control (31).

Among the most commonly used compounds in the European Union are *dapagliflozin* 5 and 10 mg film-coated tablets, *canagliflozin* 100 and 300 mg film-coated tablets, and *empagliflozin* 10 and 25 mg film-coated tablets.

Empagliflozin (fig. 9 b) is the first antidiabetic drug with clinical trials in reducing the risk of cardiovascular mortality. The EMPA-REG OUTCOME® study demonstrated a 38% reduction of cardiovascular mortality risk in patients with T2DM as compared with the placebo group. Thus, empagliflozin became the first oral antidiabetic medicine administrated as monotherapy, approved by the EMEA for the reduction of cardiovascular risk (32).



a **b**

Fig. 9. Chemical Structure of dapagliflozin **(a)** empagliflozin **(b)** (10)

CONCLUSIONS

In recent decades, oral antidiabetic drugs for T2DM has undergone a remarkable evolution from simple molecules with a hypoglycemic effect that has been accidentally discovered to complex polypeptides with multiple mechanisms of action. Although most oral antidiabetic substances are formulated into conventional (immediate) release tablets, a significant number of modified-release formulations have been introduced over the past decade, encompassing one or more drug substances thereby providing associated medication as an effective therapy in the case of patients refractory to monotherapy.

Modified release formulations generate advantages such as increasing treatment compliance, lowering the dose of drug substance, and even reducing side effects. All advances in the development of antidiabetic medication have significantly contributed to increasing the quality of life of the diabetic patient, yet T2DM remains a public health problem and a scourge affecting more and more patients.

CONFLICT OF INTERESTS

There are no conflicts of interest. All authors contributed equally to this paper.

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NEWS

REGARDING LYNCH / MUIR-TORRE SYNDROMES

A “cancer predisposing syndrome” later labeled as Hereditary Non-Polyposis Colorectal Cancer (HNPCC) or Lynch Syndrome, was firstly described by Warthin, about one century ago. An increased predisposition to the development of multiple familial tumors is described as characteristic of this syndrome where visceral and cutaneous malignancies may appear at an early age namely endometrial, gastric, small bowel, ureteral and renal pelvis, ovarian, hepatobiliary tract, pancreatic, brain (Turcot Syndrome) and sebaceous glands (Muir-Torre Syndrome). The latter, a variant of Lynch Syndrome, is characterized by the presence of sebaceous skin adenomas, carcinomas and/or keratoacanthomas associated with visceral malignancies. Both Lynch Syndrome and Muir-Torre Syndrome have been recognized due to germline mutations in mismatch repair genes *MLH1*, *MSH2* and *MSH6*. To date, 56 Lynch Syndrome founder mutations dependent on *MLH1*, *MSH2* and, although less frequently found, *MSH6* and *PMS2* are described. Some of these founder mutations, principally of *MSH2* gene, have been described to cause Muir-Torre phenotype and have been traced in large and outbreed Muir-Torre Syndrome families living in different US and European territories. Due to the evidences of highly specific Muir-Torre phenotypes related to the presence of widespread *MSH2* founder mutations, preliminary search for these *MSH2* common mutations in individuals carrying sebaceous tumors and/or keratoacanthomas, at early age or in association to visceral and familial tumors, permits cost-effective and time-saving diagnostic strategies for Lynch/Muir-Torre Syndromes (Ponti G, Manfredini M, Tomasi A, Pellacani G. Muir-Torre Syndrome and founder mismatch repair gene mutations: A long gone historical genetic challenge. *Gene* 2016; 589(2): 127-132).

ILLNESS REPRESENTATIONS OF CHILDREN WITH CHRONIC DISEASES AND THEIR IMPACT ON TREATMENT ADHERENCE AND THE QUALITY OF LIFE. A NON-CATEGORICAL STUDY

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ILLNESS REPRESENTATIONS OF CHILDREN WITH CHRONIC DISEASES AND THEIR IMPACT ON TREATMENT ADHERENCE AND THE QUALITY OF LIFE. A NON-CATEGORICAL STUDY (Abstract): The identification of the connections between illness representation, the quality of life, and treatment adherence, among the children diagnosed with diverse chronic diseases. Furthermore, we wished to see whether there were differences by gender at the level of the three variables. **Material and methods:** 94 children - 52 boys and 42 girls - with a mean age M = 13.63 ± 2.68, filled out the *Perceptions Questionnaire-Revised*, the *Self-Regulation of Medication Adherence Battery*, and the *Pediatric Quality of Life Inventory*™ Version 4.0. **Results:** Preponderantly, the correlations identified between the three variables are negative. We have not identified any significant differences by gender, in what concerns illness representations. However, girls have higher control over treatment adherence (M girls = 3.30, M boys = 3.49, p = 0.035) and lower emotional functioning compared to boys (M girls = 62.14, M boys = 73.40, p = 0.016). **Conclusions:** The higher level of illness identity, illness coherence, and personal control among children, the more likely for them to adhere to treatment. Furthermore, the higher control over the treatment, the greater motivation of children to adhere to treatment. Consequently, practical interventions should focus on instilling the children's perceived control over their treatment.
Keywords: ILLNESS REPRESENTATION, QUALITY OF LIFE, ADHERENCE, CHILDREN CHRONIC DISEASE.

According to the National Health Report for Children and Young People in Romania (1), in 2017 the prevalence of chronic diseases among children was 26.2%. The percentage varies - at international level - between 13-27%, by the definition considered for chronic disease (2, 3). When we discuss chronic disease and treatment, we must consider treatment adherence - depending on which we further model the evolution of a patient's health state (4).

Treatment adherence has significant implications on health outcomes; low levels lead to an increase in mortality and a

decrease in the quality of life (5). This is a complex construct, influenced by multiple factors, referring to the extent to which a person observes the recommendations of a health specialist. (6) Among the factors with a significant impact on treatment adherence, it is worth noting the beliefs of a person in what regards their medical condition (7). These representations are subjective perceptions, more or less close to the scientific and medical objective reality (8). The model comprising the constitution and evolution of this construct, its connections with other important variables - such as the mechanisms of adapting to illness and the outcomes obtained - is the Common-Sense Model, theorized by Leventhal and his collaborators (9). These representations are in direct connection with the coping mechanisms used by a person, further impacting treatment adherence and the outcomes obtained, such as the quality of life (8, 10).

The quality of life has been provided with a variety of definitions over time, but its central point supports the multidimensional character of the concept and the optimal functioning of a person in three main fields: physical, psychological, and social (11).

Scientific literature supports the hypothesis of significant correlations between illness representations and the quality of life (12-15), between illness representations and treatment adherence (4, 7, 10, 13, 16), and treatment adherence and the quality of life (17-22). However, among children and adolescents, the chronic disease representations are still not well understood, while their connections with treatment adherence and the quality of life are not sufficiently known (4, 6, 23). Consequently, the purpose of this study is to point out - in a non-categorical framework - the relationship between illness representations, treatment adherence, and the quality of life, as well as the gender differences in what regards the three listed variables.

MATERIAL AND METHODS

Participants

This study has included 94 children diagnosed with a chronic disease: 52 boys and 42 girls. Among the chronic diagnoses of children, the most common are kidney chronic disease, diabetes, epilepsy, scoliosis, chronic tonsilitis, and asthma. Children are aged between 8 and 18, with a mean of $M = 13.63 \pm 2.68$. For the participation in this study, the consent of the child's legal tutor was obtained beforehand.

Instruments

Illness Perceptions Questionnaire - Revised (24) contains 38 items, for which the subjects are asked - on a Likert scale - the assess the degree of agreement (from strongly disagree, to strongly agree). The items are grouped into five dimensions: timeline (two components: acute/chronic and cyclical), control (two components: personal control and treatment control), consequences, illness coherence, and emotional representation, as well as 7 subscales. The α Cronbach coefficient obtained, for all dimensions, values between 0.60 and 0.96.

The Self-Regulation of Medication Adherence Battery (25) comprises 10 items referring to the motivation to be medication adherent (3 items), perceived control of medication adherence (4 items), and support from the caregiver (3 items). The α Cronbach coefficient for the 10 items has the value 0.75.

Pediatric Quality of Life Inventory™ Version 4.0 (26) includes 23 items as-

sessing physical functioning, emotional functioning, social functioning, and school functioning of children and of adolescents. The four scales make up two dimensions: physical health and psychosocial health. Internal consistency for the entire instrument is Cronbach's $\alpha = 0.92$.

Statistical analyses

The distribution of variables has been assessed through the Kolmogorov-Smirnov test. The not normally distributed variables ($p > 0.05$) are as follows: illness identity, illness-related symptoms, personal control, treatment control, illness coherence, and support from the caregiver. We have tried to normalize these variables using logarithms; this worked only for the variable of personal control. In order to identify the relationship between the variables, we have determined Pearson's and Spearman's correlations. The differences determined by gender were identified by applying the t tests for independent samples (normally distributed variables) and the Mann-Whitney test (not normally distributed variables). Furthermore, rank regressions were performed.

RESULTS

Upon analyzing the correlations between the dimensions of illness representation and the quality of life (tab. I), we have identified the following significant relations: the dimension of illness causes correlates significantly and negatively with emotional functioning ($r = -0.359$, $p < 0.001$), social functioning ($r = -0.297$, $p = 0.004$), school functioning ($r = -0.270$, $p = 0.011$), psychosocial functioning ($r = -0.316$, $p = 0.003$) and total score of the quality of life ($r = -0.252$, $p = 0.019$).

TABLE I

Correlations between illness representation and the quality of life

Variables	Causes	Acute/chronic timeline	Consequences	Cyclical timeline	Emotional representation	Personal control
Physical functioning	-.147	-.030	-.169	-.218*	-.225*	-.200
	.165	.774	.110	.038	.032	.057
Emotional functioning	-.359**	.158	-.082	-.196	-.270**	-.270*
	.000	.132	.442	.064	.010	.010
Social functioning	-.297**	.107	-.375**	-.109	-.351**	-.050
	.004	.304	.000	.300	.001	.633
School functioning	-.270*	.122	-.089	-.152	-.136	-.167
	.011	.253	.406	.153	.200	.115
Psychosocial functioning	-.316**	.147	-.218*	-.189	-.277**	-.223*
	.003	.171	.041	.078	.009	.037
Quality of life total score	-.252*	.085	-.224*	-.230*	-.276**	-.258*
	.019	.432	.037	.032	.010	.016

**. significant at the 0.01 level (2-tailed).

*. significant at the 0.05 level (2-tailed).

The dimension of consequences correlates with social functioning ($r = -0.375$, $p < 0.001$), psychosocial functioning ($r = -0.218$, $p = 0.041$) and total score of the quality of life ($r = -0.224$, $p = 0.037$); cyclical timeline correlates significantly with

physical functioning (r = -0.218, r = 0.038) and total score of the quality of life (r = -0.230, p = 0.032); emotional representation obtained significant correlations with physical functioning (r = -0.225, p = 0.032), emotional functioning (r = -0.270, p = 0.010), social functioning (r = -0.351, p = 0.001), psychosocial functioning (r = -0.277, p = 0.009) and total score of the quality of life (r = -0.276. p = 0.010); the dimension of personal control also recorded three significant correlations with emotional functioning (r = -0.270, p = 0.010), psychosocial functioning (r = -0.223, p = 0.037), and total score of the quality of life (r = -0.258, p = 0.016). Despite our expectations, all these correlations are negative. Significant results, obtained for the Spearman correlations, were obtained between illness identity and social functioning (ρ = 0.305, p = 0.004), and psychosocial functioning (ρ = 0.260, p = 0.017); the variable of illness-related symptoms correlated significantly with emotional functioning (ρ = 0.342, p = 0.001), school functioning (ρ = 0.337, p = 0.001), and psychosocial functioning (ρ = 0.266; p = 0.013); while illness coherence correlates with social functioning (ρ = 0.255, p = 0.013). All the correlations are positive.

Upon analyzing table II, it is worth noting that the dimension of adherence motivation did not record statistically significant correlations with any normally distributed dimension of illness representation. The dimension of adherence control correlates significantly with acute/chronic timeline (r = -0.425, p < 0.001), consequences (r = -0.214, p = 0.040), and personal control (r = 0.282, p = 0.007). The total adherence score correlated significantly and negatively with acute/ chronic timeline (r = -0.263, p - 0.011). The application of the Spearman correlations have highlighted the following: the dimension of support from the caregiver correlates significantly with consequences (ρ = 0.323, p = 0.002), illness identity correlates with support from the caregiver (ρ = -0.384, p < 0.001), adherence control (ρ = -0.203, p = 0.055), and total adherence score (ρ = -0.463, p < 0.001). Illness-related symptoms correlates significantly and negatively with adherence motivation (ρ = -0.302, p = 0.004), support from the caregiver (ρ = -0.308, p = 0.003), adherence control (ρ = -0.240, p = 0.022), and total adherence score (ρ = -0.448, p < 0.001). The dimension of treatment control correlated with adherence motivation (ρ = 0.263, p = 0.010), support from the caregiver (ρ = 0.261, p = 0.012) and total adherence score (ρ = 0.235, p = 0.024). The variable of illness coherence did not record any significant correlation.

Furthermore, we have analyzed the connections between treatment adherence and the quality of life, while the significant outcomes were illustrated in third table.

Upon analyzing the significant correlations identified between the dimensions of treatment adherence and of the quality of life, it is worth noting that they are negative.

In order to identify potential differences by gender, in what concerns the dimensions of illness representations, of the quality of life, and of treatment adherence, the t tests for independent samples were used. Table IV features only the significant outcomes (p < 0.05).

Two rank regressions were performed, in order to identify the significant predictors of the quality of life and of treatment adherence. For both criteria, there were the same models, as follows: model 1 comprising illness causes, acute / chronic timeline, consequences, cyclical timeline, emotional representation, and personal control; for model 2, the variable of age was added. In

case of the adherence criterion, both models were statistically insignificant (Model 1: F = 1.876, p = 0.095; Model 2: F = 1.750, p = 0.109). For the quality-of-life criterion, both models were significant (Model 1: F = 3.861, p = 0.002; Model 2: F = 3.673, p = 0.002). Model 2 explains the highest variance percentage of the criterion, namely, 17.9%, whereas model 1 explains 16.6% of the quality-of-life variance.

TABLE II

Correlations between illness representation and the adherence

Variables	Adherence motivation	Adherence control	Adherence total score
Causes	-.035	.158	.098
	.739	.133	.360
Acute/chronic timeline	-.053	-.425**	-.263*
	.615	.000	.011
Consequences	.109	-.214*	.052
	.302	.040	.628
Cyclical timeline	-.043	-.142	-.096
	.686	.175	.369
Emotional representation	.068	.060	.053
	.517	.571	.620
Personal control	.013	.282**	.192
	.900	.007	.066

**. significant at the 0.01 level (2-tailed).

*. significant at the 0.05 level (2-tailed).

TABLE III

Correlations between treatment adherence and the quality of life

Variables	Adherence motivation	Adherence total score	Support from the caregiver	Adherence control
Quality of life total score	r = -0.440** p = 0.000	r = -0.438** p = 0.000	ρ = -0.335* p = 0.002	r = -0.246* r = 0.022
Emotional functioning	r = -0.293* p = 0.005	r = -0.384** p = 0.000	ρ = -0.197 p = .062	r = -0.393** p = 0.000
Psychosocial functioning	r = -0.367** p = 000	r = -0.432** p = 0.000	ρ = -0.316* p = 0.003	r = -0.310* p = 0.003
School functioning	r = -0.417** p = 0.000	r = -0.461** p = 0.000	ρ = -0.430** p = 0.000	r = -0.259* p = 0.014
Physical functioning	r = -0.438** p = 0.000	r = -0.320* p = 0.002	ρ = -0.228* p = 0.030	
Social functioning	r = -0.269* p = 0.009	r = -0.261* p = 0.012	ρ = -0.206* p = 0.049	

**. significant at the 0.01 level (2-tailed).

*. significant at the 0.05 level (2-tailed).

TABLE IV
Independent tests t for IV gender and DV adherence and quality of life

Variables	M	SD	t	df	p
Adherence control			-2.142	85.57	0.035
Male gender	3.49	0.87			
Female	3.80	0.52			
Emotional functioning			2.463	90	0.016
Male gender	73.40	21.31			
Female	62.14	22.44			

DISCUSSION

Most connections identified between the dimensions of variables illness representation and the quality of life are negative. The more underlying causes identified for illness, the greater number of consequences, cyclical timeline and stronger emotional representation, the higher impairment of the quality of life. It is surprising that even the connection of personal illness control correlates negatively with the children's quality of life. The need to exercise high illness control may be associated with worsened symptoms and, implicitly, with a decrease in the quality of life. However, based on the positive correlations, the clearer illness identity among children (6), the more they manage to identify the symptoms related to their illness and they record higher illness coherence and the better they function at certain levels.

On the other hand, the higher perceived personal control of the disease among children, the higher control over treatment adherence (25). An important particularity of chronic children is that they need support to follow the treatment. In this study, as the illness consequence increase, children feel that they benefit from their caregiver's support for treatment adherence. Furthermore, as treatment control improves, children are more motivated to adhere to treatment. This outcome has serious implications in adolescence because their responsibility in treatment management also increases. Consequently, interventions in practice could focus on instilling children's perceived control over their treatment. Furthermore, age is an important variable because it also improves the explanatory efficiency of the regression model, in what regards the quality of life.

In accordance with the findings reported by Barakat (27), all the connections obtained between the dimensions of the quality of life and treatment adherence variables are negative. The explanation provided by the authors is that better treatment adherence may interfere with certain activities contributing to the children's quality of life (27). Finally, we add that treatment adherence may be associated with secondary symptoms, which may explain the decrease in the quality of life; in this respect, a caregiver's support may be perceived as excessive insistence. The quality of life may be affected by the presence of illness or by its treatment; thus, it becomes necessary for it to be integrated in the assessment performed by physicians in order to determine the prognosis of a medical condition (12).

By gender, differences were identified in case of adherence control and emotional functioning variables. More precisely, girls have higher control over treatment adherence, but lower emotional functioning compared to boys. In what concerns illness perception, no significant differences have

been found (28).

An important limit is related to the non-representative sample and to the instrument assessing treatment adherence, for which we have not identified statistical data.

Although surprising, the negative correlation between treatment adherence and the quality of life has not been identified now for the first time in scientific literature. Consequently, studies clarifying the potential mechanisms and variables within this correlation are recommended. Furthermore, the coping mechanisms used by children should be studied in relation to illness representation, the quality of life, and treatment adherence.

CONCLUSIONS

Considering the connections identified between the variables featured in this study, our recommendation is to identify in practice the children's subjective illness perceptions. Furthermore, both the medical professionals and the children's caregivers should focus on instilling children's perceived control over their treatment, mostly during adolescence, when their responsibility in treatment management increases. Psychological support should focus on emotional management of the disease, especially among girls who appear to be more strongly impacted. Also, both the children's caregivers and the medical team should pay attention to the quality of life of children, even when they are adherents to treatment, and to integrate into the lives of chronically ill children activities that increase their well-being.

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CONE BEAM COMPUTED TOMOGRAPHY (CBCT) ASSESSMENT OF INCISOR ROOT RESORPTION (IRR) ASSOCIATED WITH IMPACTED MAXILLARY CANINES (IMC). LITERATURE REVIEW

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CONE BEAM COMPUTED TOMOGRAPHY (CBCT) ASSESSMENT OF INCISOR ROOT RESORPTION (IRR) ASSOCIATED WITH IMPACTED MAXILLARY CANINES (IMC). LITERATURE REVIEW (Abstract): CBCT assessment of impacted canine improves canine localization to adjacent teeth and provides higher level of confidence in the treatment plan compared to 2D images. However, there is conflicting evidence to support using CBCT as a routine first-line imaging method in patients with impacted maxillary canine and incisor root resorption. Consequently, there are doubts regarding difference in diagnostic and treatment planning between the use of conventional 2D and CBCT sets. Therefore, the present review will attempt to summarize the new aspects regarding the benefits of three-dimensional (3D) diagnostic techniques (cone-beam computed tomography, CBCT), including the accuracy of localization of impacted canine and the detection of the degree and location of root resorption of maxillary incisors. **Keywords**: IMPACTED CANINE, INCISOR ROOT RESORPTION, CONE BEAM COMPUTED TOMOGRAPHY.

Permanent maxillary canines are the second most frequently impacted teeth after the third molars. At the same time, maxillary canines are important esthetically and functionally. An impacted canine is usually diagnosed on routine examination, and it can cause additional problems during the development and eruption of the impacted tooth and the neighboring teeth (1).

Root resorption of the maxillary incisors caused by impacted canines is a relatively common phenomenon and is defined as a condition associated with either a physiological or pathological activity of the tooth resorbing cells, which results in loss of cementum and dentine. Root resorption is very difficult to treat and usually requires extraction of the affected tooth (2).

CBCT (cone-beam computed tomography) provides an alternative to traditional CT (computed tomography) systems using a significantly less radiation dose and shorter acquisition scan times, and it provides valuable information regarding impacted canines with submillimeter spatial resolution images of high diagnostic quality to better understand and improve treatment at lower cost (3-6).

The present paper attempts to review in the recent literature (last 15 years) the

three-dimensional assessment of impacted canines and incisor root resorption using cone beam computed tomography.

MATERIAL AND METHODS

We have searched the MEDLINE database through *PubMed* by using following key words in English "impacted canine" [All Fields], "incisor root resorption" [All Fields], "cone beam computed tomography" [All Fields].

The selection criteria and filters were the year of publication 2005 to 2020, longitudinal, transversal and cohort studies, systematic reviews, and meta-analysis. Excluding criteria: case reports, irrelevant articles, laboratory studies, articles in languages other than English, abstracts. The Pub Med search identified 48 articles, but only 25 met our inclusion criteria (fig. 1).

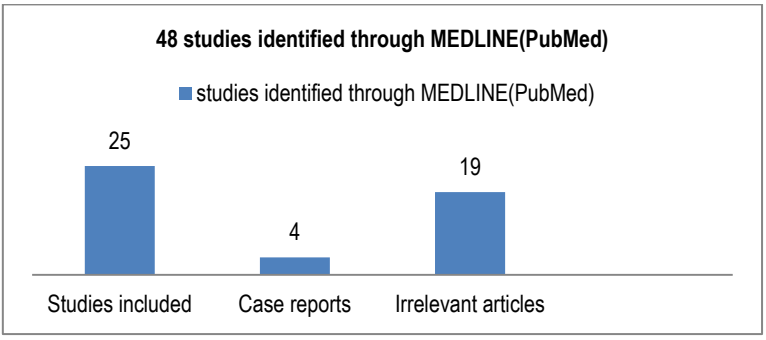

Fig. 1. Studies identified on PubMed

DISCUSSION

In a 2018 study (7) which included 608 CBCT reconstructions the pathology that was most commonly caused by impacted teeth was periodontal bone loss (44.4%), followed by root resorptions (33.3%), cysts or tumors (8.6%), and tooth decays (2.3%).

3D volumetric imaging of impacted canines can show the following: presence or absence of the canine, size of the follicle, inclination of the long axis of the tooth, relative buccal and palatal positions, amount of bone covering the tooth, 3D proximity and root resorption of adjacent teeth, condition of adjacent teeth, local anatomic considerations, and overall stage of dental development (6).

a. CBCT assessment of impacted canines (tab I.)

In the first CT study of 113 impacted canines, Bjerklin and Ericson (8) reported more buccally placed canines, with respective numbers of 42% (palatal), 40% (buccal), and 18% (in line with the arch).

Liu *et al*. (9) found in 210 patients utilizing CBCT that impacted canines were located palatally in 41%, labially in 45%, and midalveolus in 14% of cases. The location of impacted maxillary canines varied greatly in 3 planes and showed incisor root resorption in 27% of lateral incisors and 23% of central incisors.

A CBCT study by Walker *et al*. (6) found that 25 of 27 impactions (n=19 patients) were located palatially, and two were located labially. He found lateral incisor root resorption and central incisor root resorption in 18 and 3 of those cases, respectively.

A Polish study (10) suggested that CT scan allows the assessment of the degree

and extent of resorption of lateral incisor roots adjacent to impacted maxillary canines. Of a total of 54 impacted permanent maxillary canines, 41 (75.9%) were positioned palatally, 10 (18.5%) buccally, and 3 (5.6%) horizontally. There were 31 cases (64.6%) of lateral incisor root resorption, of which in 18 (58.1%) resorptions was deep, and in 13 (41.9%) it was slight. Age of patients is a significant factor which has an effect on occurrence of damage to incisor roots. Also, the position of impacted canines has an effect on the process of resorption of incisor roots.

TABLE I
Studies reporting 3D assessment of impacted canines

Authors	Year		Number of canines	Location of impacted canines					
				Palatal		Buccal		Middle	
				N	%	N	%	N	%
Walker *et al.*	2005		27	25	93	2	7		
Bjerklin and Ericson	2006		113	47	42	45	40	2	18
Liu *et al.*	2008		210	86	41	95	45	29	14
Siegel R *et al.*	2010		54	41	75.9	10	18.5	3	5.6
Yan B *et al.*	2012		170	101	59.4	69	40.6		
Kim Y *et al.*	2012		186	46	24.8	140	75.2		
Lai Cs *et al.*	2013		134	69	51.49	41	30.6	24	17.91
Almuhtaseb E *et al.*	2014		46	17	37.6	19	40.2	10	22.2
da Silva Santos LM *et al.*	2014		79	53	67.1	17	21.5	9	11.4
Dağsuyu *et al*	2015		140	76	54.3	25	17.9	39	27.8
CTT Ngo *et al.*	2018		86	58	67	24	28	4	5
Kalavritinos *et al.*	2020	Right	42	20	46.6	7	16.6	15	37.7
		Left	49	27	55.10	5	10.2	17	34.6

Another study (11) comprises 113 patients with CBCT scans, and 134 impacted canines were analyzed retrospectively. In the patients evaluated, 69 impacted canines were located palatally (51.49%), 41 labially (30.60 %), and 24 (17.91 %) in the middle of the alveolar process. Root resorptions were found in 34 lateral incisors (25.37 %), 7 central incisors (5.22 %), 6 first premolars (4.48 %), and one second premolar (0.75 %).

In a Chinese study (12), pretreatment CBCT scans of 170 Chinese subjects (ages, 12-30 years; mean, 14.5 years) with impacted maxillary canines (101 buccal and 69 palatal impactions) were used.

Of 42 CBCT reconstructions (13) of impacted canines undergoing orthodontic treatment, 17 (40.4%) had no root resorption, 15 (35.7%) showed slight root resorption, 6 (14.2%) moderate resorption, and 4 (4%) severe root resorption of the adjacent lateral incisor. In only one individual both central and lateral incisors were resorbed. The most frequent location of impacted canines was palatal, mesial, and gingival.

In a Korean study (14), the maxillary canine impaction occurred more frequently on the buccal side (140 canines) than the palatal side (46 palatally canines) and more frequently in females than in males by a 1.5-fold margin. Tooth resorption in the CT images showed a large percentage value of 49.5 %. The impacted angulation evident in the panoramic radiographic image did not have any statistically significant relations

with either the impacted position or the root resorption.

Forty-six patients with impacted teeth undergoing orthodontic treatments in Tongji Hospital (Wuhan, China) were examined (15). Most of the upper impacted canines (40.2%) were located buccally, 37.6% were located palatally, while 22.2% were located in midalveolus. Adjacent tooth root resorption of 28.26 % was in the upper lateral incisors while 17.39% in upper central incisors, but no lower root resorption was found in the samples.

In a retrospective radiographic (CBCT and panoramic) study (16) of 64 patients with 86 MICs, most of the positions of MICs were palatal (67%), followed by labial (28%) and midalveolus (5%). The association between sectors of the MICs on panoramic images and the labio-palatal position of the MICs on CBCT was statistically significant. Root resorption of adjacent incisors showed a significant difference according to sector location. Labio-palatal position of MICs and resorption of permanent incisors might be predicted using sector location on panoramic radiographs .

A retrospective study (17) examined the CBCT scans of 140 MICs in 102 patients (43 males, 59 females). Maxillary canine impaction was more frequently seen in female subjects than in male subjects. Lateral incisors were more frequently affected than first premolars, and slight resorption was more frequently seen in adjacent teeth. CBCT assessment of MICs can provide accurate measurements of angular, linear, and resorption parameters .

b. 3D position of maxillary canine impactions and the influence on adjacent incisor root resorption (tab II)

The use of CBCT in patients with impacted canines has diagnostic potential and can be a genuine method used to rectify, diagnose, and suggest a treatment for incisor root resorption (18).

TABLE II

CBCT studies reporting incidence of root resorption caused by MIC

Authors	Year		Percentage % of teeth reporting root resorption			
			Lateral Incisor	Central incisor	First premolar	Second premolar
Walker *et al.*	2005		67	11		
Liu *et al.*	2008		27	23		
Siegel R *et al.*	2010		64.6			
Yan B *et al.*	2012		27	18	10	
Kim Y *et al.*	2012		49.5 (32.8 Buccal. 17.7 Palatal)			
Oberoi *et al.*	2012		59.6	2.3		
Lai Cs *et al.*	2013		25.37	5.22	4.48	0.75
Almuhtaseb E *et al.*	2014		28.26	17.39		
da Silva Santos LM *et al.*	2014		60.7	24.7	5	
Dağsuyu *et al.*	2017		55			
Kalavritinos *et al.*	2020	Right side	13.3			
		Left side	6.4			
Alemam AA *et al.*	2020	Palatal displayed canines	74	18		
		No displayed canines	25.5			

In a recent study (19), CBCT provided improved detection rates (63%) of root resorption associated with impacted canines. There was no difference in resorption rates between buccally and palatally impacted canines. The resorption scale developed by Jawad Z *et al*. (Three-Dimensional Leeds Orthodontic Root Resorption Target Scale) (29) was intended to address the subjective nature of reporting the CBCT images taken to assess root resorption associated with impacted teeth.

In one study (20) the angulation of the canine was steeper on the labial side than on the palatal side, but root resorption of adjacent laterals was not different. There were no statistically significant differences in the amount of root resorption of the laterals when the canine was evaluated according to localization and angulation .

In contrast, another study (21) concluded that the risk of resorption of the lateral incisor is not linked to the buccal or palatal situation of the canine. The risk is greater when the canine is vertically above the lateral incisor root and close to the median palatine suture, suggesting a mechanical blockage by the apex of the lateral incisor.

Sixty-one patients presenting with unilateral or bilateral impacted maxillary canines were evaluated in a university clinic in Athens, Greece using CBCT reconstructions (22). They concluded that there is no relationship between lateral or central incisor root resorption and the shape and width of the impacted canine follicle. Lateral incisor root resorption due to canine impaction occurred in 18.5% of the examined cases. The percentage of impacted canines causing root resorption to both central and lateral incisors on the ipsilateral side is very low. Younger patients appear to have the left canine located more often in the middle of maxillary bone, whereas older patients have left canines located more often in the palatal or buccal side of the bone. There is a tendency for younger ages to demonstrate resorption in the apical or middle third of the tooth, whereas the tendency for older patients is to demonstrate resorption at the incisal third. There is a positive correlation between the appearance of resorption on the lateral incisor and the angle between the axis of canine and adjacent lateral incisor.

In a Chinese study (12) root resorption was significantly more prevalent in the canine-impaction subjects and the side of impaction, with overall prevalence rates of 27%, 18%, and 10% at the maxillary lateral incisor, the central incisor, and the first premolar, respectively.

CBCT images for 107 palatally displaced canines (PDCs) and 51 fully erupted nondisplaced canines (NDC) were analyzed for the presence of incisor root resorption (IRR) (23). Root resorption affected 74% of lateral incisors adjacent to PDCs and 25.5% of lateral incisors adjacent to NDCs. The most common location of the root resorption is lingual surface, which can be explained by the palatal position of displaced canines. The second most common location was the distal surface. Canines in contact with adjacent incisor roots was the only risk factor detected for PDC-IRR. Very good predictive discrimination (83%) emerged for PDC-IRR subjects. Predictors of PDC-IRR were canine contact with adjacent incisor, size of canine dental follicle, and presence of peg-shaped lateral incisor.

c. CBCT assessment of incisor root resorption location (tab III)

CBCT provides accurate information about the location of impacted canine and

prevalence and degree of root resorption of neighboring teeth with high interrater correlation. This information is of great importance for surgeons and orthodontists for accurate diagnosis and interdisciplinary treatment planning (11, 24).

TABLE III

Studies reporting incisor root resorption location on CBCT

Authors	Year	Incisor root resorption location		
Ericson and Kurol	2000	1/3 middle root	Labial surface	Lingual surface
Yan B *et al.*	2012	1/3 apical root	1/3 middle root	
Doğramaci EJ *et al*	2015	The apical third and palatal surface		
Schroder *et al.*	2018	All root levels and surfaces of teeth associated especially 1/3 apical root		
Alemam AA, *et al.*	2020	1/3 middle root	Lingual surface	
Kalavritinos *et al.*	2020	Young patients -1/3 apical or 1/3 middle of the root		
		Older patients -1/ 3 incisal root		

Using CT, Ericson and Kurol (25) reported that maxillary incisor resorption occurred most commonly in the middle third of the roots, on the labial or lingual surfaces, with 60% of the resorbed lateral incisors and 43% of the resorbed central incisors that have pulpal involvement.

Root resorption in maxillary incisors was correlated with their contact with maxillary canines during eruption. Delayed eruption or treatment of impacted canines may lead to resorption of the adjacent lateral and central incisors. All root levels and surfaces of teeth associated with impacted maxillary canines can be resorbed to different levels of severity, but the apical one-third with slight resorption is the most common (26).

Data from a 2015 study (27) showed that a total of 110 impacted maxillary canine teeth resorbed 120 adjacent teeth, including 14 premolars and one permanent molar. The apical third and palatal surface were commonly involved. Fifty per cent of the resorptive lesions were mild, 20% moderate and 30% severe. Neither age nor gender influences the number, location or severity of the resorption .

Predominantly affecting the apical third of all teeth, impacted canine-associated root resorption, if present, reached the pulp of the maxillary lateral incisor, the central incisor, and the first premolar at rates of 36%, 57%, and 0%, respectively. Maxillary canine impaction increases the risk of root resorption at adjacent teeth (incisors and first premolars). Physical proximity (≤1 mm) between the impacted canine and an adjacent root is the most important predictor for root resorption, and this characteristic is largely similar in Chinese patients to that in white people (12).

A Brazilian study (28) concluded that root resorption of adjacent teeth is present in most cases of canine impaction, mostly affecting the adjacent lateral incisors to a mild degree. Another study (29) associates severe incisor root resorption with female sex, enlarged dental follicles, a greater degree of canine displacement, and normal lateral incisors.

Regarding the size of canine dental follicle, no significant correlation was found between follicle width and the variables gender, impaction side, and MIC location. It could not be confirmed that increased dental follicle width of the MICs exhibited more resorption risk for the adjacent lateral

incisors (30). A Brazilian study (28) concluded that a wide dental follicle of impacted canines was not associated with a higher incidence of external root resorption of adjacent teeth . The same idea was stated in a study hypothesizing that the dental follicle of impacted maxillary canines does not cause resorption of adjacent maxillary incisors (31).

CONCLUSIONS

From this review we can conclude that impaction of maxillary canines occurs twice as often in females than in males. The palatal displacement of impacted maxillary canines is more common than labial displacement; however, it seems to be more buccal impactions of canines in the Asian population.

The most common degree of root resorption is severe on all root levels and surfaces, especially apical 1/3. Pulpal involvement includes both central and lateral upper incisors. Increased dental follicle width of the maxillary impacted canines do not cause a higher risk of lateral incisor root resorption.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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CHITOSAN-GELATINMICRO/NANOPARTICLES AS A CONTROLLED DELIVERY OF DEXKETOPROFEN TROMETAMOL FOR TOPICAL APPLICATIONS IN WOUND CARE

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CHITOSAN-GELATIN MICRO/NANOPARTICLES AS A CONTROLLED DELIVERYOF DEX-KETOPROFEN TROMETAMOLFOR TOPICAL APPLICATIONS IN WOUND CARE (Abstract): Progress in the nanotechnology field has led to the discovery of various drug delivery systems for biomedical applications in the last few decades. **Materials and methods:** Micro/nanoparticles (MPs) based on chitosan (CS) and gelatin (GEL), designed as a drug delivery system for biomedical applications, were prepared using a double cross-linking process within an emulsion-phase separation system. **Results:** The obtained MPs were characterized using scanning electron microscopy (SEM), which revealed diameters ranging from 0.467 to 4.596 µm. The swelling properties of the MPs were investigated in a neutral environment with a pH of 5.5. **Conclusions**: The applicability of the prepared materials was further evaluated for drug loading and *in vitro* delivery of Dexketoprofen trometamol (DEX), demonstrating excellent capacity, which recommends them for effective wound care, including postoperative wounds. **Keywords:** CHITOSAN, GELATIN, MICROPARTICLES, NANOPARTICLES, DOUBLE CROSSLINKING, REVERSE EMULSION, DEXKETOPROFEN TROMETAMOL, DRUG DELIVERY.

INTRODUCTION

Nanotechnology is utilized to design various particulate systems that serve as effective drug delivery supports for a range of biomedical applications due to their unique properties. Natural polymers, especially polysaccharides, are particularly advantageous for biomedical use because of their chemical structure, which impart essential qualities such as biocompatibility, non-toxicity, biodegradability, abundance, relatively low cost, and renewable nature (1, 2).

Among natural polysaccharides, CS is the most frequently used support material for polymeric particles. It is highly regarded for its exceptional properties, including biocompatibility, biodegradability, non-toxicity, mucoadhesion, and the presence of highly reactive amine groups. CS can be chemically modified to improve specific characteristics, such as solubility, mucoadhesion, and stability, thus expanding its potential applications in drug delivery (3).

Similar to CS, GEL is a biodegradable polymer that has attracted growing interest in recent years due to its diverse applications and favorable properties, such as biodegradability, biocompatibility, non-toxicity, and ease of cross linking and chemical modification (4, 5). As a natural and renewable substance, GEL is utilized in various fields, including bioengineering, pharmaceuticals, and the food industry (6, 7). It is derived from the partial hydrolysis of collagen, which is found in animal tissues such as bones, hides, and pigskin (8).

The diversity and effectiveness of CS, respectively gelatin-based particle systems for drug targeting and controlled drug release applications have been well-documented in the literature. Particles based on CS and/or GEL prepared via emulsification and crosslinking method, containing different bioactive molecules have been extensively recorded in the literature(9-13).Thus, GEL and CS particles represent a promising carrier system for controlled drug delivery technology.

The current study involves the preparation of MPs based on CS and GEL through a double crosslinking process using a water-in-oil emulsion. The MPs were characterized in terms of morphology, swelling behavior, and drug loading/release properties, which were controlled by adjusting synthesis parameters such as polymer concentration and the GEL/CS ratio. *Invitro* tests were conducted to demonstrate the MPs' capacity for drug loading and release of DEX. We can state that the prepared MPs can be used as support for post-surgical drug administration. Usually, acute wounds are associated with inflammation and in uncontrolled inflammation, proinflammatory macrophages and inflammatory mediators such as tumor necrosis factor α (TNF-α) and interleukin-1β (IL-1β) become overexpressed. As a result, acute wounds become chronic and difficult to heal, incurring additional costs to healthcare systems and especially decreasing the quality of life of affected patients (14). Therefore, a non-steroidal anti-inflammatory drug such as DEX can effectively contribute to the wound healing process and thus benefit both patients and healthcare system management.

MATERIALS AND METHODS

Materials

The following materials were used: low molecular weight Chitosan (CS, deacetylation degree 75-80%, Sigma Aldrich, Saint Louis, MO, USA), Gelatin (GEL, Sigma Aldrich, Saint Louis, MO, USA), Glutaraldehyde (GA, Sigma Aldrich, Saint Louis, MO, USA) –25% aqueous solution, sodium sulfate (Sigma Aldrich, Saint Louis, MO, USA), Acetone (Sigma Aldrich, Saint Louis, MO, USA), Hexane (Sigma Aldrich, Saint Louis, MO, USA), Dexketoprofen trometamol (DEX, Sigma Aldrich, Saint Louis, MO, USA), Tween 80 (Sigma Aldrich, Saint Louis, MO, USA), Span 80 (Sigma Aldrich, Saint Louis, MO, USA), Milli-Q ultrapure distilled water (DW, Merck).

Methods

GEL-CS Microparticles synthesis

The chosen method for MPs preparation involved a double crosslinking (ionic and covalent) technique using a water-in-oil emulsion (10, 11, 12). First, a CS solution was prepared by dissolving the polymer in

a 2% (w/w) aqueous acetic acid solution. Second, GEL was dissolved in double distilled water at 40°C. According to specific polymer ratios, the CS and GEL solutions were mixed, and Tween 80 was added to the solution (1%, w/w) and stirred vigorously for 10 minutes. Next, separately, the organic phase was prepared by mixing a 1% (w/w) Span 80 solution in toluene. The MPs were obtained by gradually adding the polymer solution to the organic phase (water/oil ratio 1/4) while homogenizing with an UltraTurrax at 20.000 rpm for 10 minutes, leading to emulsion formation. Subsequently, freshly prepared crosslinker aqueous solutions, namely sodium sulfate (10%), were added at a rate of 1 mL/min with continuous stirring for another 10 minutes. Afterward, the emulsion was transferred to a round-bottom flask and mechanically stirred at 1000 rpm. Subsequently, 10 mL of a saturated GA solution in toluene was added, and the mixture was stirred for another 1 hour. The MPs were separated by centrifugation at 10.000 rpm and repeatedly washed with acetone and water to remove unreacted compounds. After washing with hexane, the obtained MPs were dried at room temperature.

Techniques used for characterization

MPs morphology was investigated by SEM technique. SEM micrographs were recorded with a HITACHI SU 1510 (Hitachi SU-1510, Hitachi Company, Japan) Scanning Electron Microscope, MPs were fixed on an Aluminum stub and coated with a 7 nm thick gold layer using a Cressington 108 device before observation.

MPs swelling behavior in aqueous media

MPs swelling characteristics in the aqueous environment were evaluated by the gravimetric method using a buffer solution (pH 5.5) (Phosphate Buffer Solution, PBS).

0.030g of MPs were immersed in a 2 mL Eppendorf tube containing 1 mL of PBS. The suspensions were monitored and maintained at room temperature under mechanical stirring (100 rpm) for 24 hours when equilibrium swelling was attained. MPs suspensions were ultra-centrifuged (12.000 rpm), withdrawn from the buffer solutions, and weighed at specified time intervals, respectively immediately replaced with an equivalent volume of fresh buffer. The maximum swelling degree was determined with equation (Eq.) (1):

$$Q\% = \frac{w_s - w_0}{w_0} \times 100 \quad \textbf{(1)}$$

where:

w_s- the weight of swollen CPH;

w_0- the weight of dry CPH.

MPs drug loading capacity

DEX is the tromethamine salt of S-(+)-2-(3-benzoylphenyl) propionic acid, an analgesic, antipyretic, and anti-inflammatory drug that belongs to the class of non-steroidal anti-inflammatory drugs (NSAIDs)(15). DEX was utilized as a model drug for loading into the microparticle (MPs) drug delivery system. The loading process of DEX was conducted via a diffusional mechanism. Specifically, MPs (0.030g) were suspended in 1 mL of an aqueous DEX solution (c = 20 mg/mL, dissolved in PBS pH=5.5) and dispersed by sonication for 10 minutes. These suspensions were maintained at room temperature with gentle mechanical stirring for 72 hours. After, MPs samples were centrifuged (15,000 rpm, 5 min). The amount of DEX encapsulated in the MPs was quantified by determining the drug content in the supernatant, calculated using Eq. (2) derived from a previously prepared calibration curve.

$$y = 3.9395x + 0.0092; \; R^2 = 0.9917 \quad \textbf{(2)}$$

Drug encapsulation efficiency (DEE) represents the quantity of added drug (%) that is encapsulated in the formulation. The DEE of LEV from CPH was calculated with Eq. (3). The DEX entrapment efficiency of MPs was determined with Eq. (4).

$$\mathbf{DEE} = \frac{\text{Actual drug (LEV) content loaded in CPH}}{\text{Theoretical drug content (LEV)}} \times 100 \quad \mathbf{(3)}$$

$$\mathbf{Entrapment\ efficiency} = \frac{\text{The total amount of LEV} - \text{Amount of LEV from supernatant}}{\text{Total amount of LEV}} \quad \mathbf{(4)}$$

All tests were accomplished with a spectrophotometer UV-VIS Nano Drop ND-1000. DEX-loaded MPs were freeze-dried.

In vitro DEX release from MPs

To assess the *invitro* drug release properties, the release of DEX-loaded MPs was conducted in PBS with a pH of 5.5, ensuring a slower release rate of the drug. The DEX release process involved immersing the DEX-loaded MPs in a 2 mL Eppendorf tube containing 1 mL of PBS. The suspensions were maintained at 37°C with continuous gentle stirring at 50 rpm. At predetermined time intervals, the suspensions were ultracentrifuged at 12.000 rpm, and the amount of released DEX was quantified using UV-VIS spectrometry at 258 nm. Each determination was performed in triplicate, and the results were averaged.

RESULTS

The selected method for the MPs preparation was a double crosslinking (ionic and covalent) technique within a water-in-oil emulsion. The method advantage is due to a reduced amount of covalent crosslinker required, which subsequently decreases the final product's toxicity. The fundamental principle of this process involves forming an interconnected/interpenetrated network initially through predominantly ionic crosslinking, followed by a covalent crosslinking step. The experimental parameters used for the preparation of the MPs are detailed in first table.

TABLE I.
MPs Preparation parameters

Sample code	% GEL	% CS	Concentration (%)	Water/Oil ratio
M1	10	90	0.8	1/4
M2	30	70	0.8	1/4
M3	50	50	0.8	1/4
M4	70	30	0.8	1/4
M5	10	90	1.2	1/4
M6	30	70	1.2	1/4
M7	50	50	1.2	1/4
M8	70	30	1.2	1/4

The parameters taken into consideration for the preparation process were total initial concentration and the GEL/CS ratio. The initial water/oil phase ratio, surfactant concentration, and ionic and GA content in the aqueous/organic phase were kept constant

throughout the study.

The morphology of the prepared microparticulate systems was examined using SEM (fig. 1). The results indicate that the MPs exhibit a spherical shape, are well-individualized, generally possess a micron diameter, and demonstrate a dimensional polydispersity. Moreover, two primary particle populations were observed: the first consisted of particles in the micron range, characterized by rough surfaces and an approximately spherical shape, while the second population comprised submicron particles with a distinct spherical shape and smooth surfaces. Also, the images confirm the obtaining of MPs with variable diameters which ranged between 0.550 – 1.36 µm for M1-M4, respectively 0.467 – 4.596 µm for M4-M8. The adapted emulsion double crosslinking procedure effectively produces clear particulate systems with relatively low dimensional dispersity, as evidenced by the SEM micrographs.

To further understand the interaction between the MPs and the aqueous environment, swelling studies were conducted in a neutral medium (pH = 5.5) using a gravimetric method. The water swelling behavior provides insights into the interactions between double-crosslinked MPs CS-GEL and the selected environment, as well as its suitability for the drug loading and release processes. Figure 2 displays the kinetic data for all samples, while table II presents the maximum swelling degree values obtained. The results revealed that the maximum swelling degree for the M1-M4 samples ranged from 706% to 787%, and for the M5-M8 samples, it ranged from 681% to 788%. The swelling kinetics, as shown in Figure 2, indicate that an equilibrium phase is established, demonstrating the good stability of the particulate formulations. Also, was observed that when the ratio between GEL and CS is in favor of GEL the water uptake increased, demonstrating good water-CS interactions, most likely favored by the hydrophilic GEL.

DISCUSSION

Considering the swelling results which demonstrate that MPs presented a high ability to swell, DEX was loaded in neutral conditions ensuring the encapsulation of a higher amount of drug. The ability of MPs to encapsulate drugs was analyzed using DEX as a model drug. The DEX content in the supernatant was analyzed using UV-VIS spectrophotometry (at 258 nm wavelength) based on an established calibration curve. Table III presents the DEX loaded MPs amounts and loading efficiency. MPs were able to entrap DEX between 0.46 – 0.48 mg drug/mg particles. Therefore, the DEX loading efficiencies values calculated with Eq. 4 were between 98.0 – 99.0%, showing a high capacity of the MPs as support for the biologically active compound.

The DEX release capacity of MPs was analyzed by the diffusion method in PBS pH = 5.5 at 37°C. The released DEX for all samples is presented in Table IV and the kinetic data are displayed in Figure 3 a) and b). The results showed a fast release phase which is reached within the initial 6 hours followed by a slower phase (characterized by a slow release) until 72 hours. The sustained release of DEX can be explained by the fact that the released drug was adsorbed in the MPs network due to excellent hydrophilicity of the polymer network which it seems that it is maintained also after the crosslinking procedures. The maximum released amount of DEX varied between 0.146 and 0.301 DEX mg/mg MPs (Table IV, Figure 3a). Also, the release efficiency of DEX for all MPs samples, values between 30 and 65% were obtained (tab. IV, fig. 3b), the highest efficiency being noticed for the sample M4 and M8 which has been shown also a high-swelling degree.

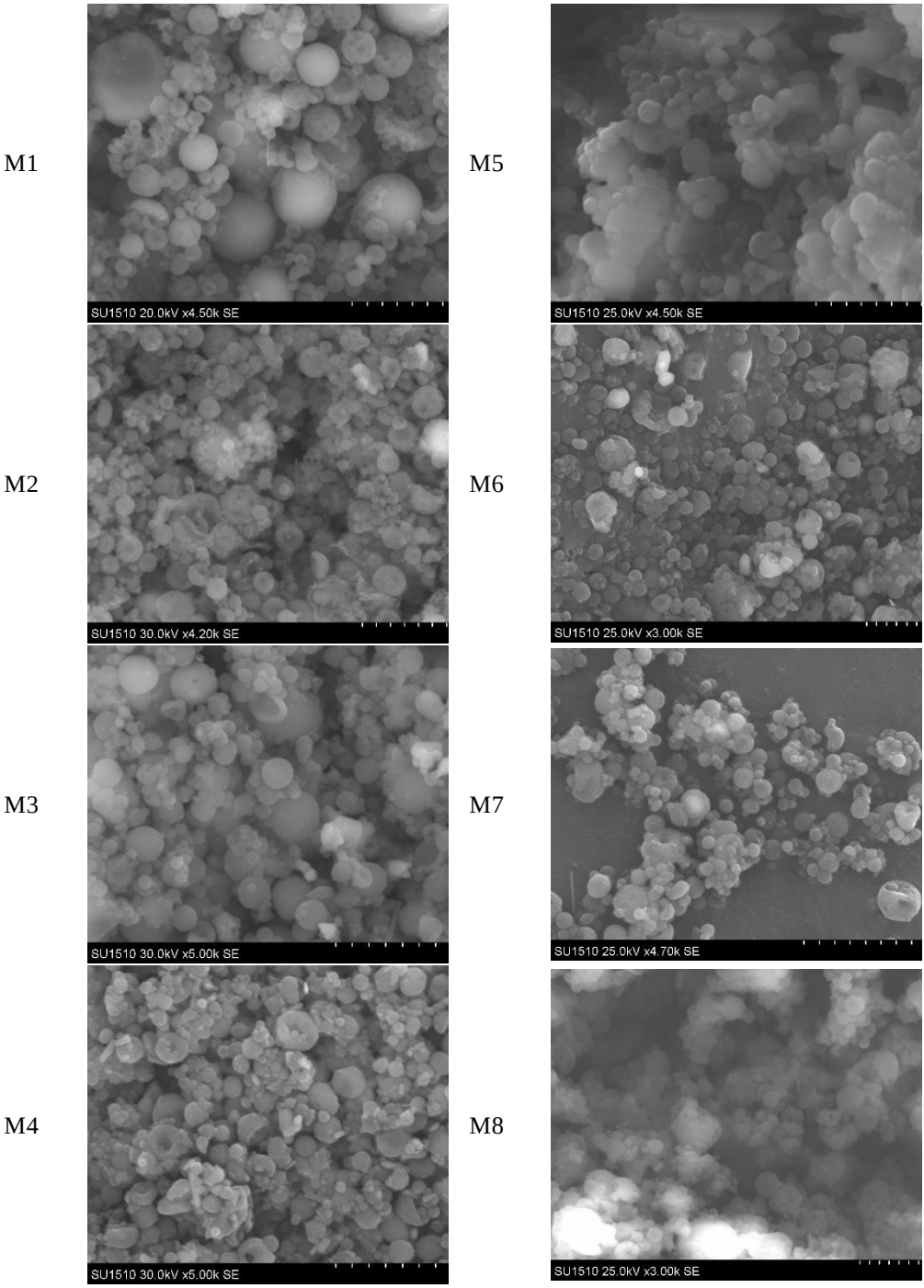


Fig. 1. Micro/Nanoparticles SEM images.

TABLE II.

Microparticle swelling degree in PBS pH=5.5

Sample code	M1	M2	M3	M4	M5	M6	M7	M8
Swelling degree, %	706	750	768	787	681	740	753	788

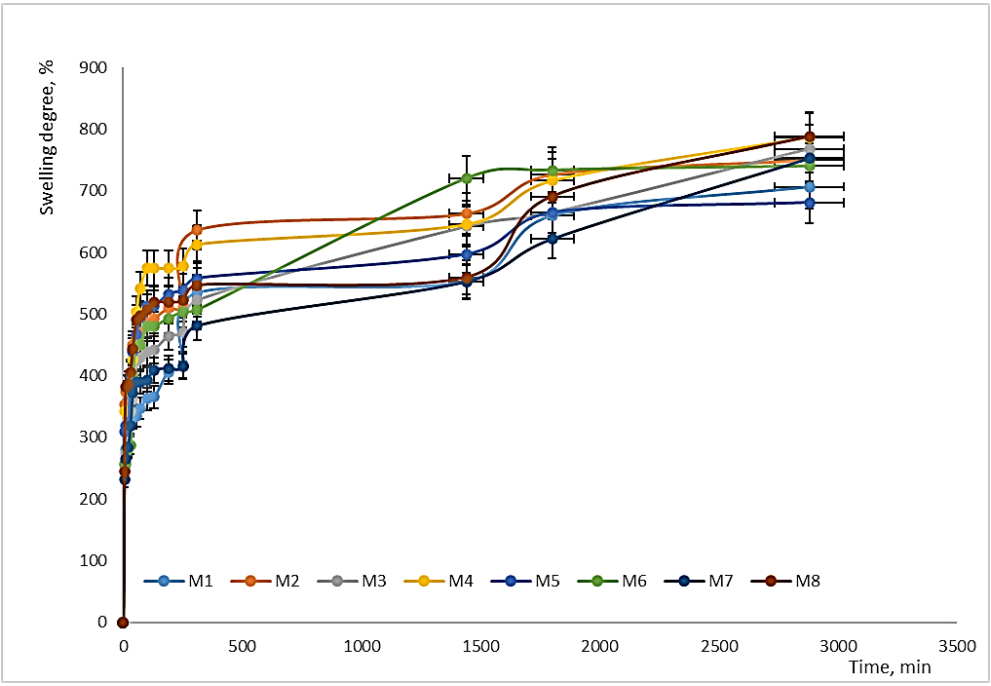


Fig. 2. Microparticles Swelling degree

TABLE III.

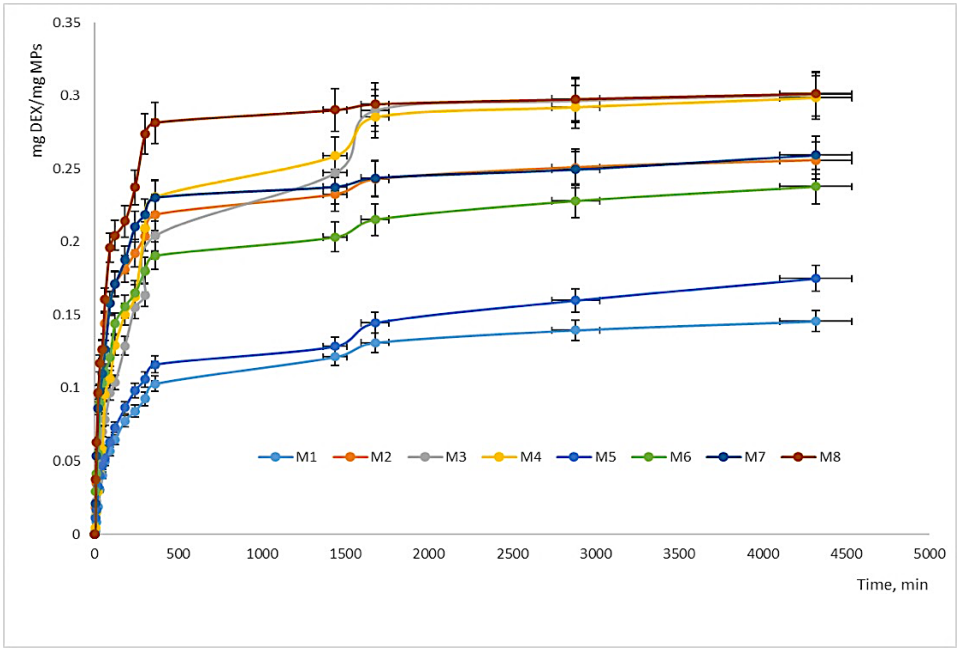
Microparticles Loading DEX

Sample code	M1	M2	M3	M4	M5	M6	M7	M8
DEX mg / mg MPs	0.479	0.477	0.511	0.478	0.48	0.481	0.481	0.464
DEX loading Efficiency, %	98.93	98.67	98.8	98.73	99.27	99.33	99.33	99.07

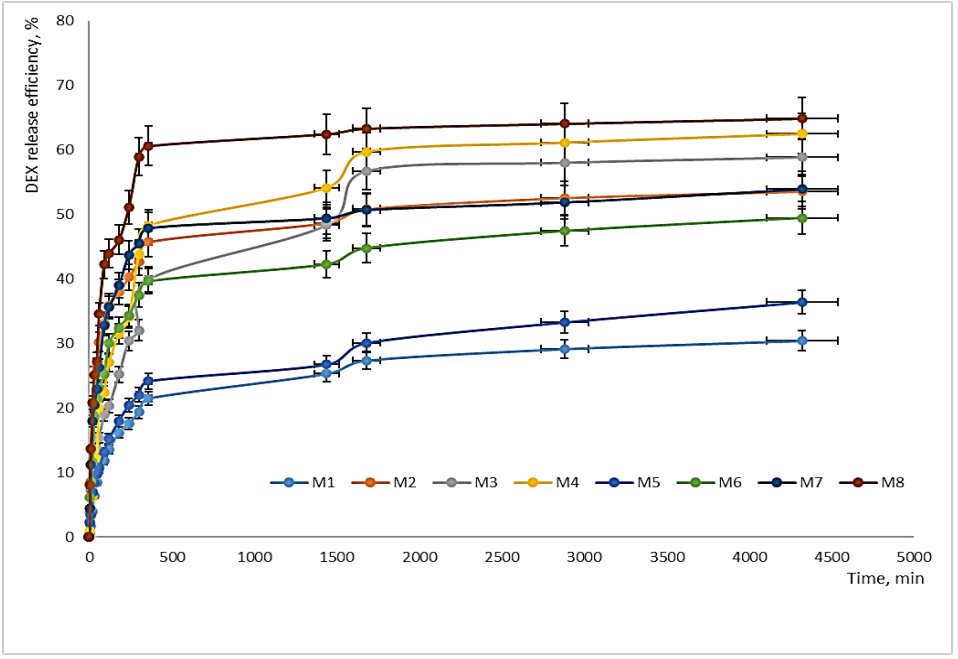
TABLE IV.

Microparticles Releasing DEX

Sample code	M1	M2	M3	M4	M5	M6	M7	M8
DEX mg / mg MPs	0.146	0.256	0.30	0.299	0.175	0.238	0.259	0.301
DEX releasing Efficiency, %	30.42	53.57	58.87	62.50	36.41	49.49	53.95	64.88



a)



b)

Fig. 3. MPs ability to *in vitro* release DEX: a) DEX mg/mg MPs;
b) DEX releasing Efficiency, %

The results of release kinetics were analyzed also based on the Korsmeyer-Peppas mathematical model. From the linear plotting of DEX release data (figs. 4a, 4b), it can be observed that (n) is smaller than 1, indicating that the drug release mechanism follows a Non-Fickian transport (16, 17).

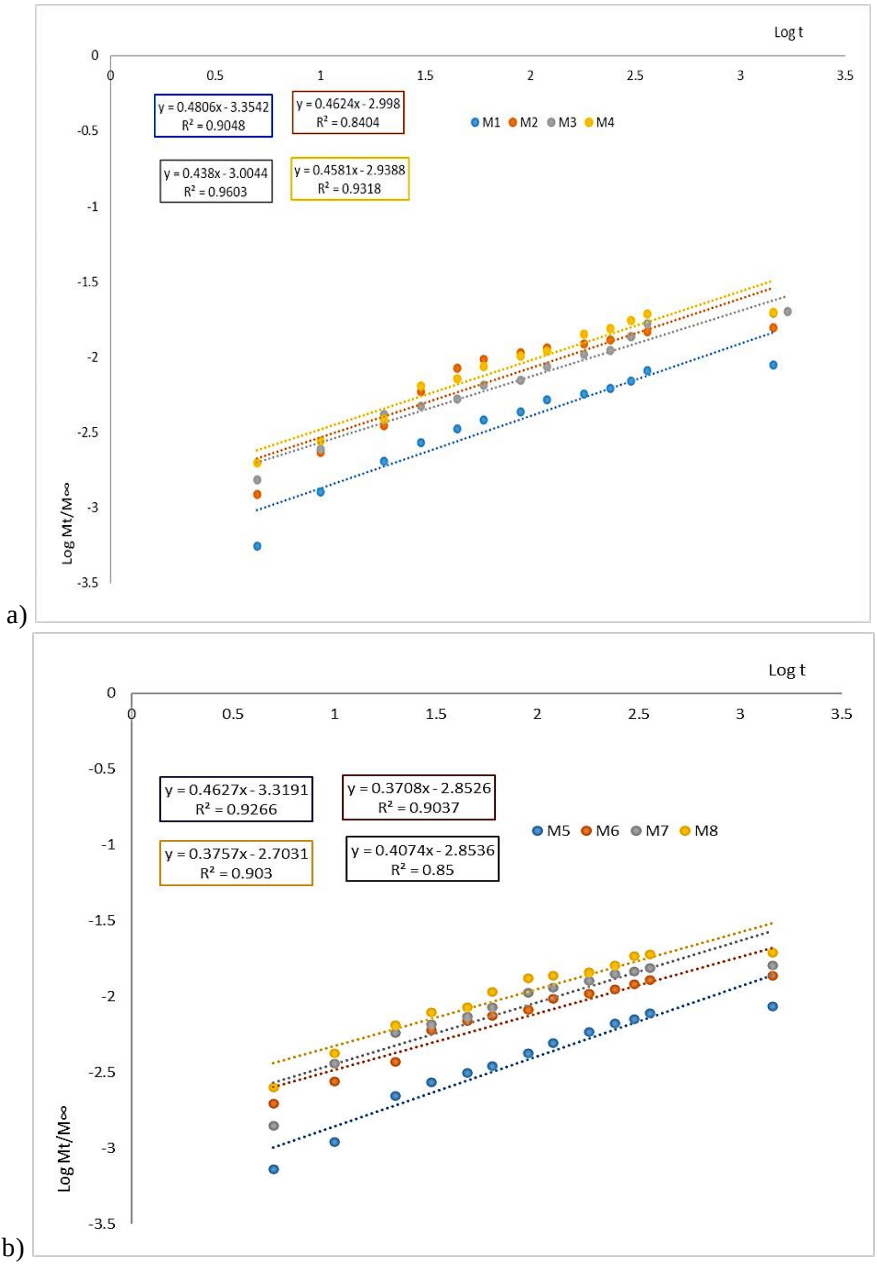


Fig. 4. Korsmeyer-Peppas model for a) M1-M4 samples; b) M5-M8 samples

The diffusional exponent (n) values, determined using the Korsmeyer-Peppas model, ranged from 0.37 to 0.48. In the case of M1 - M4 samples the diffusional exponent (n) values were between 0.43 and 0.48. It can be observed that the values of the exponent n, which characterize the release mechanism, are notably close to 0.5 ($0.5 < n < 1.0$). The results suggest that the transport/release mechanism is predominantly governed by diffusion. Moreover, in the case of samples M5 – M8 was observed that the values of the exponent n are lower than 0.5. This indicates an anomalous transport, which combines both diffusion and swelling mechanisms of the analyzed samples (18).

CONCLUSIONS

The present work demonstrates the amenability of CS-GEL for the preparation of particulate systems through a double cross-linking process into a reverse emulsion. SEM micrographs revealed two primary particle populations: the first consisted of micron-sized particles with rough surfaces and an approximately spherical shape, while the second population comprised submicron particles with a distinct spherical shape and smooth surfaces. Additionally, the images confirmed the formation of MPs with variable diameters ranging from 0.550 to 1.36 μm for M1-M4, and from 0.467 to 4.596 μm for M4-M8. MPs behavior in aqueous media with pH = 5.5 was studied, demonstrating a higher water retention capacity.

The MPs water uptake properties are significantly influenced by polymer concentration and CS-GEL ratio employed in the preparation stage, and they are correlated with the particle size. The drug loading and release capacity of MPs, tested using DEX, showed a good diffusional loading capacity, up to 0.5 mg of DEX per mg of MPs in a neutral environment. A rapid drug release was observed within the first 6 hours, followed by a slower phase, characterized by a constant release, up to 72 hours. Drug release kinetics analysis demonstrated that the drug transport/release mechanism is predominantly diffusional.

The obtained result confirms that CS-GEL-based MPs are a promising carrier system for controlled drug delivery for biomedical applications, including topical application of DEX to support the normal wound healing process, with all the advantages of prolonging the time of action and minimizing the specific side effects of NSAIDs, which will significantly improve patient compliance with this type of treatment.

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CONFLICT OF INTEREST

The authors declare no conflicts of interests.

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EDENTULISM COMPLICATIONS IN PATIENTS ELIGIBLE FOR HYBRID PROSTHETIC TREATMENT. A CROSS-SECTIONAL STUDY

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STUDY ON EDENTULISM COMPLICATIONS IN PATIENTS ELIGIBLE FOR HYBRID PROSTHETIC TREATMENT. A CROSS-SECTIONAL STUDY (Abstract). Aims of study. Patients with extended partial edentulism or complete edentulism, eligible for hybrid prosthetic solutions, are frequently affected by edentulism complications. The **aim** of our study was to find out the distribution of the edentulism complications that can impair biomechanical stability of the prosthetic treatment in patients eligible for hybrid prostheses. **Materials and methods:** A descriptive cross sectional, retrospective study, was conducted in Clinical Learning Base of Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iasi. 150 subjects with partial extended or complete edentulism (mean age 61,54 +/- 8,99 yrs.), eligible for hybrid prosthetic therapy were evaluated. Patients were divided in two groups: Group A (n=96): patients with partial reduced or extended edentulism that were eligible to hybrid prosthetic therapy, retained by fixed teeth-supported prosthetic restoration; Group B (H-PFR/IP) (n=54): patients with total or subtotal edentulism that were eligible to implant-supported hybrid prosthetic therapy, retained by fixed implant-supported prosthetic restorations. Data were collected regarding the patients' variables and edentulism complications. The need for therapeutic interventions in the pro-prosthetic and pro-implant stage was evaluated. **Results:** Significantly higher percentage of edentulous arches in Group B (90.9%) experienced severe bone resorption compared to Group A (45.6%). Temporo-mandibular joint pathology was notably more prevalent in Group B (94.4%) than in Group A (51.0%) ($p < 0.001$**). Group B had a higher prevalence of muscular disorders (77.8%) compared to Group A (32.3%) ($p < 0.001$**). Mandibular-cranial misalignments were significantly more common in Group B (88.9%) than in Group A (52.1%) ($p < 0.001$**). Temporomandibular disorders were significantly more prevalent higher in patients from Group B (94.4%) when compared to Group A (54.2%) ($p < 0.001$**). **Conclusions**: Patients eligible for hybrid prostheses consisting of removable dentures retained by fixed implant-supported bridges have significantly higher percentages of complications and need for pro-implant therapeutic interventions than patients eligible for removable dentures retained by fixed implant-supported bridges. **Keywords:** EDENTULISM, COMPLICATIONS, HYBRID PROSTHESIS.

Edentulism is a global concern, with projections indicating an increasing need for complete dentures and removable dentures in the future. Complete edentulism is the final result of a multifactorial process involving both biological and patient-

related factors. Among older adults, an increase in the number of comorbidities was found to be predictive of the onset of edentulism, with this pattern of association observed consistently across two longitudinal study periods. As the number of comorbidities rises, older adults may face a greater likelihood of tooth loss over time (1). Nonregular dental attenders were more likely than regular dental attenders to become edentulous, while smokers were more likely than non-smokers to become edentulous (2). Patients who are completely edentulous were found to be at higher risk for several health issues, including poor nutrition, coronary artery plaque formation, smoking, diabetes, rheumatoid arthritis, and various cancers (3). An increased number of missing teeth to be replaced, increases the likelihood of a removable restoration (4). Modern treatments based on combination of teeth-supported fixed bridges and hybrid removable dentures as well as implant-supported hybrid dentures helps both in minimizing the loss of residual alveolar ridges, rehabilitation of the masticatory and aesthetic functions, and the social reintegration (5).

Designing a hybrid prosthesis requires a thoughtful and systematic approach, grounded in strong clinical and technological expertise. The treatment of partially extended edentulism demands a comprehensive understanding of the case, including clinical indications and elements of maintenance, support, and stability (6). The treatment plan must take into account the aesthetic aspects, the remaining teeth on the dental arches and the tissues of the prosthetic field (7). The extraoral clinical examination reveals aspects that individualize the prosthetic treatment plan by identifying pre-existing conditions such as muscle dysfunction and temporomandibular joint (TMJ) pathology, which may restrict mouth opening and cause lateral deviation. It is crucial to assess the origin of these disorders, their etiology, and the extent of morphological and functional impairment (8). A successful hybrid prosthetic treatment requires effective approach of complications of partial and complete edentulism in the pre-prosthetic, pro-prosthetic and pro-implant stages. While residual ridge resorption remains the primary intraoral complication of edentulism, the planning of the treatment of the edentulous patient candidate to hybrid prosthetic treatment must approach complications such as need for grafting of the severe resorbed alveolar bone, and occlusal rehabilitation and rehabilitation of the cranio-mandibular relationships (5). To improve planning of the treatment of the edentulous patients in the pre- and pro-prosthetic stages, as well as in the pro-implant stages, clinical-biological indices (systemic status, dental support, periodontal support, mucosal support, alveolar bone support, occlusion, cranio-mandibular relationships) must be assessed (5). Management of the full and partially edentulous arch requires also a thorough understanding of the vertical and horizontal restorative space needed for various types of hybrid prostheses The restorative space specific for each type of hybrid restoration should be carefully considered during treatment planning to avoid lack of stability, aesthetics compromise, and inadequate contours of the future denture. For patient candidate to implant-supported hybrid prosthetic treatment, the minimum vertical space needed is as follows: fixed screw-retained (implant level): 4-5 mm; fixed screw-retained (abutment level): 7.5 mm; bar overdenture: 11 mm; fixed screw-retained hybrid: 15 mm. Regarding horizontal space, calculations must

be addressed to the discrepancy between implant and tooth positions (9). In cases of patients with complete edentulism, the physiological mandibular position must be established before proceeding with immediate loading. The use of interim removable prostheses for a few weeks can be beneficial to test the new occlusion in centric relation and to validate the prosthetic plan. Once the correct intermaxillary relationships are achieved, transferring them from provisional to final prostheses can be challenging, as impressions or scans of edentulous arches lack reference points for intermaxillary records. Anthropometric indices may be used to improve aesthetic outcome, due to reproducible results related to perioral and overall facial morphology as well as the occlusal vertical dimension (10).

Expert system-based applications allow for the integration of data from medical history, along with information gathered from clinical and paraclinical examinations, to initially evaluate the parameters of the prosthetic field. Following the pre-prosthetic, pre-implant, and pro-implant stages, as well as after completing temporary prosthetics, these applications conduct a secondary evaluation before the final prosthetic treatment to compare the prosthetic field indices recorded during the initial and secondary evaluations (11). The software applications and expert systems utilized for assessing mucosal and osseous support parameters, as well as for planning the surgical implant stage, rely on data derived from CBCT images. Applications based on CBCT image processing have demonstrated significant value in preoperative diagnostics and the planning of techniques for implant site rehabilitation, including the dental implant insertion phase (12-15). Digital workflow allows for the adjustment of the vertical dimension of occlusion, while ensuring excellent adaptation of the prosthesis to the soft tissues, and decreases the number of sessions required to install the definitive implant-supported hybrid prosthesis (16-18).

The aim of our study was to find out the distribution of the edentulism complications that can impair biomechanical stability of the prosthetic treatment in patients eligible for hybrid prostheses.

MATERIALS AND METHODS

Study groups included a total number of 150 subjects with partial extended or complete edentulism (gender: 82 males, 68 females; mean age 61.54 +/- 8.99 yrs.), selected from Clinical Learning Base of Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania, between 30.02.2023 and 30.05.2024 (tab. I). All patients were eligible for hybrid prosthetic therapy. Patients were divided in two groups: Group A (H-PFR/T) (n=96): patients with partial reduced or extended edentulism that were eligible to hybrid prosthetic therapy (maxillary and/or mandibular hybrid prosthesis using a substructure of metal covered by acrylic teeth, retained by fixed teeth-supported prosthetic restorations); Group B (H-PFR/IP) (n=54): patients with total or subtotal edentulism that were eligible to implant-supported hybrid prosthetic therapy (maxillary and/or mandibular hybrid prosthesis using a substructure of metal covered by porcelain fused to metal teeth, retained by fixed implant-supported prosthetic restorations).

Clinical and paraclinical evaluations were performed for each patient. Data were collected regarding the type of edentulism and Kennedy class, number of missing teeth, absence or presence of malocclusion, temporo-mandibular joint pathology, mus-

cular disorders, status of the cranial-mandibular relationships. Each patient was diagnosed for the presence or absence of the stomatognathic system disorders (TMD- temporo-mandibular disorders). The same methodology, clinical procedures, and evaluation criteria were used for patients in both study groups.

TABLE I.

Socio-demographic parameters of patients eligible to hybrid prosthetic treatment

		n	%
Gender	F	68	45.3
	M	82	54.7
Age groups	41-50	13	8.7
	51-60	50	33.3
	61-70	60	40.0
	71-80	27	18.0
Type of hybrid prosthesis	H-PFR	96	64.0
	H-PFR/IP	54	36.0
Total		150	100.0

Statistical analysis. Statistical analyses were conducted using *Microsoft Excel* and *SPSS version 29.0*. For qualitative data (where values represent categories), frequency distributions were created. The determined values were graphically represented using histograms (in various forms), and for qualitative variables, Pie Charts were also used. For numerical data, we calculated descriptive statistics parameters, including the mean, standard error of the mean, standard deviation, and minimum, maximum, and median values. The t-Student test was used for comparisons between samples, after verifying that the value distribution follows a normal distribution using the Kolmogorov-Smirnov goodness-of-fit test. The threshold of statistical significance was $p<0.05$.

RESULTS

Table II compares features and complications of edentulism in patients eligible for hybrid prosthetic therapy between Group A (H-PFR/D) and Group B (PFR/IP). Table III exposes features and edentulism complications related to the maxillary and mandibular arches. Percentages of patients eligible for hybrid prosthetic treatments were higher for males (54,7%) when compared with females (45,3%), with similar proportions intra-groups ($p=0.870$). In group A percentages of patients were highest in age group 51-60 yrs. (42,7%), followed by age group 61-70 yrs. (40,6%). In group B percentages of patients were highest in age group 61-70 yrs. (38,9%), followed by age group 71-80 yrs. (44,4%). Highly significant differences regarding the distribution of patients according to age were found between groups ($p < 0.001$**). Group B had a significantly higher percentage of patients with mandibular edentulism (33.3%) compared to Group A (17.7%), while higher percentage (44,4%) of patients from Group B had maxillary edentulism when compared to patients from Group A (29,2%). Additionally, maxillary and mandibular combined edentulism was more prevalent in Group A (53.1%) than in Group B (22.2%), with a p-value of 0.001**.

TABLE II.

Features and edentulism complications of patients eligible for hybrid prosthetic therapy

		GROUP A (H-PFR/T)		GROUP B (H-PFR/IP)		Total		Chi-squared	p-value
		N	%	N	%	N	%		
Demographics									
Gender	F	44	45.8%	24	44.4%	68	45.3%	0.027	0.870
	M	52	54.2%	30	55.6%	82	54.7%		
Age groups	41-50	13	13.5%			13	8.7%	47.150	<0.001**
	51-60	41	42.7%	9	16.7%	50	33.3%		
	61-70	39	40.6%	21	38.9%	60	40.0%		
	71-80	3	3.1%	24	44.4%	27	18.0%		
Edentulism parameters									
Edentulous arch	MD	17	17.7%	18	33.3%	35	23.3%	13.801	0.001**
	MX	28	29.2%	24	44.4%	52	34.7%		
	MX + MD	51	53.1%	12	22.2%	63	42.0%		
Edentulism type	PEE	85	88.5%			85	56.7%	141.689	<0.001**
	PRE	9	9.4%			9	6.0%		
	TE	2	2.1%	45	83.3%	47	31.3%		
	SE			9	16.7%	9	6.0%		
Kennedy class	I	33	34.4%						
	I + I	30	31.3%						
	I + II	12	12.5%						
	I + IV	6	6.3%						
	II	12	12.5%						
	II + II	3	3.1%						
Edentulism complications									
Medium/advanced bone resorption	Yes	47	49.0%	48	88.9%	95	63.3%	23.729	<0.001**
	No	49	51.0%	6	11.1%	55	36.7%		
Malocclusion	Yes	79	82.3%	54	100.0%	133	88.7%	10.785	0.001**
	No	17	17.7%			17	11.3%		
TMJ pathology	Yes	49	51.0%	51	94.4%	100	66.7%	29.297	<0.001**
	No	47	49.0%	3	5.6%	50	33.3%		
Muscular disorders	Yes	31	32.3%	42	77.8%	73	48.7%	28.622	<0.001**
	No	65	67.7%	12	22.2%	77	51.3%		
M-C misalignments	Yes	50	52.1%	48	88.9%	98	65.3%	20.671	<0.001**
	No	46	47.9%	6	11.1%	52	34.7%		
Dishomeostasis	Yes	40	41.7%	30	55.6%	70	46.7%	2.679	0.102
	No	56	58.3%	24	44.4%	80	53.3%		
TMD	Yes	52	54.2%	51	94.4%	103	68.7%	26.059	<0.001**
	No	44	45.8%	3	5.6%	47	31.3%		
Total		96	100.0%	54	100.0%	150	100.0%		

PEE- partial extended edentulism; PRE- partial reduced edentulism; SE- subtotal edentulism; TE- total edentulism

TABLE III.

Distribution of the maxillary and mandibular arches in patients eligible for hybrid prosthetic therapy

		GROUP A (H-PFR/T)		GROUP B (H-PFR/IP)		Total		Chi-squared	p-value
		N	%	N	%	N	%		
Gender	F	66	44.9%	27	40.9%	93	43.7%	0.295	0.587
	M	81	55.1%	39	59.1%	120	56.3%		
Age group	41-50	23	15.6%			23	10.8%	71.344	<0.001**
	51-60	62	42.2%	15	22.7%	77	36.2%		
	61-70	59	40.1%	21	31.8%	80	37.6%		
	71-80	3	2.0%	30	45.5%	33	15.5%		
Arch	MD	68	46.3%	30	45.5%	98	46.0%	0.012	0.913
	MX	79	53.7%	36	54.5%	115	54.0%		
Edentulism type	PEE	130	88.4%			130	61.0%	204.022	<0.001**
	PRE	15	10.2%			15	7.0%		
	TE	2	1.4%	48	72.7%	50	23.5%		
	SE			18	27.3%	18	8.5%		
Kennedy class	I	111	75.5%						
	II	30	20.4%						
	IV	6	4.1%						
Moderate/severe bone resorption	Yes	67	45.6%	60	90.9%	127	59.6%	38.880	<0.001**
	No	80	54.4%	6	9.1%	86	40.4%		
Total		147	100.0%	66	100.0%	213	100.0%		

Group A predominantly consisted of patients with partial extended edentulism (88.5%), whereas Group B had a higher prevalence of total edentulism (83.3%). The difference was statistically significant with a p-value of <0.001**. Regarding the distribution of the severe alveolar bone resorption, a significantly higher percentage of patients in Group B (88.9%) experienced advanced bone resorption compared to Group A (49.0%), with a p-value of <0.001**. All patients in Group B (100%) exhibited malocclusion, compared to 82.3% in Group A, with a significant difference (p-value of 0.001**). TMJ pathology was notably more prevalent in Group B (94.4%) than in Group A (51.0%), reflected by a p-value of <0.001**. Group B had a higher prevalence of muscular disorders (77.8%) compared to Group A (32.3%), with a significant p-value of <0.001**. Mandibular-cranial misalignments were significantly more common in Group B (88.9%) than in Group A (52.1%), with a p-value of <0.001**. Temporomandibular Disorders were significantly more prevalent higher in patients from Group B (94.4%) when compared to Group A (54.2%), with a p-value of <0.001**. No significant statistical difference was found in the incidence of dishomeostasis between the groups (p-value = 0.102). The comparison of parameters and edentulism complications related to the edentulous maxillary and mandibular arches, between Group A (H-PFR/T) and Group B (H-PFR/IP), ob-

served highly significant statistical differences ($p<0.001$**) between the groups in the distribution according to age, edentulism type, and presence of moderate/advanced alveolar bone resorption. According to age, Group B had a notably higher percentage of edentulous arches in the 71-80 age group (45.5%) compared to Group A (2.0%), while Group A had a higher representation in the 51-60 and 61-70 age groups. According to the edentulism type, Group A predominantly consisted of arches with extended partial edentulism (88.4%), while Group B had a much higher proportion of total edentulism (72.7%). Significantly higher percentage of edentulous arches in Group B (90.9%) experienced severe bone resorption compared to Group A (45.6%).

DISCUSSION

The objectives of our study were to investigate edentulism complications that can impair biomechanical stability of the future hybrid prosthetic treatment.

In a comparative analysis between patients from Group A (hybrid removable prostheses retained by fixed teeth-supported bridges) and Group B (hybrid removable prostheses retained by implant-supported bridges), it was observed that Group B exhibited a notably higher prevalence of several edentulism complications compared to Group A. Specifically, a substantially larger proportion of patients in Group B experienced medium to advanced alveolar bone resorption. Additionally, every patient in Group B showed signs of malocclusion, whereas a smaller portion of Group A was affected. TMJ pathology was markedly more common in Group B, with nearly all patients being affected, in contrast to a significantly lower proportion in Group A. Similarly, muscular disorders were more frequently observed in Group B, where the prevalence was more than double that seen in Group A. Mandibular-cranial misalignments were also more prevalent in Group B, with a significant difference when compared to Group A. Lastly, Temporomandibular Disorders were observed much more frequently in Group B, with the prevalence being almost twice as high as in Group A. The statistical significance of these findings underscores the considerable disparity in the health profiles of the two groups. Number and position of missing teeth, occlusal relationships, periodontal health of the remaining teeth, and the movement pattern or size of the tongue are considered local factors, while systemic factors include neuromuscular control, age, psychological status, and overall health resilience (19). Oral cavity and temporomandibular joint (TMJ), a mechanically demanding and biochemically dynamic environment, requires therapeutic approaches that can not only restore joint functionality but also adapt to ongoing changes within the joint (20). While multifactorial theory was considered to explain TMJ disorders, various treatment approaches, should be aimed to resolve possible etiology from different aspects (21). Prosthetics specialists must design a comprehensive plan of definitive hybrid prosthetic treatment, while considering the loss of occlusal relationship and adverse effects on the stomatognathic system in patients with extended partial edentulism or complete edentulism. Temporomandibular joint, as a significant component of this system, may also

be affected by edentulism in the process of supporting mandibular movement. Considering the interrelationship between the components of the stomatognathic system, the impairment of one component often leads to the damage of the others, or at least some of them, while mandibular-cranial misalignment exacerbates the severity of dysfunction, making mandibular recovery more challenging and sometimes limiting therapeutic options (22).

CONCLUSIONS

Most edentulous patients eligible have edentulism complications that will impair biomechanical stability of the future hybrid prosthetic treatments. Patients eligible for hybrid prostheses consisting of removable dentures retained by fixed implant-supported bridges have significantly higher percentages of complications (medium/advanced alveolar bone resorptions, temporo-mandibular disorders) than patients eligible for removable dentures retained by fixed implant-supported bridges.

CONFLICT OF INTEREST AND FUNDING

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THE DECISION-MAKING PROCESS BEHIND REMOVABLE PROSTHESIS: A THERAPEUTIC AND DESIGN PERSPECTIVE

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THE DECISION-MAKING PROCESS BEHIND REMOVABLE PROSTHESIS: A THERAPEUTIC AND DESIGN PERSPECTIVE (Abstract): The prosthodontics revolution, which reinstated oral function, enhanced aesthetics, and improved patient satisfaction, contains removable dentures as one of its vital parts. **Materials and methods:** This study examined a group of patients who sought prosthetic treatment to determine the factors that influence partially extended dentition treatment decisions, treatment solutions, and removable prosthesis design. **Results:** A retrospective study was carried out, including 546 patients who addressed the Dental Faculty in Iasi's Department of Partial Dentures. According to this study, the palatal plate was the most popular maxillary major connection, followed by the U-shaped connector. This result may assist in assessing the quality of removable partial dentures provided to patients. **Conclusions**: Future advancements in digital dentistry and 3D printing may facilitate the creation of customized connection designs adapted to individual requirements, while heightened understanding of gender-specific tendencies might enhance treatment outcomes. **Keywords:** REMOVABLE DENTURE, MAIN CONNECTOR, PROSTHETIC TREATMENT, ORAL HEALTH.

INTRODUCTION

Removable dentures have been an integral part of the prosthodontics revolution, which has restored oral function, improved aesthetics, and increased patient happiness. Dentures, both full and partial, can replace natural teeth when they are lost as a result of disease, decay, or accident. These devices enhance the patient's psychological well-being by restoring chewing efficiency, communication, and facial attractiveness. Clinical, functional, aesthetic, and patient-centered concerns are all part of the decision-making process when choosing a removable denture (1).

Both clinical requirements and aesthetic preferences should be considered before settling on a removable denture. Improving the patient's quality of life, restoring functioning, and preventing future oral degeneration are the key therapeutic aims. The level of tooth loss, the condition of the patient's remaining teeth and gums, and the patient's general oral health are all important considerations for clinicians. These evaluations determine the choice of the best removable denture treatment-full or partial -and the particular design elements needed to fulfil the patient's requirements (2, 3).

From a design perspective, the construction of removable prostheses must

balance functionality, comfort, and aesthetics. Modern prosthetic design considers not only the biological factors of tooth replacement but also the materials used, the fit and retention of the prosthesis, and its ability to harmonize with the patient's existing oral structures. Advances in dental materials and fabrication techniques have expanded the range of possibilities for creating durable and aesthetically pleasing removable prostheses (3).

These innovations enable more precise customization, ensuring that the prosthesis is tailored to each individual's unique oral environment (3, 4). An important aspect of the decision-making process is patient involvement. Today, a patient-centered approach is widely recognized as essential for achieving successful outcomes. Patients' expectations, preferences, and lifestyle factors must be integrated into the planning and design phases of removable dentures (5).

To ensure that patients are completely informed and satisfied with the final decision, it is vital to be able to talk honestly about the benefits, drawbacks, and possible difficulties of different prosthetic solutions. The consultation is the opportunity to address all of the patient's worries, including those about the prosthesis's look, its comfort while using it, and the amount of time it will take to adjust to it (6, 7).

Furthermore, clinicians must also consider the long-term success and maintenance of removable prostheses. The selection of materials and design features plays a crucial role in the longevity and stability of the prosthesis, while regular follow-up care is necessary to monitor any changes in the patient's oral health or adjustments needed for the dentures over time (5). The manufacturing process has been simplified with the incorporation of modern technologies like 3D printing and digital impressions, which allows for more precise fits and reduces patient pain during the adaption phase (8).

A removable prosthesis is often the result of a multi-factorial decision-making process that considers clinical requirements, patient preferences, and technological advancements in the field. When permanent treatments like implants or bridges are not an option owing to health, anatomy, or cost, removable prosthesis might be the most practical or affordable option for tooth replacement for many individuals. Because of its portability, affordability, and convenience of use, removable prostheses remain an important aspect of dentistry, even when fixed alternatives are available. This is especially true for patients who are either completely or partially missing teeth (9).

At the level of a group of patients who sought out prosthetic treatment, this study intends to assess the elements that impact the treatment decision for partially extended dentitions, as well as the impact of these factors on the treatment solution and the final design of removable prostheses.

MATERIALS AND METHODS

The proportion of Romanians 65 and older has been on the rise for some time now, mirroring a worldwide trend. Some forms of cancer, chronic kidney disease, Alzheimer's type dementia, hypertension, diabetes, chronic obstructive pulmonary disease, vascular accidents, and other chronic diseases are becoming more common as a result of these demographic shifts. Additionally, there is a correlation between one's dental health and their overall physical health, which influences treatment strategies, according to the research (10, 11).

Despite widely known statistics, our un-

derstanding of the unique requirements pertaining to the physical and dental health of patients over the age of 60 is still limited.

Consequently, improving the quality of life for older patients and providing them with more effective health care can be achieved by a comprehensive investigation of the relationship between overall physical health and oral condition (4).

The specifics of the prosthetic field are carefully examined to determine the best course of treatment, with an eye towards assessing the odonto-periodontal support, the muco-osseous support, and the presence of local, loco-regional, or systemic issues (12, 13). Reducing pressures on the supporting tissues and distributing forces over as large an area as feasible are ideal goals of the partial denture design (14).

In order to conduct this study, researchers analyzed and quantified clinical documents from patients who had professional prosthetic therapy at the Dental Faculty in Iasi's Department of Partial Dentures.

A number of factors were examined, including the design of different prosthetic structures, the ratio of current clinical-biological characteristics that impact treatment selection, and the therapies using removable partial dentures. The 873 people who sought out prosthetic therapy from 2004 to 2019 were part of the group investigated for these characteristics. After collecting all of the necessary data from various sources, including patient registration sheets, computer tomography, study casts, laboratory archives, and individual case presentations, a total of 546 patients were included in the final research.

This study was approved by the "Grigore T. Popa" University of Medicine and Pharmacy, Iasi's ethics committee (No. 9430/12.06.2020), and all participants were told that clinical and paraclinical data will be used in the study.

Inclusion criteria

Patients had to meet the following criteria in order to be included: they had to be 19 years old or older; all treatment information and protocols had to be included in the patient record; they had to be from a socially disadvantaged category and not be employed at the time; paraclinical examinations had to be done, including at least one radiological investigation (CT, MRI, or x-ray) and the initial cast study; specific laboratory data sheets had to be included, detailing all aspects of the removable dentures and how they were designed to provide stability and support.

Exclusion criteria

Patients who did not continue treatment after getting transitional dentures or who had incomplete treatment were not included in the study. Other exclusion criteria included incomplete specific laboratory data sheets, a lack of paraclinical investigations, and incomplete patient record data sheets.

The data was quantified, and statistical analysis and validation tests were run using descriptive statistics. Microsoft Access was used for parameter-specific analysis, and Excel and the database were used for statistical analysis. Parts of descriptive statistics as well as Pearson correlations were utilized in the statistical analysis.

RESULTS

From the analyzed database the following is a centralized distribution of the frequencies of local and loco-regional infections found in the patient group.

Gender characteristics

The men presented the following classification of conditions as seen in figure 1:

carious conditions- 37.6%, endodontic- 3.1%, periodontal- 41.6%, mucosal- 2.6%, underlying bone- 3.8%, loco-regional- 11.2%.

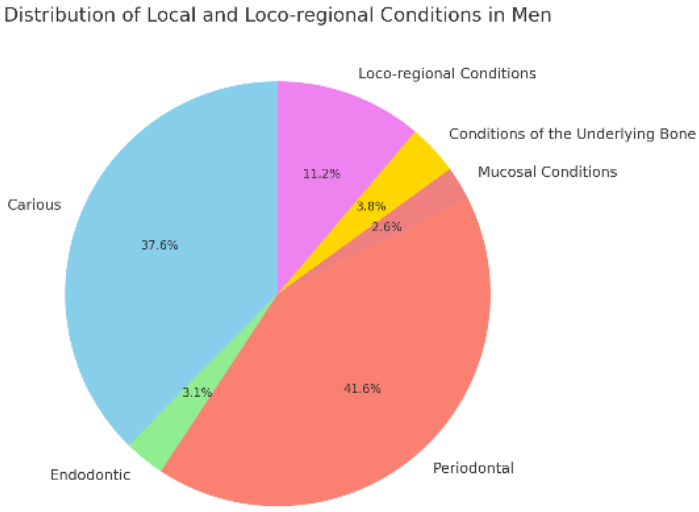

Fig. 1. Local and loco-regional conditions in men

The women presented the following classification of conditions as seen in figure 2: carious conditions - 34.3%, endodontic - 27.6%, periodontal - 16%, mucosal - 10.9%, underlying bone - 4.5%, loco-regional - 6.7%.

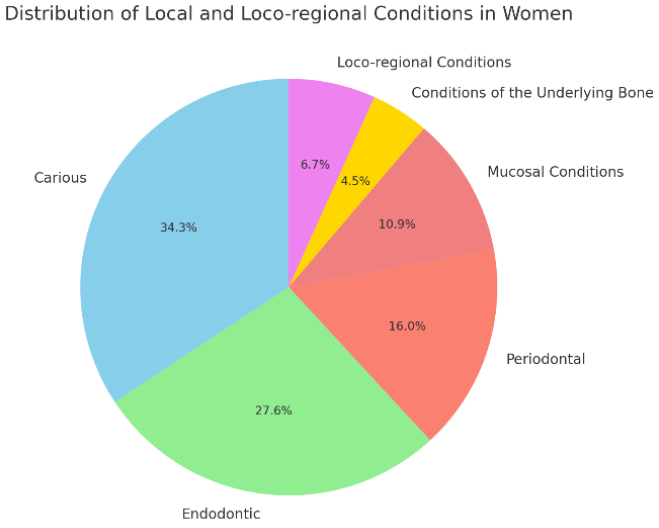

Fig. 2. Local and loco-regional conditions in women

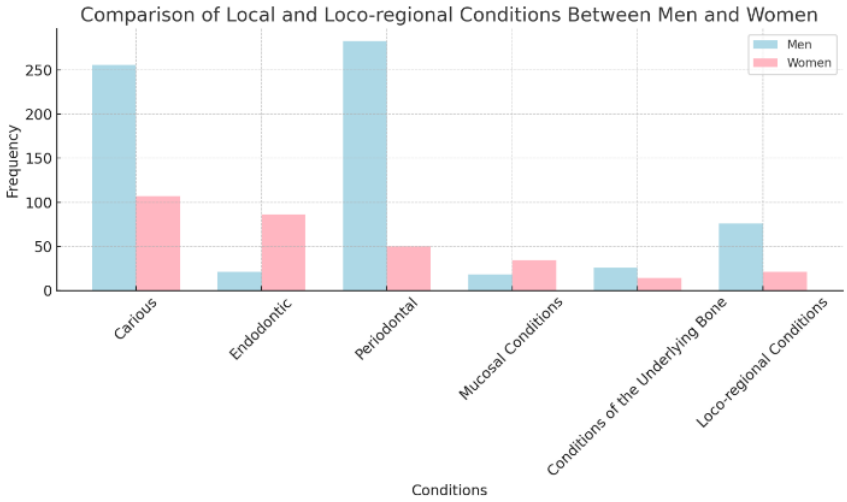

Fig. 3. Comparison between men and women of local and loco-regional conditions

Several factors could contribute to the observed differences in the prevalence of local and loco-regional conditions between men and women (fig. 3). These factors can be related to biological, behavioral, socio-economic, and healthcare access differences. Men tend to have worse outcomes in conditions like periodontal and carious diseases due to less frequent care and poorer oral hygiene, while women, despite seeking care more frequently, may exhibit higher rates of conditions like endodontic and mucosal diseases due to early detection and hormonal influences.

The frequency distributions for pre-existing general conditions were as follows in figure 4 for men: cardiac - 32.9%, renal - 7%, respiratory - 18.4%, metabolic - 10.5%, gastrointestinal - 12.1%, immunological - 0.4%, psychiatric - 6.3%, other conditions - 3.2%, good general health - 9.3%.

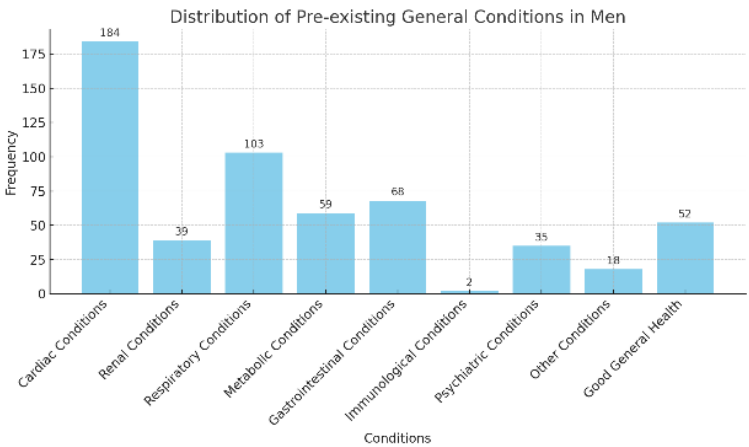

Fig. 4. Distribution of pre-existing general condition in men

Following the statistical analysis, women presented the following pre-existing general conditions: cardiac - 21.2%, renal - 1.7%, respiratory - 10.7%, metabolic - 18.1%, gastrointestinal - 16.4%, immunological - 5.6%, psychiatric - 7.9%, other conditions - 3.4%, good general health - 15% (fig. 5).

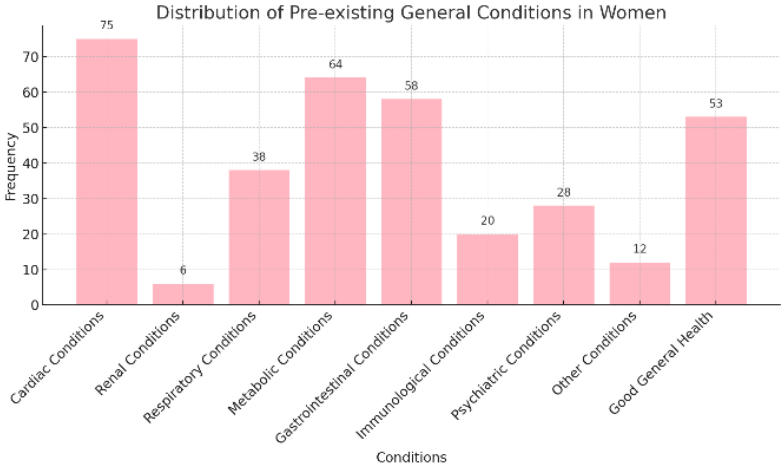

Fig. 5. Distribution of pre-existing general condition in women

The gender disparities in pre-existing conditions between men and women result from a combination of biological differences, lifestyle behaviors, societal norms, occupational exposure, and healthcare access. While men are more likely to experience cardiac and respiratory conditions, women show higher rates of metabolic and psychiatric conditions, likely due to a complex interplay of hormones, health-seeking behaviors, and social factors.

Main connectors

The frequency distributions for the types of connector designs used were as follows in figure 6:

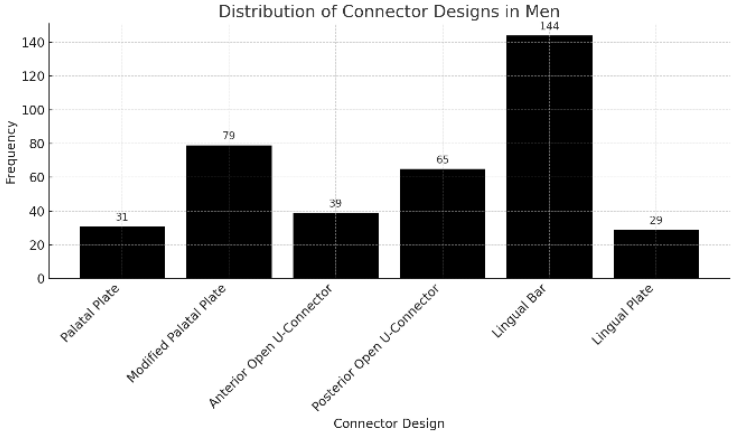

Fig. 6. Distribution of main connectors in men

The frequency distributions for the types of connector designs used in women were as follows in figure 7.

Analyzing the results, we can notice that there is a significant positive relationship between the design of the main connectors and the conditions of the present teeth (r = + 0.679 - correlation coefficient, N = 387, p < 0.0001) (tab. I). The positive value of the correlation coefficient indicates that as the values of one variable increase, the values of the other also increase. The coefficient r indicates a moderate association between the two variables.

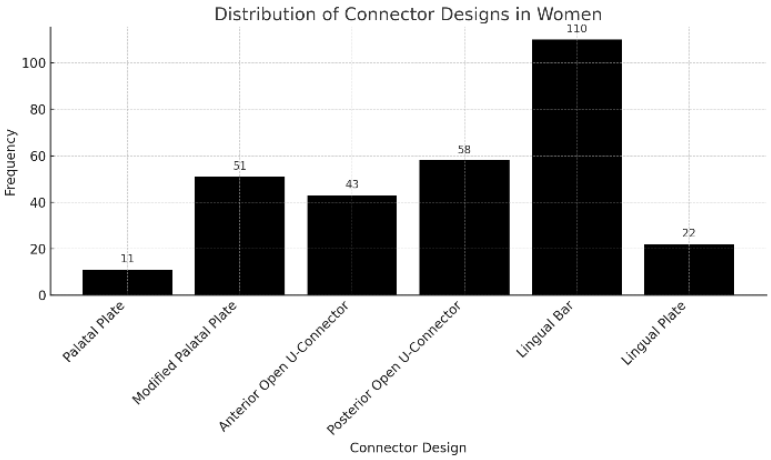

Fig. 7. Distribution of main connectors in women

TABLE I.

Correlations for men local conditions and connector design

		Design	Local condition
Connector design	Pearson Correlation	1	**.679****
	Sig. (2-tailed)		**.000**
	N	387	387
Local conditions	Pearson Correlation	.679**	1
	Sig. (2-tailed)	.000	
	N	387	560

**Correlation is significant at the 0.01 level (2-tailed).

And for women we also have a significant positive relationship between the design of the main connectors and the conditions of the present teeth (r = + 0.846 - correlation coefficient, N = 295, p < 0.0001) (tab. II). The positive value of the correlation coefficient indicates that as the values of one variable increase, the values of the other also increase. The coefficient r indicates a strong association between the two variables.

We also have a significant positive relationship between the design of the main connectors and pre-existing general condi-

tions (r = + 0.838 - correlation coefficient, N = 326, p < 0.0001) in men (fig. 8). The positive value of the correlation coefficient indicates that as the values of one variable increase, the values of the other also increase. The coefficient r indicates a strong association between the two variables.

And for women exist also a significant positive relationship between the design of the main connectors and pre-existing general conditions (r = + 0.846 - correlation coefficient, N = 295, p < 0.0001) (fig. 9). The positive value of the correlation coefficient shows that as the values of one variable increase, the values of the other also increase. The r coefficient indicates a strong association between the two variables.

TABLE II.

Correlations for women local conditions and connector design

		Design	Local conditions
Design	Pearson Correlation	1	**.846****
	Sig. (2-tailed)		**.000**
	N	295	295
Local conditions	Pearson Correlation	.846**	1
	Sig. (2-tailed)	.000	
	N	295	312

**. Correlation is significant at the 0.01 level (2-tailed).

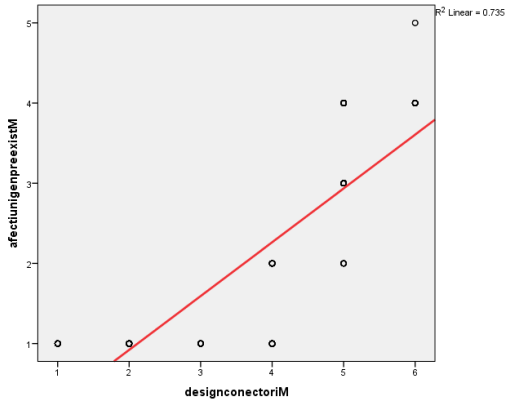

Fig. 8. Correlation between general conditions and main connectors in men.

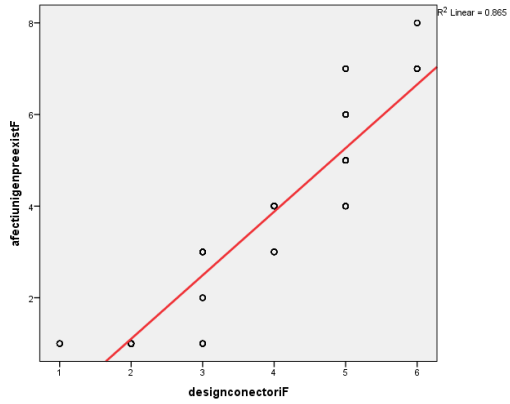

Fig. 9. Correlation between general conditions and main connectors in women.

DISCUSSION

Despite the inherent limitations, the present study had several strengths. First, the oral health status of the participants was classified based on multiple factors - local, loco-regional, and also the effect of general conditions, depending on the use of removable dental prostheses - specifically referring to types of connectors.

Based on this grouping scheme, a de-

tailed analytical and descriptive statistical analysis was conducted. Furthermore, the data in this study were collected by specialists, whereas previous studies relied on self-reported data. According to the results of this study, the palatal plate was the most dominant maxillary major connector, followed by the U-shaped connector.

The U-shaped major connector was used less frequently, in line with previous findings (15), possibly due to its mechanical deficiency and tendency to bend during handling or mastication (16,17), contrary to the results of Owall and Taylor (18), who found that the U-shaped major connector was used more (55.2%) than other maxillary major connectors.

Based on the results of the present study, the use of the lingual plate (21.2% and 16.2%) was significantly higher than that of the lingual bar. This is more likely due to the strength requirements for Kennedy Class I, which necessitates stability and indirect retention.

In the future, more personalized prosthetic treatments could be developed by considering patient-specific factors such as gender, lifestyle, and anatomical differences. Advances in digital dentistry and 3D printing may lead to customized connector designs tailored to individual needs. Further research could explore how cultural and regional preferences influence design choices.

Increased awareness of gender-specific trends may improve treatment outcomes. Lastly, integrating patient feedback into design development could enhance both function and comfort.

CONCLUSIONS

The results of this study might be helpful for evaluating the standard of removable partial dentures given to patients.

For training and educational purposes, it also provides valuable content. The data was only collected from one school, and the sample size was rather small, which are two of the study's weaknesses.

More studies on the impact of gender on oral health outcomes could optimize treatment strategies. Collaboration between engineers and dental professionals will likely drive further advancements in prosthetic design and functionality.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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CHITOSAN-STABILIZED LIPID VESICLES FOR SUSTAINED RELEASE AND PROLONGED ANALGESIC EFFECTS OF INDOMETHACIN IN A SOMATIC PAIN MODEL IN MICE

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CHITOSAN-STABILIZED LIPID VESICLES FOR SUSTAINED RELEASE AND PROLONGED ANALGESIC EFFECTS OF INDOMETHACIN IN A SOMATIC PAIN MODEL IN MICE (Abstract): The evolution of pharmaceutical nanotechnology enhances the flexibility of drug delivery by using carrier systems to target specific tissues, enabling controlled release at optimal concentrations. The inclusion of nonsteroidal anti-inflammatory drugs (NSAIDs) in nanocarriers presents a promising approach for achieving sustained release and improving the pharmacodynamic effects. We **aimed** to evaluate the in vivo kinetic release profile and the impact of indomethacin (IND) encapsulated in lipid vesicles on somatic nociceptive reactivity in mice. **Materials and methods:** Lipid vesicles were prepared using a molecular droplet self-assembly method, incorporating phosphatidylcholine and chitosan (CHIT) to encapsulate IND. The *in vivo* evaluation involved administering IND-loaded lipid vesicles to male Swiss mice and assessing their analgesic effects using the hot plate test, a standard method for measuring somatic pain. Blood samples were collected at different intervals to determine the release kinetics of IND, with concentrations measured by high-performance liquid chromatography. **Results** indicated that IND encapsulated in lipid vesicles exhibited a sustained release profile compared to the direct administration of the drug. The treatment with IND-loaded microvesicles resulted in significantly prolonged analgesic effects, as shown by extended latency times in the hot plate test. The release profile of IND from lipid vesicles, composed of phosphatidylcholine and stabilized with CHIT, demonstrated a gradual and controlled drug release, correlating with extended pain relief. **Conclusions:** These findings confirm the potential of lipid-based vesicles, as effective carriers for IND, offering sustained drug release and prolonged analgesic effects in a somatic pain model. **Keywords**: LIPID VESICLES, INDOMETHACIN, IN VIVO RELEASE, HOT PLATE, MICE.

INTRODUCTION

Nanotechnology applications in medicine, known as nanomedicine, involve the use of nanomaterials for diagnosing, monitoring, controlling, preventing, and treating diseases (1). Nanomaterials possess unique physicochemical properties distinct from their conventional counterparts due to their small size, making them valuable in drug development (2). These properties, such as altered pharmacokinetics, the ability to cross biological barriers, potential toxicity, and persistence in the environment and human body, must be carefully examined under use conditions (3, 4).

Lipid nanoparticles have emerged in the pharmaceutical industry as promising carriers for therapeutic agents. Liposomes, an early form of lipid nanoparticles, are versatile nanocarriers capable of transporting both hydrophobic and hydrophilic molecules, including small molecules, proteins, and nucleic acids. Many liposome-based drug formulations have been approved and successfully used in medical practice (5). Traditional drugs often face limitations in pharmacokinetics, low bioavailability, and high toxicity, which can restrict their use (6). Nanotechnology and nanomedicine have advanced in overcoming these issues, enhancing pharmacodynamic effects and improving disease detection, diagnosis, and treatment (5). With their small size, nanosystems reduce toxicity and improve pharmacokinetic parameters, such as distribution, prolonged circulation time, targeted release, higher intracellular concentration, and enhanced drug solubility and stability (6, 7).

A key area of research is enhancing the therapeutic profile of non-steroidal anti-inflammatory drugs (NSAIDs) with nanoparticles. NSAIDs are widely used to treat inflammatory diseases, but their long-term use can lead to side effects, especially gastrointestinal toxicity, due to repeated administration. Nanoparticle-based approaches offer an intriguing solution to improve NSAID safety and efficacy (8-10).

The 1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-indole-3-acetic acid derivative indomethacin (IND) is a nonsteroidal anti-inflammatory drug (NSAID). Its structure is characterized by an indole acetic acid core with additional functional groups that contribute to its activity. The structure includes a chlorobenzoyl group attached to the indole ring, along with a methoxy group and a methyl group, which enhance the molecule's stability and potency as an anti-inflammatory agent. It is practically insoluble in water, which limits its absorption in aqueous environments (11). IND is rapidly absorbed from the gastrointestinal tract following oral administration, reaching peak plasma concentrations within 2 to 4 hours, though this can vary based on the formulation and presence of food. It has a bioavailability of approximately 90-95%, though high variability can occur among individuals. It is approximately 90% bound to plasma proteins, predominantly albumin, which limits its free fraction in circulation but also contributes to its longer half-life and tissue distribution. The drug undergoes extensive hepatic metabolism, primarily by demethylation and deacetylation, and is eventually excreted in urine and feces both as metabolites and in small amounts as the parent compound. Its elimination half-life typically ranges between 4 to 5 hours but can vary depending on patient-specific factors such as liver and kidney function (12).

IND exerts its effects primarily by inhibiting cyclooxygenase (COX) enzymes, specifically COX-1 and COX-2, thus reducing the inflammatory response, pain, and fever, making it effective in the treatment of conditions such as arthritis, gout,

and dysmenorrhea (13, 14). However, its inhibition of COX-1 also reduces the protective effects of prostaglandins in the gastrointestinal tract and kidneys, contributing to common adverse effects like gastrointestinal irritation, ulceration, and renal impairment. Its strong inhibition of COX enzymes also explains its potential cardiovascular risks, as prostaglandins involved in vasodilation and platelet inhibition are reduced, potentially leading to increased blood pressure and risk of thrombotic events (15). Incorporating IND into nanosystems may offer significant pharmacological benefits, including reduced gastrointestinal irritation and enhanced anti-inflammatory and analgesic effects.

The **purpose** of this study was to assess the impact of IND encapsulated in nanosystems on somatic nociceptive reactivity in rats.

MATERIALS AND METHODS

Substances. The materials used for nanoparticle preparation were procured from Sigma-Aldrich Chemical Co., Steinheim, Germany (www.sigma-aldrich.com). These included CHIT (catalog code: C3646; derived from crab shells, molecular weight: 310,000 g/mol 80% degree of N-deacetylation, polydispersity index of 3.26), IND (catalog code: I7378; 98.5% purity; molecular weight: 357.79 g/mol), phosphatidylcholine (catalog code: P5638; type II-S, soy-derived, containing 14–29% choline), cholesterol (catalog code: C8667; ≥99% purity; molecular weight: 386.65 g/mol), chloroform (catalog code: C2432; stabilized with 100–200 ppm amylene; ≥99.5% purity; molecular weight: 119.38 g/mol), glacial acetic acid (catalog code: 695092; ≥99.7% purity; molecular weight: 60.05 g/mol), and ethyl alcohol (catalog code: E7148; 95% purity; molecular weight: 46.07 g/mol).

Preparation of lipid vesicles entrapping IND. Lipid vesicles containing IND were prepared using a molecular droplet self-assembly method. Ethanol was used as the solvent for the lipids, cholesterol, and IND, while CHIT was dissolved in 0.5% glacial acetic acid.

In the preparation, 0.09 g of soy lipid was mixed with 0.66 mL of ethanol, and 0.015 g of cholesterol and 0.01 g of IND were each dissolved in 1 mL of ethanol. The combined lipid and IND solution (1.66 mL) were subjected to sonication at 25% amplitude for 10 minutes at 29°C using a Bandelin SONOPULS 2450 ultrasonic homogenizer, providing 20,000 kJ of energy (16). This sonication step transformed multilamellar structures into unilamellar vesicles, with their size controlled by ultrasound amplitude. The resulting mixture was then injected into 8.33 mL of double-distilled water (22-23°C), forming a slightly translucent suspension of IND-loaded lipid vesicles (IND-vl). Separately, 1.66 mL of the ethanol mixture was added to 8.33 mL of 0.25% CHIT solution under magnetic stirring (800 rpm for 20 minutes at 22°C), resulting in the formation of IND-loaded vesicles (IND-ves). Both dispersions were further stirred for an additional 5 minutes (16). The inclusion of CHIT altered the vesicle size and morphology, improving the colloidal stability. The pH of both dispersions was measured using a Sartorius Professional PP-50 pH meter. To remove excess lipids and unencapsulated drug, the dispersions were dialyzed for 2 hours using 12,000 Da MWCO tubular membranes (catalog code: D6191-25EA, Sigma-Aldrich). This process adjusted the pH to physiological levels, with the IND suspension reaching a pH of 7.0 and the CHIT-coated vesicles (IND-ves) achieving a pH of 6.7 (16).

Animals. The experiments were carried out on male white Swiss mice (20-25 g), acquired from the National Medical-Military Institute for Research and Development, Baneasa, Bucharest, Romania, the Biobase at "Grigore T. Popa" University of Medicine and Pharmacy of Iasi and CEMEX (Advanced Research and Development Center for Experimental Medicine) in Iasi, Romania. The mice were kept in individual plexiglass cages and given a 7-day acclimatization period in a controlled laboratory setting, where the temperature was maintained at 21±2°C, humidity ranged from 50% to 70%, and a 12-hour light/dark cycle was followed. Standard granulated food and tap water were available *ad libitum*, with daily food intake recorded and the animals' behavior closely monitored.

The protocol of the experiments. For the experiment, the animals were randomly assigned to groups of five and treated orally using an esophageal-gastric device with a single daily dose as follows: Group 1 (Control) received 0.1 mL of distilled water per 10 g of body weight; Group 2 (IND) was administered IND solution at 5 mg/kg body weight; and Group 3 (IND-ves) received CHIT-based vesicles containing IND at 5 mg/kg body weight.

The release profile of IND from the lipid vesicles was determined by conducting repeated measurements of the drug concentration in the blood. The mice were anesthetized with 1% isoflurane, and 0.3 mL of blood was collected prior to the administration of IND-ves. Subsequent blood samples were taken at intervals of 15 minutes, 30 minutes, 60 minutes, 90 minutes, 2 hours, 3 hours, 4 hours, 5 hours, 6 hours, and 8 hours post-administration. The concentration of IND released into the bloodstream was analyzed using high-performance liquid chromatography (HPLC) with an Agilent 1100 HPLC system (Santa Clara, CA, United States), employing ultraviolet (UV) absorbance detection at a wavelength of 254 nm.

The somatic pain model utilized in this research involved the hot plate test (Deuis, 2017). The baseline latency, measured before drug injection, was found to be 4.2 ± 0.2 seconds (mean ± standard deviation). A cut-off time of 10 seconds was implemented to prevent any potential tissue damage. The differences observed between experimental and baseline latencies were used as indicators of analgesia; specifically, longer latency times suggested analgesic effects, while shorter latencies indicated hyperalgesia (17,18). Latency times in response to the thermal stimulus applied to the paws were assessed at several points: before the experiment and at 15, 30, 60, 90, and 120 minutes, as well as at 4, 6, and 8 hours after administration of the substances. To quantify the intensity of the antinociceptive effect, the latencies were converted into a percentage of the maximum possible effect (%MPE), calculated using the formula: % MPE = (measured latency – baseline latency) x 100 / (cut-off time – baseline latency) (19). Changes in pain threshold following administration of the lipid vesicles with IND were considered significant when they exceeded 60% MPE; values below this threshold, while statistically notable, raised concerns regarding their biological significance. In this somatic pain model, an extension of reaction time compared to baseline measurements indicated an antinociceptive effect of the substances under study, whereas a decrease in latency suggested a hyperalgesic response to the compounds tested (18).

The protocol followed the guidelines established by the Ethics Commission of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi (Ethical Approval Certificate No. 362/28.11.2023; Project

Authorization No. 69/15.01.2023), in accordance with current European ethical standards (20).

Statistical processing of data. Data were reported as mean values with standard error of the mean (± SEM). Statistical significance was evaluated using *SPSS Statistics for Windows version 13.0,* employing the ANOVA method. A P-value of less than 0.05 was interpreted as statistically significant when compared to the control group.

RESULTS

In our previous studies, we developed novel polymeric vesicles that encapsulate IND, with an average size of 317.6 nm and a polydispersity index of 0.364, demonstrating good suspension stability (Zeta potential of 24 mV). In vitro studies revealed a sustained release of IND from the lipid vesicles, unlike the non-encapsulated drug. Furthermore, the IND-loaded vesicles exhibited excellent in vitro hemocompatibility (16).

We continued the researches investigating the in vivo release profile of IND from carrier systems and evaluating the effects of the IND-loaded lipid vesicles administration on the nociceptive reactivity in mice.

The in vivo kinetic assessing of IND incorporated into IND-ves shows no detectable release of the drug during the initial 15 minutes. At 30 minutes, a small percentage begins to appear, marking the onset of release. By 2 hours, approximately 75% of the total release is achieved. The release reaches its peak at 3 hours, corresponding to 100% of the maximum release (fig. 1). Following this peak, the drug concentration decreases gradually due to metabolism and clearance from the bloodstream. By 6 hours, about 50% of the maximum release remains, dropping further to 16% at 8 hours. After 10 hours, the concentration declines close to baseline, indicating nearly complete clearance of the drug (fig. 1). This release profile highlights a controlled and sustained release of IND over time, demonstrating the efficiency of the nanoparticles in prolonging drug availability compared to free IND.

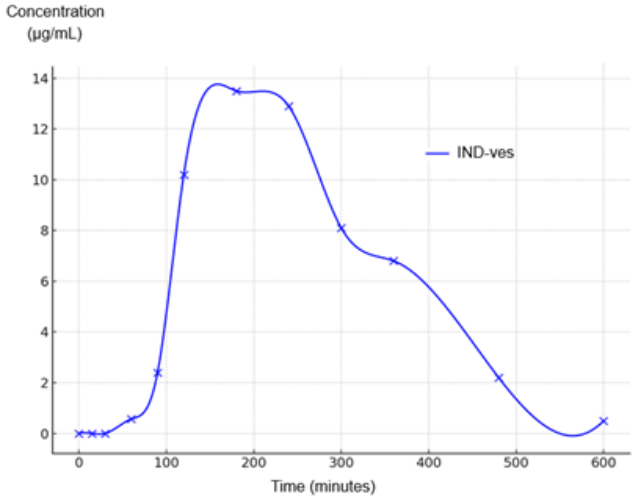

Fig. 1. The *in vivo* kinetic release profile of IND from IND-ves

The statistical analysis of the hot plate test demonstrates that free IND significantly increased the latency time of the animals' response following thermal noxious stimulation. A statistically significant increase was observed between 15 minutes and 90 minutes (**p > 0.01), as well as at 2 hours (*p > 0.05) post-administration (fig. 2). However, the effect did not persist beyond this period, as the response latency rapidly decreased and the antinociceptive effect was entirely lost by 180 minutes (fig. 2). These findings are consistent with previous studies showing the antinociceptive properties of IND in various pain models.

When IND was encapsulated in soft vesicles, it resulted in a notable prolongation of the animals' response latency to experimentally induced pain. The significant effects (**p > 0.01) were observed between 90 minutes and 6 hours, with the response latencies lasting longer compared to free IND. The antinociceptive effect of IND-ves peaked during this period, but the effects diminished after 8 hours.

Free IND significantly increased %MPE during the first 2 hours, with the peak effect observed at 60 minutes post-administration. During the initial hour of the experiment, the effect was notable, exceeding 50% MPE, indicating a submaximal antinociceptive response. However, the effect waned after 120 minutes, with %MPE dropping below 50% (fig. 3).

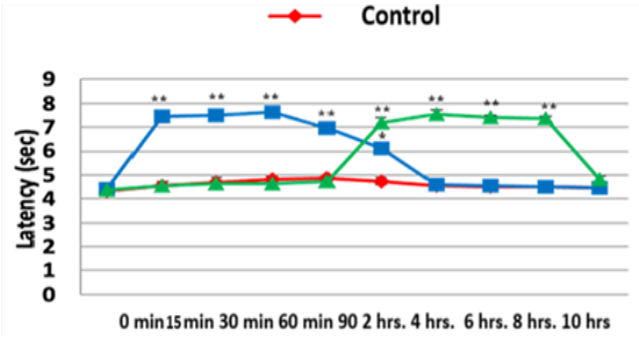

Fig. 2. The latency time response to thermal noxious paw stimulation in hot plate test in mice

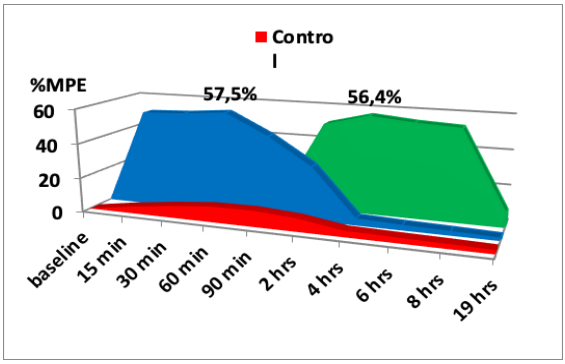

Fig. 3. Quantification of the analgesic activity of IND and IND-ves in hot plate test in mice

In contrast, IND encapsulated in soft vesicles demonstrated a delayed onset of %MPE, becoming evident around 2 hours post-administration. The %MPE exceeded 50% between 120 minutes and 8 hours during the experiment, before abruptly declining thereafter. These results indicate that vesicle-encapsulated IND offers a more prolonged antinociceptive effect compared to the free drug form.

DISCUSSION

The advancement of pharmaceutical nanotechnology expands drug application possibilities, as carrier systems enable the targeted delivery of active compounds to specific tissues, releasing them within an effective concentration range to increase the accumulation of anti-inflammatory agents. In recent years, nanosystems incorporating NSAIDs have shown the ability to enhance anti-inflammatory effects while reducing side effects (21). A diverse array of hybrid nanoparticles with anti-inflammatory agents has been synthesized, characterized, and studied. These nanoparticles act as carriers, loaded with anti-inflammatory drugs to create a controlled delivery system for active substances. Each nanoparticle structure has unique properties that optimize delivery to target tissues (22, 23).

The use of biocompatible and biodegradable polymers is a major advantage, as they allow the carrier to degrade naturally, minimizing toxicity (24, 25). Natural polymers like starch, collagen, alginate, cellulose, chitosan, and chitin are particularly appealing for developing systems to incorporate pharmacologically active agents. Among these, chitosan is a biocompatible, biodegradable, and non-toxic polymer with exceptional properties. It exhibits antibacterial effects, film-forming ability, high mechanical strength, and good thermal stability. Numerous chitosan-based bio nanocomposites with enhanced physical and chemical properties have been developed to create novel drug-loaded nanoformulations (26).

Research has presented numerous innovative approaches for creating IND-loaded systems, including phospholipid conjugates designed to improve IND's transport and controlled release. These methods focus on enhancing the solubility, stability, and release profile of IND within nanoformulations (27). Lipid-based nanocarriers, such as lipid emulsions, leverage phospholipids and surfactants have been developed to improve the solubility and bioavailability of IND (28, 29). Liposomal formulations, incorporating sterically stabilized vesicles with stearyl amines and cholesterol, enhance drug stability and provide sustained release (30).

Other biocompatible carrier systems for modified release of IND were developed by embedding IND within copolymeric networks of poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5]-undecane) and poly (aspartic acid) as a stabilizing colloid, or hyaluronic acid and four variants of poly(itaconic anhydride-co3,9-divinyl-2,4, 8,10-tetraoxaspiro[5.5] undecane) via poly-merization methods (31-33).

Some researchers developed original systems based on indomethacin-loaded microparticles composed of poly(lactic-co-glycolic acid), prepared using solvent evaporation and freeze-drying for enhanced stability (34). Keßler *et al*. prepared amorphous solid dispersions loading indomethacin by embedding the drug into polymer matrices such as poly(vinyl pyrrolidone) or poly(lactic-co-glycolic acid), employing

techniques like hot melt extrusion or solvent evaporation (35). Moreover cyclodextrin-based systems for loading IND were obtained using various techniques, such as solvent evaporation, co-precipitation, or spray-drying, for controlled and targeted drug delivery uses (36-38).

In our researches, the in vivo assessment revealed that IND, when encapsulated in lipid vesicles stabilized with CHIT, exhibited a significantly prolonged and controlled release compared to the direct administration of the drug. The IND-loaded micro-vesicles not only enhanced the duration of the analgesic effect but also extended the overall therapeutic response in the hot plate test, a common method for evaluating pain sensitivity in animals. In particular, the release profile of IND from lipid vesicles, composed of phosphatidylcholine and stabilized with CHIT, showed a sustained and gradual release of the drug over time, which correlated with a longer-lasting antinociceptive effect. This extended analgesic activity was in stark contrast to the rapid onset and quick decline of pain relief observed with the non-encapsulated drug. The onset of the antinociceptive effect of IND-vesicles after a two-hour latency period can be attributed to the release mechanism of IND from the lipid vesicles, which aligns with the in vivo drug release kinetics.

The results demonstrated a clear relationship between the in vivo release kinetics of IND from the lipid-based vesicles and the prolonged pain relief observed in the somatic pain model.

CONCLUSIONS

The administration of IND-loaded lipid vesicles demonstrated a sustained release profile in comparison to direct drug administration. Treatment with the micro-vesicles containing IND resulted in a longer-lasting analgesic effect in the hot plate test, unlike the non-encapsulated drug. A clear correlation was found between the in vivo release of IND from lipid vesicles made of phosphatidylcholine and CHIT, and the extended antinociceptive activity in mice. Overall, our results support the effectiveness of lipid vesicles as carriers for IND, facilitating controlled drug release and prolonged analgesic effects in this somatic pain model.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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DESIGN AND OPTIMIZATION OF CHITOSAN-BASED NANOFIBROUS SCAFFOLD FOR THE DELIVERY OF NATURAL BIOACTIVE COMPOUNDS

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DESIGN AND OPTIMIZATION OF CHITOSAN-BASED NANOFIBROUS SCAFFOLD FOR THE DELIVERY OF NATURAL BIOACTIVE COMPOUNDS (Abstract): The **aim** of this study was to obtain and optimize chitosan (CH)/poly(vinyl alcohol) (PVA)-based nanofibrous scaffold with controlled nanoscale characteristics, by identifying the key electrospinning parameters. The optimized scaffold was subsequently loaded with natural bioactive compounds (ACs). **Materials and methods**: To achieve an optimal polymer composition, different concentrations of each polymer and various CH/PVA ratios (v/v) were investigated until a formulation capable of generating uniform nanofibers (NFs) under experimentally optimized electrospinning conditions was obtained. Based on this optimized matrix six formulations were prepared: CH/PVA@ARG, CH/PVA@ARG-ALA, CH/PVA@ARG-RJ, CH/PVA@ALA, CH/PVA@CUR, and CH/PVA@CUR-ALA, by incorporating L-arginine (ARG), allantoin (ALA), royal jelly (RJ), and curcumin (CUR), known for their antioxidant, antiseptic, and wound-healing properties. Fiber morphology was initially assessed by optical microscopy, followed by detailed analysis using scanning electron microscopy (SEM) to confirm the fibrous architecture and determine the fiber diameter. **Results**: The optimization process yielded uniform, bead-free CH/PVA NFs at a 1:3 ratio and a total polymer concentration of 6.25%. The optimized matrix enabled the incorporating ACs at selected concentrations (10% ARG, 4% ALA, 3% CUR, 3% RJ). For synergistic combinations, concentration adjustments were required to preserve fiber morphology and avoid rheological instabilities associated with cumulative AC loading. **Conclusions**: CH/PVA@ACs NFs with uniform, bead-free fiber morphology suitable for wound healing applications, were prepared. **Keywords**: CHITOSAN, ACTIVE COMPOUNDS, OPTIMIZATION, ELECTRO-SPINNING.

INTRODUCTION

One of the main innovations in the development of advanced wound dressings is the application of nanotechnology. In this context, nanofibers (NFs) (nonwoven materials) synthesized by electrospinning represent one of the most promising and advantageous approaches. These NFs can mimic the three-dimensional (3D) architecture of the extracellular matrix (ECM) and modulate cellular behavior, thereby promoting the cascade of processes involved in tissue regeneration (1). The term wound is a general concept, that describes several types of skin damage or injury. Wounds can be classified according to several criteria, such

as: healing time, etiology, mechanism of injury, complexity, depth, degree of contamination, risk of post-operative infection and wound bed characteristics (2).

An effective wound dressing must satisfy three key requirements: it should be able to absorb exudate, alleviate pain, and protect the wound from secondary infections. In addition, the wound dressings to promote tissue repair and regeneration should have key properties, including antimicrobial activity, antioxidant potential, appropriate mechanical strength and biodegradability (3). Because the traditional gauze dressings have many disadvantages, including traumatic removal and a tendency to dehydrate the wound bed, new advance biomaterials have been developed. The modern dressings are designed to come into direct contact with the wound surface and to actively support tissue regeneration without inducing additional tissue damage. In clinical practice, NFs represent innovative dressings capable of providing controlled release of various active compounds (ACs) with important biological effects on wound healing, such as antioxidant, antibacterial, and immunomodulatory properties. In addition, NFs can maintain a moist, oxygenated, and sterile microenvironment that is essential for accelerated and effective wound healing. Furthermore, the development of composite biomaterials combining natural and synthetic polymers has led to improved biocompatibility, reduced toxicity, and enhanced wound-healing efficacy (4).

Chitosan (CH) is a polysaccharide composed of beta-(1-4)-linked 2-amino-2-deoxy-D-glucose (D-glucosamine) and N-acetyl-2-amino-2-deoxy-D-glucose (N-acetyl-D-glucosamine) units. It is obtained through alkaline deacetylation of chitin, which is extracted from the exoskeletons of arthropods as well as from the cell walls of certain fungi (5). Owing to its favorable physicochemical and biological properties, including non-toxicity, biodegradability, biocompatibility, and intrinsic bioactivity, CH is an important natural polymer for biomedical applications (5). CH exhibits significant hemostatic effects due to interactions between its protonated amino groups (NH_3^+) and the negatively charged membranes of erythrocytes. In addition, CH exhibits analgesic properties by reducing bradykinin levels at the wound site, as well as anti-inflammatory effects, which is attributed to interactions between NH_3^+ groups and wound components, leading to attenuation of the inflammatory response (6). Furthermore, CH possesses antibacterial properties by forming a polymeric film around bacterial cells that inhibits cellular exchanges and nutrient transport (7-11).

Poli(vinyl alcohol) (PVA) is obtained through the free-radical polymerization of vinyl acetate, resulting in polyvinyl acetate (PVAc), followed by partial or complete hydrolysis of the acetate groups along the polymer backbone. During this process, acetate groups are replaced by hydroxyl (-OH) groups, and the degree of hydrolysis strongly influences the solubility, crystalline and physicochemical properties of the resulting PVA (12, 13). Due to its biocompatibility and low toxicity (14), PVA is frequently blended with natural polymers such as CH during electrospinning to produce NFs for tissue engineering applications (15).

L-Arginine (ARG) is an essential α-amino acid component of the urea cycle, a precursor to many biochemical pathways involved in cell physiology. ARG plays a key role in wound healing through two main mechanisms: (i) transformation of

ARG under the influence of arginase, with the obtaining of ornithine, precursor of L-proline, an important amino acid in the process of collagen synthesis; (ii) oxidative deamination of ARG, until obtaining nitric oxide (NO), a bioactive molecule involved in the processes of reepithelialization, cell proliferation, collagen synthesis and angiogenesis (16).

Allantoin (ALA), chemically known as 5-ureidohydantoin, is a natural compound that is widely regarded as safe for topical administration and exhibits low toxicity (17). ALA can be synthesized from urea and is also found biologically as an intermediate in the metabolism of certain plants and microorganisms, as well as a final product of purine catabolism in mammals. In mammals, it is formed via non-enzymatic degradation of uric acid in the absence of uricase, under the influence of reactive oxygen species (ROS) (18,19). ALA exhibits multiple biological effects, including antioxidant properties (20), anti-inflammatory activity, manifested by the reduction of pro-inflammatory cytokines, such as interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) (21), as well as keratolytic, moisturizing, and immunomodulatory properties (22).

Curcumin (CUR), is a polyphenolic complex derived from turmeric (*Curcuma longa L.*) that exhibits a broad spectrum of biological activities (3). Curcumin I is the main component, accounting for 60-70% of the total curcuminoid content, followed by demethoxycurcumin (curcumin II, 20-27%), and bisdemethoxycurcumin (curcumin III, 10-15%) (23). The scientific data indicate that curcumin I is largely responsible for the biological activity of the curcuminoid complex (3). It is widely recognized for its antioxidant, antimicrobial, anti-inflammatory and antidiabetic properties (24, 25).

Royal jelly (RJ) is a highly valued natural product produced by honeybees (*Apis mellifera*), whose composition varies depending on bee species, botanical origin, and seasonal factors. It is primarily composed of water (50-60%), proteins (9-18%), carbohydrates (11-23%) and lipids (3-6%), and also contains vitamins (B complex, C, A, E), essential amino acids, minerals (Ca, Mg, Na, Cu, K, Mn, Fe, Zn), enzymes, hormones, polyphenols, nucleotides, and heterocyclic compounds (26-29). The beneficial effects of RJ in the treatment of skin disorders are largely attributed to its ability to reduce transepidermal water loss and to restore the lipid barrier (30), thereby maintaining an optimal degree of hydration at the wound site, which is essential for the healing process. In addition, RJ exhibits antifungal activity mainly due to royalisin (26), antimicrobial activity mediated by royalisin, jelleins (1-3), and the major RJ protein 4 (MRJP4) (22, 27-31), antibacterial effects associated with 10-hydroxy-2-decenoic acid (10-HDA), major RJ protein 2 (MRJP2), and MRJP4 (21, 22, 26), as well as antioxidant activity primarily attributed to its phenolic compounds and MRJP2 (22, 27).

The objective of this study was to design and optimize a CH/PVA-based nanofibrous matrix for the efficient encapsulation and delivery of the natural ACs such as CUR, ALA, ARG, and RJ.

MATERIALS AND METHODS

Reagents. Chitosan (CH) (medium molecular weight, 75-85% deacetylation grade), polyvinyl alcohol (PVA), 87-89% hydrolyzed, high molecular weight, aver-

age M.W. 88.000-97.000 were purchased from Alfa Aesar; L-arginine (ARG), Allantoin (≥98% purity) (ALA), curcumin (CUR) analytical standard (M.W: 368.38) were purchase from Supelco; pure Royal jelly (RJ) was purchased from Melidava with 1.59 % (wt/wt) 10-Hydroxy-2-decenoic acid; glacial acetic acid (≥ 99.7%) were purchased from Sigma Aldrich, distilled water (DW), tween 80.

Optimization of CH/PVA ratio for nanofibrous scaffold preparation

The optimization of CH and PVA blend ratio was systematically investigated to obtain nanofibrous scaffold with optimal characteristics, including reduced fiber diameter and bead-free structure. CH solutions (1%, 2%) were prepared by dissolving defined amounts of high molecular weight CH in glacial acetic acid, while PVA solutions (3%, 6%, 7%, 8%, 10%) were prepared by dissolving corresponding PVA amounts in deionized water under heating to ensure complete polymer dissolution. The two polymer solutions were subsequently combined in different volume ratios (CH: PVA - 1:1, 1:2, 1:3, 1:8, 2:6, 3:7) under continuous stirring for approximately 20 min, followed by equilibration at room temperature for 30 min to allow for air bubble removal. The resulting mixtures were stirred at room temperature until homogeneous solutions suitable for electrospinning were obtained.

Optimization of CH/PVA/ACs ratio for CH/PVA@ACs NFs preparation

After identifying the optimal CH:PVA ratio, ACs, namely CUR (1%, 2%, 3%, 4%), ALA 1%, 2%, 3%, 4%, 5%), ARG (1%, 3%, 5%, 10%, 15%), and RJ (1%, 2%, 3%, 4%), were incorporated at various concentrations. The resulting mixtures were stirred at room temperature until homogeneous solutions suitable for electrospinning were obtained.

Electrospinning process

The CH/PVA nanofibrous scaffold and CS/PVA@ACs NFs were prepared by electrospinning using a Nanospinner device (Inovenso) equipped with a high-voltage power supply, syringe pump, spinneret, and a grounded collector. The polymers solutions were loaded into a syringe serving as positive electrode, while the collector acted as the counter electrode. Electrospinning parameters, including the applied voltage, flow rate, and needle tip-to-collector distance were systematically optimized in conjunction with the polymer ratio to obtain uniform, bead-free NFs. The collected fibers were deposited on to aluminum foil and dried under room temperature prior to further characterization.

Characterization of the CH/PVA nanofibrous scaffold and CH/PVA@ACs NFs

CH/PVA nanofibrous scaffold and CH/PVA@ACs NFs, including single formulations (CH/PVA@ARG, CH/PVA@ALA, CH/PVA@CUR) as well as combined synergistic systems (CH/PVA@ARG-ALA, CH/PVA@ARG-RJ, CH/PVA@CUR-ALA), were first examined by macroscopic and low-magnification microscopic observation (40X) to verify the presence of continuous fibrous structures. Subsequently, fiber morphology and diameter were analyzed by scanning electron microscopy (SEM, Inspect S, 30 keV) (32). Prior to imaging, the samples were sputter-coated with a thin gold layer to minimize charge accumulation. Fiber diameter distributions were determined using *Phenom Fiber Metric* software, and the results are expressed as mean ± standard deviation (SD) based on three independent measurements (n = 3).

RESULTS

In order to optimize the formulation for the fabrication of CH/PVA nanofibrous scaffold with uniform morphology, bead-free structure, high yield and reduced diameter, a series of processing and formulation parameters were systematically investigated through multiple experimental trials. Specifically, the effect of CH concentration (1%, 2% wt/v), PVA concentration (3%, 6%, 7%, 8%, 10%), CH: PVA polymer ratio (1:1, 1:2, 1:3, 2:6, 3:7, 1:8), applied voltage (13-17 kV), flow rate (0.1-0.3 mL/h), tip-to-collector distance (17-24 cm) and relative humidity (22-64%) were evaluated.

Based on the obtained results (tab. I), the CH/PVA (1:3 v/v) formulation with a total polymer concentration of 6.25%, electrospun at an applied voltage of 17 kV, a flow rate of 0.25 mL/h, and a tip-to-collector distance of 20-24 cm, was identified as the optimal composition. This optimized formulation consistently yielded uniform, bead-free NFs, as confirmed by SEM analysis (fig. 1). The micrographs reveal the successful formation of an interconnected nanofibrous network with smooth fiber surfaces and homogeneous morphology, thereby validating the optimization strategy and establishing this composition as the base matrix for subsequent functionalization using natural ACs.

TABLE I.

Optimization of CH/PVA nanofibrous scaffold preparation

CH % (wt/v)	PVA % (wt/v)	CH: PVA (v/v)	Total polymer conc. (%)	Flow rate (mL/h)	Distance (cm)	Voltage (kV)	Microscopic morphology
1	3	1:1	0.6	0.1	17-22	13	polymeric fragments without a well-defined fibrous network
1	3	1:2	1.2	0.1	17-24	14	-
1	6	1:1	1.2	0.15	17-24	16	-
1	6	1:2	2.6	0.1-0.13	17-22	14	fibers with multiple beads interspersed among otherwise uniform fibers
1	6	3:7	4.3	0.13	17-22	13-15	-
1	7	1:2	3.0	0.13	20-24	14	uniform, bead-free fibers arranged in two layers, but with a reduced fiber production yield
1	8	2:6	5.0	0.3	20-24	17	-
2	8	1:8**	7.4	0.1-0.2	22-24	16	highly crowded fiber morphology
2	10	3:7**	7.6	0.1	18-20	12	-
1*	**8***	**1:3***	**6.25***	**0.25***	**20-24***	**17***	well-structured fibers with increased fiber density and ~ 100 nm diameter

*The parameters considered optimal used for obtaining the CH/PVA nanofibrous scaffold;

** One drop of tween 80 was added

For the electrospinning of the CH/PVA@ACs polymeric solutions, the same ranges of process parameters used for CH/PVA polymeric solution were applied. In this case, the only variable was the concentration of ACs incorporated into the polymer matrix (tab. II). The resulting CH/PVA@ACs nanofibers (NFs) were subsequently characterized by macroscopic and microscopic analyses (fig. 2).

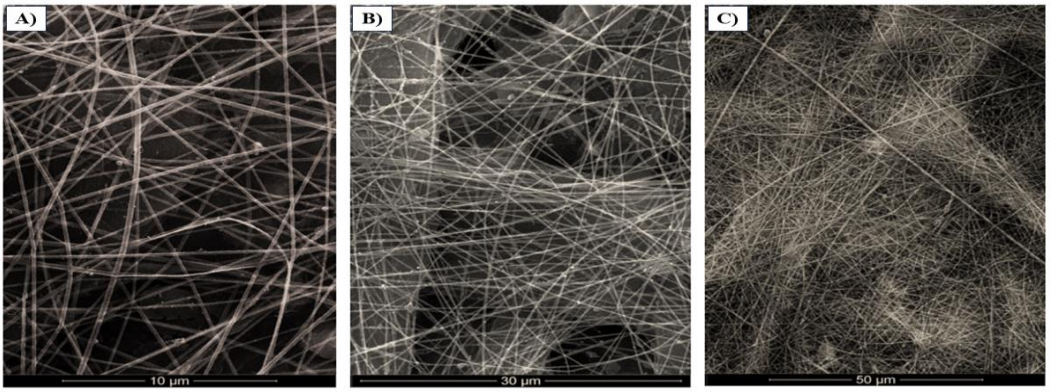

Fig. 1. Representative SEM images of electrospun CH/PVA nanofibrous scaffold at different scale bars: A) 10 μm; B) 30 μm; C) 50 μm

TABLE II.

Optimization of CH/PVA@ACs NFs preparation

CH/PVA@ACs	ACs conc. (wt/v)	Flow rate (mL/h)	Distance (cm)	Voltage (kV)
CH/PVA@ARG	ARG: 1%, 3%, 5%, **10%***, 15%	0.3	22	17
CH/PVA@ALA	ALA: 1%, 2%, 3%, **4%***, 5%	0.4	22	18
CH/PVA@CUR	CUR: 1%, 2%, **3%***, 4%	0.4	24	17
CH/PVA@ARG-ALA	ARG: 10% - ALA: 4% ARG: 10% - ALA: 3% ARG: 5% - ALA: 4% ARG: 5% - ALA: 3% **ARG: 3% - ALA: 3%***	0.4	23	18
CH/PVA@ARG-RJ	ARG: 10% - RJ: 1% ARG: 10% - RJ: 2% **ARG: 10% - RJ: 3%*** ARG: 10% - RJ: 4%	0.3	16	15
CH/PVA@CUR-ALA	CUR: 4% - ALA: 4% CUR: 4% - ALA: 3% **CUR: 3% - ALA: 3%***	0.3	26	16

*The ACs concentration (wt/v) considered optimal for obtaining the CH/PVA@ACs fibers

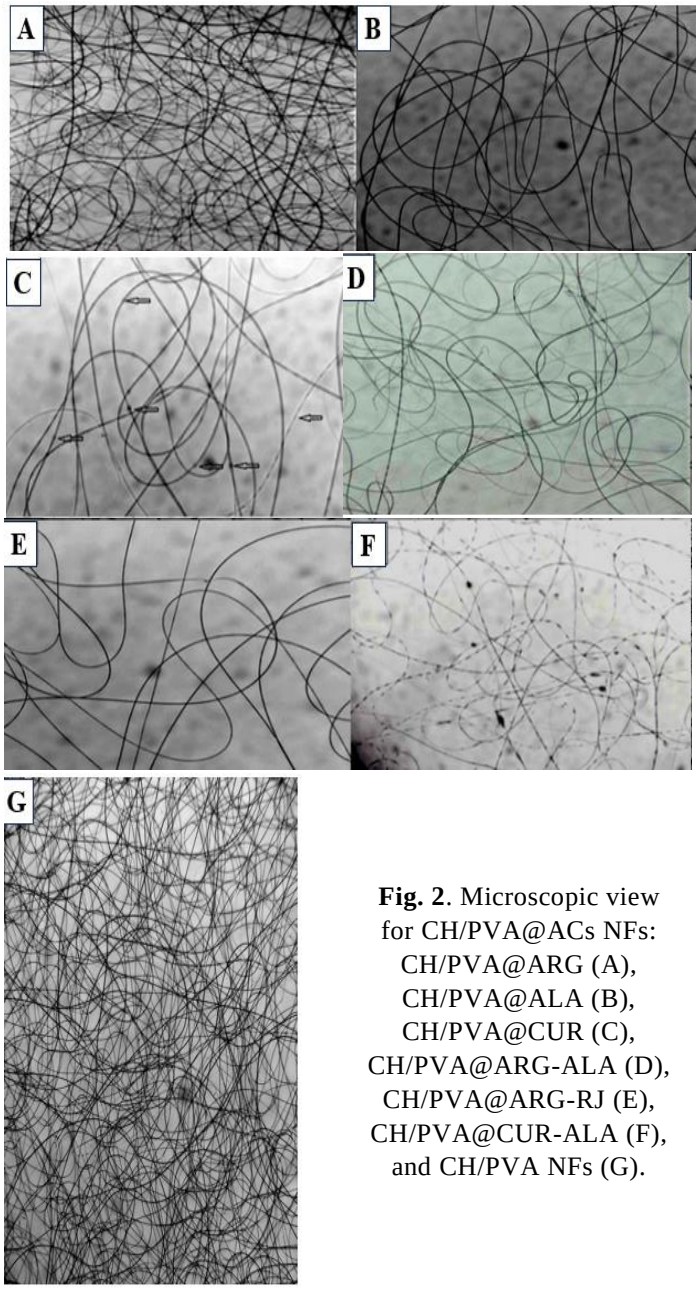

Fig. 2. Microscopic view for CH/PVA@ACs NFs: CH/PVA@ARG (A), CH/PVA@ALA (B), CH/PVA@CUR (C), CH/PVA@ARG-ALA (D), CH/PVA@ARG-RJ (E), CH/PVA@CUR-ALA (F), and CH/PVA NFs (G).

DISCUSSION

Analysis of the experimental data indicates that a minimum total polymer concentration of approximately 5% is required to obtain uniform, small-diameter NFs, above which the nanofibrous production yield increases markedly. Below this threshold, bead-like structures and polymer

fragments were predominantly observed, a behavior attributable to the low viscosity which is insufficient to stabilize the electro spun jet.

A progressive increase in the CH:PVA ratio in favor of PVA significantly improved fibers morphology. This effect can be explained by the formation of hydrogen bonds between PVA and the $-NH_2$, and -OH groups of CH, which reduces surface tension and weakens the strong intramolecular interactions within CH chains. This trend is reflected by the morphological transition from polymer fragments (CH 1% /PVA 3%, 1:1) to continuous fibers (CH 1% /PVA 8%, 1:3). Moreover, the addition of a surfactant (tween 80) further facilitated nanofiber formation by lowering surface tension. The electrospinning process parameters exhibited a complex interdependence. For low-viscosity polymer solutions, increasing the applied voltage led to pronounced jet instability in the electric field. In addition, the optimal flow rate was found to be inversely related to the total polymer concentration, with values of 0.1 mL/h for 7.6%, total polymer and 0.2 mL/h for 7.4% total polymer.

All ACs were successfully incorporated into the polymer matrix at the desired concentrations, requiring only minor adjustments of the operating parameters, which highlights the versatility and robustness of the optimized CH/PVA base formulation. For synergistic combinations, AC concentrations were selected based on individually optimized values, while considering the maximum loading capacity of the CH/PVA matrix and the need to preserve stable electrospinning conditions. Simultaneous loading of ACs at their individual maximum concentration led to cumulative rheological disruptions, resulting in process instability.

The ARG concentration was therefore fixed at 10%, since preliminary experiments showed that increasing it to 15% caused excessive solution conductivity, which destabilized the electrospinning jet and promoted droplet formation instead of continuous fibers. In addition, bead formation and surface defects were observed, while enhanced electrostatic interactions between positively charged ARG and protonated CH induced visible solution inhomogeneities, necessitating extensive re-optimization of the process parameters. The combination of 10% ARG with 3% RJ was successfully achieved, with RJ being homogeneously incorporated into the polymer matrix without altering the optimized electrospinning parameters. Increasing the RJ concentration above 3% led to significant changes in the rheological properties, mainly due to a marked increase in viscosity associated with the molecular complexity and variability of RJ, which destabilized the jet and resulted in non-uniform fibers.

For ALA, the concentration was limited to 4%, as higher amounts revealed its limited solubility in the CH/PVA system, leading to presence of undissolved particles that blocked the electrospinning needle. Moreover, the associated viscosity changes disrupted the electrospinning parameters, resulting non-uniform fibers with morphological defects. Although increasing the temperature could potentially improve ALA solubility, this approach would have induced additional instability into the system. Consequently, the combination of 3% ARG with 3% ALA was selected in order to preserve the optimized electrospinning parameters.

The CUR concentration was limited to 3% due to its hydrophobic nature, which

led to suspension formation at higher loadings, even in the presence of DMSO. Furthermore, the crystalline character of CUR interfered with jet stability and resulted in non-uniform fibers morphologies. Attempts to maintain the same ALA concentration used in the individual formulation within the synergistic CUR-containing systems were unsuccessful, as no stable electrospinning system could be achieved and defective fibers were obtained

CONCLUSIONS

The optimization of CH/PVA nanofibrous scaffold and the establishment of optimal ACs concentrations, both in individual and synergistic combinations, through balancing of solution rheological properties and electrospinning process stability, enabled the fabrication of nanofibers with the desired characteristics. This approach successfully addressed challenges related to limited solubility, viscosity variations, and cumulative rheological disruptions, while preserving the bioactivity of the incorporated natural compounds and ensuring reproducible fiber morphology.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest. The research was supported by a grant of the Ministry of Education and Research CCCDI-UEFISCDI, project no. PN-IV-PCB-RO-MD-2024-0246, within PNCDI IV.

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ASSESSMENT OF ROOT CANAL OBTURATION AND THE ADHESION OF ENDODONTIC SEALERS TO THE DENTIN WALLS OF THE ROOT

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ASSESSMENT OF ROOT CANAL OBTURATION AND THE ADHESION OF ENDODONTIC SEALERS TO THE DENTIN WALLS OF THE ROOT (Abstract)**:** This study aims to compare the quality of root canal obturation achieved using different obturation techniques and to evaluate the adaptation of the interface between epoxy resin- based sealants and gutta-percha to the dentin walls of the root canal. **Materials and methods:** Thirty-six extracted single-rooted human teeth were used, prepared according to a standardized protocol and filled with gutta-percha using different techniques (lateral condensation and vertical hot condensation), to evaluate, through radiological analysis (retro-dento-alveolar radiography) and optical microscopy, the following aspects: the quality and homogeneity of the root fillings, the adhesion of the sealer to the dentin root walls, and the apical and coronal seal of the endodontic filling. The data were statistically analyzed to identify differences between techniques ($p < 0.05$), using the F-test and ANOVA. **Results:** Radiographic analysis revealed significant differences in the homogeneity of the filling and the presence of voids between techniques, with vertical hot condensation yielding the best results, particularly in the apical third. Optical microscopy confirmed better adaptation of the sealants in the case of thermoplasticized gutta-percha techniques, while lateral condensation showed a higher incidence of marginal voids at the apical level. **Conclusions:** The obturation technique significantly influences the quality of root canal obturation. Vertical hot condensation ensures homogeneity and optimal adaptation. Combined radiographic and optical microscopy evaluation provides a comprehensive method for assessing obturation results. **Keywords:** ROOT CANAL FILLING**,** THERMOPLASTICIZED GUTTA-PERCHA, ROOT DENTIN**,** OPTICAL MICROSCOPY.

INTRODUCTION

Filling materials and techniques that facilitate the effective penetration of the sealer and gutta-percha (GP) into the complex endodontic canal system are considered effective methods for preventing microleakage and subsequent treatment failure (1). Proper root canal obturation can ensure complete sealing to prevent communication between the endodontic space and the periapical tissue (2). The quality of root canal obturations (length, homogeneity, taper) has been shown to be a risk factor for apical periodontitis following evaluations of root-canal-treated teeth, whether treated for pulpitis or prosthetic pulpectomies, or for pulp necrosis (3). The presence of carious lesions adjacent to coronal resto-

rations has been shown to be a risk factor for apical periodontitis in root-filled teeth that had previously undergone bio pulpectomy, with this condition being linked to both the patient's caries risk and poor coronal marginal seal (4).

The purpose of this study is to conduct an experimental *in vitro* comparative study to evaluate the efficacy of two root canal filling techniques - the cold lateral condensation technique and the thermoplastic gutta-percha injection technique - in order to highlight, through radiological analysis (retro-dento-alveolar radiography) and optical microscopy, the following aspects: the quality and homogeneity of root canal fillings, the adhesion of the filling material to the dentin walls of the root canal, and the apical and coronal seal of the endodontic filling

MATERIALS AND METHODS

Sample preparation. The study was conducted in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Grigore T. Popa University of Medicine and Pharmacy in Iași (334/16/07/2023). To calculate sample sizes, we used the G*Power program (Heinrich-Heine University, Düsseldorf, Germany). We used an effect size of 0.5, considered a large effect according to Cohen's classification, a significance level of 0.05, and a power of 0.8. The results obtained indicate the need for a minimum total of 42 samples. Therefore, to obtain statistically relevant results, we will use a minimum of 14 samples for each study Lot.

Forty-two single-rooted teeth, free of carious lesions, extracted for orthodontic or periodontal purposes, were selected upon the recommendation of specialist physicians. The inclusion criteria used were as follows: single-rooted teeth, free of carious lesions, extracted for orthodontic or periodontal purposes, upon the recommendation of specialists, from patients aged between 18 and 40 years. Exclusion criteria: devitalized teeth, teeth with calcifications, internal or external resorption, incompletely formed apices, as observed clinically and radiologically.

The impacted teeth were divided into three lots (n=14) as follows:

Lot I: Teeth treated with endodontic filling using the cold lateral condensation technique with standardized gutta-percha points having a 2% taper (Gutta Percha Points, 0.02, Meta Biomed Europe GmbH, Mulheim an der Ruhr, Germany) and an epoxy resin-based endodontic sealer (Adseal Plus, Meta Biomed Europe GmbH, Mulheim an der Ruhr, Germany).

Lot II: Teeth endodontically treated using the hot vertical condensation technique with standardized gutta-percha points having a 4% taper (Gutta Percha Points, 0.04, Meta Biomed Europe GmbH, Mulheim an der Ruhr, Germany), the endodontic sealer (Adseal Plus, Meta Biomed Europe GmbH, Mulheim an der Ruhr, Germany), the heated plugger (Op-FILL, China), in combination with thermoplasticized gutta-percha using a device (Fast-Fill, Eighteeth, Nuremberg, Germany) to inject the gutta-percha in its plastic form.

Lot III (Control Lot): Anterior teeth with root canal fillings performed using various condensation techniques (monocon technique).

For each study Lot, new sets of rotary files (Orodeka Plex – V) were used, adapted to the motor (Endo-Radar, Wodpacker) using the crown-down technique. The teeth were shaped for root canal treatment using 5.25% NaOCl – 5 mL (CHLORAXID 5.25% – 400 mL) and a

sonic endoactivator (EA) (Endoactivator, Maillerfer, Dentsply Sirona, Germany), with a 15/0.2-sized tip (Refill Type, Maillerfer, Dentsply Sirona, Germany) for 60 seconds. Final irrigation with 20% EDTA solution – 5 mL (20% liquid EDTA – 20 mL) – 1 min. The irrigation protocol was the same for all study Lots. Subsequently, they were endodontically obturated using the cold lateral condensation technique and the hot vertical condensation technique in combination with thermoplasticized gutta-percha (Fast-Fill, Eighteeth, Nuremberg, Germany) in combination with an epoxy resin-based endodontic sealer (Adseal Plus, Meta Biomed Europe GmbH, Mülheim an der Ruhr, Germany). The final stage of specimen preparation was to perform the coronal filling using a resin-modified glass ionomer cement-based material (Ionofeel-F, Flow, Schulzer, Hanau, Germany) and a light-cured composite (Charisma, Kulzer GmbH, Hanau, Germany) in shade A3, as the final coronal restoration.

Radiological evaluation. Postoperatively, 2D radiographs were taken (using a Carestream CS 2200 machine, Kodak, Carestream Health, Inc., NY, USA) with the following parameters: 0.071 mGy, 7 mA, 70 kV, from two angles (vestibulo-oral and mesio-distal) to determine whether the root filling was tight and homogeneous, both apically and coronally.

TABLE I.

Parameters and scores for evaluating the quality of root fillings

Parameters	Criteria	Description	Score
Density	Appropriate	Absence of fill material in the root filling or between the root filling and the walls of the root canal	**0**
	Weak	Presence of fill material in the root filling or between the root filling and the walls of the root canal	**1**
Length	Appropriate	The root filling extends to within ≤ 2 mm of the radiographic apex	**0**
	Weak	The root filling extends beyond the radiographic apex or is more than 2 mm from the radiographic apex	**1**
Conicity	Appropriate	Conical and continuous root canal preparation, from the canal entrance to the apex, with the cross-sectional diameter becoming narrower at each point toward the apex.	**0**
	Weak	Inconsistent conicity from the canal entrance to the apical part of the filling or root filling deviated from the original canal	**1**

The analysis of endodontic fillings was performed according to the criteria for evaluating the quality of root canal fillings (Nur *et al*., 2014; El Ouarti *et al*., 2021), assigning scores of 0 or 1 based on the identified criteria (tab. I). Regarding coronal seal, scores of 0 (sealed coronal filling, absence of gaps between the filling and the coronal walls) and scores of 1 (coronal filling with gaps between the filling and the coronal walls) were assigned.

Microscopic analysis. These teeth were sectioned transversely at the amelocemental junction and longitudinally under water jet cooling using a 0.17 × 22 mm diamond disc (Flexible Double Side, BesQual Diamond Disc, Meta Dental), mounted on a straight handpiece, at

20,000 revolutions per minute. This diamond disc was used for both sectioning and finishing the specimen, which was then analyzed using a Zeiss Imager microscope (a1M Carl-Zeiss, Jena, Germany) equipped with an Axiocam digital camera. The images were obtained using AxionVisionRelease 4.7.1 software. A magnification of 50× was used in dark field (DF) mode. Measurement of the marginal gaps was performed using the 500/1000 µm scale provided by the Axion Vision Release software.

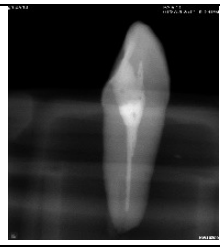
Statistical analysis. To analyze the parameters, the F-test was used—calculating the difference in mean and intra Lot standard deviation; this technique is an extension of the Student's t-test applied to the means of two or more Lots. After applying the ANOVA test, a Bonferroni correction (post-hoc Bonferroni) was performed. This correction reduces the error rate when testing multiple hypotheses.

RESULTS

Qualitative results-radiological images. Table II presents radiographic images of the analyzed samples from various angles and an evaluation of root canal obturation based on specific criteria.

TABLE II.

Radiographic images of the specimens obtained at different angles and evaluation of root canal obturation based on specific criteria.

Study Lots	Vestibulo-oral incidence	Evaluation criteria and scores	Meso-distal incidence	Evaluation criteria and scores
LOT I Tooth 4.4		Density **score 0** Length **score 0** Conicity **score 0** Coronal sealing **score 0**		Density **score 1** Length **score 0** Conicity **score 0** Coronal sealing **score 0**
LOT II		Density **score 0** Length **score 0** Conicity **score 0** Coronal sealing **score 0**		Density **score 0** Lenght **score 0** Conicity **score 0** Coronal sealing **score 0**
LOT III		Density **score 0** Lenght **score 1** Conicity **score 1** Coronal sealing **score 0**		Density **score 1** Lenght **score 1** Conicity **score 1** Coronal sealing **score 0**

Regarding Lot I, where the cold lateral condensation technique was applied to tooth 4.4, a compact, homogeneous, and tight root filling can be observed from the vestibulo-oral view, with a score of 0 obtained for all evaluated criteria. However, the mesio-distal view reveals the presence of air voids in the root filling, between the root filling and the root canal walls (density scored as 1), while the other criteria (length, taper, and coronal seal) scored 0. Thus, in the case of Lot II, tooth 2.2, where root canal obturation was performed using the vertical technique and thermoplasticized gutta-percha injection, the vestibulo-oral view reveals a tight, homogeneous filling throughout the working length, with evaluation criteria such as density, length, and taper receiving scores of 0. Regarding coronal sealing, the coronal filling is tight against the coronal walls without air gaps, resulting in a score of 0. From the mesio-distal view, a root and crown filling are thus observed that is tight and adequately compact, with scores of 0 for all evaluated criteria. In contrast, following the analysis from the vestibulo-oral and mesio-distal views of tooth 2.1, Lot III (already obturated using a monocom condensation technique), a non-homogeneous root obturation is observed, with the presence of air pockets along the entire length of the root canal (score 1), inconsistent taper from the canal entrance to the apical portion of the filling (score 1), the root canal filling ends approximately 2 mm from the radiographic apex (score 1), although the coronal seal has a score of 0.

Optical microscopy results. Analysis of the images obtained via optical microscopy (tab. III) highlights the level of adaptation between the coronal and root canal fillings, with a tight seal of the root canal filling at the coronal level achieved for all three Lots. Additionally, representative images for each Lot studied can be observed in the three areas of the root canals. Thus, for the sample from Lot I, there is adequate marginal adaptation of the endocanal filling at the level of each analyzed root third; however, large amounts of sealer are observed between the gutta-percha cones, indicating insufficient condensation of the cones, although a tight apical seal is achieved in the apical third. At the same time, in the sample from Lot II, good adaptation to the dentin walls is observed in the three analyzed zones, with a compact mass of gutta-percha and less sealer noted on the root walls and within the gutta-percha mass. For the samples in Lot III, a loss of integrity of the root canal filling is observed, especially in the middle third, with insufficient adaptation at the dentin walls, and air voids present in the gutta-percha mass. In the apical third, an enlargement of the apical orifice can be observed, with the apical closure being achieved more with sealer than with solid gutta-percha, posing an increased risk of microleakage.

To assess the marginal adaptation of the filling material to the root walls, we used the 500 µm or 1000 µm scale provided by the AxionVisionRelease 4.7.1 software.

Statistical results. The statistical results obtained indicate a statistical analysis between Lots based on the root third; thus, the paired comparison test values for the crown third (tab. IV) indicate significant differences between Lot II and Lot I ($p = 0.033$) and between Lot II and Lot III ($p = 0.017$), suggesting notable variations

among these Lots. The comparison between Lot I and Lot III is not statistically significant (p = 1.000), indicating similarity between these two Lots. Adjusting the p-values using the Bonferroni correction confirms the robustness of these conclusions.

For the median third (tab. V), significant differences are observed only between Lot II and Lot III (p = 0.007). The other comparisons (Lot II *vs.* Lot I and Lot I *vs.* Lot III) do not reach the adjusted significance threshold (p > 0.05). These results suggest that only Lot II shows a distinct variation from Lot III, while Lots I and III are relatively homogeneous.

TABLE III.

Representative images of a sample from each study Lot, obtained by optical microcopy

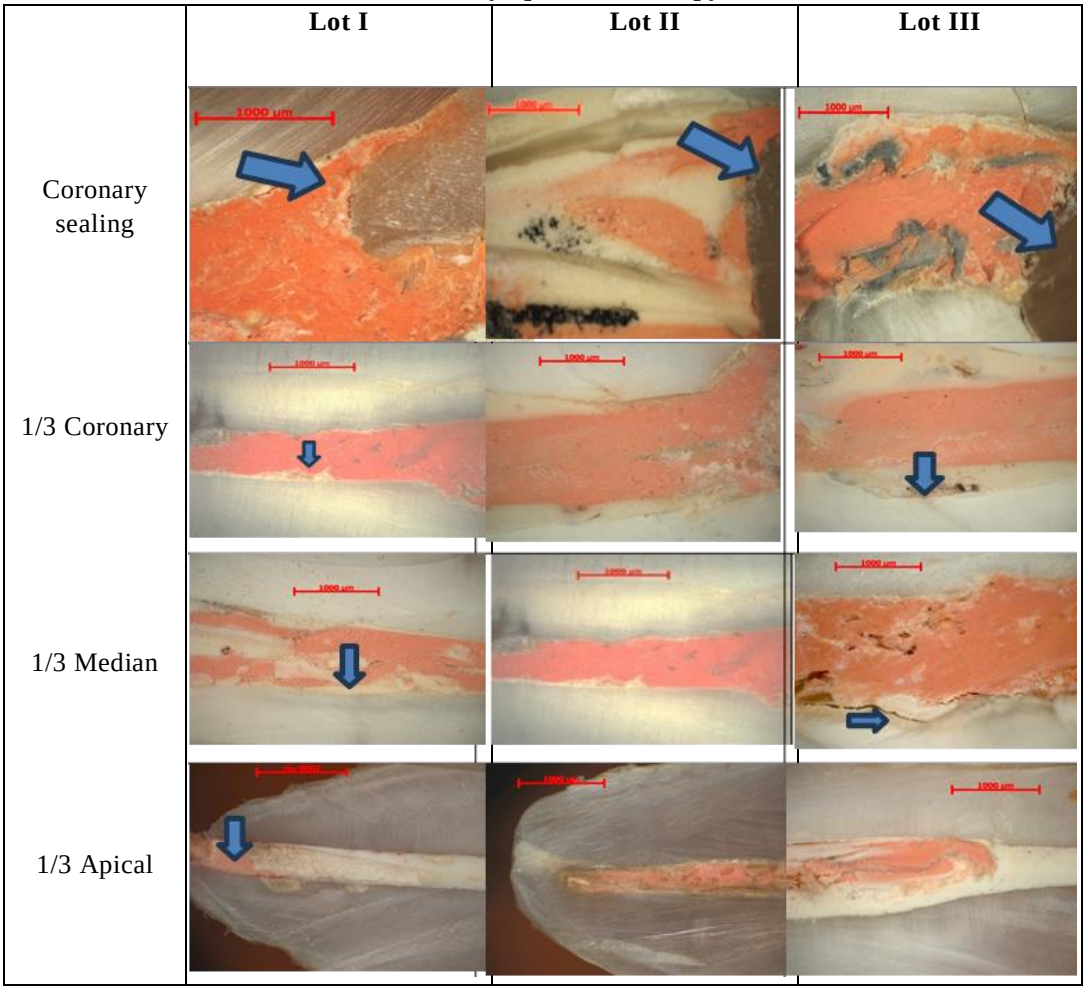


TABLE IV.

Paired comparison of Lots for the third coronary using the Mann-Whitney test with Bonferroni correction

Pairwise Comparisons of Lot					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Lot II- Lot I	7.200	2.828	2.546	.011	.033
Lot II- Lot III	-7.800	2.828	-2.758	.006	.017
Lot I- Lot III	-.600	2.828	-.212	.832	1.000

TABLE V.

Paired comparison of Lots for the median third using the Mann-Whitney test with Bonferroni correction

Pairwise Comparisons of Lot					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Lot II- Lot I	5.200	2.828	1.838	.066	.198
Lot II- Lot III	-8.600	2.828	-3.041	.002	.007
Lot I- Lot III	-3.400	2.828	-1.202	.229	.688

The analysis of the apical third (tab. VI) reveals a significant difference between Lot II and Lot III ($p = 0.002$), suggesting a clear distinction between these two Lots. Although the comparison between Lot II and Lot I approach significance ($p = 0.169$), it does not reach the 0.05 threshold. Similarly, the difference between Lot I and Lot III is not statistically significant ($p = 0.413$). These results indicate a more pronounced variation in Lot II, which differs from Lot III.

TABLE VI.

Paired comparison of Lots for the apical third using the Mann-Whitney test with Bonferroni correction

Pairwise Comparisons of Lot					
Sample 1-Sample 2	Test Statistic	Std. Error	Std. Test Statistic	Sig.	Adj. Sig.[a]
Lot II- Lot I	5.400	2.828	1.909	.056	.169
Lot II- Lot III	-9.600	2.828	-3.394	<.001	.002
Lot I- Lot III	-4.200	2.828	-1.485	.138	.413

Regarding the analysis of the degree of adaptation between the root-canal filling and the crown, the mean results obtained from the evaluation of radiographic scores and from the measurement of voids using a micrometer scale on optical microscopy images, as well as their standard deviations, are presented in table VII.

TABLE VII.

Mean values and standard deviations of radiological scores, and mean values of the voids measured using a micrometer scale on optical microscopy mages

	Lot I	Lot II	Lot III	p-value
Median (radiological scores)	0.70±0.47	0.55±0.21	0.80±0.41	0.232
Median (μm)	86±12	61±19	105±	0.175

The values presented in table VII show that, for both measurement methods, the lowest mean values were recorded in Lot II, followed in ascending order by the values obtained in Lot I and, finally, Lot III. Although the values varied across the Lots, statistical analysis using the parametric ANOVA One-Way test revealed the absence of any statistically significant differences between them.

DISCUSSION

The quality of root canal obturation certainly depends on the technique used. The results of our study showed that the vertical warm condensation technique with thermoplasticized gutta-percha ensures better adaptation of the material to the canal walls, especially in the apical third, compared to cold lateral condensation, which exhibited larger marginal voids and a more uneven distribution of gutta-percha. These observations are consistent with the literature, which reports difficulties in achieving a uniform filling in the apical area when using lateral or cold condensation techniques (5-8).

Microscopic and radiographic evaluations have shown that the three-dimensional adaptation of thermoplasticized gutta-percha reduces the risk of microleakage and improves the seal of the filling. Although epoxy resin-based endodontic sealants contribute to achieving root canal seal, some studies have shown that the use of the warm condensation technique improves the homogeneity of the root canal filling, with similar results observed in this study as well, particularly in the apical third. These observations suggest that optimal sealing of root canals depends not only on the materials used but also on the technique of compacting and adapting the gutta-percha, a finding confirmed by data from the specialized literature (9, 10).

Clinically, choosing an effective root-filling technique is essential for preventing the entry of microorganisms into the endodontic system and, consequently, for reducing the risk of reinfection and the occurrence of long-term failures (11). The control lot, which included samples obturated using various techniques, showed the greatest variability in results, supporting the importance of standardizing the procedure for predictable success. The statistical results indicate significant variability among the Lots studied, with Lot III exhibiting the largest marginal gaps and Lot II the smallest, confirming the superiority of the hot-compaction technique in terms of root canal obturation sealing. This trend of

increasing marginal gaps from the coronal to the apical third is consistent with the observations of other studies (12-14).

In addition to apical sealing, a crucial

step in endodontic treatment is coronal sealing, which prevents microorganisms from entering the already obturated canals. This step can be achieved by using a glass ionomer cement as the base material for the coronal obturation, thereby contributing to the long-term success of endodontic treatment (15). In our study, coronal sealing was performed using a composite restoration consisting of a base filling with a resin-modified glass ionomer cement (Ionofeel F) and a light-cured composite (Charisma) for Lots I and II, providing a tight coronal seal for all samples, an aspect observed radiographically and via optical microscopy. The success of root canal obturation depends not only on the technique used but also on the methods of debridement and disinfection of the root canals; thus, procedures for cleaning and decontaminating the canals are essential for ensuring an optimal environment for obturation, preventing the subsequent development of periapical infections (16, 17). In the study, an identical irrigation protocol was applied for Lots I and II and consisted of using 5 mL of 5.25% NaOCl, sonic endoactivation for 60 seconds to enhance the irrigate, followed by a final irrigation with 5 mL of 20% EDTA solution for 1 minute per irrigation. The 1-minute duration is necessary for the irrigants to soften the dentin walls. However, uneven surfaces could promote bacterial colonization and biofilm formation (18, 19).

Furthermore, in the root canal preparation process, the shaping technique used plays a very important role in ensuring an effective endodontic obturation. In the present study, the crown-down technique was used for canal shaping, employing rotary files adapted to an endomotor. This allows for progressive cleaning from the coronal to the apical direction, facilitating the removal of dentin debris and microorganisms (20, 21).

Limitations of the study include it's in vitro nature, which does not fully replicate clinical conditions (masticatory pressure, saliva, biological tissue response), the sample size, and the exclusive use of optical microscopy for evaluation. The integration of additional methods, such as SEM or micro-CT, could provide more detailed information about the fit of the filling materials (22, 23, 24).

Furthermore, the study's results confirm that the vertical hot condensation technique is superior to the lateral cold condensation technique in terms of the adaptation and seal of the root canal filling, reducing the risk of microleakage and ensuring a homogeneous three-dimensional filling. The choice of compaction method must be guided by the characteristics of the root canal and the need to achieve optimal seal integrity, which is essential for the long-term success of endodontic treatment.

CONCLUSIONS

Vertical hot condensation with thermoplasticized gutta-percha provided superior sealing and a better fit to the root walls, particularly in the apical third, reducing the risk of microleakage. The cold lateral condensation resulted in larger marginal voids and uneven distribution of gutta-percha, indicating an increased risk of microleakage. The size of marginal distribution increased progressively from the coronal to the apical third, suggesting greater difficulties in apical sealing. The coronal sealing was achieved through coronal sealing with adhesive materials (resin - modified glass ionomer cement and light - cured composite), providing an effective seal confirmed

radiographically and microscopically. Vertical hot condensation, using thermoplasticized gutta-percha and an epoxy resin-based sealer, is the optimal method for the long-term success of endodontic treatment. Microscopic and statistical results underscore the necessity of thermoplastic techniques for achieving homogeneous and airtight three-dimensional obturations.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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EXPERIMENTAL EVALUATION OF THE ACTION OF ETHEPHON AND SODIUM SELENITE ON SOME HEMATOLOGICAL PARAMETERS IN RATS

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EXPERIMENTAL EVALUATION OF THE ACTION OF ETHEPHON AND SODIUM SELENITE ON SOME HEMATOLOGICAL PARAMETERS IN RATS **(**Abstract): Ethephon (2-chloroethylphosphonic acid) is one of the most intensely used plant growth regulators. **Aim**: Assessment of hematological parameters during subacute intoxication with ethephon (200 mg/kg bw/day) in rats and evaluation of the potential protective effect of selenium (1 mg/kg bw/day) (administered as sodium selenite) in rats during the experimental intoxication by oral route with high doses of ethephon (200 mg/kg bw/day). **Material and methods:** Male Wistar rats were randomly assigned to four groups: Control group, Ethephon group (200 mg ethephon/kg bw/day, 14 days), Selenium group (1 mg Se/kg bw/day, 14 days), Selenium+ethephon group (200 mg ethephon/kg bw/day and 1 mg Se/kg bw/day). Blood samples were collected on the 15th day and a complete blood count was conducted by using an automated hematology analyzer. **Results**: Mean red blood cell count was $7.06x10^6$/µl in the Ethephon group and $7.18x10^6$/µl in the Selenium group, as compared to $7.16x10^6$/µl in the Control group. Platelet count decreased from $1028.66x10^3$/µl in the Control group to $999.66x10^3$/µl in the Ethephon group. Total leukocyte count was $7.82x10^3$/µl in the Ethephon group and $8.40x10^3$/µl in the Selenium+ethephon group, as compared to a mean of $8.07x10^3$/µl in the Control group. **Conclusions**: The modification of some of the hematological parameters in the groups to which ethephon was administered can be suggestive of the harmful effect of this substance (which is present as a residue in food products) on consumer health. Orally administered selenium (as sodium selenite) at a dose of 1 mg Se/kg bw, seems to have acted as a toxic and not as a protective agent during the intoxication with ethephon. **Keywords**: ETHEPHON, PLANT GROWTH REGULATOR, SELENIUM, HEMATOLOGICAL PARAMETER.

Ethephon (2-chloroethylphosphonic acid) (fig. 1) is one of the most intensely used plant growth regulators, due to its wide action spectrum (1).

In pomiculture and vegetable growing, ethephon can be used to hurry up flower formation, ripening or coloring of fruits, etc. (2, 3). Ethephon is also used as a treatment for cereals, to increase their mechanical resistance, by shortening and

hardening the straw (4). In some countries, ethephon is extensively used for post-harvest acceleration of ripening of bananas and mango fruits (5).

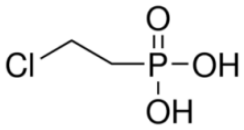

Fig. 1. Chemical structure of ethephon

In practice, ethephon is mainly formulated as solutions in concentrations between 20 and 720 g/L and is marketed under multiple brands, which correspond to specific uses. Formulations which have ethephon as active substance are registered in more than 60 countries worldwide (6, 7). The mechanism of action is because it is easily absorbed by plants and transformed in ethylene (the main metabolite), which is a natural hormone for plants (8).

In case of oral administration to laboratory animals (rats and mice), ethephon can induce digestive manifestations (gastroenteritis, gastric necrosis), respiratory and renal diseases, cardiac fibrosis, brain damage (mineralization processes) and liver damage (necrotic hepatitis). Ethephon is also teratogenic (9, 10). Other authors (11, 12) reported for ethephon direct cholinergic effects and indirect effects on the contraction of intestinal musculature. El-Okazy (13) demonstrated that ethephon perturbs some hematological and biochemical parameters in laboratory animals.

Selenium is an important biological antioxidant, because it is a constituent of glutathione peroxidase, the selenoenzyme that catalyzes the reduction of lipid peroxides and hydrogen peroxide, thus preventing the harmful effects of lipid peroxidation and protecting erythrocytes from hemolysis; in addition, selenium protects cells and cell membranes from oxidative processes (14, 15, 16).

The aim of this study was: a) to assess the hematological parameters during subacute ethephon poisoning (200 mg/kg bw/day) in rats and b) to evaluate the potential protective effect of selenium (1 mg/kg bw/day, administered as sodium selenite) in rats during experimental poisoning by oral route with high ethephon doses (200 mg/kg bw/day).

MATERIAL AND METHODS

Sodium selenite (purity 99%; CAS 10102-18-8) and ethephon (purity 96%; CAS 16672-87-0) were purchased from Sigma Aldrich. All other reagents were of analytical grade.

The animal study protocol was approved by the Research ethics committee of "Grigore T. Popa" University of Medicine and Pharmacy Iaşi, Romania. All procedures were conducted according to the 86/609/EEC directive and internal regulations of the university.

Male Wistar rats purchased from the "Cantacuzino" National Institute for Research and Development in Microbiology and Immunology were used. The animals were housed in collective cages, in relative constant environmental conditions (temperature 18-22°C; light-dark cycle: 12 hours/12 hours) and were given standard feed for rats and water *ad libitum*.

Rats, weighing 180-230 g, were randomly assigned to 4 groups of 6 animals each, to which the following treatments were given by gavage after 12 hours of fasting: Control group (C) - physiological saline; Ethephon group (ETH) - ethephon 200 mg/kg bw/day as aqueous solution; Selenium+ethephon group (SE+ETH) -

ethephon 200 mg/kg bw/day as aqueous solution and selenium 1 mg/kg bw/day as sodium selenite aqueous solution; Selenium group (SE) - selenium 1 mg/kg bw/day as sodium selenite aqueous solution.

All animals were weighed again on the 15th day, after which they were anesthetized with intraperitoneally administered ketamine (100 mg/kg bw, as injectable solution for veterinary use). Blood samples were collected in vacutainers containing anticoagulant (K_2EDTA). A complete blood count was performed by using a Sysmex XT 1800 (Sysmex Corporation, USA) automated hematology analyzer. The experimental data were processed by using *Microsoft Excel* 2013 with Data analysis add-on. A paired Student's t-test was used ($p < 0.05$ was considered significant).

RESULTS AND DISCUSSION

The results obtained for the erythrocyte count (RBC) (fig. 2) show that in animals treated with ethephon the mean RBC was slightly lower ($p = 0.398$) than in the Control group (C). An insignificant increase in mean RBC was seen in the Selenium group when compared to the Control group ($p = 0.882$). At the same time, in the Selenium+ethephon group mean RBC was slightly higher than that recorded in the Ethephon group ($p = 0.611$), but much lower than in the Control group ($p = 0.830$).

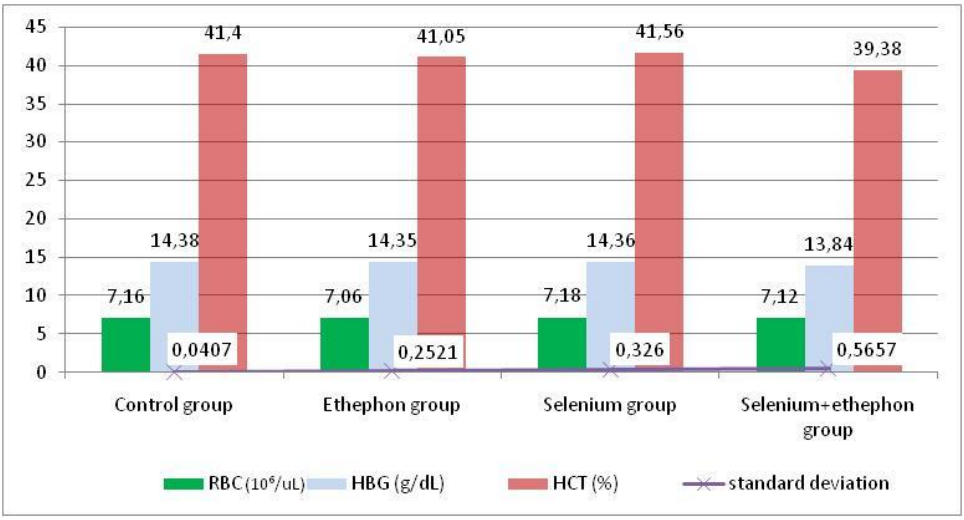

Fig. 2. Mean levels of red blood cell count (RBC), hemoglobin concentration (HBG) and hematocrit (HCT)

Hemoglobin concentration (HBG) and hematocrit (HCT) are parameters on which the investigation related to the quality of erythropoiesis is based on. Thus, mean HBG level (fig. 2) for the group treated with ethephon was almost like that for the control group ($p=0.923$). After comparing the levels recorded for the group treated with selenium and ethephon, for which the mean value was 13.84 g/dL, with those obtained for the group treated with selenium alone (mean value 14.36 g/dL) a slight decrease in mean HBG was noticed ($p= 0.451$). The results obtained from this experimental study agree

with those reported by Al-Fartosi (16), who highlighted a decrease in HBG in the animal groups treated with ethephon. For the group treated with selenium, the decrease of mean HBG and of other parameters that characterize the process of erythropoiesis is in total agreement with the results obtained by other authors (17, 18), who assessed the action of sodium selenite on hematological parameters in rats. El-Okazy (13) reported that no significant variations in HBG ($p>0.05$) were observed at low doses of ethephon; the decrease was significant when the doses were increased ($p<0.01$). Also, the RBC decreased, in correlation with the dose of ethephon ingested by the animals.

Regarding HCT, parameter which expresses the volume percentage of red blood cells in blood (fig. 2), the mean levels were in accordance with mean HBG concentrations; thus, mean HCT for the Ethephon group was slightly decreased when compared with the Control group, but without statistical significance ($p>0.05$). In the case of the group to which ethephon was administered together with sodium selenite, mean HBG was insignificantly lower when compared to the Selenium group ($p=0.508$) and Control group ($p= 0.161$). This evolution, in direct correlation with the variation of mean HGB levels suggests a reduced interference of ethephon and/or selenium with the erythropoiesis process in rats.

After analyzing the data obtained for erythrocyte indices (tab. I), very low group variations have been recorded, in accordance with the other erythrocyte indices; thus, mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) for the groups treated with an association of selenium and ethephon were lower than those for the Ethephon and Selenium group, respectively ($p<0.05$), and lower than those obtained in the Control group ($p>0.05$). Mean corpuscular hemoglobin concentration (MCHC) and red cell distribution width (RDW-SD) did not show statistically significant differences between groups ($p>0.05$).

The data obtained by us are generally in agreement with those reported in the scientific literature. Thus, Al-Fartosi (16) reported that sodium selenite reduces erythrocyte levels and hemoglobin concentrations in a dose dependent manner. This evolution of erythrocyte count and hemoglobin concentration was confirmed by other authors (18). In addition, Jia et al (19) confirmed that sodium selenite administered to rats in a sub chronic experiment led to a dose dependent decrease in red blood cell counts and hemoglobin concentration. The mechanism by which selenite reduces the RBC may be hemolysis, which leads to depletion of circulating red blood cells (18).

TABLE I

Values of erythrocyte indices

Group	MCV (fL)			MCH (pg)			MCHC (g/dL)			RDW-SD (fL)		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Control	57.30	58.10	57.63	20.10	20.70	20.36	34.90	35.40	35.2	31.00	31.00	31.00
Ethephon	56.30	61.00	58.10	19.50	21.00	20.30	34.40	35.70	34.96	30.70	34.00	32.26
Selenium	56.00	59.50	58.46	19.10	21.20	20.51	34.20	35.50	34.86	29.70	32.40	31.50
SE+ETH	53.20	56.80	55.32	18.80	19.90	19.42	34.60	36.70	35.12	29.40	31.90	30.45

MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin;
MCHC = mean corpuscular hemoglobin concentration; RDW = red cell distribution width;

Platelets are anucleate cytoplasmic fragments, rich in granules, involved in hemostasis and in the initiation of tissue repair processes and vasoconstriction after vascular lesions and during inflammatory processes. Our research showed that mean platelet count (PLT) for the group treated with ethephon (tab. II) was significantly decreased when compared to the Control group (p=0.050). For the group treated with selenium, mean PLT was decreased in comparison with both the Control group (p >0.05) and Ethephon group, but the decrease was not statistically significant. Platelet count for the group treated simultaneously with Ethephon and sodium selenite was higher compared with the Selenium group, without approaching the levels recorded in the Control and Ethephon groups, respectively; in both cases, the drop in mean PLT was statistically insignificant (p>0.05).

Platelet indices, mean platelet volume (MPV), respectively, which indicates the variations in platelet volume, and platelet distribution width (PDW), which indicates the uniformity in size of platelets, are very useful for the differential diagnostic of thrombocytopenia (tab. II). The Control group had a mean MPV value of 6.96 fL and a dispersion of values given by a standard deviation of 0.294. For the other three groups, the mean values are higher than those observed for the Control group; for the association ethephon-selenium the value was significantly decreased when compared to the Selenium group (p=0.003), but increased in comparison with the Control group (p=0.023). These results are in accordance with the data published by El-Okazy (13), who reported that ethephon altered the hematopoiesis process, depending on the dose, exposure period and animal species. The results obtained for PDW, which reflects the variation in platelet size, correlated with the distribution of PLT values and with the percentage of macro thrombocytes (P-LCR), respectively (tab. II). The increase in mean PDW values was statistically significant when compared to the Control group, both in the ethephon group and in the Selenium group (p=0.029 and p=0.004, respectively). In the group treated with an association of selenium and ethephon, PDW decreased significantly (p=0.021), when compared to the group treated with selenium alone, but it did not reach the levels in the control group.

TABLE II

Mean values for platelet count and for thrombocytes indices

Group	PLT (x10^3/ µL)			MPV (fL)			PDW (fL)			P-LCR (%)		
	min	max	mean	min	max	mean	min	max	mean	min	max	mean
Control	939	1103	1028.66	6.50	7.30	6.96	7.10	7.70	7.40	4.90	6.90	5.75
Ethephon	847	1118	999.16	7.10	7.50	7.33	7.50	8.00	7.78	6.00	8.90	7.66
Selenium	697	1006	930.83	7.40	9.30	8.53	7.60	10.5	9.46	7.70	18.40	15.00
SE+ETH	848	1146	964.85	7.00	7.90	7.45	7.20	8.80	7.92	5.60	11.20	8.21

PLT = platelet count; MPV = mean platelet volume; PDW = platelet distribution width; P-LCR = platelet large cell ratio;

An increase in the percentage of macrotrombocytes was observed in all groups, in comparison to the Control group, but especially in the group treated with selenium; the increase was statistically significant for all groups, but especially for the group

treated with selenium ($p > 0.05$). These results would suggest an influence of selenium at the doses administered to rats on the process of hemostasis, by increasing the percentage of macrotrombocytes.

White blood cells (WBC) are involved in the immune defense processes of the body. Depending on their structure, leukocytes are classified in granulocytes or polymorphonuclear leukocytes (neutrophils, eosinophils and basophils) and agranulocytes or mononuclear leukocytes (lymphocytes and monocytes). Their role is complex and different, depending on their type. In our research, the mean values of total WBC count (tab. III) for the Ethephon and Selenium groups are very near, but slightly decreased in comparison to the Control group ($p>0.05$ for both groups). Conversely, a statistically insignificant increase was observed in WBC for the Selenium-ethephon group ($p>0.05$) when compared to the Control, Ethephon and Selenium groups. The mean values for this parameter are suggestive for the intervention of ethephon, but also of selenium (administered as sodium selenite) as xenobiotics, intervention manifest through the alteration of the leukocytic formula.

The results obtained in this experimental study are in contradiction with those reported by El-Okazy (13), who found that WBC showed a significant, dose dependent increase ($p < 0.01$) in the groups treated with ethephon, when compared to the Control group. Pisek et al. (20) confirmed the dose dependent reduction of WBC in laboratory animals after administering sodium selenite, a phenomenon which was also observed during our research. Sadeghian et al. (21) suggested that when administering selenium in supranutritional, but subtoxic doses in sheep, the WBC does not vary or barely increases; the explanation could be the antioxidant properties of selenium and its protective effects towards oxidative stress. Other authors (22) reported that selenium at oral doses of 1 mg/kg bw, administered to mice for 10 days, slightly increased WBC.

Basophils were detected only in the Selenium and in the Selenium+ethephon groups (tab. III), suggesting that their occurrence is due exclusively to selenium. For the groups treated with ethephon, respectively selenium, the mean values are lower than those observed for the Control group ($p<0.05$). On the other hand, in case of the Selenium+ethephon group, the mean value is approximately 70% higher when compared to the Ethephon group and 64.5% higher when compared to the Selenium group. In both cases, the increase is not significant ($p>0.05$). These results would suggest that the association of selenium with ethephon tends to normalize the decrease in eosinophils determined by the two substances, administered individually. Our results are in accordance with those reported by Aghwan (23), who highlights the role of selenium, in low doses, in normalizing the hematological parameters in laboratory animals.

Lymphocytes are mononucleate cells with role in antibody systems and immunological reactions. The number of lymphocytes is an easily accessible parameter for examining the potential immunotoxic chemical substances. Within our current research, the mean percentage of lymphocytes (fig. 3) for the groups treated with ethephon, respectively with selenium, are higher than those observed for the Control group. In both cases, the increase is statistically significant ($p<0.05$). The percentage of lymphocytes for the group treated with ethephon and selenium is lower than that for the Selenium group

($p>0.05$). According to a study conducted on mice, Wang et al (24) reported that for the group treated with a dose of 47.7 mg ethephon/kg bw/day, the percentage of lymphocytes from peripheral blood rose by 37% and in the group treated with 143 mg ethephon/kg bw/day, the increase was 54%.

Surprisingly, the percentage of lymphocytes from the total number of white blood cells from blood did not show any difference between the Control group and the groups treated with ethephon. This means that not only the lymphocyte count rose, but also the number of granulocytes. It could be suggested that ethephon may also influence all nucleate cells from blood.

TABLE III

Mean WBC levels

Group	WBC (10^3/ µL)			W-LCC (x10^3/ µL)			Baso (%)			EO (%)			W-LCR (%)		
	Min	max	Mean	min	max	mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
Control	8.03	8.13	8.07	1.12	1.63	1.32	0	0	0	0.50	1.41	0.86	16.51	20.12	17.46
Ethephon	6.85	8.91	7.82	0.80	1.76	1.15	0	0	0	0.10	0.63	0.30	10.00	19.80	14.76
Selenium	7.86	8.23	8.01	1.15	1.57	1.30	0	0.10	0.030	0.10	0.50	0.31	13.91	19.12	15.20
Selenium+ ethephon	5.84	10.61	8.40	1.10	2.75	1.60	0	0.01	0.004	0.10	2.12	0.51	10.40	28.50	19.34

WBC = white blood cell (leukocyte) count; W-LCC = white large cell count; Baso = basophils;EO = eosinophils;

Monocytes are white blood cells with the capacity of phagocytosis; they are formed in the bone marrow, they reach the blood stream and invade various tissues during an infectious process. Mean monocyte counts (fig. 4) are practically identical for the Ethephon and Selenium+ethephon groups and slightly decreased compared with the Control or Selenium group ($p>0.05$ for all groups).

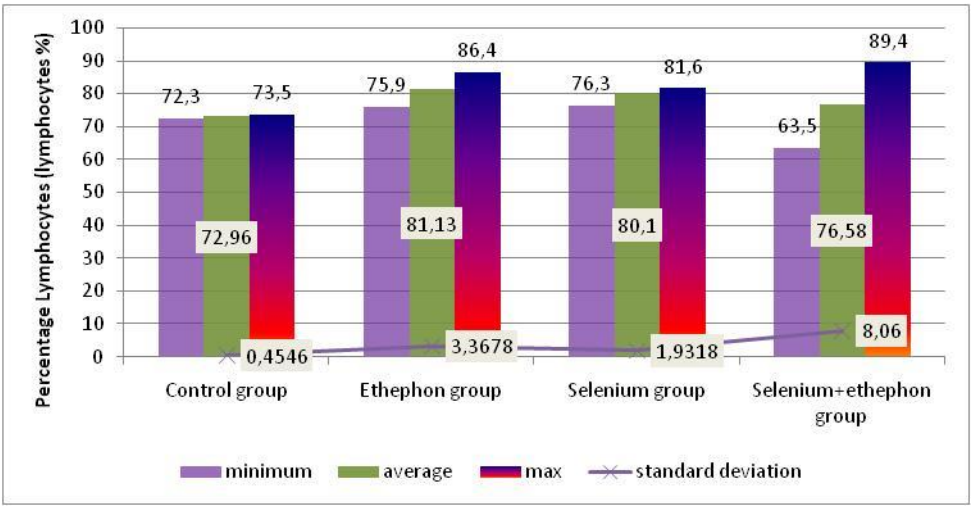

Fig. 3. Percentages of lymphocytes in the experimental groups

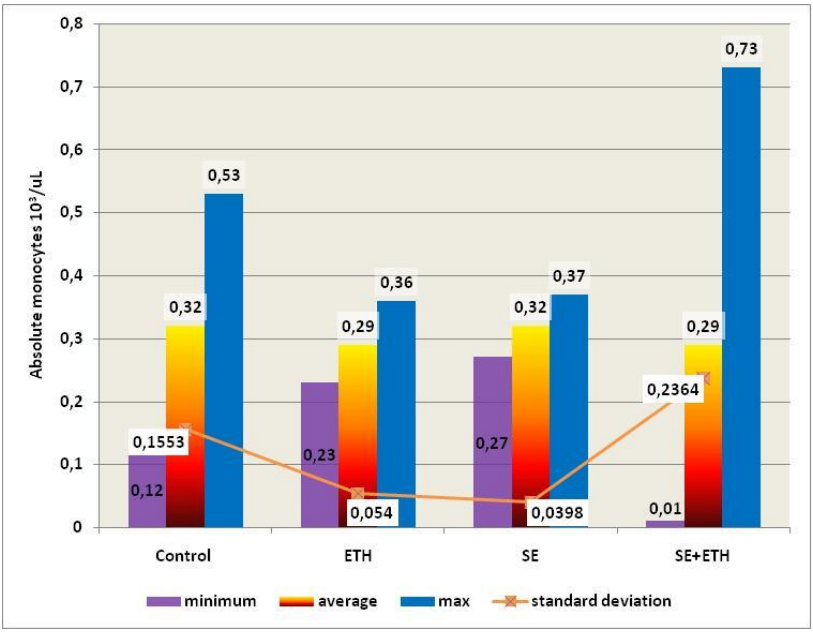

Fig. 4. Monocyte count for the experimental groups

CONCLUSIONS

The results of this study highlighted the effects of orally administered ethephon on hematological parameters in rats; the changes in the values of some complete full blood count parameters in the groups treated with ethephon may be suggestive of the harmful effect on consumer health of this plant growth regulator, present as a residue in food products. Selenium, administered orally as sodium selenite, at a dose of 1 mg/kg bw, seems to have acted as a toxic and not as a protective agent during ethephon poisoning.

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AN OVERVIEW ON SCREENING FOR BREAST CANCER AWARENESS IN ROMANIAN GENERAL POPULATION

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AN OVERVIEW ON SCREENING FOR BREAST CANCER AWARENESS IN ROMANIAN GENERAL POPULATION **(**Abstract): Trends in breast cancer incidence has been decreasing worldwide since several years, meanwhile screening programs were properly implemented in countries with financial support for healthcare infrastructure and population awareness to choose prevention. **The study aim** was to evaluate the level of knowledge regarding risk factors and screening for breast cancer in general population from Romania. **Material and methods**: The study was conducted on a group of 470 individuals aged between 21 and 55, during 1st October and 31st November 2016. The study was based on fulfilling an online questionnaire of 20 items, sent by e-mail. **Results:** Participants chose "breastfeeding" as a protective factor (84.47%), "family history" as a risk factor (78.09%), lobular carcinoma *in situ* as precancerous lesion (20.00%), mammography as a screening method (94.04%), performed "once a year in 45-54 aged women" (90.21%). A breast screening program would be useful in Romania because of the "high incidence" (98.30%) for "early detection of the disease" (97.87%), and nothing could deter people from screening participation (84.34%), but "fear of detecting a cancer" (12.64%). **Conclusions**: A larger study on a representative population group would provide better information and could be a capable guidance for national strategies on breast cancer prevention and screening. **Keywords**: BREAST CANCER, SCREENING, PREVENTION, QUALITY OF LIFE, QUESTIONNAIRE.

Readers of a paper on such a common topic for our decade as breast cancer are usually accustomed with an introduction about prevalence and mortality, as well as risk factors or an overview on screening programs worldwide. Same as responders below, we chose to skip them and took into consideration some information regarding healthcare systems and indices performed by worldwide professionals, just because of the quality the above-mentioned notions depend on.

By the year 2000, Romania ranked 99th amongst world healthcare systems (1). Many countries have recently introduced reforms in the healthcare sector with the

explicit purpose of improving their performance. There is an extensive literature on healthcare system reform and recent debates on the best way to measure performance so that the impact of reforms can be assessed. The performance of a healthcare system has been a major concern for decision-makers over many years. Performance measurement requires an explicit framework that defines the objectives of a healthcare system, on which performance can be evaluated and quantified. To assess overall effectiveness, the first step was to combine individual achievements with all five health system targets into a single number, called the composite index. The composite index is an average of the objectives of the five components, reaching the country in all five indicators such as health, health inequality, response, reactivity-distribution and fair financing (2).

One of the recently introduced indicators for assessing the state of development of a country is *Human Development Index* (HDI). This indicator considers that factors such as the health and educational level of the population are intangible objectives of population development, but not instrumental assessment purposes. The composite index is based on a similar idea, measuring the overall achievement of the intrinsic goals of the healthcare system (2).

An indicator developed by the research group involved in the *Global Burden of Disease Study* 2015 (GBD 2015) is the *Healthcare Access and Quality Index* (HAQ). It has been found that in the past two decades, in almost all regions of the globe; the values of this index have increased, ranging from 28.6 to 94.6. Thus, varied patterns appeared in regions and degrees of development, depending on the type of specific pathology. For example, oncologic pathology for developed countries has influenced HAQ values. In countries with medium levels of development, there are kidney coronary diseases, diabetes, diarrheal diseases and lower respiratory infections, but measles and tetanus in less developed countries. The authors of the study showed that if every country had reached the development average, then the overall HAQ would have reached 73 (out of 100 maximum) in 2015 (2-4).

Breast cancer is the most common cancer in women with a second-place frequency in oncologic pathology and more than 1.6 million new cases diagnosed in 2012, affecting women in all regions of the globe with any level of economic development. The lowest incidence rates were recorded in West Africa and East Asia (27 per 100,000 inhabitants), and the highest in Western and North America (over 92 per 100,000 inhabitants). Breast cancer is the most common cause of death by oncology pathology in women in developing regions (14.3% of total), and in the developed ones, the second place after lung cancer (15.4% of the total) (5, 6). In this context, screening programs for early detection of breast masses and population awareness about screening principles and methods should be considered.

The study aim was to evaluate the level of awareness regarding risk factors and screening for breast cancer in general population of Romania.

MATERIAL AND METHODS

The study was conducted on a group of 470 individuals aged between 21 and 55, during 1st October and 31st November 2016. The study was based on an online questionnaire of 20 items, sent by e-mail.

The applied questionnaire was based on models of WHO and CDC, Romanian versions. The inclusion criteria were both males and females, from general population of Romania. The exclusion criteria were graduation from medical schools, colleges and universities. All patients were informed about the confidentiality and privacy of data. The data were processed using *MS Excel 2010.*

RESULTS

1. Questions 1-5:

Demographic characteristics

The demographic structure of questioned group highlighted that most of participants were young adults (26-30 years old-35.53%) and women (77.45%), from urban area (79.46%), graduated from a university (41.06%) and employed (84.26%) (tab. I, fig. 1).

TABLE I.

Demographic and socioeconomic characteristics (n=470)

Characteristics	Frequency %
Age (years)	
21-25	24.89
26-30	35.53
31-35	28.30
36-40	6.17
41-45	2.77
46-50	2.13
51-55	0.21
Gender	
Female	77.45 (n=364)
Male	22.55 (n=106)
Residence area	
Urban	79.36
Rural	20.64
Education	
Primary	1.7
Secondary	37.02
University	41.06
Post-university	20.21
Occupation	
Employee	84.26
Student	9.57
Unemployed	6.17

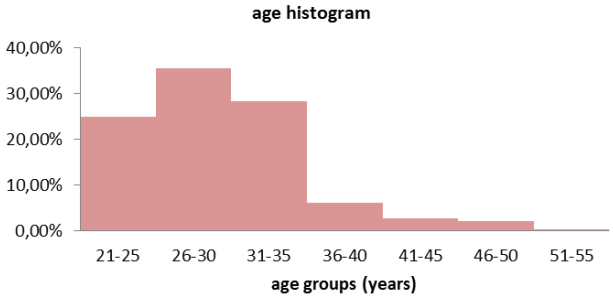

Fig. 1. Age histogram

2. Questions 6-11:

Breast cancer awareness

Item 6: "Protective factor in breast cancer". Most of the participants chose the variant "breastfeeding" (84.47%), but there were people who believed that "smoking" (11.06%) or "overweight" (2.34%) or even "alcohol consumption" (2.13%) could protect against breast cancer in women. *Item 7*: "Risk factor in breast cancer". 78.09% of responders ticked "family history of breast cancer", but 19.57% considered that "breast implants" could be a risk factor, or even "physical activity" (1.7%). *Item 8*: "Premalignant lesions". Responders thought that breast cysts (62.34%), lobular carcinoma *in situ* (20.00%) or mastitis (13.40%) became malignant. Lipoma was ticked by 4.26% only. *Item 9*: "Symptoms of breast cancer". Participants answered, "nipple retraction" as a sign in 46.38%, then "fatigue" in 45.74%. "Nausea" or "weight gain" chose by < 5% of the study group. *Item 10*: "Breast changes". 59.15% chose "orange peel", but other 20.00% ticked "pain before and after menstruation", but "decreasing" (19.57%) or even "increasing" (1.28%) in breast volume. *Item 11*: "First degree relatives with breast cancer". Answers were *no* for most of the participants (93.62%), but 6.38% of total did (tab. II).

TABLE II.

Protective & risk factors. Premalignant lesions *(correct answers only)*

Items	%
Protective factor	
breastfeeding	84.47
Risk factor	
family history of breast cancer	78.09
Premalignant lesions	
lobular carcinoma *in situ*	20.00
Symptoms	
nipple retraction	46.38
fatigue	45.74
Breast changes	
orange peel	59.15

3. Questions 12-20:

Breast cancer screening awareness

Item 12: "Breast screening methods". Most of participants answered mammography (94.04%), but 5.11% chose "computed tomography". Few of them chose "endoscopy" (0.64%) and even "colposcopy" (0.43%). *Item 13*: "Clinical exam in 20-20 aged women". "Once a year" was the most ticked variant (67.87%), but 19.79% chose "two-yearly", 6.81% - "triennially", 5.53% - "quinquennially". *Item 14*: "Mammography in 45-54 aged women". "Once a year" for 90.21% of participants, but 7.02% - "two-yearly", 1.91% - "triennially", 0.85% - "quinquennially". *Item 15*: "Self-examination". Rarer than "once a year" - 44.51% of women involved in questionnaire, "twice a year" – 28.02%. *Item 16*: "Screening purpose". Most of responders (97.87%) believed in "early detection of a cancer", but there are people (0.43%) who chose "mutilating surgical treatment" or "little purpose". *Item 17*: "Necessity of screening program in Romania". Most of participants answered, "yes because the incidence is very high" (98.30%), but few thought that "it isn't, because it could not influence women health" or "Romania has other priorities" (0.85%). *Item 18*: "Deterring from screening participation". 84.34% believed that "nothing, it is useful for health monitoring". 12.64% had "fear of detecting a cancer", 1.92% "fear of doctor" or "waste of time, it cannot help". *Item 19*: "Choosing prophylactic mastectomy". "Most of participants chose "yes" (84.89%) and the others "no" (15.11%). *Item 20*: "Frequency of breast cancer in Romania". A half of responders chose "frequent" (50.00%) and the most frequent cancer in women (46.17%), but few answered "rare" (3.62%) or "not at all" (0.21%) (tab. III).

TABLE III.

Breast cancer screening awareness (*correct answers only***)**

Items	%
Breast screening methods	
mammography	94.04
Mammography in 45-54 aged women	
once a year	90.21
Screening purpose	
early detection	97.87
Necessity of screening program in Romania	
yes, because the incidence is very high	98.30
Deterring from screening participation	
nothing, it is useful for health monitoring	84.34

DISCUSSION

Austria was the first EU state member state which implemented a breast cancer screening program, in 1974. At that time, screening programs for breast cancer were appropriate in many European countries, with mammography being indicated in women over the age of 40, however, with-

out two incidents. Other countries included in screening programs only women with medical insurance. Sweden was one of the first European countries which managed breast screening programs with a high performance on national level. Women aged 50-69 are recommended for biannual mammograms and those of 40-49 years of age to perform mammographic tests every 18 months. In about 50% of cases, a biannual mammography is recommended for women aged 70-74 (7-9).

The European Commission has recommended screening for breast cancer in females aged 50-69 in all member states since 2003. Such active national programs, according to the European Council guidelines, were carried out in 22 states, in 2007. Various models of national screening programs, including online invitations, have been able to adapt and improve existing detecting resources and facilities, and the adaptation of protocols to the risk profile and clinical status of every woman. However, several national and European studies showed that screening participation due to online invitation achieves a higher and faster level of coverage, as well as lower costs for healthcare providers (9).

In Romania, in accordance with the Regional Oncology Institute of Iași, a local screening program is performed following algorithms depending on the age of women. For young women aged 20-40 years, self-examination is recommended monthly, then clinical consult annually. For them aged 40-50, a mammogram is recommended to be performed in two incidents for each breast, every two years. Depending on the aspect of mammography, the physician may recommend completing breast imaging investigations with mammary ultrasound and / or breast MRI. In women aged 50-70, mammogram is recommended to be perform in two incidents per breast, yearly. For young females with an oncogenetic risk, it is recommended to alternate classical mammogram with breast MRI (mammography followed by breast MRI after six months) (10).

The issue of adherence to breast screening programs plays a substantial role in every country. National healthcare services should guarantee access and participation in screening program of all eligible women, even if this would entail an increasing in healthcare costs. For this purpose, awareness and information campaigns performed in general population are needed to improve awareness of breast cancer risk factors, screening and prevention methods, as well as screening protocols. Population gets old and this fact has led to a steady increase in the incidence of oncological pathology. A well-implemented and monitored national cancer registry for every state allows monitoring the effectiveness of the fight against breast cancer, too. A breast screening national program could mean an increased diagnostic rate. Any change in such screening program active for years could also have an impact on the incidence of the disease. For example, the introduction of digital mammography in the Netherlands has led to increase values of breast cancer incidence, but mortality rates were not affected. This could be explained by the effect of increasing survival rate due to improved diagnostic methods, surgical techniques and more effective treatments (10, 11).

Statistics highlighted that participation in mammographic screening for early detection reduced breast cancer mortality by 20%. For example, in UK population, mammographic screening saved about

1,300 women yearly or the equivalent of about 22,000 years of life. However, we must also consider the negative effects that screening may have, especially the risk of over-diagnosis. Short-term screening trials show a rate of over-diagnosis higher than 10%. The excess mortality resulting from the investigation and treatment of breast cancer is insignificant and outweighed by the benefits of treatment. Other harm effects such as increased anxiety and emotional discomfort can occur frequently. In the current situation, when breast cancer treatment has improved considerably, the question that arises is whether screening would be relevant or not. We need to look at the benefits of screening independently and the benefits of improved treatments. To weigh up the benefits of screening and current treatments, there is not a reason to stop breast screening programs (12-14). Further research, including quality of life studies, will be able to answer more promptly to this issue.

Breast reconstruction after surgery for cancer is a way to improve body image, aesthetic and emotional aspects of women who experience such a disease as cancer. Studies highlighted that there is no single reconstruction procedure that could be discussed with a patient from the beginning, but a careful selection of techniques must be made, allowing for optimal results. Thus, many elements, such as incision lines, tegument or asymmetrical differences in unilateral reconstruction, should be considered (15). However, this information should not avert the patient from choosing postoperative reconstruction procedure, as a subsequent positive attitude that could contribute to increase the survival rate and improve scores in health-related quality of life (16).

Thus, the wellbeing of every woman considering breast oncologic condition safety has also a cost. In addition to increase the survival rate, by early detection and treatment, mammographic screening identifies tumor masses that would have been never detected during the woman's lifetime if she had not been ever screened. Here is the problem of over-diagnosis (12). Another eligible question is whether this improved treatment would have been necessary for the woman to extend her lifetime or she can live as well with breast tumor or even better, considering that quality of life scores change considerably in a woman who underwent surgical treatment for breast cancer.

CONCLUSIONS

As soon as the questionnaire was uploaded, we were surprised that both women and men from general population of Romania were very receptive to participate in. The responders had no proper information either about breast cancer risk factors, or screening. Many participants misinterpreted breast implants as a risk factor, claimed that fear of detecting cancer would deter them from participating in screening, refusing prophylactic mastectomy for high oncogenetic risk and having no idea about breast reconstruction after that surgical procedure.

We believe that our study is important because it provides information regarding breast cancer level of awareness in a general population of a country never ranked amongst 50th world healthcare systems. A larger study on a representative population group would provide better information and could be a capable guidance for national strategies on breast cancer prevention and screening.

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LATE CERVICAL NODAL TUBERCULOSIS RECURRENCE MIMICKING METASTASIS OF PAPILLARY THYROID CARCINOMA. A CASE REPORT

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LATE CERVICAL NODAL TUBERCULOSIS RECURRENCE MIMICKING METASTASIS OF PAPILLARY THYROID CARCINOMA. A CASE REPORT (Abstract): Although papillary thyroid carcinoma (PTC) represents the most usual form of thyroid neoplasia the association with cervical tuberculous lymphadenitis (CTL) is rare and diagnosis remains a challenge. **Case report**: We report the case of a 78-years-old female patient with personal history of cervical fistulizing tuberculous gumma diagnosed with right lateral cervical mass and Hashimoto nodular thyroiditis. The neck ultrasound and CT exam revealed an enlarged right thyroid lobe containing a nodule of 2/2 cm and right later cervical multiple masses resembling lymphadenopathies. The patient underwent to surgical treatment which consisted in total thyroidectomy and modified neck dissection with resection of internal right jugular vein adherent to the lymph node mass. **Results**: The final pathology report demonstrated a pT3mN0LV1Pn0 right lobe papillary thyroid carcinoma on Hashimoto thyroiditis while lymph nodes presented giant epithelioid granulomas with giant multinuclear Langhans cells and central necrosis consistent with the diagnosis of tuberculous adenitis. The postoperative course was uneventful and the patient was referred to both TB and endocrinology specialist. **Conclusions**: Cervical tuberculous lymphadenitis can mimic metastatic adenopathy in papillary thyroid carcinoma and frequently the definitive diagnosis is achieved by excisional biopsy and histopathological examination. **Keywords**: PAPILLARY THYROID CARCINOMA, CERVICAL TUBERCULOUS LYMPHADENITIS, THYROIDECTOMY, LYMPHADENECTOMY.

Papillary thyroid carcinoma (PTC) represents the most generic form of thyroid neoplasia. Total thyroidectomy eventually followed by radioiodine is the treatment of choice while cervical lymphadenectomy is performed in case of apparent or histologically proven lymph node metastases (1). On the other hand, tuberculosis (TB) remains a major health problem today. It mainly involves the lungs but can cause infection in almost all tissues in the body. Lymph node tuberculosis (LNT) is a common cause of lymphadenopathy in areas in which TB is endemic. Although new diagnostic methods have been developed, especially in patients without a history of tuberculosis, the cervical tuberculous lymphadenitis (CTL) diagnosis remains a challenge.

CASE REPORT

We report the case of a 78-years-old female patient who has been referred to our surgical unit by the endocrinologist with right lateral cervical mass and Hashimoto nodular thyroiditis. Significant elements in the history of the patient were a cervical fistulizing tuberculous gumma almost 50 yrs. ago, left mastectomy for in situ breast cancer (2014), arterial hypertension and atrial fibrillation under treatment. The physical examination revealed a good general appearance patient, of normal weight, vitals stable, apyrexial. Neck examination reveals a right lateral aspect elastic tender mass from the top of posterior triangle extending behind clavicle and palpable nodular right thyroid lobe; suprasternal scar from previous treated skin TB. Laboratory tests showed autoimmune thyroiditis (ATgAB-629 IU/ml, APOAB-446 IU/ml) with mild hypothyroidism, iron deficiency anemia. The neck ultrasound exam revealed an enlarged right thyroid lobe containing a nodule of 2/2 cm, a micronodular normal sized left lobe and right later cervical multiple masses resembling lymphadenopathies. Thyroid scintigraphy described a heterogenous goiter with hypo/affixation of the right lobe nodules and inferior pole retrosternal, right lobe is compressed by a cervical mass with no uptake of Tc. CT scan showed along right internal jugular vein- multiple cystic tumors forming a mass of 43/48/73 mm (fig 1, 2).

FNAC from the largest thyroid nodule showed a benign aspect. Chest X-Ray, abdominal ultrasonography and ENT – indirect laryngoscopy examination were all normal. Patient underwent to surgical treatment which consisted in total thyroidectomy and modified neck dissection with resection of internal right jugular vein adherent to the lymph node mass. The frozen section of lymph nodes revealed TB adenitis.

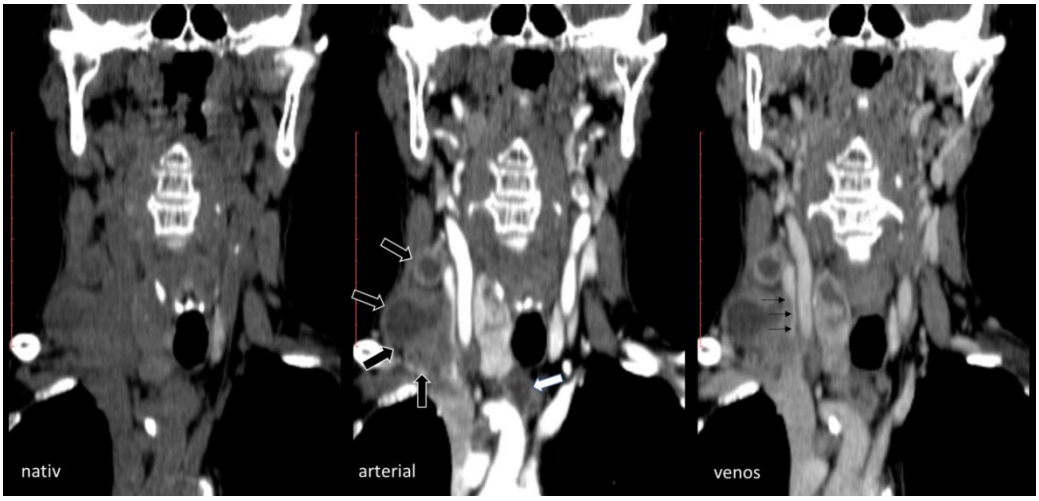

Fig. 1. Cervical coronal section: supraclavicular and right inferior jugular cystic tumors (black arrows). Similar lesion in contact with right thyroid lobe (white arrow). Compression on right jugular vein (thin arrows).

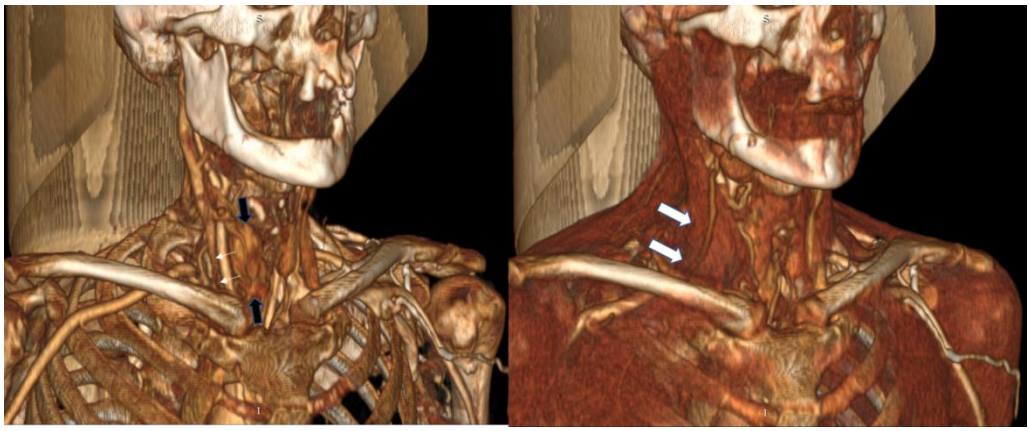

Fig. 2. 3D reconstruction – right jugular vein compression (thin white arrows), right thyroid lobe increased in volume (black arrows), jugular supraclavicular tumor mass (thick white arrows)

The final pathology report demonstrated a 2.5 cm papillary thyroid carcinoma on the right lobe and Hashimoto thyroiditis, capsular invasion (fig. 3), left lobe Hashimoto thyroiditis, all lymph nodes without tumor cells, giant epithelioid granulomas with giant multinuclear Langhans cells and central necrosis - TB adenitis (fig. 4). The staging of the tumor was pT3mN0LV1Pn0.

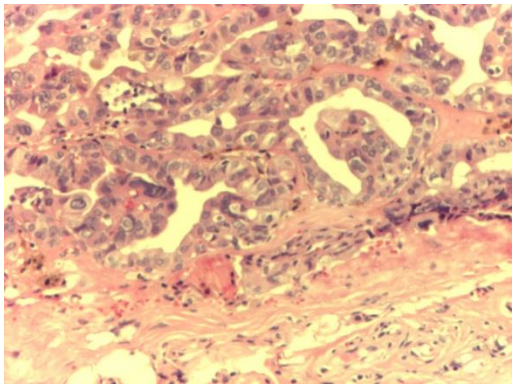

Fig. 3. Papillary thyroid carcinoma with capsular invasion, HE, x10

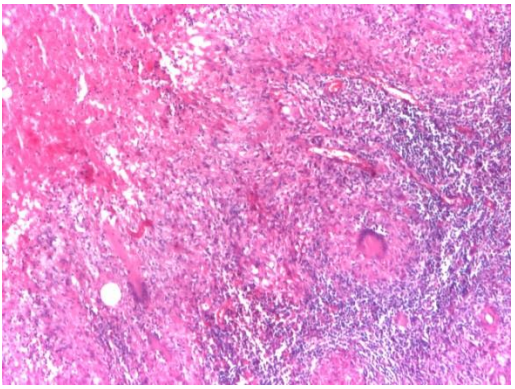

Fig. 4. Giant epithelioid granulomas and central necrosis, HE, x10

The postoperative course was uneventful and the patient was discharged in the 5th postop day and referred to both TB and endocrinology specialist.

DISCUSSION

One of the most common clinical presentation of extrapulmonary tuberculosis is cervical lymphadenitis. In a study published in 2000, Kanlikama et al. demonstrated that the history of TB contact is in only 21.8% and tuberculosis infection occurs in only 16.1% of the cases with cervical lymphadenitis (2). Cervical tuberculous lymphadenitis

may appear either a unilateral single or multiple painless mass and is usually found in the supraclavicular area (3). Partially, these clinical features are common to those of the metastatic adenopathy from PTC. The sonographic characteristics of tuberculous adenopathy are very like metastatic cervical nodes from PTC namely intranodal cystic necrosis and calcification. CT scan can be a valuable complementary test in cervical tuberculous lymphadenitis but is not sufficient to make a certain diagnosis. The most common pattern on CT is a node with main area of necrosis but in our case the features were not consistent. Fine-needle aspiration cytology could have shown a well-formed epithelioid granuloma and the presence of caseous necrosis but, again, in our case the appearance was uncertain. Coexistence of thyroid cancer and cervical tuberculous lymphadenitis is rare. However, some similar cases have been reported to date (4, 5) but preoperative diagnosis was impossible for most cases. As the other case reports, we could not distinguish preoperatively whether cervical lymph nodes enlargement was due to PTC metastasis or tuberculosis infection either by clinical findings or imaging studies.

CONCLUSIONS

In our experience, cervical tuberculous lymphadenitis can mimic metastatic adenopathy in papillary thyroid carcinoma. Preoperative diagnosis can be difficult but is valuable in avoiding unnecessary and complications prone neck dissection. In the presence of enlarged cervical lymph nodes on a patient with TB history, tuberculous lymphadenitis should be considered. The definitive diagnosis of tuberculous lymphadenitis is done by excisional biopsy and histopathologic examination if all other techniques fail.

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SEPTIC COMPLICATIONS AFTER EMERGENCY SURGICAL PROCEDURES CONSENSUS OF ITALIAN SOCIETY ON TRAUMA AND EMERGENCY SURGERY

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SEPTIC COMPLICATIONS AFTER EMERGENCY SURGICAL PROCEDURES CONSENSUS OF ITALIAN SOCIETY ON TRAUMA AND EMERGENCY SURGERY (Abstract). **Background**: Emergency surgery is an independent factor among the postoperative morbidity risks. The most frequent complications after emergency abdominal surgical procedures occur in the case of digestive tract surgery and they manifest themselves as intestinal dehiscence or abdominal sepsis. Delay on recognition of one of these conditions is an important variable in the outcome. Moreover, a general agreement in this field is currently lacking, thus SICUT society designed a consensus study aimed to define their optimal workout**. Material and methods**: The electronic search was performed using the following databases Medline, PubMed, Scopus, Embase and Cochrane Library. Inclusion criteria were date of publication after 2006, and type of work randomized clinical trials and prospective, prospective observational studies, systematic reviews and meta-analysis. Papers were reviewed and selected by two experts with the help of a third in the role of tutor per the PRISMA methodology. Experts have indicated levels of evidence and grades of recommendations using the method of "*Grading of Recommendations Assessment, Development, and Evaluation"* (GRADE). **Results:** The only method of reducing the morbidity rate is to follow the septic complications of postoperative surgery, including the early diagnosis and the individual treatment following precise criteria. **Conclusions**: This consensus is in line with current international strategies and guidelines, and it could be a useful tool in the management of these postoperative complications after emergency abdominal surgery. Early recognition and multidisciplinary management of these septic complications is recommended. **Keywords:** COMPLICATIONS AFTER EMERGENCY SURGICAL PROCEDURES, MANAGEMENT OF SEPTIC COMPLICATION, EMERGENCY SURGERY, CONSENSUS.

Emergency surgery is an independent factor for postoperative morbidity risk. A recent study published on *JAMA Surgery* highlighted 7 emergency surgery at greater risk, involving, almost exclusively, the intestinal disease and in which the main complication is constituted by intestinal dehiscence or abdominal sepsis (1). Delay on recognition of one of these conditions is an important variable in the outcome (2). The symptoms and signs associated with postoperative abdominal sepsis can be

subtle and/or difficult to recognize in a critical patient with a complex clinical course, so our goal was the definition of a clinical pathway that leads to early control of septic source.

MATERIAL AND METHODS

The electronic search was performed using the following databases Medline, PubMed, Scopus, Embase and Cochrane Library. Research keywords were: emergency surgery, acute care surgery, abdominal sepsis, anastomotic leek, abdominal abscesses and peritonitis. Inclusion criteria were date of publication after 2006, and type of work randomized clinical trials and prospective, prospective observational studies, systematic reviews and meta-analysis.

Papers were reviewed and selected by two experts with the help of a third in the role of tutor per the PRISMA methodology. Our research produced 1,446 articles from which 621 were included for review. Experts have indicated levels of evidence and grades of recommendations using the method of *"Grading of Recommendations Assessment, development, and Evaluation"* (GRADE) (3) under assessment by the members of ITALIAN SOCIETY ON TRAUMA AND EMERGENCY SURGERY (SICUT) and revision of two experts appointed by the Board of SICUT.

RESULTS

The first step was to define the terminology.

Secondary peritonitis is defined as an acute infection of the peritoneum due to loss of integrity of the gastrointestinal tract (SP) are spontaneous perforations (e.g., due to diverticulitis, appendicitis, cholecystitis) traumatic perforation of a visceral organ, or iatrogenic causes (e.g., perforation, anastomotic leek).

Tertiary peritonitis (TP) is an uncommon disease in comparison with secondary and primary ones. It is defined as a severe recurrent as persistent intra-abdominal infection after apparently successful and adequate surgical source control of secondary peritonitis. In approximately 80% of the patients with SP, the initial surgical intervention may sanitize the abdominal cavity from the septic source (4, 5). The remaining patients develop a persisting peritonitis characterized by a prolonged systematic inflammation and organ dysfunction leading to high rate of SIRS, sepsis, severe sepsis, or septic shock. TP usually occurs in ICU settings at least 48-72 hours following surgical adequate intervention for SP (6). Thus, mortality of TP ranges between 30% and 64%. The latest ICU consensus conferences guideline provides a precise definition of TP as intra-abdominal infection that persists or recurs ≥ 48 hours following successful and adequate surgical source control. This definition contains two essential conditions, which should be met: ***the time period ≥ 48 hours and the successful surgical source control (6)*** (GoR A).

Abdominal abscesses may be visceral and non-visceral, intra or extraperitoneal. Non-visceral abscesses arise after resolution of diffuse peritonitis in which a loculated area of infection and suppuration is ~walled off~ and persists, or after a perforation of a viscerus (or an intestinal anastomosis) that is effectively localized by peritoneal defenses.

The definition of **anastomotic leakage (AL)** is widely agreed as a breach in a surgical joint between two hollow viscera, with or without active leak of luminal contents (although there are some minor deri-

vations (7). The definition of AL adopted the classification that was proposed by the International Study Group of Rectal Cancer. The severity of AL was classified into three grades according to the following criteria:

- grade A, AL requiring no active therapeutic intervention,
- grade B, AL requiring active therapeutic intervention without relaparotomy,
- grade C, AL requiring relaparotomy.

On the basis of this study SICUT experts answered the following questions and authored a guide for good practice:

1. What clinical and biological indicators can be used to suspect the early onset of a complication in the presence of a suboptimal clinical course and with devious clinical signs without no obvious condition of sepsis or leakage of enteric juices through the abdominal drainage?

Statement A: The most reliable biological indicators to evaluate the occurrence of major abdominal septic complication are the increase and trend of PCR and/or procalcitonin between the 3rd and the 5th postoperative day (GoR C**)** (8-11).

Statement B: The combination of a postoperative modification of Mannheim Peritonitis Index and Apache II is an indicator of risk of septic complication (GoR C) (12). The mean Mannheim Peritonitis Index (MPI), which was recorded at the revealed significant higher values for TP patients compared to SP patients (4) (GoR A).

Marshall score is more suitable for SP. The sum score of the APACHE II and MPI presented an increased percentage of sensitivity (77%) compared to isolated APACHE II and MPI. Both prognostic scores were statistically correlated to mortality and tertiary peritonitis (GoR C) (12). Indicators to assess: New Definition Sepsis (Quick Sofa Score and Lactates), DULK Score and Early Warning Score (13, 14).

2. What is the indication and the role of computed tomography (CT) in the early diagnosis of postoperative sepsis where there is not an obvious clinical condition of dehiscence or peritonitis?

Statement A: In adult patients not undergoing immediate laparotomy, CT scan is the imaging modality of choice to determinate the presence of an intra-abdominal infections and its source (GoR B).

Statement B: The indication for CT scan is determined by the onset of abdominal symptoms associated with the changes of clinical and biological indicators referred to in paragraph 1 so it is recommended within 24 hours of the onset of these changes (GoR B).

3. Once the septic complication was diagnosed as managing antibiotic therapy, which bacteriological monitoring is to be implemented?

Statement A: In relation to the treatment in progress the aim is to implement the empirical coverage of likely pathogens and gram negative multiresistant (GoR C), while the use of antifungal is indicated only in case of their isolation (GoR B).

Statement B: Cultures in patients at risk of nosocomial infections should always be performed on:

- intra-abdominal material during surgery or percutaneous drainage,
- blood in case of fever over 38°C or after invasive device removal (GoR C).

The presence of postoperative septic complications requires a consultation by infectiologist.

4. What kind of monitoring and what complementary therapy should be implemented in the presence of septic complication?

Statement A: The level of monitoring should be evaluated according to the NEWS system (14).

Statement B: The volume of perioperative fluids must be managed according to the criteria of the "goal directed therapy" (15).

Statement C: Nutritional support should be appropriate starting with early enteral therapy, according to the specific clinical condition.

5. What therapeutic approach per the diagnosed medical case should be chosen:

5.1. What factors influence the therapeutic approach?

Statement: the clinical approach is recommended as a function of the following factors:

- patient at high risk per ASA and P-POSSUM scores;
- systemic conditions for progressive clinical deterioration or worsening of sepsis and MODS indicators (SOFA Score);
- presence of signs of abdominal guarding;
- location and grading of anastomotic leakage;
- presence and localization of abscess collections.

5.2. When do we implement the interventional treatment, or limit the treatment to only drainage of previously placed?

Statement: The conservative management can be seen as a choice only in the presence of intra-abdominal abscesses localized and/or low flow localized dehiscence, drained, without abdominal guarding either signs of systemic involvement (16) (GoR C).

5.3. What indications recommend a surgical approach?

Statement: It is recommended a surgical approach to:

- an ostomy must be created in the presence of anastomotic dehiscence;
- the prompt treatment of the primary foci in the presence of sepsis with clinical deterioration and/or signs of abdominal guarding.

Type of surgical approach: this consensus does not provide any evaluation about the technique (open or laparoscopic) to perform an ostomy or a direct approach to the abdominal septic source.

5.4. Which surgical approach can be implemented in the presence of gastroduodenal dehiscence?

Statement A: Dehiscence of gastroduodenal suture requires surgery in the presence of an uncontrolled fistula (GoR C).

5.5. Which surgical approach can be implemented in the presence of intestinal or ileum - colic dehiscence?

Statement A: Ileal or ileo-colonic anastomotic leakage should be treated with resection and primary anastomosis according to clinical judgement (GoR C).

To be evaluated: in case of a second dehiscence according to the clinical condition and the grade of disease consider to implement damage control strategy.

5.6. Which surgical approach can be implemented in the presence of colonic or colorectal leakage?

Statement A: The anastomotic leakage with systemic and/or peritoneal involvement require early execution of Hartmann' procedure with cleaning and drainage of septic focus (GoR C).

Statement B: The anastomotic leakage can be treated with stomy and drainage if it is a extraperitoneal leak or grade A and B of fistula (GoR C) (7).

6. When it may be necessary to use the technique of open abdomen?

Statement A: Damage Control strategy can be recommended in a high-risk patient with not complete peritoneal cleaning, visceral hypo perfusion, inability to perform an ostomy and/or tertiary peritonitis (GoR C).

To be evaluated: the opportunity of choosing a damage control strategy with an open abdomen to facilitate early recanalization.

Open abdomen management: This consensus has not developed any guidelines for the management of the open abdomen for abdominal sepsis, and postpone this issue topic to a subsequent evaluation, inside the SICUT, about these main topics: criteria, perioperative management, implementing approach, risk of complications and closing techniques.

DISCUSSION

This study, aiming presenting a consensus agreement regarding the management of septic complications following emergency abdominal surgery, was carried out by an Italian group of experts with a very rich experience in the field of emergency surgery, appointed by SICUT.

SICUT is the most powerful and best organized European scientific society in the field of emergency surgery. It has collaboration link with the International Society of Emergency Surgery and with other similar European societies, including the Romanian Society of Emergency and Trauma Surgery, which is concretely helped by taking part in conferences of emergency surgery organized in Bucharest, Sinaia and Iasi. SICUT, a model scientific society has expertise in the management of emergency surgery, including postgraduate learning in the field. SICUT, the oldest society of this kind in Europe and a model to be followed, has expertise in the management of emergency surgery, including postgraduate learning in the field. SICUT has a rich tradition in implementing consensus, studies and guides for good practice. During the presidency of Prof. Franco Stagnitti three consensus studies have been reached at. They have been promoted by national emergency conferences in Italy and a successful postgraduate live course at Matelica, in December 2016.

A consensus was preached often average a period of six months and 45 experts and 37 experienced surgical centers, including university were included. The consensus under discussion was presented and debated at the National Congress of Surgery from Rome in September 2016. After the definition of secondary and tertiary peritonitis, of postoperative abscess and anastomotic leaks the study group reached a powerful consensus of the clinical, biologic and imagistic diagnosis of septic complications in emergency surgery and multidisciplinary complex treatment of these complications. The only method of reducing the morbidity rate is to follow the septic complications of postoperative surgery, including the early diagnosis and the individual treatment following precise criteria.

After defining secondary and tertiary peritonitis, postoperative abscesses and anastomotic fistulae, the study group reached a strong consensus regarding clinical s, biological and imaging diagnostic of septic complications after emergency surgery and a complex multidisciplinary treat-

ment. Careful monitoring of postoperative evolution of emergency surgery, an early diagnosis and individualized treatment of postoperative complications, using precise criteria are the only method that can reduce mortality. An unfavorable evolution on the third postoperative day, accompanied by fever and abdominal signs, ineffective abdominal drainage, leukocytosis, increased PCR and procalcitonin require an early diagnosis, both imagistic (ultrasound, CT with contrast material) and bacteriological (culture of secretion drained, blood cultures). Decision criteria in choosing a complex multidisciplinary treatment are include: the clinical risk assessed by ASA, P-Possum, deterioration of general condition (New Sepsis Score, MODS-SOFA), peritoneal signs, presence of anastomotic dehiscence, characteristic of abdominal sepsis.

Treatment begins with broad-spectrum antibiotics (attention being paid to nosocomial infections; antifungal therapy is indicated only in the presence of fungi). If no general signs are present, anastomotic leek points to type A and the abscess is drained, while antibiotics continuing to control drainage. In case the peritoneal cavity is not drained, abscess is multiple and therefore interventional radiology is recommended. In the presence of systemic signs, dehiscence is of type B and the abscess is drained, is recommended a stoma. In the presence of systemic signs, the dehiscence is of type C and the identification of peritoneal signs require iterative lavage and peritoneal drainage. In case of uncontrolled gastro-duodenal fistula or in the case of ileo-colic leek, resection and anastomosis are recommended (in the case of iterative fistula, damage control strategy is to be followed). Hartmann operation is the solution in the case of colon - rectal fistula.

CONCLUSIONS

This paper reports the consensus agreement of a panel of Italian surgical experts - on the behalf of SICUT - covering early recognition and management of septic complications after emergency surgical procedures.

It is important to remark that although this consensus is in line with current international strategies and previous published guidelines, this study summarizes the agreement reached at by a panel of experts and further developed by adhering to common clinical practices and by using a well-defined methodology. Ongoing studies aim at identifying criteria for establishing a damage control in the case of open abdomen to facilitate early recanalization.

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NEWS

STRUCTURE-BASED DESIGN, SYNTHESIS AND ANTITUMORAL EVALUATION OF ENULOSIDES

The purpose of this research is to designe and obtaine in high yields a new class of antitumoral agents with lower structural complexity. The general strategy for the synthesis of enulosides is based on the use of glycals as starting materials. From the pharmacological point of view, some synthetic enulosides exhibited activity against Mycobacterium tuberculosis H37Rv and they can also act as glycosidase inhibitors. All compounds exhibited high antitumoral activity in micromolar range against the tested tumor cell lines. (Santos JAM, Santos CS, Almeida CLA, et al. Structure-based design, synthesis and antitumoral evaluation of enulosides. *European Journal of Medicinal Chemistry* 2017, 128: 192-201).

Oana Dragostin

ADENOSIN TRIPHOSPHAT BIOLUMINISCENCE IN HOSPITAL CLEANING–A METHOD TO INFLUENCE INVASIVE FUNGAL INFECTIONS

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ADENOSIN TRIPHOSPHAT BIOLUMINISCENCE IN HOSPITAL CLEANING-A METHOD TO INFLUENCE INVASIVE FUNGAL INFECTIONS (Abstract). Invasive fungal infections are infections determinate by fungal invasion and can be endogenous from patient mycobiome or through horizontal transmission from medical workers' hands and from patients hospital environment especially candidemia with *C. parapsilosis*. Candidemia is one of leading bloodstream infections associated with healthcare situated on fourth position and USA with high mortality rates especially for immunosuppressed patients. Demonstrating the level of cleaning using adenosine triphosphate bioluminescence technique we can improve the cleaning and hand hygiene and can influence decreasing of invasive fungal infections transmitted by hands. **Material and methods:** We have evaluated 200 samples from hands and patient environment on 2 hospitals with pneumology and infectious diseases profile (I and II) using bioluminescence to establish the status of hygiene and to determine measures of cleaning. **Results:** Regarding hand hygiene the level of relative light units over the values recommended by producer (FAIL) was 66% (6 of 9) in unit I and 75% in unit II (3 from 4). Regarding the level of cleaning in other places in direct contact with patients like toilets, bed bar, commodes the results were FAIL over 60%. **Conclusions:** In this moment in hospitals the hygiene is evaluated visually and no objective method was implemented beside the sanitation tests in Romania. ATP Bioluminescence evaluates in 15 seconds the level of cleaning in comparison with other methods and generates input in cleaning process, influencing indirectly decreasing hospital acquired infections including invasive fungal infections, together with other measures like education, antibiotic stewardship, increasing patient safety. In hospitals evaluated cleaning of hands and surfaces need improvement and investment in cleaning products and processes monitoring.
Keywords: ATP BIOLUMINISCENCE, INVASIVE FUNGAL INFECTIONS, HAND HYGIENE, CANDIDA PARAPSILOSIS, PATIENT SAFETY.

Invasive fungal infections (IFI) are the effect of the advances in healthcare. Candidemia is on the top level of healthcare associated infections being in fourth place in hospital acquired infections. Five species are the most species found responsible of 92% of the candidemias *C. albicans*, *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* (1). There are multiple factors which can determine invasive fungal infections

(risk factors) like immunosuppression, prolonged antibiotic use, totally parenteral nutrition, corticotherapy, chemotherapy and immunosuppressive therapy post-transplant, invasive devices. *C. parapsilosis* is associated more with hand hygiene and especially on extreme ages, elderly and neonates. Bioluminescence is a special method which measures the level of adenozintriphosphate (ATP) on surfaces after interacting with an enzyme named luciferases. The method was used in food industry where the security of hands and environment are on high level. ATP is present in fungal cells, bacteria and other organic compounds. Identifying ATP we receive indirectly data about fungal charge in hands and surfaces. ATP is an organic molecule as a source of energy. Animals, plants, bacteria, yeast and molds are producing and destroying ATP for biological processes. ATP is the premium molecule for energy transfer inside the cytoplasm. ATP identified on a surface indicates that this surface is not clean and can support the growing of fungal and bacterial colonies. Researchers from Brasil have compared the genotype of *C. parapsilosis* of patients in an outbreak and they identify the same species on patients with healthcare workers (2) and using bioluminiscence ATP hygiene monitoring and improving cleaning in United Kingdom the researchers decrease hospital acquired infections (HAI) with *Staphilococcus aureus and Clostridium difficile* (3, 4). Our epidemiology data between 2010-2016 show an 10.6% involvement of *C. parapsilosis* in invasive fungal infections (IFI) in INBI Matei Bals Bucharest (5). We can estimate that monitoring ATP we can have a clear image about the cleaning of one surface or hands or instruments and improving cleaning of hands and patient in hospitals we influence directly decreasing of *C. parapsilosis*, increasing the patient safety.

MATERIAL AND METHODS

We are using one device called luminometer called System Sure Plus (trademark of Hygiena company) (6) and a swab (Ultrasnap) for collection the probe on 10 cm^2 for gathering organic material from healthcare workers and patient environment (bed, commode, toilet, spaces in the hospital) in two units on different wards in infectious diseases and pneumology. The swab has on top one reserve with luciferases enzyme which we will open after we swab the surface and close it to interact enzyme with organic material from swab. Enzyme with organic material generates light and we immediately measure this value with one luminometer (fig.1) which counts *Relative Light Units* (RLU).

The more RLU we have the more contaminated is the surface. Proving, we can take immediately actions in cleaning. Hand hygiene is hard to implement in hospitals and the most resistant are doctors with 30% compliance even hand hygiene can decrease with 20-50% hospital acquired infections (7).

We have evaluated 200 swabs from 2 hospital units I and II (100 swabs for each hospital) from different areas–hands of healthcare workers and different surfaces. We have compared the values with the recommendation of producers for different areas for PASS/FAIL result to evaluate the level of contamination (tab. I).

For hand hygiene 60 RLU was the limit for a clean hand recommended by producer of a device to qualify as a clean hand. Centers for Diseases Control and Prevention (CDC) started one campaign “Clean hands,

Save Lives" (8) to create awareness about the capital importance of hand hygiene. 1 of 25 patients acquired at least one healthcare associated infection at any given day.

TABLE I
Values of RLU recommended for PASS/FAIL result

Applying places	Limits	
	PASS (RLU)	FAIL(RLU)
Public places in hospital	< 150	> 300
Sterilization	< 10	> 20
Washing disinfectors	< 5	> 10
Meal preparation	< 10	> 30

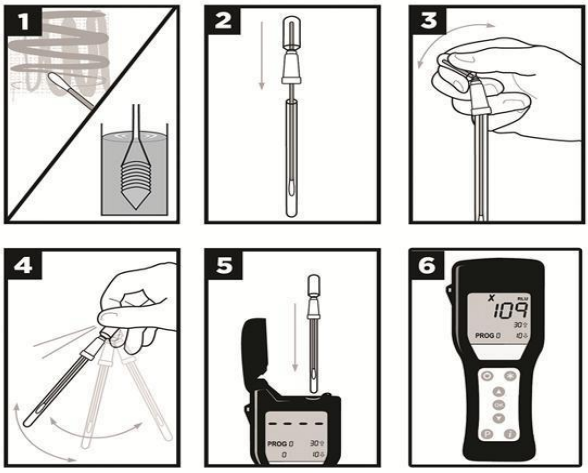

Fig. 1. Luminometer swab and measure steps
(System Sure Plus manual - Hygiena LLC (6)

Hand hygiene should be one part of a multimodal promotion strategy and strong measures and studies showed increasing of compliance significantly among nurses and nursing assistants, but remained poor among doctors in a study done by Pittet et al.(9). Healthcare associated infections decreased (prevalence of 16.9% in 1994 to 9.9% in 1998), MRSA transmission rates decreased (2.16 to 0.93 episodes per 10,000 patient-days;), and the consumption of alcohol-based hand rub solution increased from 3.5 to 15.4 L per 1000 patient-days between 1993 and 1998 (9).

Even education and questionnaires and conferences rises the compliance, nothing is more clear that showing to somebody in a few seconds about the level of his hands following by wash and show again. We say that bioluminescence is similar with speed radar for detecting and transform behavior and self-consciousness.

RESULTS

If 60 RLU is the value recommended by manufacturer we can notice that 6 from 9

results (66%) are FAIL ranking and 3 from 9 (33%) are PASS. We can conclude that most of the hands are dirty at one moment of the day (10.00) (tab. II).

Regarding the toilet of patients 2 swabs from 7 (28.57%) were considered PASS and the rest FAIL (71.43%) (tab. III).

In the second hospital 25% of swabs are PASS, 75 % were FAIL and the same result was on patient toilet. Regarding patient environment, we have taken swabs from patient bed bar and commode and we had the following results: 3 /6 (50%) samples are PASS regarding bed bar and 5/6 (83%) samples are PASS for commode (tab. IV, V).

TABLE II

Values obtained from hands of healthcare workers and toilets on Hospital I

Hospital I Wards	RLU Healthcare workers' hands	RLU Patients toilets
1	179	694
2	36	-
3	14	157
4	261	230
5	415	95
6	92	281
7	158	-
8	465	119
9	49	245

TABLE III

Values obtained from hands of healthcare workers and toilets of patients on Hospital II

Hospital II Departments	RLU Healthcare workers' hands	RLU Patients toilets
1	1500	301
2	32	371
3	749	1014
4	222	71

TABLE IV

Values obtained from patient environment in Hospital I

HOSPITAL I wards	RLU bed bar	RLU commode
1	186	67
2	158	100
3	78	58
4	36	94
5	195	171
6	32	11

TABLE V

Values obtained from patient environment in Hospital II

Hospital II wards	RLU bed bar	RLU commode
1	191	6
2	448	240
3	552	403
4	92	560

One sample from 4 (25%) were PASS and 3 from 4 were FAIL regarding bed bar and the same values for commodes.

DISCUSSION

There is no consensus about monitoring of cleaning and in our country: visual inspection, Aerobic Colony Counts (ACC) is used and ATP. The literature has shown that inadequate cleaning identified by visual inspection, ACC, and ATP was 11.8%, 20.0%, and 50.6% before cleaning and 4.7%, 5.9%, 21.2% after cleaning (10).

From our study, we have demonstrated difference between visual inspection and ATP monitoring, after cleaning process, obtaining on hand hygiene failure on 66% (6 of 9) in unit I and 75% in unit II (3 from 4). Regarding the level of cleaning in other places in direct contact with patients (toilets, bed bar, commodes) the results were failed over 60%. In the meta-analysis of Almodio et al., the percentage of surfaces exceeding the chosen cut-off limit showed a failure rate varying from 21.2% to 93.1% before cleaning and from 5.3% to 96.5% after cleaning. The benchmarks were between 0-500 RLU (11).

This method has opponents because is not a direct identification of germs. The method is an indirect estimate of presence of ATP from fungal cells, but also bacterial cells and other cells alive and the low demands identification of germs. Because this identification in the microbiology laboratory needs 24-48-72 hours this method can give us an immediately orientation and can guide the swabs orientation used in microbiology.

Taking in consideration that candidemia is the fourth place in bloodstream infections, and *C. parapsilosis* was identified on the staff hands, implementing this technology in monitoring the hand hygiene and patient environment will decrease the level of these infections and all healthcare associated infections.

CONCLUSIONS

Our study analyzed the level of cleaning on hands and patient environment and values showed failure in most of the swabs. We estimate that determining cleaning using ATP followed by cleaning processes can decrease of invasive fungal infections. These results create us the input to propose to the Ministry of Health a project for all the emergency hospitals and control departments to have this technology implemented as the “radar” of cleaning.

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NEWS

COFFEE INTAKE DECREASE THE MULTIPLE SCLEROSIS RISK

Health benefits and health risks of coffee drinking were evaluated by numerous studies that used human or animal models. Some studies have linked coffee consumption with neuroprotective effects by decreasing inflammation and demyelination. The influence of coffee consumption on the risk of multiple sclerosis (MS) has been investigated in two retrospective case–control studies conducted in Sweden and the US. Both trials included people aged over 15 years who were diagnosed with MS and have investigated consumption habits of caffeinated coffee in different periods of age. The two studies have involved a total of 2779 cases and 3960 controls. The risk of MS among participants which consumes coffee was compared with the risk of MS among participants who never drank coffee, by calculating ORs with 95% CIs. The results showed a reduced risk of MS among those who reported a consumption of coffee exceeding 6 cups daily (OR 0.70 in the Swedish study, and OR 0.69 in the US study) in comparison with participants who never drank coffee. Additional studies are needed to determine which of biologically active constituents of coffee determines these results, to use them in multiple sclerosis therapy (A K Hedström; E M Mowry; M A Gianfrancesco; X Shao; C A Schaefer; L Shen; T Olsson; L F Barcellos; L Alfredsson. High consumption of coffee is associated with decreased multiple sclerosis risk; results from two independent studies. *Neurol Neurosurg Psychiatry* 2016; 87: 454-460).

Cătălina Luncă

SURGICAL MANAGEMENT OF TYPE A ACUTE AORTIC DISSECTION IN PATIENTS WITH MARFAN SYNDROME: A SINGLE CENTER EXPERIENCE

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SURGICAL MANAGEMENT OF TYPE A ACUTE AORTIC DISSECTION IN PATIENTS WITH MARFAN SYNDROME: A SINGLE CENTER EXPERIENCE (**Abstract)** Introduction Acute aortic dissection is the most common cause of death in patients with Marfan syndrome and untreated aortic root enlargement. Emergency surgery for replacement of the ascending aorta has the potential of life saving procedure, but is associated with high morbidity and mortality. Long-term outcomes after surgical repair of acute aortic dissection type A in patients with Marfan syndrome are limited. **Material and methods**: We made a retrospective study concerning emergency surgical intervention for acute aortic dissection type A, by Bentall procedure, performed in Emergency Institute for Cardiovascular Diseases „Prof dr. C. C. Iliescu" Bucharest between January 2005 and July 2014. We included 332 patients with type a acute aortic dissection divided into two groups: group A - 16 patients with Marfan syndrome and group B - 316 patients with other etiologies. We analyzed differences between these two groups regarding perioperative characteristics, surgical technique and short and long-term morbidity and mortality. **Results**: The patients from group A were significantly younger than those in group B (35.1±12.7 years *vs.* 56.8±7.1 years; $p<0.001$). Arterial hypertension was three times more common in group B as compared to group A ($p<0.001$). The incidence of postoperative morbidity, and intraoperative and 30-days mortality death rates were similar between groups, but overall mortality at 10 years was lower in group A (31.3%) *vs.* group B (44.9 %). **Conclusions**: Emergency surgical in Marfan syndrome, by Bentall procedure could improve perioperative morbidity and mortality of patients with acute aortic dissection, but need an early diagnosis, proper medical therapy and imagistic surveillance. **Keywords**: MARFAN SYNDROME, TYPE A ACUTE AORTIC DISSECTION, BENTALL PROCEDURE.

The aortic disease is a severe disorder with bad prognosis. This term encompasses a lot of different of conditions like aneurysm, dissection, atherosclerosis and a general stiffening of elasticity associated with ageing. The etiology is complex and include injury, arterial hypertension, bicuspid aortic valve or genetic disorders like Marfan syn-

drome (MS), Ehler–Danlos syndrome, osteogenesis imperfecta, or Turner syndrome. The aorta has a high degree of elasticity, necessary for the propulsion of blood in the systemic circulation. This feature is allowed by connective fibers, where the major components are elastin and collagen. Any alteration of quantity and/or architecture of these fibers induces mechanical changes that generate aortic disease.

The Marfan syndrome is a disease of connective tissue. It presents an autosomal dominant inheritance and affects 1/5,000 individuals. The majority of cases presents a *FBN1* gene mutation. Other mutations (around 10% of cases) concern *TGFbR1/2, COL3A1* or *ACTA2* genes. The diagnostic evaluation for MS is difficult due to the phenotypic variability, different age depending manifestations, the absence of well-established criteria, and a difficult differential diagnosis. The diagnostic criteria improve the clinical early diagnosis, but accuracy is also important to diminish the deleterious and irreversible consequences of diagnosis errors. The main clinical signs interest ocular, cardiovascular and skeletal systems, but also could be discovered changes in skin, lung or dura. The diagnosis of MS is based on so called Ghent criteria, established in 1991. The revisited Ghent criteria help the clinician in diagnosis of MS. Thus, when is identified a single case in a family, the diagnosis of MS could be established if is present one of the following associations: dilatation of aortic root (Z=2) and *ectopia lens*; dilatation of aortic root (Z=2) and *FBN1* gene mutation; dilatation of aortic root (Z=2) and a clinical score more than 7 or *ectopia lens* and pathogenic *FBN1* gene mutation for dilatation of aortic root. Where the diseases are present in other members of the same family, the diagnosis of MS is sustained by the association between familial history of MS and *ectopia lens*, dilatation of aortic root (Z=2) or clinical score more than 7. Excepting the dilatation of aortic root and *ectopia lens* the clinical score contains elements of skeletal, cardiovascular, ocular or skin. The main component of clinical score are: wrist and thumb sign, *pectus carinatum*, hindfoot deformity, pneumothorax, dural ectasia, and *protrusio acetabuli*.

The *FBN1* gene is located 15q21 and comprises 65 coding and 3 alternatively spliced exons. It encodes a glycoprotein - fibrillin-1 - with a molecular weight of 350 kD. Fibrillin-1 is a major component of the extracellular micro fibrils, which are connected to extracellular matrix to form sheaths around elastic fibers (Bonetti, 2009). Fibrillin-1 present three types of domains: an epidermal growth factor (EGF) like motif (many of these have a highly conserved calcium-binding sequence (cbEGF)), a latent transforming growth factor beta (TGF-β) binding protein motif and a fusion module. The main type of mutation (>2/3) is a missense mutation that affects the cbEGF modules, but also were described frameshift, splice site and nonsense mutations [Turner et al., 2009]. Except the mutations that concern exons 24–32 usually associated with a severe phenotype (“neonatal Marfan syndrome”) it is impossible to establish a genotype-phenotype correlation in MS.

The major cause of death in MS is acute aortic dissection (1). Without surgical treatment, this condition leads to death in more than 95% cases. Despite advances in surgical technique, the early mortality remains high. Treatment of patients with type A acute aortic dissection and Marfan syndrome is an emergency lifesaving proce-

dure (2). In a type A aortic dissection, simple replacement of ascending aorta is usually the best option, but when Marfan syndrome is the underlying cause of dissection, this option is not feasible because of important aortic root dilatation (3, 4). Under these circumstances, replacement of the ascending aorta and the entire aortic root represents the adequate surgical treatment (5). This study aimed to identify differences between perioperative characteristics, surgical technique and early and late mortality and morbidity in a group of patients operated for acute aortic dissection with and without Marfan syndrome.

MATERIAL AND METHODS

Patients cohorts

We made a retrospective study on a cohort of 332 patients underwent an emergency surgical intervention for acute aortic dissection type A. This surgical procedure was applied in Emergency Institute for Cardiovascular Diseases "Prof dr. C. C. Iliescu" from Bucharest, between January 2005 and July 2014. We excluded all patients with type B acute aortic dissection and those with type A acute aortic dissection complicated with mesenteric ischemia. The 332 patients were divided into two groups: group A including 16 patients with Marfan syndrome and Group B including the remaining 316 patients with acute aortic dissection by other etiology. We analyzed demographic data and perioperative features of the patients from available patients' medical records. Information regarding long-term survival was obtained from the National Institute of Statistics/The Register of Persons Records.

Surgery procedures All patients were evaluated before surgery by transthoracic and transesophageal echocardiography, in order to identify the location of intimal tears and the extension of the dissection.

The surgical strategy implied resection and replacement of the dilated segment (aortic root, ascending aorta or aortic arch) with a prosthetic graft, and exclusion of access intimal tear. Surgery was performed via standard median sternotomy with total cardiopulmonary bypass (CBP). Depending of the integrity of the peripheral vessels, cannulation is initiated via right axillary artery or right femoral artery for arterial access and right atrium for venous return. A vent was placed in the left ventricle via the right superior pulmonary vein. Myocardial protection with cold blood cardioplegia was initiated through retrograde route after cross clamping the ascending aorta. Completion of cardioplegia is achieved after transverse aortotomy at the level of ascending aorta and administration of the remaining dose of cold blood cardioplegia via direct cannulation of both coronary ostia. Inspection of the ascending aorta and the aortic valve was performed, together with the resection of the segment including the proximal entry tear, followed by repair or replacement of the aortic valve, as needed. After reaching a mean temperature of 25-28°C, during circulatory arrest the aortic clamp was removed and the entire aortic arch was examined and replaced whenever an aortic arch tear has been identified. The distal anastomosis with prosthetic collagen impregnated low porosity graft was then completed under circulatory arrest with anterograde selective cerebral perfusion. Teflon strips and sometimes biological glue were used to reinforce the distal suture lines. After completion of the distal anastomosis, the prosthetic graft and the great vessels are de-aired, and extracorporeal circulation is restarted after a cross clamp is placed on the prosthetic graft. Finally, in

the supracommissural aortic repair the proximal anastomosis is performed in a similar fashion using Teflon strips and biological glue. In the Bentall procedure we use a premanufactured conduit with Valsalva sinuses and a mechanical valve, each coronary button was sutured with Prolene 6.0 to the Valsalva part of the premanufactured conduit. Eight surgeons performed the operations throughout this period, using a similar perioperative and surgical strategy according to current available guidelines and hospital`s established protocols.

Bentall procedure

As the abnormalities found in patients with Marfan syndrome often compel to replacement of the ascending aorta and the aortic valve with a composite prosthesis containing a mechanical or bio prosthetic cardiac valve enclosed in a tubular polyester graft, operation referred to as the Bentall procedure. The patient is placed on cardiopulmonary bypass using a single venous two-stage cannula, with the arterial cannula in the femoral artery. The ascending aorta is clamped, retrograde cardioplegic solution is administered in the coronary sinus and a vent catheter is placed through the right superior pulmonary vein to the left atrium and left ventricle. The ascending aorta aneurysm is opened via a vertical incision. Traction stitches are placed above each aortic valve commissure to expose the aortic root and the aortic valve is excised. The coronary arteries are mobilized with buttons of sinus aorta and the remaining sinus aorta is removed. After calibration of aortic annulus, the composite prosthesis is sewn to the aortic annulus using mattress stitches of 2-0 braided polyester with PTFE felt pledges, starting from the right coronary sinus. Reimplantation of the coronary arteries requires direct anastomosis of the coronary ostia to the graft, but it can also involve a bypass graft or a polyester extension depending on the position of the ostia in relation to the aneurysm or dissection. The right coronary anastomosis is performed after completing the left coronary anastomosis, with deep bites into the aorta using continuous stitches of 6-0. The distal end of the graft is then shortened to allow a direct end-to-end anastomosis.

Statistical methods

Continuous variables are presented as mean ± standard deviation and compared using the Student t-test. Discrete variables were expressed as counts and percentages and comparisons between groups were done with the χ^2 or Fisher`s exact test, when appropriate. Five-year survival rates were estimated using Kaplan-Meier analysis and comparisons between groups were performed with the log-rank test. A two-sided P-value of 0.05 was considered significant for all tests. All analyses were conducted using *SPSS 21.0* (*SPSS*, Inc., Chicago, IL, USA).

RESULTS

Preoperative data are presented in Table 1. Only two statistical significant differences were discovered: patients with Marfan syndrome were significantly younger than patients of group B (35.1±12.7 *vs.* 56.8±7.1 years; $p<0.001$) and arterial hypertension was more common among patients' form group B (79.7% in group B *vs.* 25% in group A; $p<0.001$).

The operative data are reported in Table 2. These data showed only two significant differences. In Marfan syndrome group the replacement of aortic root and first portion of ascending aorta was more frequent applied (81.3% *vs.* 33.2%, $p<0.001$). Similar

value concerned the replacement of aortic root and entire ascending aorta more frequent applied in Marfan syndrome (56.3% *vs.* 18.4%, p=0.001).

TABLE I

Preoperative characteristics

Preop. characteristics	Group A (n=16)	Group B (n=316)	p value
Gender (female)	37.5% (6)	39.9% (126)	0.85
Age (years)	35.1±12.7	56.8±7.1	<0.001
Arterial hypertension	25% (4)	79.7% (252)	<0.001
Diabetes mellitus	0% (0)	5.7% (18)	1.00
Renal impairment	18.8% (3)	45.9% (145)	0.033

TABLE II

Operative data

Intraoperative characteristics	Group A (n=16)	Group B (n=316)	p value
Arterial cannulation sites			
Axillary artery	56.3% (9)	69.0% (218)	0.29
Ascending Aorta	12.5% (2)	2.5% (8)	0.08
Femoral artery	25.0% (4)	22.5% (71)	0.76
Axillary and Femoral artery	0.0% (0)	2.2% (7)	1.00
CPB time (min)	253.3±81.1	247.0±113.2	0.80
Cross clamp (time)	168.0±54.8	144.8±71.9	0.18
Cerebral perfusion (%)	33.3 % (5)	43.1% (129)	0.45
Circulatory arrest (min)	20.3±26.0	24.3±32.5	0.70
Temperature Circulatory arrest (ºC)	24.4±5.4	24.0±4.1	0.81
Aortic segment replaced			
Aortic root and first portion of ascending aorta	81.3% (13)	33.2% (105)	<0.001
Ascending aorta	6.3% (1)	30.7% (97)	0.046
Aortic root and entire ascending aorta	56.3% (9)	18.4% (58)	0.001

*CPB – cardio-pulmonary bypass.

All patients with Marfan syndrome had aortic insufficiency at the time of diagnosis, and the majority of them had grade 4 aortic regurgitation (11/16 patients). The Bentall modified operation (BMO) was performed in 11 patients, while in the rest cases were applied other type of surgical interventions.

Postoperative characteristics were evaluated but we did not found any significant differences between groups concerning mechanical ventilation time (106.6±252.5 hours in group A *vs.* 211.4±397 hours in group B, p=0.15) or length of stay in the intensive care unit (8.9±15.6 days in group A as compared to 13.8±19.5 in group B; p=0.25).

Follow-up was 100% complete as data were received from the National Institute of Statistics / the Register of Persons Rec-

ords. The mean follow-up of the study was 4 years (standard error ±2.76 years, min 1 day, max 10.2 years, and median 3.8 years) with x patients at the end of the study. Intraoperative death rate was similar in both groups without statistical significance (12.5% in group A *vs.* 6.0% in group B; p=0.27). The incidence of postoperative morbidity was not significantly different between the two groups, with the main incidents being: stroke, mediastinitis, and multisystem organ failure in both group. The only major difference was the acute kidney disease present in only 18.8% patients from Marfan syndrome group compared with 58.2% in group B (p=0.002). Also, we not found statistical significance differences regarding 30-day mortality (18.3% in group A compared with 30.1% in group B; p=0.41). Similar results we found the one-year mortality (7.7% in group A *vs.* 9.2 % in group B; p= 0.87) and mortality at 10 years (31.3% in group A *vs.* 44.9 % in group B; p= 0.28).

DISCUSSION

Marfan is a systemic connective-tissue, autosomal-dominant inherited disorder characterized by mutations in the fibrillin-1 gene (FBN1), with a prevalence of approximately 1 in 5000 individuals (6). There is no sex, racial, or ethnic predilection, and one third of cases present without previous family history (7). Management of Marfan syndrome with cardiac symptoms includes medical treatment with beta blockers and regular screening to assess the progression of the aorta dilatation to a significant diameter aneurysm, when surgical treatment is required to prevent to prevent dissection or rupture (8). Abnormalities frequently found in patients with Marfan syndrome include aortic root dilatation, associating aortic insufficiency due to aortic annulus dilation or bicuspid aortic valve.

Replacement with a composite graft containing a mechanical valve and a polyester tube graft - the modified Bentall operation - is the gold standard for such patients with corresponding aortic valve cusps pathology (9-11). For young patients with isolated aortic insufficiency with pliable aortic valve cusps, however, valve sparing root replacement might be a good option in experienced institutions (12). Yacoub and colleagues in 1983 introduced the aortic valve remodeling technique with replacement of the ascending aorta and the sinuses of Valsalva (13). David described replacement of the aorta with native aortic reimplantation into a prosthetic tube graft (12). For the past several years, the David aortic valve reimplantation operation has been the procedure of choice for patients with aortic root enlargement and pliable aortic valve cusps (14). Patients with severe damaged cusps are not good candidates for valve sparing operations (15).

Surgery for acute type A aortic dissection remains associated with high operative mortality and morbidity, and adding a long and complex aortic valve sparing may not be the safest approach (10). Current ESC/EACTS guidelines for the management of valvular heart disease state that, surgery should be considered in patients with Marfan syndrome with risk factors (family history of dissection, size increase 2 mm/year in repeated examinations) or who have aortic root disease with a maximum ascending aortic diameter of ≥45 mm (Class II a, Level C evidence) (16).

In a study analyzing the impact of surgical intervention in patients with Marfan syndrome, Ohsubo et al. showed that valve preserving aortic root reconstruction result-

ed in improved postoperative quality of life compared with Bentall operation, by reducing late complications related to anticoagulants (17). Unfortunately, in patients presenting in the acute setting of aortic dissection, valve-sparing root replacement or reconstruction is often not possible. After initial repair of an ascending aortic aneurysm, a significant number of patients have subsequent surgeries at other sites throughout the aorta, indicating that Marfan syndrome is a disease involving the entire aorta. Some studies show that patients with Marfan syndrome who had a dissection at the time of the first aortic surgery were more likely to require subsequent aortic surgery than those who underwent prophylactic composite graft repair of an aortic aneurysm (18).

As with any reconstructive procedure, these operations must be performed with intraoperative echocardiography to assess valve morphology and function at the end of the procedure. Restoration of normal aortic cusp geometry is the single most important technical aspect of aortic valve–sparing operations. In addition, to have a competent valve with no or only a minimal central AI at the end of the procedure, no cusp should be prolapsing, and the coaptation height has to be well above the level of the nadir of the aortic annulus.

Finally, patient selection also is important. Patients with severe AI before surgery often have damaged cusps and are not good candidates for this operation. Most of our patients had normal or near-normal cusps on.

Study limitations

This study has several limitations, especially related to the retrospective design of the study and the small number of patients in group A. Under these circumstances, advanced analysis, such as multivariable analysis, is unreliable. Nevertheless, our study provides meaningful information on management of patients with Marfan syndrome presenting with acute aortic dissection.

CONCLUSIONS

Due to the unstable condition of patients with Marfan syndrome presenting with acute aortic dissection, the Bentall operation was the most common surgical procedure performed in our cardiac surgery hospital. The operative mortality was relatively high, but not different in comparison with group without Marfan syndrome. The long-term survival of the patients with type A acute aortic dissection and Marfan syndrome was suboptimal. Early diagnosis and regular CT and EET surveillance of patients with Marfan syndrome and a proper medical therapy could reduce the incidence of the aortic dissection or aortic rupture, and may improve the results by making elective valve sparing operation feasible.

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LEFT ATRIAL, LEFT ATRIAL APPENDAGE AND PULMONARY VEINS ANATOMICAL VARIANTS IN PATIENTS WITH ATRIAL FIBRILLATION VERSUS PATIENTS IN SINUS RHYTHM

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LEFT ATRIAL, LEFT ATRIAL APPENDAGE AND PULMONARY VEINS ANATOMICAL VARIANTS IN PATIENTS WITH ATRIAL FIBRILLATION VERSUS PATIENTS IN SINUS RHYTHM (Abstract): The aim of the study is to evaluate by multidetector computed tomography (MDCT) the prevalence, location and size of LA (left atrial) diverticula and accessory appendages, the prevalence of PVs anatomical variants and LAA (left atrial appendage) shapes in patients with atrial fibrillation (AF) compared to patients in sinus rhythm (SR). Images obtained by MDCT in a group of 100 patients with <1 year paroxysmal or persistent AF prior to radiofrequency catheter ablation and in a group of 100 patients in SR with unconfirmed or insignificant (<50%) coronary arteries stenoses were compared. The prevalence of LA diverticula (22% in AF group, 19% in SR group) and accessory appendages (6% in AF group, 5% in SR group) was not significantly different between the two groups. Similar sizes of LA diverticula and accessory appendages were registered in patients with AF and in SR. The prevalence of a common left venous trunk was significantly higher in patients with AF (30%) compared to patients in SR (18%) (p=0.047) and proved to be an independent predictor for AF (odds ratio (OR) 1.32; 95% confidence interval (CI) 0.93–1.51; p=0.002) at multivariate logistic regression. LAA had a complex shape but no significant differences concerning the prevalence of the 4 major types were registered between the groups. In conclusion, the only parameter associated with AF was the presence of a left common trunk suggesting that it is either a consequence or a predisposing factor for the development of AF. **Keywords:** ATRIAL FIBRILLATION; SINUS RHYTHM; LEFT ATRIUM; LEFT ATRIAL APPENDAGE; LEFT ATRIAL DIVERTICULUM.

The left atrial appendage (LAA) is a complex morpho-anatomical structure distinct, from an embryological, anatomical and pathophysiological point of view, from the rest of the left atrium.

Atrial fibrillation (AF) is a well-known supraventricular arrhythmia but its exact relation to the LAA shape, left atrium (LA) accessory appendages, LA diverticula and anatomical variants of pulmonary veins (PVs) still remains unclear. In up to 94% of patients with AF, ectopic foci with electrical activity triggering AF have been found in myocardial muscle extensions located at the junction of PVs with the LA (1). Specialized conduction tissue with pacemaker activity

remains at the specified level after the looping process of the heart tube during development (2). Due to its high spatial and temporal resolution, multidetector computed tomography (MDCT) is the non-invasive imaging method of choice for evaluation of PVs and LA prior to radiofrequency catheter ablation (RFCA) in AF. Anatomical variants of LA, LAA shape and PVs with unknown electrical impact are frequently identified when performing cardiac MDCT.

The aims of the current study were to evaluate by means of MDCT the morphology of the LAA; to determine the prevalence, location, and size of LA diverticula and accessory appendages and to analyse PVs anatomical variants in patients with AF compared to patients in sinus rhythm (SR) in order to determine if the incidence of particular aspects of these structures is higher in patients with AF.

MATERIAL AND METHODS

Study design

The current study was designed as a retrospective evaluation carried out in the Radiology Department, Cardiovascular Diseases Institute, Iasi, Romania between September 2012-January 2014. Informed consent prior to examination was obtained in all cases.

Anatomical considerations

LAA is an embryological remnant of the primitive left atrium, morphologically described as a long, narrow, tubular hooked structure situated in the left atrioventricular sulcus close to the proximal part of the left circumflex artery. Posteriorly, it is closely related to the left superior PV, their ostia being separated by a ridge of variable width. This area is travelled by the oblique vein (OV) of the LA that can be sheathed by a myocardial sleeve – a potential electrical communication with the myocardium over the coronary sinus. From a clinical standpoint, LAA develops a distinct pattern of contractions different from the rest of the LA thus explaining its arrhythmogenic role in triggering AF (3) and its association with an increased thromboembolic risk. Wang et al. (4) classified LAA shapes in 4 groups, classification later improved by Kimura et al. (5): "*cactus*": a main lobe of less than 4 cm long, with more than 2 additional lobes; "*cauliflower*": a main lobe of less than 4 cm long, without any forked lobes; "*chicken wing*": a main lobe of more than 4 cm long, with a folded angle of less than 100^0; "*windsock*": a main lobe of more than 4 cm long, with a folded angle of more than 100^0, over 1 cm (fig. 1).

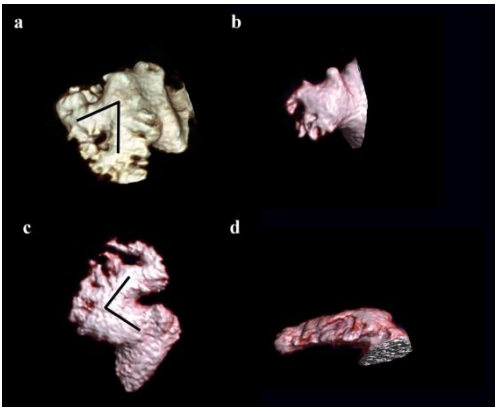

Fig. 1. LAA shapes: (a) "cactus", (b) "cauliflower",(c) "chicken wing", (d) "windsock"

A saccular structure protruding from the LA wall is considered a diverticulum (fig. 2a) if it has smooth walls and a broad-base ostium, or an accessory appendage (fig. 2b) if it has a narrow-base ostium and an irregular inner contour suggesting the presence of pectinate muscles. These saccular structures can occur anywhere on the surface of the LA and are classified according to the involved wall (anterior, posterior, right or left) and their position onto this wall (superior or inferior).

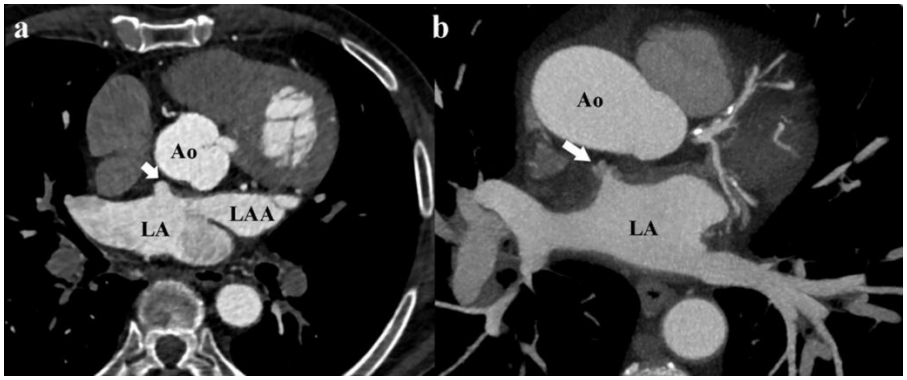

Fig. 2. Appearance of left atrial diverticula (a) and accessory appendage (b) in two different patients

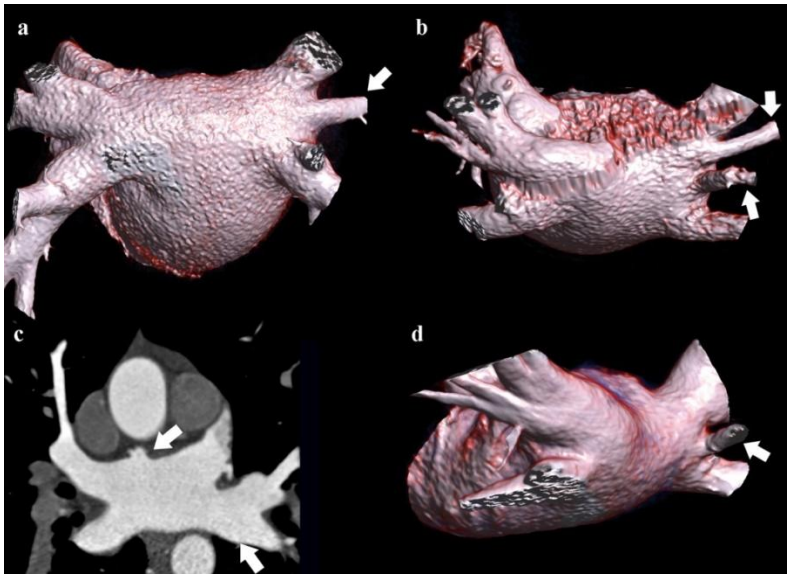

Fig. 3. (a) Separate right middle PV and short common left trunk, (b) Accessory intersegmental PV associated with a right middle PV, (c) long common left trunk and accessory appendage, (d) accessory right intersegmental PV

Most patients present two superior (right and left) and two inferior (right and left) PVs that have a distinct course from that of arteries or bronchi. Normally, the right superior PV drains the right superior and the middle lobes, the left superior PV drains the left superior lobe (culmen and lingula), and the inferior PVs drain the correspondent inferior lobes. Superior PVs ostia are larger than those of the inferior PVs. A minority of cases display variation in number (single antrum for the left/right-sided PVs with a long or short common trunk, supernumerary PVs), size and orientation of PVs. Kato et al. described 6 branching patterns of the PVs: 1) typical

branching pattern (two superior and two inferior PVs), 2) short common left trunk, 3) long common left trunk (fig. 3c), 4) one right middle PV, 5) two right middle PVs, 6) right middle PV and right "upper" PV (fig. 3b) (6). In the current study there were found 2 additional combinations: short or long common left trunk associated with a right middle PV (fig. 3a), and the presence of accessory intersegmental PVs directed towards the apical or a basal segment of the right inferior pulmonary lobe (fig. 3d).

Study groups

The authors retrospectively reviewed a group of 100 patients diagnosed with AF (paroxysmal or persistent) for less than 1 year (63 men and 37 women aged between 23-78 years with a mean age of 56.75 years) and no underlying structural heart disease that underwent cardiac CT examination prior to RFCA and a group of 100 patients in SR (52 men and 48 women aged between 25-79 years with a mean age of 58.74 years) referred for CT evaluation of suspected coronary arteries disease (CAD) but with no or mild (less than 50% diameter stenosis) coronary lesions and no other structural heart disease.

Clinical characteristics of study groups

Patients' clinical characteristics are summarized in Table I. Patients with previous cardiac surgery, other cardiac diseases (except mild CAD) or prior RFCA were excluded from the study.

TABLE I

Clinical characteristics of patients included in the study groups

	AF	SR	p
Number of patients	100	100	-
Age (mean ± SD)	56.75±11.39	58.74±11.23	<0.05
Sex ratio (M/F)	63/37	52/48	<0.05
Body mass index (kg/m^2)	25.6±4	26.1±3.5	n.s.
Diabetes mellitus	4 (4%)	8 (8%)	<0.05
Type of AF	65 paroxysmal, 35 persistent	-	-
Duration of AF (years)	5.2±3.5	-	-

Image acquisition protocol

All 200 patients were examined using a 2nd generation 256-slices dual source MDCT scanner (Siemens Somatom Definition Flash, Siemens Medical Solutions, Germany) with the following scan parameters: 100 or 120 kV tube voltage, tube current modulated by CareDose 4D algorithm (320 mAs reference), 128 x 0.6 mm collimation, gantry rotation time 280 ms. Two imaging protocols were used depending on the heart rhythm and cardiac pathology:

a) High pitch retrospective scanning in patients with high (more than 71 beats per minute) or irregular heart rates in order to perform end-systolic phase reconstructions;

b) High pitch prospective scanning in patients with stable heart rate less than 70 beats per minute.

Metoprololum (Metroprolol LPH 50 mg, LaborMed Pharma S.A., Romania) was administered orally to all patients the morning of the examination if no contraindication was present. Test bolus injection protocol was used for optimal contrast timing. A volume of 20 ml of contrast agent (Iomeron 400, Bracco, Milan, Italy) followed by 25 ml of saline chaser were injected via an 18G cannula placed in the right mediobasilic or mediocephalic vein at

a flow rate of 6ml/sec. A series of dynamic, low-dose monitoring scans were performed (region of interest placed in the ascending aorta) beginning 10 seconds after initiation of contrast agent injection in order to determine the time interval between the start of the test bolus injection and the peak of aortic enhancement. After the aortic peak time evaluation (APT) 2 puffs of nitroglycerin (0.8 mg) were administered sublingually for coronary artery dilation, thus allowing the start of the main examination. An average volume of 85 ml of contrast media and 50 ml of saline chaser were injected and scanning in a single breath hold began after a delay based on the previously determined APT+5 seconds. Optimal reconstructions at different R-R interval percentages were performed (thickness 0.75 mm). Images were reconstructed at 30-80% of the R-R interval in 5% increments and optimal reconstruction interval was chosen for image analysis on a Syngo.via workstation (Siemens Medical Solutions, Germany) by two independent radiologists in order to obtain more objective results.

Statistical analysis

Statistical analysis was carried using IBM SPSS Statistics 22.0 and Excel 15.20 (Mac OS X platform). Quantitative data was tested for normality and presented as mean value ± standard deviation (SD). Student's t test was used to compare means between the AF group and SR group. Qualitative data was presented as percentages and compared using Pearson chi-square test. Multivariate logistic regression analysis was performed to identify independent predictors of AF after adjusting for potential confounders with univariate analysis. A p value of less than 0.05 was considered significant.

RESULTS

AF group

Most of the patients in the AF group had a "*chicken wing*" shaped (43%) or a "*cauliflower*" shaped (25%) LAA. Seven LA accessory appendages were identified in 6 cases, one patient having 2 LA accessory appendages. Six of them were located on the right anterosuperior wall and one on the right anteroinferior wall. Twenty-four diverticula were identified in 22 patients, 2 patients presenting 2 diverticula.

As in the case of LA accessory appendages, most of the diverticula (18 – 75%) were located on the right anterosuperior wall, 4 (16.67%) on the right anteroinferior wall and 2 on the left anteroinferior wall (8.33%). LA accessory appendages had a narrower base (7.42 mm) and were longer (9.38 mm) compared to diverticula (8.8 and 7.1 mm respectively). Considering PVs variants, a common left trunk was the most frequently accounted (30%) followed by separate right middle lobe PV(s) (single or double in 11% of cases). Accessory intersegmental PVs were noted in 6% of cases, while an association of variants was identified in 5% of cases.

SR group

As in AF group, most patients presented a "chicken wing" shaped LAA (55%). Five single accessory appendages and 19 diverticula were found, none of the patients had multiple accessory appendages and in one case 2 diverticula were identified. The most common location for both accessory appendages and diverticula was the right anterosuperior wall, one accessory appendage was located on the left anterosuperior wall and one diverticulum, on the left anteroinferior wall. A common left PVs trunk was identified in 18% of cases, followed by separate right middle lobe PV(s) (12%). An

accessory intersegmental PV was present in 5 cases (5%), and an association of variants in 3 cases (3%). The only parameter the proved to be associated with AF at univariate analysis was the presence of a left common venous trunk (tab. II).

TABLE II
Characteristics of patients with AF and in SR

Parameter	AF	SR	p
LAA shape:			
-"cauliflower"	25/100	21/100	
-"cactus"	16/100	14/100	n.s.
-"windsock"	16/100	10/100	
-"chicken wing"	43/100	55/100	
LA accessory appendage:			
-prevalence	6/100	5/100	
-number	7	5	
-width	7.42±3.15 mm	7.34±2.97 mm	n.s.
-length	9.38±4.28 mm	9.94±3.97 mm	
-single	5/6	5/5	
-multiple	1/6	-	
LA accessory appendage location:			
-right anterosuperior	6/7	4/5	n.s.
-right anteroinferior	1/7	-	
-left anterosuperior	-	1/5	
LA diverticula:			
-prevalence	22/100	19/100	
-number	24	14	
-width	8.8±3.1 mm	8.1±3.62 mm	n.s.
-length	7.1±2.5 mm	6.5±1.7 mm	
-single	20/22	12/13	
-multiple	2/22	1/13	
LA diverticula location:			
-right anterosuperior	18/24	13/14	n.s.
-right anteroinferior	4/24	-	
-left anteroinferior	2/24	1/14	
PVs variants:			0.047
-common left trunk	30/100	18/100	
• short common left trunk	21/100	14/100	
• long common left trunk	9/100	4/100	
-right middle PV(s)	11/100	12/100	n.s.
• 1 right middle PV	9/100	9/100	
• 2 right middle PVs	2/100	3/100	
-accessory right intersegmental PV	6/100	5/100	n.s.
-association of variants	5/100	3/100	n.s.

AF – atrial fibrillation; LA – left atrium; LAA – left atrial appendage; PV – pulmonary vein; SR – sinus rhythm

Multivariate logistic regression analysis demonstrated that the only parameter that acted as an independent predictor for AF was the presence of a left common trunk

(odds ratio (OR) 1.32; 95% confidence interval (CI) 0.93–1.51; p=0.002) suggesting that it is either a consequence or a predisposing factor for the development of AF.

DISCUSSION

LA accessory appendages and diverticula are frequently identified in patients undergoing cardiac CT examinations for various reasons, without any association with a congenital pathology. In some cases, it is difficult to differentiate accessory appendages from diverticula as irregularities of the inner wall might be too subtle or the base too wide. Histologically, all cardiac layers form diverticulum's wall, but it can contain less myocardium than normal LA wall thus being translucent. Wan et al. consider that LA diverticula represents an acquired condition secondary to the atrophic thinning of the atrial wall due to various cardiac and systemic pathologies (atherosclerosis, arrhythmias, systemic thrombosis, occlusion of the arteries supplying the atrium, mitral valve disease) (7). Unfortunately, the authors couldn't identify any study published to date to actually certify those findings. Contrary to diverticula, accessory appendages contain pectinate muscles and have contractile properties as it has been demonstrated by Killen et al. Lee et al. that suggested and sustained a common embryonic origin of LA accessory appendages and LAA (8,9).

In the current study groups LA accessory appendages and diverticula were common structures with a cumulated incidence of 28% in the AF group and 24% in the SR group. Diverticula were identified 3.7 more frequent than accessory appendages in the AF group and 4.2 more frequent in the SR group. The prevalence of diverticula in the group with AF (22%) was not significantly higher than in patients without AF (19%) and correlates with previous researches that have stated a prevalence of 23.5 - 36% in patients with AF and 16.7 – 41% in general population or patients in SR (1,7,10-15). Both diverticula and accessory appendages were most frequently located in a right lateral position on the anterosuperior wall. The size of accessory appendages and diverticula was similarly between the two groups and within the wide range reported by other studies (tab. III) (1,7,10-16). Absence of a statistical significant difference in size, location and prevalence of diverticula and accessory appendages between patients in AF and SR pleads against a relation between these structures and the genesis of AF.

Nevertheless, the presence of diverticula and accessory appendages should be signalled to electrophysiologists performing RFCA. Their ostia could be mistaken to those of PVs during procedure, and isolation attempts could determine a perforation of the atrial wall with subsequent hemopericardium. During the current analysis no thrombus was visualized inside these structures.

Anatomical variants of PVs are frequently encountered both in patients with AF and in SR as proved by the results of the current study (prevalence of 52% in AF group and 38% in SR group) and the reviewed literature (tab. III). The predominantly encountered variants were a common left trunk (30% in AF group and 18% in SR group) and 1 or 2 accessory right middle PVs (11% in AF group and 12% in SR group). Our findings are similar to those reported by other authors (6,17-23). A precise knowledge of the anatomy of PVs is mandatory prior to RFCA as non-isolation of a vein could lead to failure of

the procedure, PVs stenosis or recurrence of AF. Cronin et al. state that, given the small diameter of the right middle PV, it is often difficult to identify this vein during procedure, so it might be overlooked (24).

Bittner et al. reported a higher prevalence of a common left trunk in patients with AF as compared with controls. These results were reproduced during our study. We also identified a significant difference between the prevalence of a common left trunk in patients with AF and patients in SR. In order to explain this finding Bittner et al. proposed two hypotheses: 1) congenital variant that constitutes a risk factor for developing AF; 2) acquired condition secondary to the remodelling of LA geometry associated with AF that "pushes" the carina between the two left PVs thus creating a common left trunk (18). In either case, the presence of a common left trunk is associated with the occurrence of AF. Concerning the other PVs variants, prevalence was not significantly different between the two studied groups.

Several authors (tab. III) studied the morphology of LAA in general population, patients with AF and patients with AF and a history of stroke and found it to be complex and heterogeneous (3-5). Di Biase et al. and Kimura et al. proved the existence of a relation between the morphology of LAA and the risk of thrombotic stroke in patients with FA, "chicken wing" shaped LAA being associated with a lower risk of stroke compared to "cauliflower" shaped LAA that was considered an independent predictor of stroke (3,5). In our study group, there was identified no significant statistical difference between the prevalence of different LAA morphologies in patients with FA and patients in SR, thus allowing us to affirm that the shape of the LAA could be of importance only in patients with AF, already at risk for stroke.

TABLE III

Review of the literature concerning analysed structures

Study	Prevalence of diverticula	Width of diverticula	Height of diverticula	Dominant location
Peng et al. 2012 (10)	36% in AF 32.7% in SR	5.3±2.9 mm in AF	5.6±3.3 mm in AF	Right anterosuperior
Abbara et al. 2009 (11)	19.4% in GP	6.2 ± 3.9 mm in GP	6.4 ± 2.5 mm in GP	Superior anterior
Lazoura et al. 2012 (1)	23.5% in AF 20.5% in SR	8.1 mm in AF 7.2 mm in SR	8.1 mm in AF 7.8 mm in SR	Right anterosuperior
Troupis et al. 2012 (12)	40% in AF 36% in SR	3.6 mm in AF 5 mm in SR	4.7 mm in AF 6.2 mm in SR	Right anterosuperior
Shin et al. 2011 (13)	18.3% in GP	4.7 ± 2 mm in GP	4.7 ± 2.1 mm in GP	Right side of the upper left atrial wall
Incedayi et al. 2012 (14)	41% in GP	5.2 mm in GP	-	Anterosuperior
Poh et al. 2008 (15)	28% in GP	-	-	Anterosuperior
Wan et al. 2009 (7)	16.7% in GP	4.9 ± 3.2 mm in GP	5.4 ± 2 mm in GP	Anterior

Study	Prevalence of accessory appendages	Width of accessory appendages	Height of accessory appendages	Dominant location
Duerinckx et al. 2007 (16)	10% in GP	9 mm in GP	6 mm in GP	Right upper atrial wall
Abbara et al. 2009 (11)	8.5% in GP	3.9 ± 2.4 mm in GP	4.9 ± 2.1 mm in GP	Left lateral inferior posterior
Lazoura et al. 2012 (1)	6.5% in SR 6.5% in AF	6.3 mm in AF 5.7 mm in SR	8 mm in AF 9.4 mm in SR	Right anterosuperior
Troupis et al. 2012 (12)	8.5% in AF 10% in SR	4.7 mm in AF 4.6 mm in SR	6.9 mm in AF 12 mm in SR	Right anterosuperior
Incedayi et al. 2012 (14)	3.08% in GP	-	-	Inferolateral
Poh et al. 2008 (15)	14% in GP	-	-	
Study	**"Cauliflower"**	**"Cactus"**	**"Chicken wing"**	**"Windsock"**
Di Biase et al. 2012 (3)	2% in AF	28% in AF	50% in AF	19% in AF
Kimura et al. 2013 (5)	17.5% in AF	2.5% in AF	13.8% in AF	28.8% in AF
Wang et al. 2010 (4)	29.1% in AF+SR	5.9% in AF+SR	18.3% in AF+SR	46.7% in AF+SR
Study	**Prevalence PVs variants**	**1st most frequent variant**	**2nd most frequent variant**	**Study**
Kato et al. 2003 (6)	38% in AF	Common left trunk	Middle PV(s)	Kato et al. 2003
Anselmino et al. 2010 (17)	60% in AF	Common left trunk	Middle PV(s)	Anselmino et al. 2010
Bittner et al. 2011 (18)	44.6% in AF 29.5% in SR	Common left trunk	Middle PV(s)	Bittner et al. 2011
Kaseno et al. 2008 (19)	24% in AF	Middle PV(s)	Common left trunk	Kaseno et al. 2008

AF – atrial fibrillation; GP – general population; SR – sinus rhythm

CONCLUSIONS

LA accessory appendages and diverticula are frequently identified in patients undergoing cardiac CT examinations for various reasons, without any association with congenital pathologies. In the current study groups LA accessory appendages and diverticula were common structures with a cumulated incidence of 28% in the AF group and 24% in the SR group. Diverticula were identified 3.7 more frequent than accessory appendages in the AF group and 4.2 more frequent in the SR group. No difference in prevalence, size and location of LA accessory appendages and diverticula between patients with recurrent AF referred for RFCA and patients in SR was identified in the present study. The prevalence of a left common venous trunk was higher in patients with AF compared to patients in SR and proved to be an independent predictor for AF suggesting that it

is either a consequence or a predisposing factor. The prevalence of LA shapes was similar in patients with AF compared to patients in SR. Absence of a statistical significant difference in size, location and prevalence of diverticula and accessory appendages between patients in AF and SR pleads against a relation between these structures and the genesis of AF. The presence of diverticula and accessory appendages should be signalled to electrophysiologists performing RFCA

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NEWS

CORRELATION BETWEEN C REACTIVE PROTEIN LEVEL AND DEPRESSION

The purpose of this study was to examine the association between C reactive protein and depressive symptoms in an elderly Korean population. Among participants aged 60 or over recruited from a rural community, 224 men and 345 women without a history of stroke, angina pectoris, myocardial infarction or CRP≥20 mg/L were employed for cross-sectional analyses. As a marker of systemic inflammation, CRP was measured.In men, CRP levels had significant associations with depressive symptoms. However, in women, the association between CRP and depressive symptoms was not significant. Our findings suggest that elevated CRP levels are independently associated with the presence of depressive symptoms in elderly Korean men (Song BM, Lee J-M, Choi W et al. Association between C reactive protein level and depressive symptoms in an elderly Korean population: Korean Social Life. *BMJ* Open 2015;5: e006429. doi:10.1136/bmjopen-2014-006429. Epub 2015 Feb 3).

Raluca Mihaela Struț

EFFECT OF TRANEXAMIC ACID USE ON POSTOPERATIVE BLOOD LOSS IN TOTAL KNEE ARTHROPLASTY

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EFFECT OF TRANEXAMIC ACID USE ON POSTOPERATIVE BLOOD LOSS IN TOTAL KNEE ARTHROPLASTY (Abstract). **Aim**: To monitor the effect of tranexamic acid use (Exacyl) on postoperative bleeding (aspiration), autologous blood transfusion (autotransfusion), allogeneic blood transfusion and postoperative anemia (difference between preoperative and immediate postoperative hemoglobin levels) in total knee arthroplasty. **Material and methods**: This retrospective observational study was conducted on a sample of 457 patients who underwent endoprosthetic knee arthroplasty in the interval January 1, 2008-July30, 2014 at the Traumatology-Orthopedics Clinic of the Iasi Rehabilitation Hospital. The mean age of the study group was 66 years (range 32-84 years), 74.4% were female, 39.8% presented *varus* and 6.1% *valgus* malalignment. The study group was subdivided into two groups: **Group 1** served as controls and underwent surgery without the administration of tranexamic acid and **Group 2** received tranexamic acid. **Results:** In group 1 the average amount of blood lost was significantly higher than in group 2, 1168.94 ml *vs* 452.9 ml ($p < 0.001$). The use of a cell saver was required in 70% of group 1 patients with an average amount of auto transfused blood of 480.70 ml, significantly more frequent and in greater amounts than in group 2 (below 10%) with an average of 15.05 ml ($p < 0.001$). In this study tranexamic acid had not a statistically significant favorable effect on blood transfusion requirements and anemia syndrome. **Conclusion:** Tranexamic acid has a favorable effect on postoperative blood loss and its associated complications, with a favorable impact on both early clinical and functional recovery. **Keywords:** TRANEXAMIC ACID, TOTAL KNEE ARTHROPLASTY, TOURNIQUET, BLOOD LOSS.

Knee osteoarthritis is one of the most common causes of pain and loss of joint function, the risk of disability being as high as that attributable to cardiovascular diseases and higher than in any other disease in the elderly (1). The disease is more common in women (2). In the advanced stages of knee osteoarthritis endoprosthetic arthroplasty is the gold standard treatment, the technique being customized according to the associated deformity and individual joint architecture (bone and ligaments) (3).

The use of a tourniquet induces local fibrinolysis in the operated lower leg and increased fibrinolytic activity causes significant postoperative blood loss. Blood loss causes such postoperative complications as anemia (4), cardiovascular complications, post transfusion reactions (post transfusion shock, transfusion transmitted diseases, postoperative infection) (5.6), with a direct

impact on the early postoperative course and functional recovery. Tranexamic acid, a synthetic analogue of lysine which competitively inhibits the activation of plasminogen to fibrin (7.8) is used to reduce postoperative blood loss. The aim of this study was to assess the effect of tranexamic acid (Exacyl) use on postoperative bleeding (aspiration), autologous blood transfusion (use of cell saver) and allogeneic blood transfusion on postoperative anemia syndrome (difference between preoperative and immediate postoperative Hb levels, *D* Hb).

MATERIALS AND METHODS

The retrospective observational study was conducted on a sample of 457 patients diagnosed with advanced knee osteoarthritis who underwent endoprosthetic knee arthroplasty in the interval January 1, 2008 - July 30, 2014 at the Traumatology-Orthopedics Clinic of the Iasi Rehabilitation Hospital. The mean age of the study patients was 66 years (range 32-84 years), 74.4% of them being female. The study group was subdivided into two groups: group 1 (78.11%) - patients who were not given Exacyl and group 2 (21.89%) - patients who received intravenous tranexamic acid intraoperatively and immediately postoperatively.

Also evaluated was blood loss depending on the presence or absence of frontal plane deformities, as evidenced by the calculation of hip-knee-ankle (HKA) angle on long leg X- ray (fig.1.a, b). 53.6% of the patients had proper knee alignment, while varus and valgus malalignment was present in 39.8% and 6.1%, respectively.

Sagittal plane malalignment, in flexum, was associated with varus misalignment in 97% of the cases, being the sole finding in only two cases. Of the study patients with genu varum 44% presented a mild deformity of 8 degrees and 18% of over 15 degrees. In genu valgum patients, the three categories were in almost identical percentages.

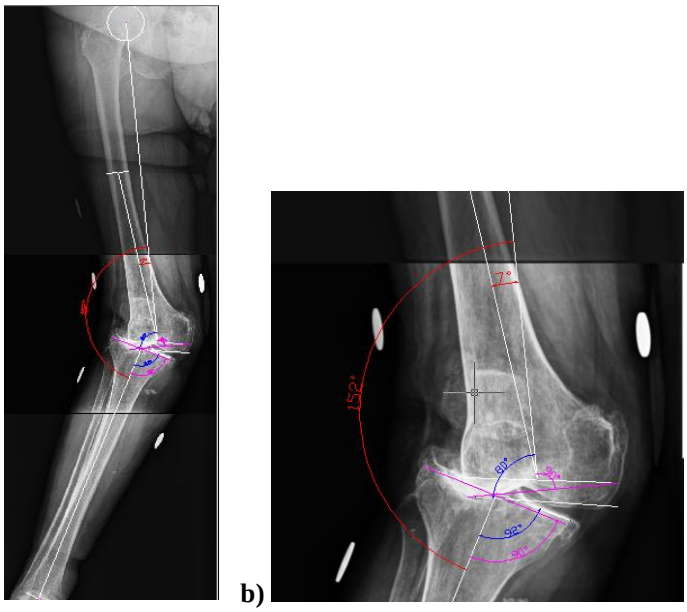

Fig. 1. a) Preoperative planning: Digital long leg X-ray of the lower limb in upright position; b)Advanced secondary knee osteoarthritis on *genu valgum* (HKA- 152^0)

RESULTS

1. **Aspiration:** Comparing the blood loss in the two groups we found a statistically significant difference ($p < 0.001$). If in group 1 the average blood loss was of 1168.94 ml (highest: 4150 ml), the analysis of data from group 2 showed an average blood loss of 452.9 ml (highest: 1100 ml) (fig. 2.) Blood loss was not influenced either by age ($p = 0.336$) or sex parameters ($p = 0.512$).

2. **Autotransfusion**: The need for a cell-saver and the amount of autotransfused blood were significantly higher in group 1 ($p < 0.001$). In group 1 a cell-saver was used in 70% of the cases and the average amount of autotransfused blood was 480.70 ml. In group 2 the average amount of autotransfused blood was of only 15.05 ml, and cell saver was used in less than 10% of patients.

3. **Blood Transfusion**: The difference between the two groups was not statistically significant ($p = 0.436$). A lower rate of allogeneic blood transfusion was recorded in group 1 (20% of cases, average amount 68.91ml) compared to group 2 (32% of cases, average amount 99.5ml).

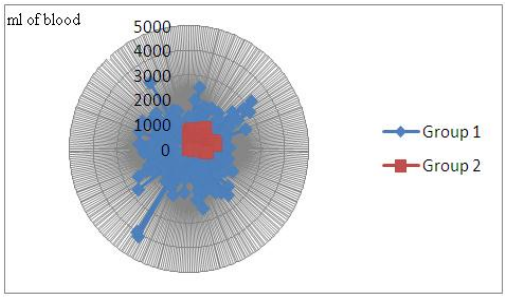

Fig. 2 Graphical representation of blood loss in the two study groups

4. **Anemia syndrome**: Mean *D Hb* was 3.27 mg/dl in group 1 and 2.74 mg/dl in group 2.The highest level was 8.10 mg/dl in group 1compared to 4.90mg/dl in group 2. Despite the values differed, the difference did not meet statistical significance ($p = 0.514$)

5. **The presence of deformities:** These variables for quantifying blood loss were also checked within the context of present or absent joint deformities, without noticing significant differences (tab. I). We noted that frontal plane deformities greater than 15 degrees were often associated with an increase in the amount of blood loss.

TABLE I

Average values of blood loss variables according to the presence/absence of diformity

Diformity Y N		No	Mean	Std. Deviation	Std. Error mean
Aspiration	No diformities	245	1001.14	597.456	38.170
	Diformities	212	1025.09	604.920	41.546
Autotransfusion	No diformities	245	357.22	388.491	24.820
	Diformities	212	403.75	387.336	26.602
Transfusion	No diformities	245	77.55	158.522	10.128
	Diformities	212	73.35	149.278	10.252
Hb difference	No diformities	245	3.0718	1.31196	0.08382
	Diformities	212	3.2521	1.24505	0.08551

DISCUSSIONS

Endoprosthetic knee arthroplasty is accompanied by minimal intraoperative bleeding due to the use of pneumatic tourniquet but by important postoperative blood loss.

This study shows that the use of tranexamic acid results in a significant decrease

in postoperative blood loss and hence in the use of cell saver, but has no impact on allogeneic blood transfusion. Our results are in agreement with literature data in terms of the amount of blood collected during suction drainage (4,7,9,10-12) but not in terms of the amount of blood needed for allogenic blood transfusion (11,12). In our study group the amount of transfused blood did not differ significantly between the two groups, the decision to resort to this therapy being more frequent in group 2, probably due to the fact that cell-saver in more rarely used in this category of patients. It is worth mentioning that although in group 2 the average blood loss was small, anemia syndrome was not statistically more significant in group 1. This mismatch between the amount of drained blood and postoperative anemia raises the suspicion of an additional blood loss. Sehat (13) speaks of a hidden blood loss of 700 ml, probably from hematoma formation.

Tranexamic acid, a synthetic inhibitor of fibrinolysis which acts by competitively blocking the lysine binding sites on plasminogen (7), reduces blood loss and financial cost in orthopedic surgery (hip and knee replacement surgery, spinal surgery) (4,9) and cardiovascular surgery (cardiopulmonary bypass) (14) but only insignificantly the hidden blood loss. This phenomenon probably represents the extravasations of red cells after tourniquet release, hemostasis being by primary hemostatic mechanisms, before fibrinolysis has any effects (15).

CONCLUSIONS

Our study highlights the importance of using tranexamic acid in knee arthroplasty as it significantly decreases the postoperative blood loss and financial cost given the fact that the use of cell-saver is less frequently required. This research demonstrates that tranexamic acid does not have a significant impact on allogeneic blood transfusion and anemia syndrome. Tranexamic acid is an essential drug product in total knee arthroplasty as it reduces postoperative blood loss and its associated complications, with a favorable impact on early recovery of joint function.

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NEWS

ANTIMICROBIAL ROLE OF FILAGGRIN-2 PROTEIN

Filaggrin-2 (FLG2) is a water-insoluble 248 kDa S100 fused-type protein found in the upper epidermis and eccrine sweat glands. A study by Hansmann *et al.* tested the antimicrobial activity of filaggrin-2 against *Pseudomonas aeruginosa* and other Pseudomonads and found that recombinant FLG2 C-terminal protein fragments have potent antimicrobial activity against *P. aeruginosa* and after cultivation of *P. aeruginosa* on stratum corneum, antimicrobially active FLG2 fragments are released from insoluble material by the bacteria themselves. These FLG2 C-terminal fragments have an alternative mode of action different from most other antimicrobial peptides, as they do not induce pore formation, but membrane blebbing. According to Hansmann et al., FLG2 fragments interfere with bacterial replication, inhibit their growth on skin surfaces and contribute to the skin's antimicrobial defense, explaining why *Pseudomonas* infections of healthy skin are very rare. Also, the absence of filaggrin 2 at certain body surfaces and in the case of burned skin seems to be responsible for the higher susceptibility to these infections. The study concluded that FLG2 C-terminal fragments could represent new *Pseudomonas*-targeting antimicrobials (Hansmann B, Schröder JM, Gerstel U. Skin-Derived C-Terminal Filaggrin-2 Fragments Are Pseudomonas aeruginosa-Directed Antimicrobials Targeting Bacterial Replication. *PLoS Pathog*. 2015;11(9):e1005159. doi: 10.1371/journal.ppat.1005159).

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ETHICAL AND DEMOGRAPHICAL ISSUES IN SCREENING FOR CERVICAL CANCER –AN OUTLINE IN NORTH EASTERN REGION OF ROMANIA

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ETHICAL AND DEMOGRAPHICAL ISSUES IN SCREENING FOR CERVICAL CANCER –AN OUTLINE IN NORTH EASTERN REGION OF ROMANIA (Abstract)**: Aim**: This study complements the national screening program for cervical cancer, implemented in Romania. **Material and Methods**: Inclusion criteria for this program of active detection of cervical cancer are women aged 25 to 64 years and exclusion criteria were other age groups and the diagnosis confirmed of cervical cancer. The testing was free and used the method of colored cervical smear Pap. **Results**: In 2013, 23.680 women were tested, that means only 10.13% of the total female population eligible for screening. In 2014, the test group was 6337 (one smear required retesting), of which 5852 were negative results and 484 positive results. Squamous cell abnormalities were detected in 433 women (6.84% of total group who attended the screening). Cervical smears with atypical squamous lesions were present at the most women included in the screening, regardless of age group (67.43%). The screening detected 21 lesions HSIL (4.84%), 2 HSIL with suspicion of invasion (0.46%) and 5 lesions of squamous carcinoma (1.15%). **Conclusions**: The presence of a small number of people compared to the female population eligible to the test screening may show the need for a better spread of notions about th screening and the afferent national program, for the population in northeastern Romania, considering the ethical issues that this test assumes. **Keywords**: CERVICAL SCREENING, CANCER, ETHICS, CERVICAL SMEAR, HUMAN PAPILLOMAVIRUS.

Cervical cancer is the fourth in frequency between the woman`s malignancy worldwide and the seventh of all cancers in both sexes, with 528,000 new cases detected in 2012, as GLOBOCAN reported. Regarding mortality, it is estimated 266,000 annual deaths worldwide in 2012, representing 7.5% of all deaths in women (1).

In the European region, Romania ranks first in the incidence and the mortality of cervical cancer. Regarding the incidence, it was 39.4% ooo in 2012, according to GLOBOCAN, with 4343 new cases registered, a standardized age rate of 28.6 and a cumulative risk (0-74) 2.8. The distribution by age showed that the most affected were women 50-54 years (83.3% ooo), then those 55-59 years (80.9% ooo) (2).

There is a regional variation in the incidence of cervical cancer, with higher rates of over 30% ooo women in Maramures, Hunedoara, Arad, Constanta, Timis, Mehedinti, compared with rates below 10%ooo recorded in Ilfov, Bucharest and Vrancea (3).

Regarding the mortality, its values were 17.3% ooo in 2012, according to GLOBOCAN, with 1909 deaths, a standardized age rate of 10 and a cumulative risk (0-74) 1.2. Most deaths were recorded among women in the 70-74 years age group (39.2% ooo), then those 65-69 years (38.2% ooo) (2).

Cervical Intraepithelial Neoplasia (CIN) is a precancerous condition that may exist in one of three stages: CIN1, CIN2 and CIN3. If it is untreated, CIN2 or CIN3 (together CIN2 +) can progress to cervical cancer (4).

It is estimated that approximately 1-2% of women have CIN2 + every year. This rate is reported to be close to 10% of HIV-positive women (5).

Standard screening test is colored cervical smear Pap, and when cytology results are positive for CIN, the diagnosis is based on subsequent colposcopy, biopsy of suspicious lesions, followed by treatment only when CIN2 + lesions was confirmed by histopathology. This traditional method of screening requires high-skilled human resources and a substantial amount of laboratory equipment. In countries with low and medium socioeconomic level, because of the high cost of such screening programs based on cytology, national coverage is very low. Further, the monitoring of women with positive cytologic test and their subsequent testing by colposcopic and biopsy requires resources and trained personnel, whicih is a major problem in many such regions. Other blockages in cytology-based screening programs includes long distances between the woman's residence and equipped hospitals or clinics. An alternative approach to the detection and the treatment of lesions of CIN may be the method "Screening and Treatment" ("screen-and-treat"), where the treatment decision is based on a screening test rather than a histologically confirmed diagnosis of CIN2 + (4).

Molecular epidemiology data showed in 1991 that human papilloma viruses (HPV) are the main etiologic agent of cervical cancer, however, only in 1997 was established this fact. The study of HPV carcinogenic role continues to be a priority in research on the molecular biology of cervical cancer, especially as the number of viral genotypes identified and characterized is rising. According to a study of the female population in the North-East of Romania (Iasi), HPV16 was the prevalent oncogenic genotype (43.12%), followed by HPV18 (10.34%) and HPV31 (10.34%), and the distribution of age groups showed that the percentage of HPV positive patients was approximately the same (65% in the 17-29 years, 66.15% in the 30-39 years and 70.83% in the 40+ years). The authors of this study concluded that, although cytological screening test will not be replaced by HPV, the results on genotypes circulating will be used to develop an algorithm for effective monitoring and proper management of this type of infection (6).

Cervical Neoplasia has a progressive development, slight dysplastic changes progressing to severe dysplasias, then to carcinoma *in situ*, and without therapeutic intervention, to an invasion of squamous cell carcinoma. Screening is one of the methods of prevention (in the literal sense

of the word, screening is a protection against the danger), being a test of orientation that allows presumptive identification of the people affected previously by unknown diseases or who are exposed to risk factors. The success of screening methods and the increase of women's compliance on cervical cancer measures of prevention encourages the health systems makers to make these methods more accessible (7).

MATERIAL AND METHODS

The study comes as a complement to The National Screening Program for Cervical Cancer, implemented in Romania. *Criteria for inclusion* in this program of active detection of cervical cancer are women aged between 25 and 64 years and *criteria for exclusion* were the other age groups and the confirmed diagnosis of cervical cancer. Testing was free and used the cervical colored smear Pap method.

The program started in October 2012, and the number of women tested by the end of that year was 2915. In 2013, 23,680 women were tested, compared with eligible women population for cervical screening, of 233.615 (census provided by those networks validated until the present and it has been included in the budget estimates for 2013, as it stated in official documents of the Iasi Regional Oncology Institute) (10.13% of the total tested).

Total group of patients tested in 2014 was 6337 (1 smear required retesting), of which 5852 results were negative and 484 results were positive. In the first quarter were tested 2355 women (37.16% of the total group), in the second quarter were tested 1713 women (27.03%) and in the third quarter were tested 1119 women (17.66%) and in the last quarter of 2014 were tested1150 women (18.15%).

The statistical analysis was performed using these software: MS Excel 2010 and EPI/ INFO 7.

RESULTS

The distribution by age of the women group who presented at the IRO, for cervical smear collection indicated a preponderance of women by 40-44 years (936 - 14.77%) and 35-39 years (927 - 14.63%), and the least represented age group was the women by 50-54 years (587 - 9.26%) (fig. 1).

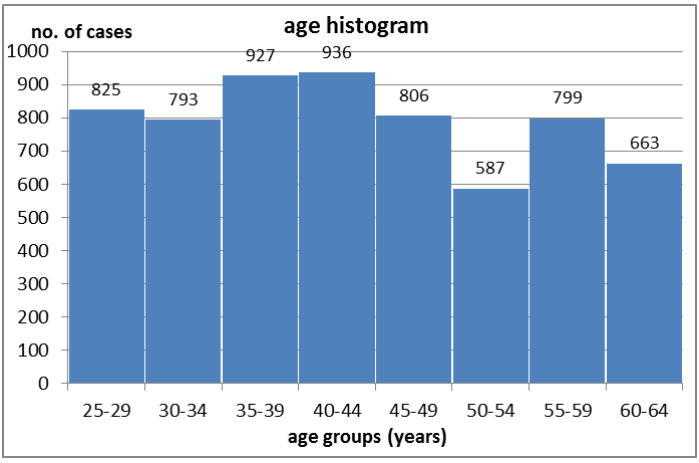

Fig. 1. Age histogram

Of all smears examined, 8.29% were positive for various neoplastic and atypical lesions (485 patients). The distribution by age groups of positive smears showed a predominance in women by 40-44 years and 55-59 years (15.87% each). Of the total harvested smears, the positive ones had the highest frequency in women by 55-59 years (9.64% of total smears collected at this age group) and 50-54 years (8.35%) (fig. 1, tab. I).

Squamous cell abnormalities were detected in 433 women (6.84% of total screening group). Cervical smears with atypical squamous lesions were present at the most womenincluded in the screening, regardless of age group (67.43%). Even at 25-29 years, they (ASC-US) accounted for 56.51% of all smears. They were most often present in women by 55-59 years (73.91%). LSIL were present in 9.69% of the total group, and most often in women by 55-59 years (11.59%). It is noted that LSIL with atypical HPV (8.77% of total) were most often present in young women by 25-29 years (34.21%). The screening detected 21 HSIL lesions (4.84%), 2 HSIL with suspicion of invasion (0.46%) and 5 squamous cell carcinoma lesions (1.15%) (fig. 2).

TABLE I

Screening test results

Indicator pentru leziuni intraepiteliale sau malignitate		Grupa de vârstă (ani)								Total
		25-29	*30-34*	*35-39*	*40-44*	*45-49*	*50-54*	*55-59*	*60-64*	
Negativ	Nr.	777	745	858	859	740	538	722	613	**5852**
	%	94.19	93.95	92.56	91.78	91.82	91.65	90.36	92.31	**91.71**
Pozitiv	Nr.	48	48	69	77	66	49	77	51	**485**
	%	5.81	6.05	7.44	8.22	8.18	8.35	9.64	7.69	**8.29**
Total		***825***	***793***	***927***	***936***	***806***	***587***	***799***	***663***	***6336***

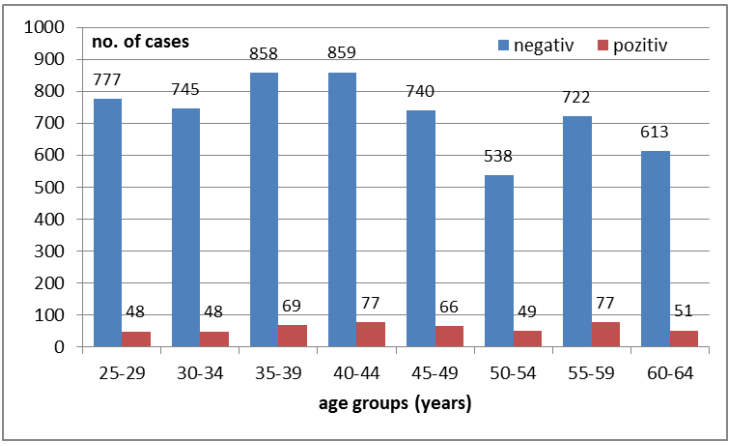

Fig. 2. Results of cervical screening

With reference to *abnormalities of glandular cells* (ACG), the harvested cervical smears showed 51 lesions (0.8% of all women participating in screening),

being more common in women by 35-39 years. Was found a case of adenocarcinoma in the 60-64 years age group and 2 anomalies in the favor of neoplasia (ACG-FN). However, most of these lesions types were ACG endocervical - NOS (significance unspecified) - 56.86% of ACG total lesions (fig. 3).

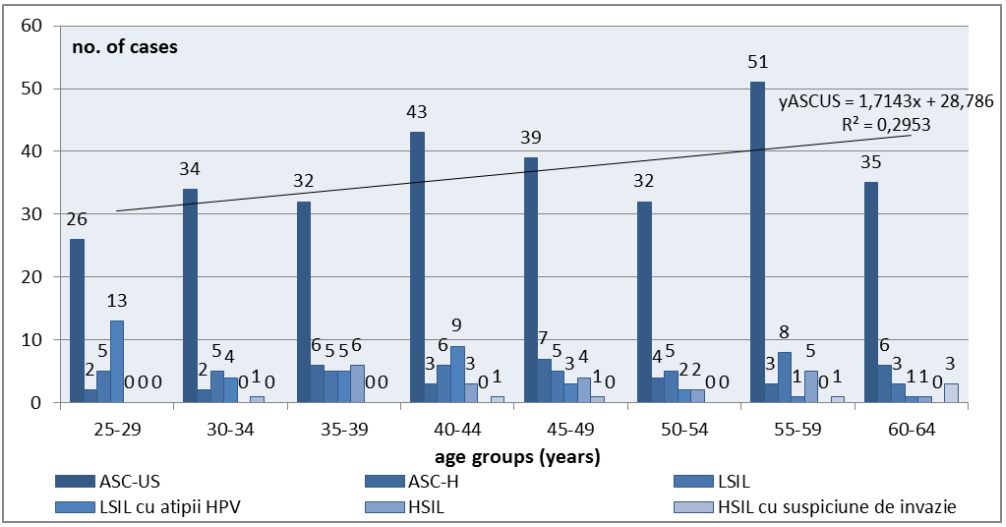

Fig. 3. Age group distribution of the anomalies of squamous epithelial cells in Pap's test of the patients

DISCUSSION

The mission facing doctors specialists and family physicians, refers to presenting the benefits of the screening test, the patients included in the list or who come to medical examination. However, must be considered that the Pap test of cervical smear can have false negative results or false positive results or can be inconclusive, therefore, when planning to guide the patient to screening, doctors should reflect the ethical responsibilities of this test (8, 9).

Some researchers in social psychology and in ethics have mentioned the existence of a degree of emotional involvement of patients, reflected in particular by increased anxiety related to performing cervical screening. False positive results can cause importantly distress to patients, including on unnecessary treatment, the false negative results may cause emotional changes when diagnosis will be made later, and the false assurance of the woman that it would be in a state of full health. These negative effects of screening are probably quite frequent and can be the cause of anxiety and a long-term distress. To minimize these problems, the patient should be well informed and involved in cognitive processes to really take the best decision regarding the test screening (8, 10).

It was found that the reduction in incidence and mortality rates of cervical cancer was more pronounced in women who were tested for HPV compared to those who carried out only cytology followed by colposcopy. Treatment may still be overestimated in groups with high prevalence of HPV and therefore, this can get greater harm at the individual level. Adding col-

poscopy, however, require a second visit to the specialist. However, in countries where there is already an adequate strategy for high quality screening, cytology (done in women with ASC-US or other more advanced lesions) is followed by colposcopy, regardless that the HPV test was previously performed (4) .

The general reaction of women to a positive test screening or HPV testing was one of distress, anxiety and anger. Women have described that they felt shocked at receiving the news that they had a genital infection, that had previously acknowledged a little or not at all, due to lack of symptoms. This was combined with anxieties about the risk of cancer and concerns about further investigation or treatment for cervical intraepithelial neoplasia (CIN) and about fertility. These problems have been common in the groups of women with abnormal Pap smears, even in the absence of HPV testing. Some women have described the degree of anxiety associated with intrusive thoughts and somatic reactions in the period in which they received their first screening test result. Much of the concern that arose related to the test result was positive was on the issue of sexual transmission of infection, this distress affecting their social, emotional and behavioral dimensions. Shock, embarrassment to the partner, feeling of shame and blaming, the stigma of having a sexually transmitted disease were common reactions in women with a positive Pap test, they used terms such as unclean, dirty, embarrassing, disgusting, immoral. Searching of a case and trying to understand what caused the positive result was a frequently behavior in women with abnormal Pap smear. Some women described the feeling of annoyance that a previous partner would be infected with a serious and carcinogenic virus, others can be considered guilty of acquiring infection because of negligence or an previously promiscuous behavior. Other women may accuse breach of trust in a partner who subsequently proved unfaithful. However, most of these women finally say that the source of the infections is another one, such as a previous partner (11, 12, 13).

The social, cultural, especially economic, religious and historical differences under the pressure whose live the women from anywhere in the world, reflect deeply on the issues and the ethical dilemmas that they carry with them when they are put in a position to consult a doctor on a gynecologist or gynecologic oncologist problem. The doctor must learn to recognize the social and ethical particularities of the community it cares for. These issues include the right to knowledge (the right to know), and inequalities in health insurances and services from that the patient can benefit (14).

Contrary to many significant advances purchased, the cervical cancer screening still may be associated with adverse events in national screening programs, requiring a balanced view of risk - the benefit of participating women. Thus, Weller notes that it would be appropriate to cover the following areas: social inequality, informed choice, the cost of screening when screening did not go well, given the new challenges of HPV testing and HPV vaccination (15).

Adding the answers to these dilemmas in a time capsule, along with the successes and the shortcomings of the program, known from past and present, can be interesting to anticipate the exciting directions and the ethical challenges that cervical

cancer screening will be up to future generations.

CONCLUSIONS

Through our study, we found that a small number of people compared to the female population eligible, was present at the screening test, which shows the need for better spread of notions screening and the national program related, on the North-East Romania population, taking into account the ethical issues of this test.

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ANIMAL MODELS OF OSTEOPOROSIS: CRITICAL CONSIDERATIONS

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ANIMAL MODELS OF OSTEOPOROSIS: CRITICAL CONSIDERATIONS (Abstract) Animal models are necessary to study the pathogenic mechanisms of osteoporosis and to test new drugs. The aim of this study was to critically evaluate the experimental models of osteoporosis in rats or mice for an appropriate use in new drug testing. There are five distinct groups of experimental methods for inducing osteoporosis: surgical, immobilization, pharmacological, dietary and transgenic models. Combinations such as ovariectomy plus immobilization or administration of glucocorticoids or diet changes, have the advantage of reducing the time when bone mass loss becomes apparent, especially for cortical bone. No experimental model can reproduce human osteoporosis; therefore, for testing new drugs the selection of a suitable model is essential. **Keywords**: OSTEOPOROSIS, EXPERIMENTAL MODELS, RAT/MICE, DRUG TESTING.

The multifactorial character of this disease, its incompletely known pathogenesis, the negative impact on the quality of life, with a high incidence of complications and mortality, are arguments for the need of further researches in this field (1, 2). Osteoporosis is characterized by low bone mass and deterioration in bone tissue micro architecture, with a consequent increase in bone fragility and susceptibility to fracture. Affecting both genders, with a higher incidence and prevalence in women, osteoporosis is a progressive systemic skeletal disease with a great social impact, associated with long-term implications, severely decreased quality of life and high mortality. Clinical studies involve high costs and need long periods of surveillance; therefore, experimental animal models play an important role in osteoporosis research, especially for testing new drug treatments (3).

The aim of the study was to critically evaluate the experimental models of osteoporosis in rats or mice for an appropriate use in new drug testing. Data sources were relevant preclinical studies on experimental osteoporosis from PubMed, published in English or French, from 1980 up to March 2015. Searched terms included "experimental osteoporosis", "rat/mice", and "biomar-kers". From 4.355 papers about experimental osteoporosis published in the interval 1980-2015, there were selected 65% of the original articles and critical

reviews (1.947 papers on rat models and 885 papers on mouse models) describing the methods for inducing osteoporosis and biomarkers. The studies with incomplete data about the criteria for osteoporosis, letters to the editors and commentaries were excluded. The methods used for inducing osteoporosis in rats or mice were: surgical intervention (4-7), immobilization (7-9), drug-induced osteoporosis (10-17), dietary interventions (18-21) and transgenic models (22-24).

BIOMARKERS IN OSTEOPOROSIS

Osteodensitometry, a non-invasive method, evaluates the bone loss which occurs immediately after ovariectomy (bone resorption exceeds bone formation); later, bone remodeling reaches a steady state where bone formation and bone resorption are in balance (3). The markers that indicate cancellous bone loss after ovariectomy are: earliest time of bone loss (14 days for the proximal tibial metaphysis, 60 days for lumbar vertebral body, 30 days for femoral neck), time of 50% bone loss (30-60 days for the proximal tibial metaphysis, 180-270 days for lumbar vertebral body and for femoral neck), earliest to achieve steady state (90 days for the proximal tibial metaphysis, 270 days for lumbar vertebral body and for femoral neck), and earliest decrease in bone strength (7). The markers that indicate cortical bone loss bone after ovariectomy are the enlargement of the marrow cavity (the site for bone loss analysis is the inner half of the shaft adjacent to the marrow), the earliest changes in the femoral and tibial cortex (90-120 days) and steady state (180 days) (4).

Ovariectomy in mice is not very popular because the markers that point the changes in cortical bone are not clear defined (4). The trabecular and cortical response to ovariectomy are variable among inbred mouse strains, which is in favor of the hypothesis that pre- and postmenopausal bone activity is partly genetically regulated (5).

Comparative with ovariectomy, in the immobilization model the earliest time of cancellous bone loss is 14 days for the proximal tibial metaphysis, 30 days for the distal tibial metaphysis and the time to achieve steady state is 126 days for the proximal tibial metaphysis and 45 days for the distal tibial methapysis (3). In the immobilization model, the earliest time of bone loss in the cortical bone is 21 days for the femoral cortex and 42 days for the tibial cortex; the time of 10% bone loss is 26 weeks (3).

Other biochemical markers useful in evaluating osteopenia are: blood and urinary levels of phosphorus, calcium, magnesium, markers of bone formation (alkaline phosphatase, osteoclacin) and markers of bone resorption (urinary type I collagen cross-linked N-telopeptides, pyridinoline, tartrate-resistant acid phosphatase).

Histomorphometry, an invasive method, provides information about: number of osteoblasts, osteoclasts, osteocytes, active osteoblasts relative to bone perimeter, trabecular thickness (3).

Mechanical strength testing, an invasive method, assess the mechanical quality of the bone and is applied to diaphyses of long bones (three-point bending, 4-point bending, torsion), vertebras (compression testing), femoral head (cantilever testing) (3).

SURGICAL METHODS FOR INDUCING OSTEOPOROSIS

Bilateral ovariectomy versus orchiectomy is the surgical intervention preferred by most researchers for obtaining osteoporosis (rat, 1691 *vs.* 99 studies; mice, 491 *vs.*

28 studies). Ovariectomy in rats, the most frequently used technique as model for postmenopausal osteoporosis, simulates the clinical criteria of the osteoporotic human skeleton and reproduces the response of therapeutic drugs. Orchiectomy induces androgen deprivation which promotes an osteoporotic model (5).

Alternative interventions in rats for obtaining osteoporosis are hypophysectomy (3 studies), pinealectomy (3 studies) or below-knee amputation (6 studies).

IMMOBILIZATION FOR INDUCING OSTEOPOROSIS

Immobilization for inducing osteoporosis may be realized by non-invasive methods such as: tail suspension (54 studies on rats, 33 studies on mice), confinement (1 study on rats, 1 study on mice) or administration of botulinum toxin (9 studies on rats, 4 studies on mice). Surgical resections of spinal nerve (3 studies on rats), tendon (4 studies on rats) or spinal cord (13 studies on rats, 3 studies on mice) are invasive methods. Immobilization combined with ovariectomy provides a severe bone loss in a shorter period than either method alone (9).

The favorite immobilization model is tail suspension because it causes hypertension and adrenal hypertrophy (6, 7). Botulinum toxin type A-induced immobilization determines similar osteoporotic effects as ovariectomy (8).

DRUG-INDUCED OSTEOPOROSIS

There are several drugs used for inducing osteoporosis (tab. I): corticosteroids (25 studies on rats; 20 studies on mice), gonadotropin-releasing hormone (GnRH) agonists (4 studies on rats) or aromatase inhibitors (1 study on rats). Corticosteroid-induced model of osteoporosis (with Prednisone or Dexamethasone) has a good correlation between drug administration and bone loss (10, 12). The administration of Buserelin, a GnRH agonist, leads to estrogen deprivation, resulting in bone loss similar to bilateral ovariectomy (15). The administration of aromatase inhibitors (Vorozole) in aged male rats induces a net trabecular bone loss in the appendicular and axial skeleton (16).

Association of methods induces osteoporosis more rapidly, using lower doses. One schedule starts 2 weeks after ovariectomy in rats, with dexamethasone 0.3 mg/kg b.w./day once every two weeks (17).

TABLE I

Drugs and schedules for inducing osteoporosis (rats or mice)

Drugs	Rat		Mouse	
	dose	schedule	dose	schedule
Prednisolone (mg/kg)	3.5 mg	s.c., daily, 4 wk	1.4-2.1 mg	s.c., daily, 4 wk
Dexamethasone (mg/kg)	2.5mg	i.m., x2/wk, 9 wk	50 mg	i.p., daily, 5 wk
Buserelin (µg/kg)	25 µg	s.c., daily, 4 wk	-	-
Vorozole (mg/kg)	17 mg	p.o., daily, 15wk	-	-

wk: week.

DIETARY CHANGES FOR INDUCING OSTEOPOROSIS

Dietary changes are non-invasive methods less used to obtain osteoporosis in animals: diet deficient in Ca^{2+}/vitamin D_2/D_3 combined with ovariectomy for

achieving osteoporosis more rapidly (18); prolonged Mg^{2+} deficiency (19); high fat diet (20); excessive alcohol (21).

TRANSGENIC MODELS FOR INDUCING OSTEOPOROSIS

Transgenic models of osteoporosis (36 studies on rats, 305 studies on mice) are useful for understanding the pathogenesis and for identifying new therapeutic targets for the treatment of osteoporosis. These models use transgenic mice carrying the human genomic region (e.g., TghuRANKL mice carrying receptor activator of NF-kappaB ligand RANKL – a region which plays a key role in osteoclast-induced bone resorption) (22), transgenic mice that inappropriately expresses the cytokines (e.g., IL-4 and IL-13 – involved in the regulation of bone remodeling) (23) or premature accelerated aging in mice (a suitable model for involutional osteoporosis and its treatment) (24).

CONCLUSIONS

In our observations we aimed at demonstrating that animal models have a crucial role in osteoporosis research, especially in the development of new therapeutic strategies. Combination of methods (e.g., ovariectomy + immobilization, + glucocorticoid or + diet changes) has the advantage of reducing the time when bone mass loss becomes apparent, especially for cortical bone. There is no ideal animal model for osteoporosis; the selection of an appropriate model is the key to well-done promising researches. Essential in selecting the best model for research and especially for testing new drugs for the treatment of osteoporosis is to know the limits and strengths of various animal models.

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TRAUMATIC SCAPHOLUNATE DISSOCIATION - CASE REPORT

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TRAUMATIC SCAPHOLUNATE DISSOCIATION- CASE REPORT (Abstract): Perilunate injuries occur after an impact trauma to the wrist. The recognition of these lesions and immediate treatment are the two essential conditions to ensure the best possible outcome. The first therapeutic step is the restoration of anatomical joint relationships of the carpus followed by percutaneous pinning or internal fixation required for maintaining the congruence this segment. Despite correct diagnosis and approach in these cases the prognosis is often encumbered by decreased range of motion, loss of grip strength and finally the onset of osteoarthritis. Scapholunate dissociation is the most frequent pattern of carpal instability. We report the case of a 53-year-old woman diagnosed with scapholunate dissociation resulting from an injury caused by fall from the same level on the outstretched right hand (dominant hand). Surgery was performed 48 hours post-injury and consisted in external reduction followed after the restoration of the anatomical joint relationships in the right wrist by percutaneous pinning. Postoperatively the wrist was immobilized in brachipalmar cast for 8 weeks and arthrosynthesis pins were removed. The patient was reassessed at 6 months postoperatively by clinical and radiological evaluations and the functional outcome was good. **Keywords:** SCAFOLUNATE ANGLE, CAPITOLUNATE ANGLE, TERRY THOMAS SIGN, SCAPHOLUNATE DISSOCIATION, ROTATORY SUBLUXATION OF THE SCAPHOID.

Scapholunate dissociation is a ligament injury of the wrist which causes the loss of normal alignment between the scaphoid and lunate bones due to partial or complete injury to the scapholunate interosseous ligament and extrinsic ligaments with a role of secondary stabilizers of this segment. This pathology is frequently under diagnosed being misdiagnosed as simple wrist sprain. The occurrence of carpal instability leads to biomechanical changes and favors the occurrence of arthrosic lesions and ultimately of scapholunate advanced collapse finally (SLAC) wrist. The dissociation is also accompanied by rotatory subluxation of the scaphoid bone. Wrist arthroscopy allows the visualization of the scapholunate space and has become a standard method useful for both the diagnosis and management of this condition.

CASE PRESENTATION

A 53-year-old woman presented to the emergency unit following an injury occurred on the same day by fall from the

same level on the right hand (dominant member) with the wrist in hyperextension. Physical examination revealed a moderately diffuse edema over the dorsal aspect of the right wrist and hand, diminution of grip strength and antalgic restriction of active movements. The scapholunate interval was identified by palpation as the point of maximum pain intensity and the scaphoid shift test revealed the presence of scapholunate instability. Neuro-vascular examination revealed no changes in the radial artery and median nerve territory. The patient had a non-pathological personal history and routine laboratory investigations were within normal ranges.

As first-line imaging investigation bilateral wrist X-rays in anteroposterior, lateral and oblique views were preformed, later supplemented by CT scanning. Anteroposterior X-rays showed the increase in scapholunate interval to 4 mm (positive Terry Thomas sign) and rotatory subluxation of the scaphoid. At the level of radial styloid apophysis a fracture line without displacement was identified. Profile X-ray revealed a scapholunate angle value of 80° (normal 30-60°) and a capitolunate angle of 40° (normal 20°) (fig.1).

For comparison X-rays of the contralateral wrist were performed and these showed a scapholunate space within normal range and the correct orientation of the semilunate and scaphoid bones (fig. 2).

Fig. 1. X-ray appearance of the right wrist

Fig. 2. Normal articular relationships in the contalateral wrist

Standard radiological examination showed the presence in both wrists of a risk factor reported in the literature, namely ulna minus configuration (1).

Computed tomography confirmed the diagnosis of scapholunate dissociation showing an unevenly increased scapholunate joint space, more pronounced posteriorly, of up to 7 mm, anterior semilunate subluxation, and the presence of a bony fragment of 4/2/ 4mm resulting from ligament avulsion. Similar millimetric frag-

ment were seen on the posterior margin of the radial epiphysis (1.4 / 3.4 / 2.5 mm) and volar aspect of the distal radioulnar joint adjacent to the radius (2/1.4/2.8 mm) (fig. 3, 4).

The intervention consisted of percutaneous pinning under locoregional anesthesia (supraclavicular block). The semilunate was repositioned by wrist flexion followed by temporary stabilization to the radius with a 1.2 mm Kirschner pin. Scaphoid position was corrected by wrist-extension maneuver and was maintained with two pins percutaneously driven into the semilunate and from the scaphoid into the big bone, respectively (fig. 5).

Postoperatively the wrist was immobilized in neutral position with a brachipalmar cast for 8 weeks. Periodic radiological controls confirmed the preservation of normal joint relationships and the correct pin positioning which were removed after 8 weeks.

Clinical and radiological reassessment at 6 months postoperatively showed regional osteoporosis without indicating the onset of avascular necrosis at the carpal bones but dorsal intercalated segment instability (DISI) deformity was observed. Grip strength in the right hand was determined and was 70% of the contralateral side. Capito-lunate angle was 30^0, scapholunate angle 60^0, active flexion 50^0, and active extension 60^0 (fig. 6).

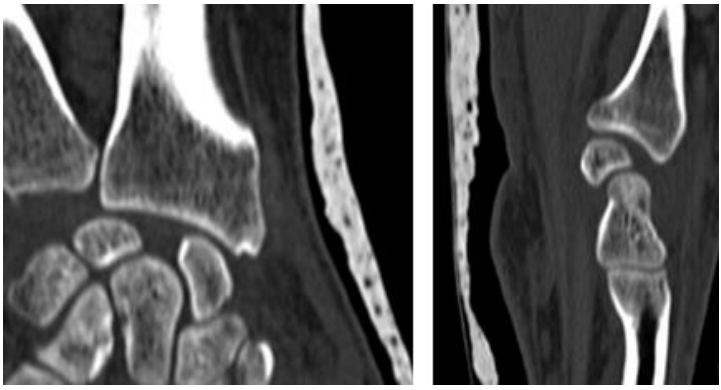

Fig. 3. CT scan of the right wrist

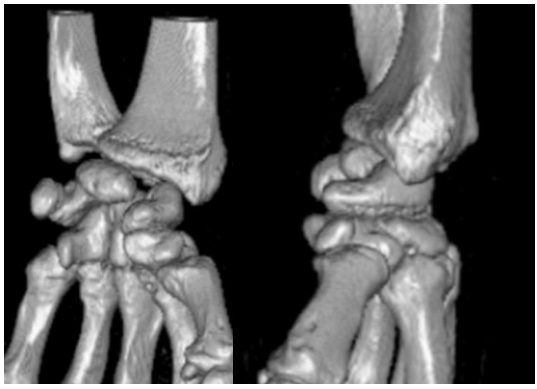

Fig. 4. Three-dimensional reconstruction of the right wrist

Fig. 5. Postoperative result

Fig. 6. X-ray control at 6 months

DISCUSSION

Intracapsular ligaments of the wrist are classified into intrinsic and extrinsic. Scapholunate and lunotriquetral ligaments are the most important intrinsic ligaments each being composed of three fascicles (dorsal, palmar and proximal). Scapholunate interosseous ligament is considered the primary stabilizer of this joint and its dorsal portion represents the area of maximum strength while the lunotriquetral ligament is more prominent on the palmar side (2).

The presence of secondary stabilizers represented by the extrinsic ligaments of the wrist makes that an isolated lesion of the dorsal fascicle of the scapholunate ligament to determine in the worst case dynamic instability of this segment (seen only on stress radiographs) without a resulting static scapholunate dissociation seen on standard radiographs (3).

In the palmar area strong radiocarpal ligaments which are secondary stabilizers are identified: the radioscaphocapitate, the radioscapholunate ligament, long and short radiolunate ligaments. The radioscaphocapitate ligament originates from the radial

styloid process, crosses the body of the scaphoid and inserts distally into the palmar aspect of the capitate bone, acting as a fulcrum around which the scaphoid rotates. The radioscapholunate ligament, also called the ligament of Testut, also has the role of stabilizing the scaphoid and neurovascular pedicle being connected with the radial artery, anterior interosseous artery and anterior interosseous nerve (4).

Scapholunate dissociation is the first stage of perilunate instability and results from injury to the scapholunate interosseous and palmar radioscaphocapitate ligaments which bring the semilunate in extension while the scaphoid flexes and pronates. Mayfield et al. describe the application of impact force to the hypothenar region of the hand with the wrist in extension and ulnar deviation as the main mechanism of action. As the impact force amplifies, the ligaments and bones of the wrist are injured in a circumferential direction around the semilunate and in the radioulnar direction (5).

Ligament injury is often accompanied by scaphoid fracture without displacement or fracture of the radial styloid apophysis. Scapholunate dissociation occurs only when injury to the scapholunate ligament is accompanied by injury to the secondary stabilizers. Incongruous joint relationships determine changes in biomechanics and load concentration at certain points resulting in the occurrence of scapholunate advanced collapse (SLAC) and development of degenerative arthritis. Patients diagnosed with scapholunate dissociation may present preexisting generalized ligamentous laxity (6).

Conservative treatment is of limited value for this type of lesion being currently abandoned due to the loss of reduction that most often occurs. Although semilunate extension can be easily corrected by wrist flexion, this will accentuate the vicious position of the scaphoid, and thus there is not an ideal position for immobilization. Surgery remains the correct option and is recommended to be performed within the first 3 days (7, 8).

If reduction is easily obtained and maintained percutaneous pinning combined with immobilization for a minimum of 8 weeks is an important therapeutic resource. Most authors recommend the use of two pins, both passed through the scaphoid and advanced into the semilunate and capitate bone, respectively. Scapholunate joint reduction can be easily achieved by the insertion of two pins into the scaphoid and lunate and used as joysticks. The intervention is facilitated by arthroscopic control. Whipple (9) reported an 85% success rate in maintaining scapholunate reduction in patients with a scapholunate interval greater by 3 mm than the contralateral wrist if surgery was performed within the first 3 months. Despite early diagnosis and correct treatment radiological revaluations reveal in over half of the operated patients the onset of wrist osteoarthritis whose treatment requires such difficult and debilitating reinterventions as proximal row carpectomy and wrist arthrodesis (10).

CONCLUSIONS

Scapholunate dissociation remains a complex pathology both in terms of diagnosis and especially of treatment. In our patient reduction by external maneuvers followed by percutaneous pinning provided good functional and radiological outcomes. No changes, such as avascular necrosis of the carpal bones were present but a dorsal intercalated segment instability (DISI) persisted.

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NEWS

DYNAMIC OBSTRUCTION OF THE LEFT MAIN CORONARY ARTERY OSTIUM BY A PAPILLARY FIBROELASTOMA

Benign tumours of the heart are usually detected as incidental findings during echocardiography. Most cases are intracardiac tumours, with myxoma being the most frequent entity. There are reported images of a patient with acute myocardial infarction and a concomitant extracardiac benign tumour, consisting in fibroelastoma in the aortic root. Papillary fibroelastoma is the second benign tumour of the heart. It is a rare intracardiac tumor, with rather high risk of embolization. It usually originates from the aortic and mitral valve. Extracardiac, as observed in the reported case, is very rare and searching the lesion as a cause of acute coronary ischemia identification is needed (Napp LC, Baraki H, Bredt M, et al. Dynamic obstruction of the left main coronary artery ostium by a papillary fibroelastoma. *Cardiovascular Pathology* 2014, 23: 57-58).

Doina Butcovan

UROGENITAL PROLAPSE – CURRENT METHODS OF SURGICAL TREATMENT

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UROGENITAL PROLAPSE – CURRENT METHODS OF SURGICAL TREATMENT (Abstract): Several combined surgical procedures have been described, but connective tissue deficiency required the use of some "prostheses" instead of conventional methods based exclusively on correction by suture. **Aim**: Assessment of the techniques used for the repair of stress urinary incontinence (SUI) and anterior vaginal wall prolapse, as well as the incidence of intraoperative and postoperative incidents and complications. **Material and method**: The study was conducted between January 2007 and December 2011 at the 1st Clinic of Obstetrics and Gynecology Iaşi. The diagnosis was made on clinical criteria. Menopausal patients received estrogens prior to surgery. Vaginal meshes were used only in patients with grade III and IV cystocele. The patients were followed up at 1½, 6, 12 and 36 months (first local evaluation for meshes at 2 weeks after surgery). **Results:** During the study interval 400 patient with a mean age of 57.45 ±5 years (range 29 - 81 years) were surgically treated in our clinic. Of these, 150 women underwent classic surgery, 243 women transobturator tape procedure - TOT (107 TOT alone for SUI and 136 TOT combined with classic surgery), 4 women tension-free vaginal tape (TVT) procedure, and 3 patients prepubic and transobturator mesh. In 12 patients meshes with 2 or 4 arms for cystocele (7 and 5 cases, respectively) were used. Vaginal mesh extrusion was recorded in 4 patients. **Conclusions:** The use of prostheses allows a better standardization of procedures, shortening of surgical time and a better postoperative recovery, the patients being able to resume their normal activities. **Keywords**: UROGENITAL PROLAPSE, SUI, TOT, VAGINAL MESH.

Prolapse greatly affected women's personal and professional activities causing some women to adjust routines or stop activities (1). Up to 50% of multiparous women are diagnosed with urogenital prolapse and 10-20% of them require surgical treatment because of their symptoms (2). The health care impact of prolapse is likely to expand, based upon estimates of an increasing prevalence in the growing population of elderly women.

Taking into account that for the reconstructive surgeries an accurate diagnosis must be done in order to correct the defect, we can use stiches, biologic or synthetic meshes**.** The aim of reconstructive procedures is to maintain the physiological length, diameter and vaginal axes for the conservation of sexual, urinary and bowel function. The necessity of simultaneous

correction of all pelvic floor dysfunctions must have a good anatomical and functional result, and for this the physician must to know a few of surgical techniques, to take a final optimal decision for the patient.

In clinical practice we will observe that damage to the supporting tissues can cause prolapse and urinary incontinence. The deficiency of connective tissue is a favoring factor in the pathogenesis of these conditions, but also for postoperatve recurrences of urogenital prolapse. The rate of recurrences varies between 24-43% depending on surgical procedure. The involvement of connective tissue had determined the use in recent years of some "prostheses" – synthetic polypropylene meshes, which have important advantages over conventional technique exclusively based on correction by suture. This technique with meshes for recurrences was firstly used in general surgery and now has a great success in urogynecology (3). For SUI several therapeutic approaches have been tried. Nowadays transobturator sling is becoming more and more important. TOT described for the first time in Netherlands (1998), and introduced by Delorme in France in 2001, has been accepted in the US since 2003 (4).

MATERIAL AND METHODS

This retrospective and prospective study was conducted between January 2007 and December 2011 at the 1st Clinic of Obstetrics and Gynecology Iasi. The diagnosis was made on history and physical examination, without urodynamic tests. We collected a series of data from patients regarding their health status and medical history. Symptomatic anterior compartment prolapse was determined by self-report of a feeling of bulge, pressure, or protrusion or a visible bulge from the vagina. There is resulting protrusion of the vaginal wall. Anterior compartment status was assessed by the most caudal bladder point and the internal urinary meatus. Transperineal ultrasound for urethra angle and abdominal ultrasound for the bladder for chronic retention were used for some cases.

Local estrogen preparations were used to improve preoperative vaginal thinning (atrophy) for minimum three-four weeks for all women in menopause. We recommended vaginal mesh only for cystocele grade III or IV.

The postoperative course was evaluated at 1½, 6, 12 şi 36 months (first local examination for meshes at 2 weeks).

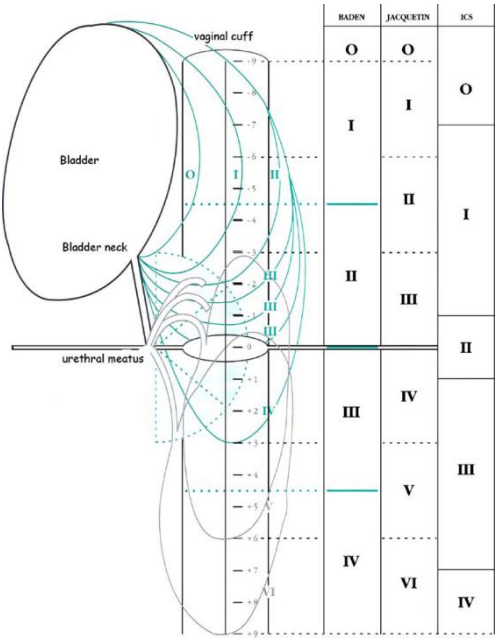

Fig. 1. Comparison cystocele quantification after Baden şi Walker, Jacquetin, International Continence Society (ICS)

The grade of anterior vaginal wall was appreciated with the patients in the lithotomy position at rest and during a cough test and Valsalva maneuver. The classification

used by us in the study was Jacqetin, which modifies the system sistemul Baden şi Walker (fig.1)

- Stage I - the leading edge descend suntil to the first 1/3 of the vagina
- Stage II – descend until the mid-vagina
- Stage III – descend at the hymen level
- Stage IV – outside the hymen

RESULTS

During the study interval a total number of 400 women were surgically treated; mean age at admission was 57.45 ±5 years (range 29-81 years). The greatest number of cases were recorded in 2008 – 108 patients (fig. 2).

The majority of patients were post-menopausal and in the 6th decade of life (fig. 3).

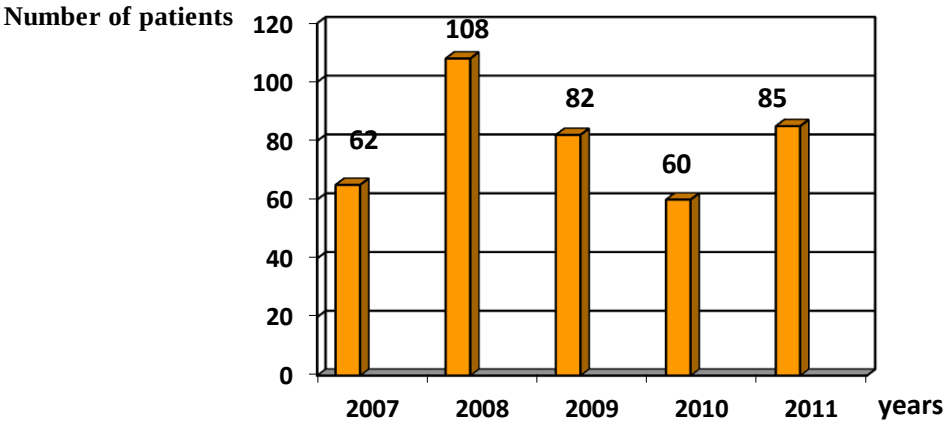

Fig.1 Distribution by years of cases

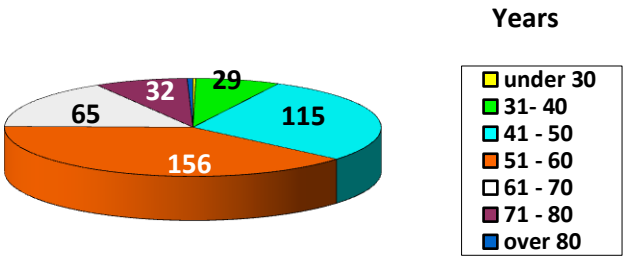

Fig. 3. Distribution of cases by age

The following surgical procedures were performed: classic pelvic reconstructive surgery (150 cases), TOT (243 cases – TOT alone for SIU in 107 women and TOT combined with classic surgery in 136 patients), TVT (4 cases) and suprapubic arc sling and transobturator mesh (3 cases). In 12 women the transvaginal repair of the cystocele was done with 2 or 4 arm meshes (7 and 5 patients, respectively) (fig. 4,5).

We mention that 25 women who had undergone previous surgery for SUI or anterior vaginal wall prolapse, had recurrence between 6 months and 5 years, and with TOT procedure in our clinic during the study interval obtained good results.

Fig. 4. Mesh with 2 arms TOT

Fig. 5. Mesh with 4 armes TOT

A small number of intraoperative complications were recorded: in 5 women the sling had a transvaginal position and the tapes were replaced during the surgery and in 2 cases the tape arrived inside the bladder during the TOT procedure and this was seen at cystoscopic examination (these were accidents durig the learning curve). As to postoperative complications, there were 2 cases with mesh exposure in the vagina – one case with insulin-dependent diabetis mellitus, one case with important atrophy (fig. 6), other 2 with vaginal mesh erosion after 6 months and 1 year, respectively, and only 1 case with erosion after mid-urethral sling. In these patients we performed the excision of the exposed mesh (fig. 7) with general and local antibiotic treatment and hormone therapy (one case with secondary suture).

Fig. 6. Mesh exposure

Fig. 7. Excision of the exposed mesh

No uterthral lesions were recorded. All patient except two had urinated spontaneously when bladder catheter was removed. After sling procedure, only 3 patients complained of persistent urine loss, but all other women – 98.78%, were satisfied with the results of surgery.

DISCUSSIONS

It was estimated that a women has an 11% lifetime risk of undergoig a single operation for urinary incontinence or pelvic organ prolapse (POP). It is considered that the risk of urogenital prolapse is duplicated in every single decade of the life, fact not supported by our study. A study estimated that the number of American women with at least one pelvic floor disorder will increase from 28.1 million in 2010 to 43.8 million in 2050. During this time period, the number of women with urinary incontinence (UI) will increase by 55% from 18.3 million to 28.4 million (5). All these underline the importance of using efficient surgical methods with a small rate of recurrence (6).

In 1996, a standardized terminology for the evaluation of pelvic organ prolapse (POP) was established by the International Continence Society, the American Urogynecologic Society, and the Society of Gynecologic Surgeons. This terminology replaced terms as cystocele, rectocele, enterocele, and urethrovesical junctions with precise descriptions relating to specific anatomic landmarks.

The first points are on the anterior vaginal wall and categorize anterior vaginal wall prolapse accordingly. Point (Aa) is a point located in the midline of the anterior wall 3 cm proximal to the urethral meatus and is roughly the location of the urethrovesical crease. Point (Ba) represents the most distal position of any part of the anterior vaginal wall. Point (C) represents either the most distal edge of the cervix or the leading edge of the vagina if a hysterectomy has been performed. Point (D) represents the location of the posterior fornix (pouch of Douglas) in a woman with a cervix. Point (Bp) is a point most distal of any part of the upper posterior vaginal wall, and point (Ap) is a point located in the midline of the posterior vaginal wall 3 cm proximal to the hymen. To record measurements, these points should be expressed in centimeters above or below the hymen. It is important for the examining individual to express the position and other circumstances of the examination (e.g., straining or not, patient flat on table or in examining chair).

Staging of Pelvic Floor Prolapse Using International Continence Society Terminology (POP Quantification):

- Stage 0: No prolapse is demonstrated. Points Aa, Ap, Ba, and Bp are all at -3 cm and either point C or D is between total vaginal length -2 cm;
- Stage I: Criteria for stage 0 are not met, but the most distal portion of the prolapse is >1 cm above the level of the hymen;
- Stage II: The most distal portion of the prolapse is less or equal to 1 cm proximal or distal to the plane of the hymen;
- Stage III: The most distal portion of the prolapse is >1 cm below the plane of the hymen but protrudes no further than 2 cm less than the total vaginal length in centimeters;
- Stage IV: Essentially complete eversion of the total length of the lower genital tract.

We did not use in practice this quantification, but it is important because it introduces a common language for all surgeons,

and much more age <60 years and preoperative POP quantification stage 3 or 4 were associated with a grater likelihood of recurrent prolapse at 1 year (7).

TOT procedure rapidly and with great succes replaced TVT technique and today is the gold standard in the surgical treatment of SUI. For understanding why this procedure was created it is neccessary to know the anatomy, and this shows us that the method is without risks because it avoids all major structures. In our clinic TOT procedure was preferred to TVT due to its shorter shorter operative time (no need of control cystoscopy after surgery) and minimal risk for bladder lesions

The treatment of pelvic static disorders might also vary depending on age, clinical conditions and sexual life prospects for the future. A comparative study of postoperative results of anterior colporrhaphy and mesh procedure showed that pain during sexual intercourse appears in 2% after colporrhaphy and in 7.3% after transvaginal repair with mesh (8). For that reason the management in our clinic recommended the use of meshes only for anterior vaginal compartment grade III and IV. Contraindication of using prosthetics for the pelvic floor are not established yet, and the interaction with bladder either. Patients must be informed about these new methods of treatment with a grate rate of anatomical repair, even though there is a risk of postoperative complications.

Data in the literature reveal that preoperative treatment for three weeks with estrogens reduce the incidence of cystitis within the first four postoperative weeks but this unexpected finding needs confirmation from larger trials. Our study supports this finding. Also tibolone, a selective estrogenic activity regulator, per vaginal administration had better results on vaginal dryness versus per os regimen (9). Some studies indicated a rate of cure about 80.64% and 87% (10,11). It seems that for our group of patients results were better – 98.78%.

CONCLUSIONS

Our study ascertains that using syntetic prosthesis allows a good standardization of procedures, decrease the operative time, a better postoperative recovery, and women resume usual activities. We reserved the technic with vaginal meshes only for cases with important anterior vaginal compartment prolapse and patients in which the techniques basesd solely on suture of destroyed structure would results in an increased risk of recurrence. In our group of patients the rate of complications was low 3.05%, possibly because of the use of preoperative local estrogens and postoperative antibiotic prophilaxis. TOT procedure offered to our patients a good urinary continence for a long period.

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NEWS

MUIR-TORRE SYNDROME

First described by Muir et al in 1967 and Torre in 1968, Muir-Torre syndrome (MTS) is a rare autosomal dominant genodermatosis characterized by at least one sebaceous gland neoplasm and at least one visceral malignancy. Muir-Torre syndrome follows an autosomal-dominant pattern of inheritance in most cases with high penetrance and variable expression, but sporadic cases have also been reported. Because MTS is associated with germline mutations in the DNA mismatch repair (MMR) genes hMSH2 and hMLH1, it is considered a phenotypic subtype of Lynch syndrome (also known as hereditary nonpolyposis colorectal cancer syndrome), in which inherited defects in DNA MMR genes result in microsatellite instability. Although germline disruption of hMLH1 and hMSH2 is evenly distributed in Lynch syndrome, more than 90% of MTS patients show hMSH2 disruption. Although MTS is rare, pathologists have an important role in the early detection and initial diagnosis of MTS: identification of at-risk individuals (affected patients and family members) allows appropriate screening and surveillance for visceral malignancies, thereby reducing morbidity and mortality (Bhaijee F, Brown AS. Muir-Torre Syndrome. *Arch Pathol Lab Med*. 2014; 138: 1685–1689).

Doina Butcovan

MEASLES: MANIFESTATIONS AT THE STAGE OF ELIMINATION OF INFECTION AND DIRECTIONS FOR EFFECTIVE MANAGEMENT OF THE EPIDEMIC PROCESS

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MEASLES: MANIFESTATIONS AT THE STAGE OF ELIMINATION OF INFECTION AND DIRECTIONS FOR EFFECTIVE MANAGEMENT OF THE EPIDEMIC PROCESS (Abstract): Controlling measles is still important despite the significant progress achieved through routine immunization. **Aim:** to identify the weak points of the infection control system and to determine the directions for effective management of the epidemic process using the example of the local epidemic situation. **Material and methods:** The materials for the study were data from the official registration of the incidence of measles among residents of a large industrial city in 1988-2016 and the results of serological screening for anti-measles IgG of health care workers during the outbreak of measles in 2016. The epidemiological, clinical, serological and statistical research methods have been used. **Results:** In conditions of a high level of vaccination against measles in the metropolitan population, significant quantitative and qualitative changes occurred in the epidemic process of this infection. Between 2001 and 2015, on the territory of the city, local cases of measles were not recorded, and its single penetration from endemic territories did not cause the spread of the infection. However, in 2016, a measles outbreak was registered, due to the spread of infection mainly in medical settings among patients and staff. Serological immunity tests for measles conducted among health care workers showed that up to 80% of those who underwent the tests had a protective level of anti-measles antibodies, with the highest proportion of people with seroprotection being detected in the age group over 50 years old. Up to 40% of workers with an interval of 10 years and more after the last vaccination were included in the cohort of persons with anti-measles IgG below 0.18 IU / ml, i.e. below protective level. **Conclusions:** Measles remains an important infection, which has a possibility to spread among children and adults who are not protected against it. To effectively control the epidemic situation for measles during the elimination stage of the infection, it is necessary to implement a system of continuous monitoring of the intensity of measles immunity in the indicator groups and to perform routine immunization against measles once every 10 years up to 50 years of age. In the outbreaks where the first patient was not previously vaccinated, it is necessary to carry out booster immunization with live measles vaccine for contact people without preliminary screening. **Keywords:** MEASLES, EPIDEMIC PROCESS, CONTROL.

Until the middle of the 20th century, the importance of measles was universally determined by the high incidence rate among young children, the ubiquitous spread of infection and high mortality (1, 2).

The introduction of live measles vaccine into the health care practice in 1967 made it possible to significantly change the situation: morbidity and mortality rate from this infection decreased, and the age distribution of the sufferers and the focality changed (3, 4).

Due to the constantly increasing coverage of the population with vaccinations, at the end of the 20th century and the beginning of the 21st century there was a pronounced tendency to reduce morbidity in most countries of the world and even to stop the circulation of the virus in separate time intervals (5, 6, 7).

However, since 2013, in many countries, the incidence of measles has been observed with the active involvement of adolescents and adults in the epidemic process, the emergence of measles outbreaks in community and the focality. Cases of measles were reported even in countries where measles had not occurred for a long time (USA, Canada, Australia and most European countries) (8, 9, 10, 11, 12, 13).

In several administrative territories of the Russian Federation, the cases of measles penetration and its spread with the formation of epidemic foci in families, medical settings and educational institutions began to be reported (14, 15).

The aim of the study is to identify the weak points of the infection control system and to determine the directions for effective management of the epidemic process using the example of the local epidemic situation.

MATERIAL AND METHODS

The study was conducted in 2016-2017 at the Department of Epidemiology of Ural State Medical University. The materials for the study were data from the official registration of the incidence of measles in a large industrial city for the period from 1988 to 2016: forms of federal state statistical surveillance № 1, 2; materials of the state report "On the state of sanitary and epidemiological welfare of the population in the Sverdlovsk region"; expedited reports, form 058 / y (1218).

To determine the main characteristics of the measles epidemic process, a more detailed analysis of the local epidemic situation which was formed in the megalopolis in 2016 was carried out. The analysis was based on reports on epidemic emergency situations (83), cards of epidemiological investigations of measles cases or suspected cases of this infection (72) and medical records of inpatients, form 003 / y (74).

Additionally, the results of serological tests for anti-measles IgG of health care workers were analyzed (369). Serological tests were performed at the accredited laboratories using the method of enzyme immunoassay (ELISA). The concentration of anti-measles antibodies of IgG class below 0.12 IU / ml was considered as the negative result of ELISA, 0.12-0.17 IU / ml was considered as doubtful and more than or equal to 0.18 IU / ml was positive. The analysis of the results was carried out considering age, position and vaccination history.

The epidemiological, clinical, serological and statistical research methods have been used. The study was retrospective. Generally accepted statistical techniques were used to assess the results obtained. The study of the trend of long-term morbidity pattern was carried out using the standardized range (estimated Hurst exponent) and the average growth / decline rate in morbidity. To evaluate the activity of epidemic foci of measles,

the indicators such as the index and the factor of focality, as well as the indicators of infection reproduction (reproductive indices) were calculated.

The calculations were made using the Microsoft Office 2007 application package. The statistical significance was assessed using the Student's test and the Fisher test. Differences were considered significant at $p < 0.05$.

RESULTS

With the high coverage of measles vaccinations in the city inhabitants of 95.0-97.0%, there have been significant changes in the epidemic process of this infection. Between 1988 and 2001 a steady downward trend in morbidity was reported in the city. The estimated Hurst exponent for the period studied was 0.6, which also allowed to determine the multi-year dynamics in measles as trend resistant, with an average decline rate of 10.87 per cent.

Between 2001 and 2015, on the territory of the city, local cases of measles were not recorded, and its single penetration from endemic territories did not cause the spread of the infection. The situation changed at the end of 2016 when the measles outbreak was recorded in the city. 72 clinically and laboratory confirmed cases of measles were identified within 11 weeks.

In the laboratory test using ELISA method, specific M antibodies were detected in all sufferers. In the PCR study, the antigen of the measles virus of genotype D8 was isolated, which circulated in previous seasons in the countries of South-East Asia, and in 2015 season it circulated in Europe and Russia.

In the age structure of the sufferers, children and adolescents up to the age of 18 (68.1%) predominated, and adults accounted for 31.9%.

The average age of the sick children was 3.8 years. Children of younger age groups, namely, up to 2 years old and 3-6 years old (46.9% and 40.9%, respectively) were mainly involved in the epidemic process, whereas children over 7 years old made up only 12.2%.

In adults, the average age was 33.2 years, and the greatest number of cases fell on the age group of 20-29 years (39.1%) and 30-39 years (30.4%). The proportion of people aged 18-19 was 4.4%, and over 40 years was 26.1%. There were no differences in the gender structure of measles cases.

According to the vaccination history, 68.1% of the cases had not previously been vaccinated against measles, including because of the refusal of vaccination (38.8%), medical exemption (34.7%), and age (16.3%). In 10.2% the reasons for the lack of vaccination were not documented. Among the measles sufferers, who were previously vaccinated, 30.4% were vaccinated once, 47.9% were vaccinated twice and 21.7% were vaccinated three times.

It should be noted that those who got measles among the previously vaccinated persons did not become active sources of infection for those who contacted them. The incidence rate in persons who contacted them was 0.53 ± 0.15, whereas in foci in contact with previously unvaccinated sufferers it was 7.94 ± 0.56 ($t = 12.78$, $p < 0.05$).

According to clinical manifestations, in 90.3% of cases the typical measles is described with the presence of all classical symptoms and the stage of their appearance. Among the sufferers who were not previously immunized, typical manifestations of measles were observed in 98.1%, and they were observed among vaccinated sufferers only in 70%.

The change the rash (macular and roseoseous), the lack of staging of its appear-

ance and pigmentation were reported in atypical forms of measles, in the previously vaccinated sufferers. In the unvaccinated sufferers, the atypical forms of measles were characterized by the absence of symptoms of intoxication and unmarked catarrhal manifestations.

It should be noted that the clinical manifestations of measles in previously unvaccinated patients were more pronounced: hyperemia of the throat, rhinitis, cough and fever up to febrile figures were reported more often.

During the outbreak, 59 local foci were formed with a different number of sufferers, including 36 family-home, 14 in organized communities and 9 in medical settings. In 30.6% of family-home foci there was a spread of infection, including 8 - within the same apartment, in one - in the block and in two foci - both in the apartment and in the block to the higher floors. The index of focality in family-home foci was 1.33, the factor of focality was 30.6%, and the infection reproduction index was 1.1.

In 14 organized communities were involved in the epidemic process, including 7 preschool educational institutions, 5 schools and 2 higher educational institutions, including one with the spread of infection among students (3 people). The index of focality in organized communities was 1.14, the focal factor was 7.1%, the infection reproduction index was 3.0.

The most active spread of the infection occurred in medical settings, where the index of focality was 7.1, the factor of focality was 55.6 per cent, and the infection reproduction index reached 10.2.

DISCUSSION

Five multidisciplinary city hospitals, one of the departments of the children's anti-tuberculosis center, an ambulance station, a private clinic and a medical and sanitary unit at the university were involved in the epidemic process. There was a spread of the infection in more than half of the hospitals. The total number of subsequent cases of measles was 51.

The spread of infection in the medical settings was due to the presence of children and adults who were not previously vaccinated against measles, late diagnosis of measles in the first and subsequent cases, and violations in the organization of anti-epidemic measures.

When analyzing the results of a serological test of the health care workers for anti-measles IgG, it was found that 79% of workers had sufficient seroprotection level.

In the 18-19 age group, the proportion of persons with and without seroprotection was approximately equal. However, for individual age groups, the proportion of persons with seroprotective antibody levels at the age over 50 was the largest and constituted 90.5% (fig. 1).

Among the different categories of health care workers, there were no significant differences in the proportion of persons with seroprotection (tab. I).

Considering the vaccination history, among those who were vaccinated once, the proportion of people with seroprotection was 82%, among those who were vaccinated twice and three times it was 76.6% and 77.8%, respectively. Among those who had had measles earlier, all examined persons showed a sufficient level of seroprotection, and in persons with a lack of data on measles vaccination the positive results in ELISA were about 80%

It should be noted that with an increase in the number of years elapsed since the date of the last application of the vaccine, the proportion of persons without seroprotection increased from 17 to 40% (fig. 2).

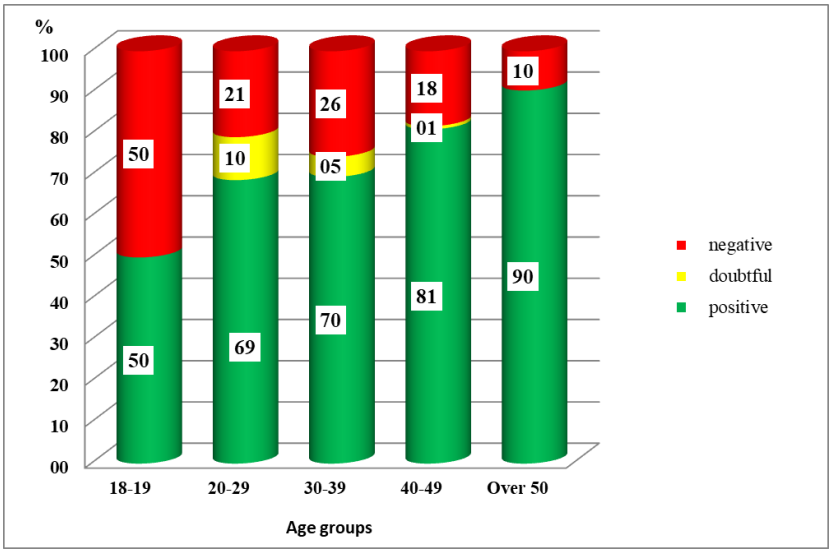

Fig. 1. Results of serological tests for anti-measles IgG in health-care workers in the selected age groups

TABLE I

Levels of protective concentrations of anti-measles IgG among health care workers

Position	Result of the study						Total	
	positive		doubtful		negative			
	No.	%	No.	%	No.	%	No.	%
Doctors	81	77.1	4	3.8	20	19.0	105	100.0
Nursing staff	98	78.4	6	4.8	21	16.8	125	100.0
Paramedical staff	42	85.7	0	0.0	7	14.3	49	100.0
Administrative staff	72	80.0	0	0.0	18	20.0	90	100.0

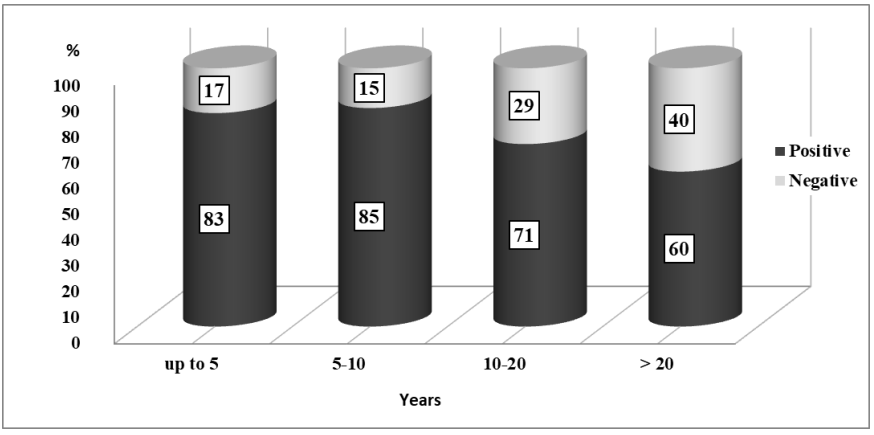

Fig. 2. Proportion of persons with seroprotection and interval from vaccination against measles

CONCLUSIONS

1. Despite positive changes towards the elimination of measles, it remains an important infection which can spread among children and adults who are not protected against measles.

2. The significance of measles cases in previously vaccinated sufferers as potential sources of infection in the foci is low, the spread of the infection upon exposure to them was 0.53 ± 0.15, whereas it was 7.94 ± 0, 56 in contact with those who were not vaccinated and got measles.

3. A peculiarity of the outbreak of measles was its active spread in medical settings with the formation of secondary foci along the routes of sick children and adults.

4. According to the results of serological monitoring, it was established that up to 80% of health care workers had a protective level of specific antibodies, and the greatest number of persons with seroprotection was found in the age group over 50 years. In 30-40% of workers who do not have a protective level of antibodies, the interval from the last vaccination was more than 10 years.

To optimize the system of epidemiological surveillance of measles, it is necessary to:

- Carry out scheduled revaccination against measles at intervals of 10 years up to 50 years of age.
- Carry out booster immunization with live measles vaccine to the contact persons as soon as possible without prior screening as an infection control measure in the foci where the first sufferer was not vaccinated against measles.
- Implement a system for the continuous monitoring of the intensity of measles immunity in the indicator groups recommended for screening by WHO.

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NEWS

NEW DATA FOR TUBERCULOUS MENINGITIS IN HIV-POSITIVE PATIENTS

Tuberculous meningitis (TBM) ranks second in frequency in Africa, with a mortality of over 50% due to diagnostic difficulties. A recent study by Bahr et al. evaluated the diagnostic performance of the new Xpert MTB/RIF Ultra (Xpert Ultra) for TBM in the cerebrospinal fluid (CSF), comparing the Xpert MTB/RIF (Xpert) results with the results of cultures. The study group included 221 HIV-positive patients suspected of meningitis (headache, nuchal rigidity, altered mental status), of which 129 suspected of TBM were tested with Xpert, Xpert Ultra and culture. The results confirmed the diagnosis of TBM in 22 subjects (17%). Of the 21 cases positive by Xpert Ultra, 13 were positive by culture, Xpert or both, and 8 were only positive by Xpert Ultra. The study showed that Xpert Ultra had a 95% sensitivity for TBM, while the sensitivity of culture and Xpert was 45%. By using Xpert Ultra rifampicin resistance was undetected in 13 cases and undetermined in 9. In conclusion, this research demonstrated that Xpert Ultra detected more cases of MTB. The limitation of this paper is that the results cannot be extrapolated to the general HIV-negative population, as the study group consisted of a relatively small number of HIV-positive patients (Bahr NC, Nuwagira E, Evans EE et al. Diagnostic accuracy of Xpert MTB / RIF Ultra for tuberculous meningitis in HIV-infected adults: a prospective cohort study. *Lancet Infect Dis* 2017 Sept. 14 doi: 10.1016 / S1473-3099 (17) 30474-30477).

Anca Maria Adavidoaiei

SURGICAL TECHNIQUES IN THE TREATMENT OF MULTILEVEL ARTERIAL OCCLUSIVE DISEASE

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SURGICAL TECHNIQUES IN THE TREATMENT OF MULTILEVEL PERIPHERAL ARTERIAL OCCLUSIVE DISEASE (Abstract): **Aim** of the study was to report the surgical techniques for multilevel arterial lesions of the lower extremities and to evaluate the clinical outcomes. In patients with multilevel arterial disease, the treatment consisting of sequentially surgical intervention on the same vascular axis is one of the methods indicated in order to obtain an adequate inflow and outflow. **Material and methods**: we have performed a non-randomized study during a 44-month period (January 2010 – September 2013) in a number of 58 patients treated by classical revascularization techniques. All the patients included in the study have been post-surgically surveyed at well-established intervals (1, 3, 6, 9, 12, 24 and 36 months) by: clinical examination, laboratory tests, Duplex ultrasound, and, as needed, CT Angiography. **Results**: the 6 months primary patency in each studied group (corresponding to the years of 2010, 2011 and 2012) was 95%, 93.33%, and 91.3%, respectively; the 12 months primary patency was 85%, 80%, and 82.6%, respectively. Clinical improvement has been noticed in 44 patients (75.86%). There have been registered 14 amputations (24.13% of the cases): 7 majors (thigh and below the knee), representing 12.06% of the total number of cases and 7 minors (toe or transmetatarsal), representing 12.06% of the total number of cases. Limb salvage rate for the patients in stage III and IV Leriche-Fontaine was 85.10%. **Conclusions**: the surgical techniques are a feasible option for the multilevel arterial disease, with favorable patency and limb salvage rates. **Keywords**: MULTILEVEL ARTERIAL DISEASE, SURGICAL REVASCULARIZATION.

Multilevel involvement is typically observed in critical limb ischemia. Surgical interventions are a well-accepted strategy for revascularization in patients with critical limb ischemia due to multilevel arterial occlusive disease (1). A staged approach to multilevel arterial disease was the standard for numerous years, consisting of revascularization of the aorta or iliac artery followed by interval infra inguinal surgery. This approach was a rational strategy at a time when open surgical techniques were only performed in separate settings. Usually, the procedures are performed successively, although individual patient anatomy plays a part in the decision of whether to perform the two procedures simultaneously or not. The aim of the treatment is to obtain an improvement of both the inflow and the outflow.

The optimum treatment of multilevel arterial occlusive disease is often difficult to establish. There is often the need to decide if or when a concomitant or successive outflow procedure must accompany an inflow procedure. The most important decision factor is probably the severity of the distal ischemia. Both an outflow and an inflow procedure are necessary whenever there are large areas of tissue loss associated with the occlusive disease (2, 3).

MATERIAL AND METHODS

We have performed a non-randomized study in one center. The data was gathered retrospectively for a 20-month period (January 2010 – September 2011), and prospectively for a 24-month period (September 2011 – September 2013) for a number of 58 patients treated by surgical revascularization techniques at the Vascular Surgery Clinic, "St. Spiridon" Hospital, Iaşi, Romania.

The including criteria for the patients were: aged 18 and over, multilevel peripheral arterial disease in any of the Leriche-Fontaine stages, with uni- or bilateral pathology, and had an indication of revascularization by open technique (bypass or endarterectomy, with or without angioplasty). The study included 58 patients (female 3.4%, male 96.6%, mean age 62.6 years).

The patients were divided into two groups: 1 – included patients who had the surgical techniques done in one procedure; 2 – included patients treated in two successive procedures.

Peripheral arterial disease was localized at a minimum of two levels on the same vascular axis: aortic, iliac, and inguinal (involving the common, deep femoral artery and the origin of the superficial femoral artery), or infra inguinal (supra- or infra genicular).

All patients were pre-operatively evaluated by physical examination, blood tests, Duplex scan, angiography or CT angiography. Also, the risk factors and associated co morbidities were noted.

Postoperative surveillance at well established time intervals (1, 3, 6, 9, 12, 24 and 36 months), consisted of: blood tests and Duplex scans, clinical examination (improvement of the claudication distance, amelioration of rest pain, healing of trophic lesions). We evaluated the primary and secondary patency of all procedures, initial technical success, complication rate, morbidity and mortality associated with each technique, amputation-free survival rate, symptomatic improvement, and the risk factors that influenced the postoperative outcome and recovery period.

The statistic analyses were performed in *SPSS, version 15.0* (SPSS Inc., Chicago, IL, USA). A p value of less than 0.005 was considered statistically significant. During the study, the primary and secondary patients were analyzed using the Kaplan-Meier estimator.

RESULTS

For the 58 patients included in the study, the open techniques consisted of an autologous or synthetic bypass graft and/or femoral endarterectomy. Twenty-five patients had concomitant revascularization procedures, and 33 had successive procedures (fig. 1).

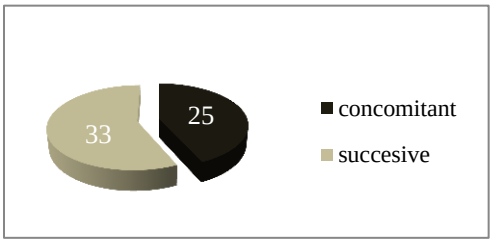

Fig. 1. The distribution of cases according to the sequence of revascularization procedures

Most patients in the given cohort were in the fourth stage of disease (53.4%). Although we observed a better primary patency for this subgroup of patient, compared with those in the second and third disease stages, the difference wasn't statistically significant. Inflow procedures followed by outflow procedures were the most often used ones, most of them being successive (58.69%), rather than simultaneous (fig. 2).

Synthetic grafts were used for most inflow procedures, whereas autologous material was the main choice for outflow procedures.

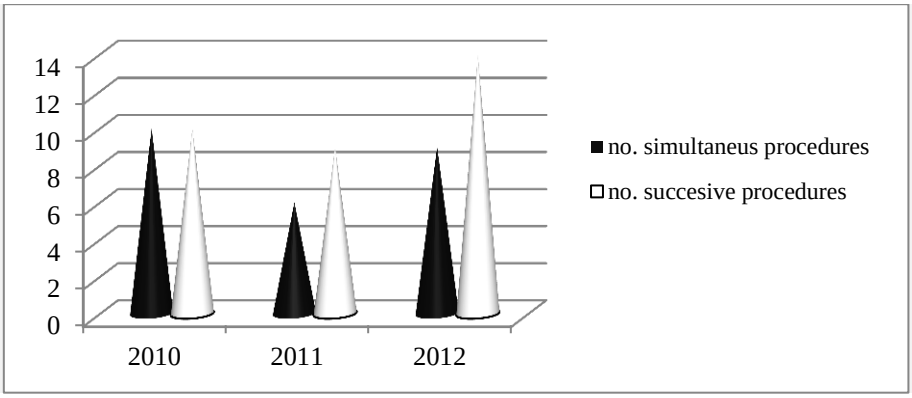

Fig. 2. Yearly distribution of multilevel occlusive disease surgical repairs

For the studied period of time, we constructed infra inguinal extension grafts for 27 aortobifemoral, 4 aortofemoral, one axilobifemoral and 19 iliofemoral bypasses. Three femoropopliteal bypasses required extension grafts and four common femoral artery endarterectomies required a redo with femoropopliteal bypasses.

The study included 13 graft thrombectomies using Fogarty catheters, 20 proximal anastomosis patch angioplasties and 6 vein Miller cuff angioplasties for distal anastomoses.

Elective procedures were performed for 77.58% of the patients and emergency procedures for the rest of 22.41% of the patients, with acute or subacute ischemia. The emergency procedures consisted of graft thrombectomies with or without simultaneous extension grafts. The initial technical success for all procedures was 100%.

We had no early artery or bypass occlusions (less than 30 days) and only one case with a postoperative local hematoma which required a reintervention for haemostasis and evacuation.

We had two graft infections (one case required an additional myoplasty, with favorable postoperative recovery at two years, and the other case required extraction of the infected synthetic graft and construction of a new bypass using the great saphenous veins).

We registered 13 late occlusions (after more than 30 days), which entailed follow-up procedures for reestablishing the patency.

There were 14 (24.13%) amputations recorded: 7 majors (thigh or below-knee), representing a rate of 12.06% of the total number of cases and 7 minors (toe or trans metatarsal), representing a rate of 12.06% of all cases. Limb salvage rate for the patients in stage III and IV Leriche-Fontaine was 85.10%. The amputation-free survival

period ranged between 1 and 36 months, with an average of 16.33 months. Clinical improvements were noticed in 44 (75.86%) patients. The mortality rate for the entire studied period was 5%.

After six months primary patency in each studied group (corresponding to the years of 2010, 2011, and 2012) was 95%, 93.3%, and 92.3% respectively, comparable with those published in the literature (Shilagy). After twelve months primary patency was 85% in the 2010 studied group, 80% in the 2011 group of patients, and 82.6% for the 2012 group (fig. 3, 4, 5).

We did not find statistically significant differences between the averages of the primary patients of various disease stages. Most of the patients who benefited from simultaneous revascularization procedures were in Leriche IV disease stage.

As far as the connection between the type of revascularization procedure and the primary patency is concerned, the average primary patency for the successive procedures is the same as the one for simultaneous procedures. The primary patency did not differ between the elective and emergency procedures subgroups.

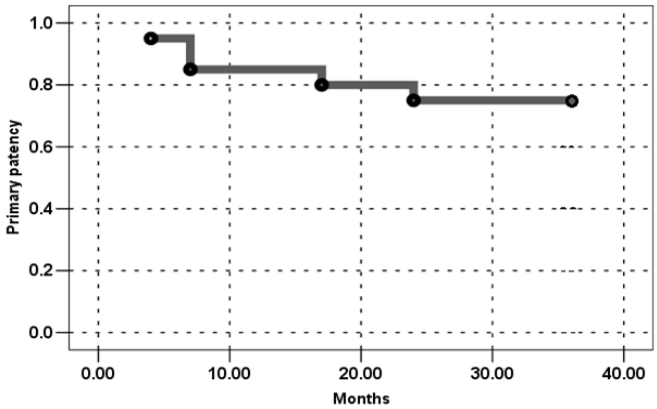

Fig. 3. The Kaplan-Meyer diagram of primary patency for the 2010 patient group

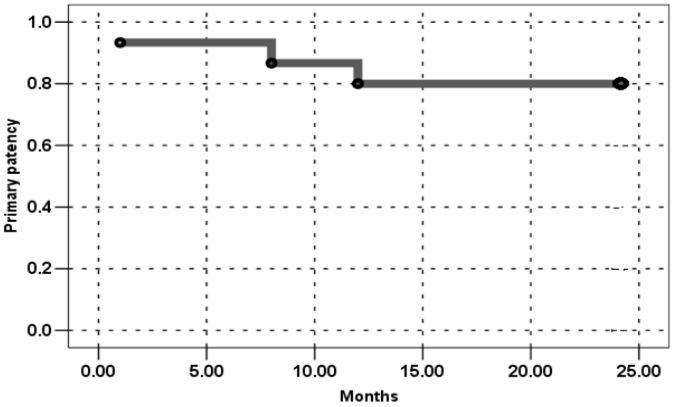

Fig. 4. The Kaplan-Meyer diagram of primary patency for the 2011 patient group

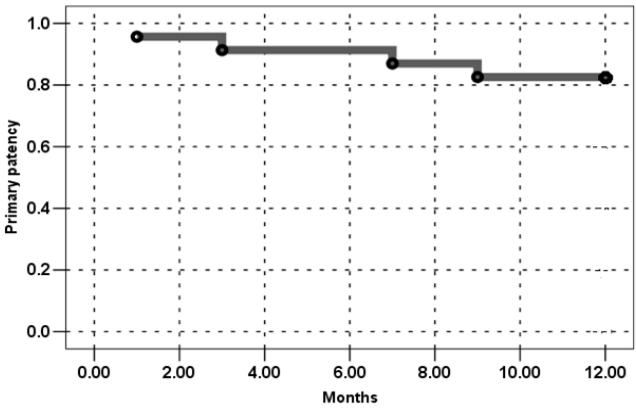

Fig. 5. The Kaplan-Meyer diagram of primary patency for the 2012 patient group

The difference in primary patency between the patients who benefited from synthetic grafts revascularizations and the ones with autologous material is statistically non-significant.

The statistical analysis identified the only risk factor which significantly influenced the primary patency in the studied group: *arterial hypertension*.

DISCUSSION

Critical ischemia due to multi-level arterial occlusive disease may necessitate both an inflow procedure and an outflow procedure, if limb salvage is to be accomplished. Direct surgical repair is the optimum choice for fit patients with a life expectancy of more than two years (4).

Simultaneous multi-level revascularization is a safe and efficient procedure for critical ischemia (5).

In a 15 years retrospective study published in 2014, Sharples and co. report on a single center experience with the treatment of multi-level occlusive disease using simultaneous aortoiliac and infrainquinal bypasses done between 1996 and 2011. Limb salvage rate was 96.9%, 85.7% and 75.9% at 30 days, one year and 5 years respectively. These results demonstrate that multi-level bypasses can be constructed with good long term results. For patients with critical ischemia and tissue loss due to both aortoiliac and infrainquinal occlusive disease, significant benefits exist if an initial multi-level revascularization procedure is accomplished (6).

Following their study on 121 patients (with 201 revascularized limbs) Horstmann and co. have concluded that combined aortofemoral and profound femoral artery reconstruction was desirable in treating multi-level occlusive disease and that infrainquina*l* extension grafts were needed only for critical ischemia (7).

Dalman and co. reported on a study of 62 patients that multi-level procedures offered a complete resolution of symptoms for a greater percentage of patients than single level procedures did (8).

Edwards et al agrees that multi-level aortoiliac and femoral occlusive disease necessitates profound femoral artery angioplasty or an outflow extension graft in order to secure pelvic and distal arterial perfusion (9).

For our surgical cases, the profound femoral artery (PFA) had the most important role. This was the distal anastomosis site for the inflow procedures, with or

without venous or arterial patch angioplasty in the case of 14 patients. Constructing an angioplasty enhanced anastomosis on the PFA for aortobifemoral bypasses yields superior early and long term patency, compared to a distal anastomosis on the common femoral artery (10). The profound femoral artery angioplasty proves very useful when the superficial femoral artery is thrombosed. We observed significant benefits throughout the study from utilizing it adequately. Using for distal anastomosis venous or arterial cuffs on the profound femoral artery decreases the rate of infrainquinal extension grafts. We used simultaneous extension grafts for patients with tissue loss (Leriche IV disease stage) and critical ischemia due to type III aortoiliac disease.

In order to achieve secondary patency, graft repermeabilization was achieved with Fogarty catheters and extension grafts were constructed with distal anastomosis on the profound femoral artery or more distally. These procedures require extensive surgical experience. Adequate surgical exposure and use of the profound femoral artery is the key for aortoiliac disease surgical reinterventions (11).

CONCLUSIONS

In our study simultaneous or successive surgical procedures are a feasible option for multilevel peripheral arterial occlusive disease, with favorable patency and limb salvage rates. The observations of the current study indicate that the profound femoral artery plays an important role in conventional surgery.

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LONG TERM BIOLOGICAL EFFECTS INDUCED BY IONIZING RADIATION – IMPLICATIONS FOR DOSE MEDIATED RISK

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LONG TERM BIOLOGICAL EFFECTS INDUCED BY IONIZING RADIATION – IMPLICATIONS FOR DOSE MEDIATED RISK (Abstract): Ionizing radiations are considered to be risk agents that are responsible for the effects on interaction with living matter. The occurring biological effects are due to various factors such as: dose, type of radiation, exposure time, type of biological tissue, health condition and the age of the person exposed. The mechanisms involved in the direct modifications of nuclear DNA and mitochondrial DNA are reviewed. Classical target theory of energy deposition in the nucleus that causes DNA damages, in particular DNA double-strand breaks and that explanation of the biological consequences of ionizing radiation exposure is a paradigm in radiobiology. Recent experimental evidences have demonstrated the existence of a molecular mechanism that explains the non-targeted effects of ionizing radiation exposure. Among these novel data, genomic instability and a variety of bystander effects are discussed here. Those bystander effects of ionizing radiation are fulfilled by cellular communication systems that give rise to non-targeted effects in the neighboring non irradiated cells. This paper provides also a commentary on the synergistic effects induced by the co-exposures to ionizing radiation and various physical agents such as electromagnetic fields and the co-exposures to ionizing radiation and chemical environmental contaminants such as metals. The biological effects of multiple stressors on genomic instability and bystander effects are also discussed. Moreover, a brief presentation of the methods used to characterize cyto- and genotoxic damages is offered. **Keywords:** IONIZING RADIATION, GENOMIC INSTABILITY, BYSTANDER EFFECTS, STOCHASTIC EFFECTS, SYNERGISTIC EFFECTS.

The linking bridge between ionizing radiation and risk (expressed in various signs, symptoms and illness) has existed since the early age of X-ray discovery by Röntgen. In less than a year (1896) skin burns produced by X-rays exposure have been reported by Stevens (1) and Gilchrist (2) and the first radiation induced skin cancer has been reported 7 years later by Frieben (3).

Our body is daily bombarded by energetic quanta of various origins and energies. From the secondary cosmic particles and nuclei, radio waves, infrared and ultraviolet light we are the subjects of a permanent intake of energy by collisions. All the energy supplied by these quanta is accumulated in our cells, tissues and organs. Of course, not all of them are fulfilling the required

threshold for producing biological effects, but it is possible that they are involved in a *tunneling effect* whose final result is the caption and deposit of the transferring energy inside our body, through elastic and inelastic (non-elastic) collisions. Each and every radiation energy carrier won't produce deterministic effects, but they are preparing and facilitating the action of future bombardments energy quanta, which, in this case, (why not?) will produce effects responsible for triggering of illness starters.

BIOLOGICAL EFFECTS OF IONIZING RADIATION (IR)

Two types of biological effects have been observed: deterministic effects that occur in the early stages of irradiation (few hours to few days) for high (5-15 Gy) and very high doses (above 15 Gy), and stochastic effects (which appear 4 to 10 years after irradiation) and they are in connection with low doses (0.05 – 0.5 Gy).

Naturally, the mean energy received by each of us is approximately 2.5 mSv per year, from γ-rays action only (4). The energy exposure to other types of radiations is variable for each individual and yet unknown, due to its difficulty to be measured. What we know for sure is that a higher absorbed dose will increase the risk of potential cellular injury. There have been described several dose modifying factors: the type of radiation (α-particles and other nuclear particles - like neutrons - perform a higher ionizing power and they are more damaging hazards than the less densely ionizing radiation, such as X-rays and γ-rays), the intensity of those radiations (the risk to develop DNA damages rises with the increase of the radiation intensity), the exposure time (energy transferred on a short period of time has a greater potential for DNA damage), the part of our body which has been irradiated (the gonads, blood-forming organs and the lens of the eye are much more sensitive to radiation damage) and, of course, the age of the exposed person (children are more sensitive to radiation effects than the adults are) (5).

More than the above mentioned dose (2.5 mSv/year), for diagnostic purposes, patients are irradiated either from outside of the human body (that happens when methods using X-rays are involved – radiography and CT-scan – or γ-rays are involved like in radiotherapy) or from the inside of the human body like γ-rays used in scintigraphy. These procedures, performed once or, some time, more than once a year, add an extra statistically calculated mean of 1 mSv for each of us every year (4). If you want to have a quantitative representation of the dose received during an imagistic method like PET-CT, notice that a 250 MBq injection of ^{18}F-fluorodeoxyglucose which is normally administered to the patients leads to an effective dose of 5.8 mSv (equal to the dose that would be received in 1.7 years from nature!) (5). This amount of radiation is considered to be a low dose in the classification of doses made by ICRP (International Commission on Radiological Protection) (6). Nevertheless, those small doses (and perfectly legal) implies supplementary processes of radiation exposure which will always involve stochastic mechanisms.

Concerning of human risk at low doses of ionizing radiation (mainly X and gamma) has been expressed by various authors in past decades. In a review (7), Kam and Banati showed that due to a citation analysis of published literature performed on 22 May 2013 through Web of Science/ Thomas Reuters, from 2003 to 2013 decade more

than 120 articles have been published each year in respect of implications for low doses. This shows the immense interest for the theme of the possible and probable effects of ionizing radiation that covers a wide area of mechanisms that include (without being limited to) non DNA targeted effects (NTEs) with genomic instability (GI) as a part of it, and a variety of bystander effects (BE) with abscopal effects and bystander mediated adaptive response as the principle mechanisms (8).

CHEMICAL EFFECTS OF IONIZING RADIATION

Exposure of living cells to ionizing radiations initiate direct and indirect cellular effects on macromolecules. The direct mechanisms imply the breaking of the atomic structures producing chemical and biological changes. Indirect effects imply both excitations and ionizations leading to the radiolysis of cellular water with a resulting plethora of free radicals. The free radicals can initiate modifications on other critical molecules (indirect effect). By stimulation of oxidases and nitric oxide synthases they will generate a major source of ROS/RNS in irradiated cells under ambient oxygen. The indirect cellular effects of ionizing radiation cause lipid peroxidation, protein oxidation, oxidative alterations to nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) and inactivation of enzymes (9).

TARGET THEORY

In the target theory, in order to produce effects the ionizing radiation must hit a critical site. It is unanimously accepted that nuclear DNA is the critical target for radiation in determining cell death as well as non death-related effects. Recently, scientists have proven that the target theory must be taken into consideration when referring to the mitochondrial DNA (8).

NON-TARGETED EFFECTS

According to Khadim et al. non-targeted effects (NTEs) are those processes that occur in the non-irradiated cells and had received signals from a cell previously irradiated (8). NTEs are not observed in all experimental models and variability characterizes the genotoxic stress responses (10). As mentioned above, NTEs include bystander effects, genomic instability and abscopal effects.

Radiation induced bystander effects (RIBE) have been observed in non-irradiated cells but which previously existed in the same physical environment with the irradiated cells (11). RIBE was also observed in the cells that were placed in a medium that has been transferred from cell cultures that have been previously irradiated (12). Moreover, W.F. Morgan has observed RIBE in both *in vitro* cell culture (13) and *in vivo* model systems (14).

Radiation induced genomic instability (RIGI) represents the radiation effects that were observed in the non irradiated progeny that are clonally descended from an irradiated cell (15).

Abscopal effects or distant out-of-field mechanism is a bystander effect associated with radiation therapy (16). During oncologic radiotherapy the tumor is irradiated with a dose of about 60-70 Gy, which represents a very high dose according to M. Khadim (8) and the healthy normal tissue in the field of the γ-rays beam receives a lower radiation dose for about 4-6 Gy (17). The abscopal effects are generated by the radiation from the environment of the patient, from the device contribution and from the internal scatter (18). Abscopal

effects must be considered low dose radiation effects and therefore not NTEs mechanism. In fact, abscopal effects are contributing to stochastic effects.

STOCHASTIC EFFECTS

The amount of radiation used in medical diagnosis (with γ-rays and X-rays) are well below what is it known to produce "radiation sickness". But those low doses are of peculiar interest for the long term effects. The most important of these stochastic effects are: i) genetic damage of the cell (mutagenesis), ii) damage of the chromosomes (clastogenesis) and iii) carcinogenesis.

Until now, there is not a complete understanding of the relationship between exposure to low dose of radiation and the resulting effects consecutive to its action. The regulations and guidelines currently used in nuclear medicine laboratories all over the world are mainly based on a *linear nonthreshold* (LNT) model. This model states that there is "no threshold dose" for long term effects and the risk of an exposed person (patient or nuclear medicine laboratory personnel) increases linearly with radiation dose.

Unfortunately, in opposition with that model there are facts, reliable experiments, scientific data that had led to different radiation injury models that are inconsistent with the LNT model. There are consistent studies that emphasize that low doses of radiation have a beneficial effect on health, resulting from the stimulation of the immune system (19, 20).

BIOLOGICAL DOSIMETRY

Due to the fact that exposure to ionizing radiation causes chromosome rearrangements and, it was well established, that high doses of ionizing radiation are carcinogenic (21), a method was needed to characterize the molecular transformations. To emphasize the exposure to ionizing radiation, the method of chromosome aberrations as a biological marker has been widely accepted. This method also known as FISH (fluorescence *in situ* hybridization) has the capability to identify and quantify chromosome damages determined by the exposure to IR. In a particular way, this method is important because it can express detectable levels of translocation in medical exposures at low doses of radiation long after the cell has been irradiated. It can detect the resulting transformation month or even years after the exposure episode happened (21).

Other tests used to characterize the changes in number and structure of chromosomes and genetic mutations are sister chromatid exchange (SCE) that involves DNA breakage and reunion (22), and micronucleus method (MN) that evaluates numerical and structural chromosome damages (23).

SYNERGISTIC EFFECTS

Another important aspect regards the exposure of ionizing radiation at environmentally and/or therapeutically relevant doses together with a co-exposure to other physical and chemical agents. Very few things are known in the respect of the cumulative effects of non-ionizing electromagnetic fields on short or long term action, combined with ionizing radiation exposure (24). Even so there are controversial reports on the co-exposure to physical stressors. Early studies have focused on the usage on a large scale of the electromagnetic fields based methods in curative medicine and for diagnostic purposes. Thus, in an experiment conducted by Geard et al.

(25) on the effects of the exposure of magnetic resonance on mouse C3H 10T1/2 cells, there was not recorded an ionizing radiation – like effectiveness for cell killing, transformation and chromosome aberrations. Several other *in vitro* studies using extremely low-frequency electromagnetic fields from 0.7 to 400 mT for different periods of time, combined with a single dose exposure to X-rays or γ-rays (between 3 to 12 Gy) figured out controversial results (26). Lagroye and Poncy reported a significant enhancement of genomic damage by an increased formation of micronuclei (MN) in normal cells from rat tracheal epithelium (27). Jian et al. have been reported an induced apoptosis in a hepatoma cell line due to an increase of aneuploidy (28), while Mairs et al. recorded an increase of the mutations rate induced by ionizing radiation, even at very low doses (29). On the other hand, some authors have reported no detectable changes due to exposures to both ionizing radiation and extremely low frequency magnetic field in a human amniotic fluid cell line (30), breast cancer cells (31), or rodent repair-proficient cells (32).

Moreover, there is a lack of conclusive data regarding the biological effects of co-exposure to chemical contaminants and ionizing radiation. Acute contamination with chemical agents is very rare (except drugs cancer therapy). So, of particular interest is the case of chronic exposure to chemical agents combined with ionizing radiation exposure. There are works that show an increase of genomic instability due to chemical agents (33) while in the estimation of cancer risk Preston notified that the non-targeted effects (NTEs) have been enhanced by co-exposure to chemicals and radiation (34). As a result of the numerous works on that field of interest, an official organization UNSCEAR (United Nations Scientific Committee on the Effects of Atomic Radiation) recognized that in biological systems ionizing radiation is the main physical genotoxic and carcinogenic agent to which we all are exposed and also that low levels of chemical genotoxins such as UV-mimetic agent 4-nitroquinoline 1-oxide (4-NQO) and the metalloid arsenic (As), one of the most widespread element in drinking water, could generate a multiplicative or synergistic effect if an environmental and/or occupational ionizing radiation exposure occurs (35). More recently, the same synergistic effect associated with genomic instability was recorded in human small airway epithelial cells by *in vitro* chronic exposure to As (36). The authors have demonstrated that the key role for mediating the response to DNA damage and for its surveillance and/or maintenance of genome stability was assured by the p53 encoded protein. Another study on a heavy metal Cr (VI) and ionizing radiation synergistic effects have proved the induction of genomic instability in human fibroblasts (37). Moreover, the synergistic modulation of water metal contaminants like cadmium and aluminum and the induced bystander effect of ionizing radiation have been demonstrated in an *in vivo* experiment on salmon (38).

CONCLUSIONS

New studies are needed in order to clarify the mechanisms of the combined exposures scenarios for short and long term cumulative effects. Cancerogenesis, genomic instability and premature cellular senescence (a non-cancer late effect) must be investigated in pluriparameter experiments in which the everyday life co-

exposures to ionizing radiation, electromagnetic fields such as microwave fields associated with mobile telephony and Wi-Fi technology and chemical contaminants in environmentally normal concentration must be taken into consideration.

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SPLIT-THICKNESS SKIN GRAFTING IN ONCOLOGIC SURGERY OF THE LIMB – A CASE REPORT

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SPLIT-THICKNESS SKIN GRAFTING IN ONCOLOGIC SURGERY OF THE LIMB – A CASE REPORT (Abstract): Large excision of tumor soft tissues is often complicated by infections, seriously undermined blood supply and venous drainage, tendon exposure, functional impairment. We report the case of a 67 years old woman admitted for a sarcoma of the right forearm occurring 14 years after a modified radical mastectomy and adjuvant chemotherapy for carcinoma of the right breast. A wide excision of the lesion and split-thickness skin graft was performed with uneventful recovery and satisfactory healing of the graft tissue and functional aspect of the arm. In the last few decades, there has been a search for solutions in tissue repair without need for auto grafts, such as biological substitutes that could repair or improve the function of tissue. In case none of these products are available, auto graft is a good choice and it may be commonly used in the surgical treatment of postoperative soft tissues defects after oncologic surgery. **Keywords**: SKIN GRAFT, MULTIPLE NEOPLASM, LYMPH EDEMA

Direct closure is the reconstruction of choice for surface soft tissue defects. Major causes of recipient site morbidity after skin grafting a postoperative defect of the upper limb are lack of skin graft take to complete loss, functional impairment, infection, hematoma/ seroma and paresthesia. Long-term cosmesis remains poor. In the last few decades, there has been a search for solutions in tissue repair without need for auto grafts. This has produced a development of biological substitutes that could repair or improve the function of tissue.

The use of these products enlarges the spectrum of therapeutic resources available for the treatment of skin lesions, thereby changing the approach to many diseases (1). In case none of these products are available, auto graft is a good choice and it may be commonly used in the surgical treatment of postoperative soft tissues defects after oncologic surgery.

CASE REPORT

A 67 years old woman was hospitalized in our clinic for a swelling of the right forearm that appeared 9 months ago.

She has a history of modified radical right mastectomy (Madden operation) 14 years ago for infiltrating ductal carcinoma

of the right breast- $T_2N_0M_0$; followed by 6 cures of chemotherapy and antiestrogen treatment (Tamoxifen) for 5 years; no radiotherapy was commenced. She also declares the presence of mild lymphedema of the upper limb in the last 8 years.

The patient noticed the gradual increase in size of the swelling described and complains of non-radiating, moderate burning-like pain of the area; further on she noticed the appearance of several nodules along the anterior face of the forearm that ulcerated in the last 6 weeks. An ultrasound and CT scan of the forearm performed on outpatient basis 6 months ago revealed chronic cellulite; therefore she received a topical anti-inflammatory treatment. The local physical examination when presenting in our service showed the presence of mild lymphedema of entire right arm and purpuric spots from the elbow to the wrist. On the anterior face of the forearm, a 12 × 8 cm purple area with multiple nodular lesions, tending to confluence, some of them ulcerated, with surrounding erythema and induration was noted; apparently, on clinical examination, the subcutaneous tissue of the described area was infiltrated (fig 1) We did not find any motor, vascular (peripheral arteries) or sensitivity changes from the right shoulder joint to the right fingers.

Fig. 1. Lesion aspect on admission

The actual blood tests showed no modifications, abdomino-thoracic CT scan and bone scintigraphy did not reveal any pathological modification related to the current condition or to the breast cancer from her history. PET scan and MRI scan were not available.

The anatomopathological examination on the tissue prevailed by biopsy showed conventional high-grade angiosarcoma and microscopically examination exhibited a mixture of vasoformative features, including intravascular infiltration tumoral vascular lumen, papillary projection into the lumen of the vessels; therefore, given the history, symptoms and pathology, our first diagnosis was angiosarcoma developed on upper limb lymphedema. But the

great dilemma was brought by the immuno-histochemical tests showing specific aspects of Kaposi sarcoma (positive CD_{31}, CD_{34} and HHV_8), without succeeding a final, reliable differentiation between the two forms of sarcoma. Under these circumstances, an oncologic wide excision of the lesion and free split skin graft in tied over manner was performed under general anesthesia (fig 2).

a. **b.**

Fig. 2. Excision of the cutaneous lesion (a) and aspect of the skin graft on the 5-th postoperative day (b)

The immediate postoperative recovery was uneventful with satisfactory healing of the graft tissue and functional aspect of the arm and the patient was discharged 8 days postoperatively in order to initiate oncologic treatment recommended by the oncology board. On the 3rd week postoperative the patient was one again hospitalized for a local relapse represented by the same aspect cutaneous nodules on the edge of the graft. A local re-excision was performed; even if there was a great probability that the lesion would invade the antebrachial aponeurosis, we did not excise it because of the risk of harming the radial vessels which would not bring the patient any benefit considering the disease's fulminant evolution. We used the same type of skin graft to cover the defect with graft application after receiving a granular bed on the recipient site. Although the patient begins chemotherapy, there were no postoperative complications or delay in maturation of the graft. Evolution of the patient was unfavorable due to the emergence of many skin nodules along the upper limb and lung metastases, the patient passing away in 8 months after surgery.

DISCUSSION

Large excision of tumoral soft tissues is often complicated by infections, seriously undermined blood supply and venous drainage, tendon exposure, functional impairment. That is why repairing a postoperative skin defect continues to be a challenging problem for plastic surgeons. Direct closure by skin grafting may not be suitable for larger defects due to extensive tension. A variety of techniques are available for achieving tension free closure, including split-thickness skin grafts, skin

flaps, and internal or external tissue expansion (2).

Difficulty of the case is enhanced by diagnostic ambiguity; there are two serious diseases entering into discussion. First, with patient's symptoms and history giving a perfect match is the Stewart Treves syndrome: angiosarcoma, a rare and aggressive tumor with a very poor prognosis associated with lymph edema occurred after ipsilateral mastectomy for breast cancer. Concerning the surgical treatment, there are studies that show that there is no significant difference in survival comparing those initially treated with wide excision and those treated with amputation (3).

Giving the aggressive nature of the tumor, high rate of local recurrence, and tendency for early and multiple metastases, long-term survivorship is rare with a median survival of 2.5 years after diagnosis, most patients dying within 2 years from metastatic disease (4). Although liver and bone metastases may occur, spread of disease to the lung or chest wall is often the cause of death in patients with Stewart-Treves syndrome. We consider that, even if there is no evidence of distant metastases at diagnosis, the neoplasic disease is advanced because of the long time elapsed between the onset of the disease and the time of diagnosis. Surgical treatment can be preceded or followed by radiation therapy. Locally advanced tumors or metastatic forms can be treated with mono or polychemotherapy, on systemic or local administration.

Cases with good response using courses of intra-arterial mitoxantrone (MX) and paclitaxel (PTX) are reported (5, 6). Knowing that long-term survivorship is rare with a median survival of 2.5 years after diagnosis, most patients dying within 2 years from metastatic disease(4), wide oncologic excision with skin graft will give the patient a better quality of life than amputation. Moreover, if the final immunohistochemical test would have shown the tumor is Kaposi sarcoma, knowing that it has a better prognosis than angiosarcoma, the large excision with this type of skin graft would give the patient a satisfactory local healing and comfort.

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RETROSPECTIVE STUDY ON THE ETIOLOGICAL SPECTRUM AND ANTIBIOTIC RESISTANCE IN INFECTIONS ASSOCIATED WITH OSTEOSYNTHESIS MATERIALS

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RETROSPECTIVE STUDY ON THE ETIOLOGICAL SPECTRUM AND ANTIBIOTIC RESISTANCE IN INFECTIONS ASSOCIATED WITH OSTEOSYNTHESIS MATERIALS (Abstract): **Aim**: To determine the prevalence of infections according to their etiologic spectrum and antibiotic sensitivity in patients with or without implants and endoprostheses. **Material and methods**: This retrospective study was conducted based on a customized study protocol on 773 cases admitted to the Clinic of Orthopedics – Traumatology of the Iași "Sf. Spiridon" Emergency Hospital in the interval January 1, 2003 - December 31, 2011 for postoperative infectious complication. **Results**: *S. aureus* was more frequently found in the patients without implant (58.2%) and in those with subcutaneous implant (45.5%); the lowest frequency was noticed in the patients with endoprosthesis (27.1%), the frequency distributions showing marked statistically significant differences (p=0,001). *P. aeruginosa* was more frequently detected in the patient group with endoprosthesis (30.2%), while in the other groups it was found in approximately 7% (p=0.001). The presence of *Acinetobacter* spp. was significantly higher in patients with deep implant (12.9%), with the lowest frequency noticed in the patients without implant (4.5%) (p=0.029). Of the total isolated and identified types, 59.6% were non-multidrug-resistant (non-MDR), the remaining 40.4% being MDR types. **Conclusions**: *S. aureus* was the most frequent etiological agent in patients with or without cutaneous implant (45.5 and 58.2%, respectively), and *P. aeruginosa* (30.2%) in the endoprosthesized patients. Implementation of a preoperative screening protocol for the identification of healthy *S. aureus* carriers (Methicillin-sensitive *S. aureus* - MSSA / Methicillin-resistant *S. aureus* - MRSA) and subsequent decolonization would be an efficient way of reducing the incidence of infections caused by this bacterium. **Keywords**: PERIPROTETIC INFECTIONS, BIOFILM, IMPLANT, MRSA.

Postoperative infections still represent a major issue, both for clinicians and general population. In particular, traumatology is a branch of surgery that uses a large variety of implants to treat various fractures (1). Recent data suggest that the proportion of infection is most of the times underestimated. This is accounted for by the fact that

the causative agents may escape detection, either because they are not found in a sufficiently large number to be identified or are strongly adherent to the implant and thus difficult to diagnose using phenotypic microbiological methods (2).

The fact that antibiotic prophylaxis improves the results of endoprosthetic replacement and lowers the complication rate, aseptic loosening and osteolysis included, supports the current hypotheses which state that microorganisms might play a larger part than initially thought (3). Certain comorbidities, such as rheumatoid arthritis, hyperglycemia, obesity, deep vein insufficiency, defective diet, immunosuppression, and prior surgical interventions on the affected limb further increase the risk of infection (4).

MATERIAL AND METHODS

A retrospective analysis of the 773 cases with postoperative infections complications admitted the interval January 1, 2003 - December 31, 2011 to the Clinic of Orthopedics – Traumatology of the Iasi "Sf. Spiridon" Emergency Hospital. A customized study protocol was used to reviewed data regarding: sex, rural/urban residence, type of surgical intervention, causative agent, spectrum of antibiotics resistance, and associated risk factors.

The study patients were divided into two groups according to the absence or presence of an implant. Depending on the fracture site and type o surgical intervention, the patients in the first group (with implant) were divided into three study subgroups: subgroup 1 – 257 patients who had superficially located osteosynthesis materials (clavicle, elbow, tibial plateau, ankle, foot); subgroup 2 – 286 patients who had deeply situated osteosynthesis materials (humeral, femoral, tibial); subgroup 3 – 96 patients with knee and hip arthroplasty. The patients in the second group (without implant, 134 patients) formed subgroup 4.

The samples for bacteriological testing were processed in the Bacteriology Laboratory of the Iaşi "Sf. Spiridon" Emergency Hospital following the standard protocol. At a later stage, depending on the result of antibiogram carried out using the Kirby-Bauer method, the identified bacterial types were classifies as non-MDR and MDR (multidrug resistant) using the CLSI (*Clinical and Laboratory Standard Institute*) criteria. The basic criteria used for this classification came from an international group of experts who, at the initiative of the European Center for Disease Prevention and Control (ECDC) and of the Center for Disease Control and Prevention from USA (CDC) created a standardized international terminology in which the resistance profile for *S. aureus*, *Enterococcus* spp., *Enterobacteriaceae*, *P. aerugonosa* and *Acinetobacter* spp are described (5).

SPSS 17 was used for the statistical data analysis. The data were analyzed and expressed in terms of average values, percentages, and standard deviation.

RESULTS

In the study group 479 patients (62%) were males. Gender distribution showed a statistically significant difference (χ^2 = 44,276; GL = 1, $p < 0.01$).

Of all strains isolated from patients, depending on the microscopic classification, 61.84% were Gram positive cocci, 35.19% Gram negative bacilli, and 2.98% Gram positive bacilli. According to tinctorial affinity, a statistically significant difference was recorded (χ^2 = 402,926; GL = 2, $p < 0.001$).

In the study cases there was an increased rate of *S. aureus* infection (43.5%), a rate of approximately 10% being recorded for *P. aeruginosa* (9.7%) and *Acinetobacter* spp. (9.6%), and below 10% for the other causative agents (between 0.1% for *Morganella* and 7.8%, for *Enterococcus*) (fig. 1).

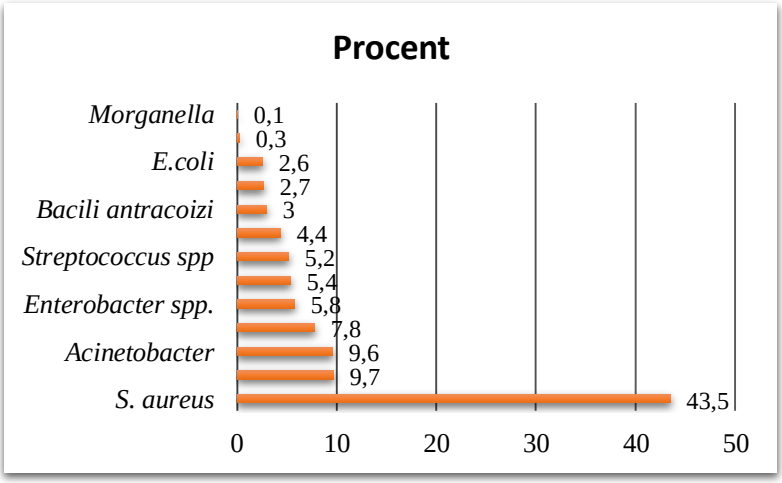

Fig. 1. Distribution of cases according to the causative agent

The proportion of cases with closed fractures infected with *S. aureus* was significantly higher compared to open fractures (44.7% *vs.* 31.4%; p=0.045). The majority of patients infected with *Enterococcus* spp. (7.1% *vs.* 14.3%; p=0.033) and anthracoid bacilli (2.6% *vs.* 7.1%; p=0.049) had open fractures.

With regard to antibiotic resistance, only 59.6% of all isolated and identified strains were non-MDR, the remaining 40.4% being multidrug resistant types (fig. 2).

Of the total number of patients who underwent implant surgery, 29% required the ablation of osteosynthesis material due to the impossibility to eradicate infection by irrigation, debridement and intravenous antibiotic therapy which had good results in most cases (71%). The distribution of patients according the ablation of osteosynthesis material showed a statistically significant difference (χ^2 = 111,56; GL = 1, p < 0.01). Of the total number of implanted patients, the highest rate of ablation was recorded in subgroup 2 (17.20%), followed by subgroup 1 and 3 (8.3% and 3.6%, respectively).

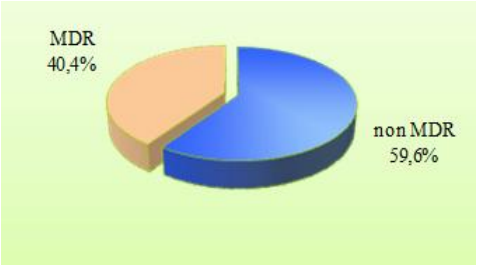

Fig. 2. Distribution of cases depending on the antibiotic resistance

One of the reasons was the involvement in infection etiology of a MDR strain. In all the three implant subgroups, the frequency of MDR strains was higher than that of non-MDR strains: 5.01% as compared to

3.29% in subgroup 1, 9.37% as compared to 7.82% in subgroup 2, and 2.5% as compared to 1.1% in subgroup 3.

As to the presence of the causative agent, assessed depending on the study subgroup, the following differences stand out:

- *S. aureus* was more frequently identified in the subgroup without implant (58.2%) and in the subgroup with subcutaneous implant (45.5%); the lowest frequency of *S. aureus* was noticed in the subgroup with endoprosthesis (27.1%), the frequency distributions showing statistically significant differences (p=0.001);
- the presence of *S. epidermidis* was reduced in all subgroups (p=0.566), the lowest frequency being recorded in the subgroup without implant (3%), and the highest in the subgroup with subcutaneous implant (6.2%);
- *P. aeruginosa* was more frequent in the subgroup with endoprosthesis (30.2%), while in the other subgroups the rate was of approximately 7% (p=0.001);
- the presence of *Acinetobacter* spp. was significantly higher in the subgroup with deep implant (12.9%), while the lowest frequency was noticed in the patients without implant (4.5%) (p=0.029);
- *Enterococcus* spp. was identified with frequencies ranging between 6.7 – 8.4% in all study groups (p=0.942);
- *Enterobacter* spp. was found in 10.4% of the patients with endoprosthesis and in 6.3% at those with deep implant, but the percentage differences were not statistically significant (p=0.138);
- the frequency of *Klebsiella* spp. presence was of 5.1% in the subgroup with subcutaneous implant and below this value in the other study subgroups (p=0.390);
- anthracoid bacilli were more frequently found in the subgroup with subcutaneous implant (4.7%) while in the subgroup without implant it was of 1.5%, with no statistically significant differences (p=0,249);
- in the study cases, *Streptococcus* spp. was found with a frequency ranging between 4 and 7%, without significant differences between the study groups (p=0.696);
- *Proteus* spp. was found most frequently in patients with deep implant (3.5% of the cases) (p=0.703);
- the frequency of *E. coli* was reduced in all subgroups (0.7%, in the subgroup without implant and 3.8% in patients with deep implant) (p=0.210);
- the highest antibiotic resistance (47.2%) was demonstrated by the study in the subgroup with deep implant, while the rate of antibiotic resistant cases in the subgroup without implant was of 29.9% (p=0.006) (tab. I).

DISCUSSION

The diagnosis and treatment of infections is complicated on one hand by the bacterial biofilm formation on implant surface and on the other hand because of the increase in the number of multidrug-resistant bacteria as causative agents.

The general incidence of these complications seems to be sex-dependent, with a much higher risk for the male patients.

The frequency of causative agents is variable according to the recently published studies (2012-2013) (tab. II).

The most frequently isolated was *S. aureus,* followed by the gram negative bacilli (*P. aeruginosa* and *A. baumanii*) and *Enterococcus* spp. The bacterial spectrum related to fracture fixation devices seems to differ from that in hip and knee arthoplasty, resembling more to infections occurring in

patients who do not associate fixation devices, and being characterized by a relatively high incidence of *S. aureus* (over 40%) and *P. aeruginosa*.

Species of the *Streptococcus* genus and *Enterobacteriaceae* family are frequently associated with hip endoprosthesis, where surgical incisions in the proximity of the perineum not surprisingly modify the entire etiologic spectrum.

TABLE I

Antibiotic resistance on types of implant

Resistance	Subcutaneous implant (n=257)		Deep implant (n=286)		Endoprosthesis (n=96)		Without implant (n=134)		p
	n	%	n	%	n	%	n	%	
non-MDR	155	60.3%	151	52.8%	61	63.5%	94	70.1%	0.006
MDR	102	39.7%	135	47.2%	35	36.5%	40	29.9%	

TABLE II

Frequency of causative agents compared to data in the literature

Causative agent	Our study	Dresing and col. 2013 (6)	Fernandes and col. 2013 (7)	Mabit and col. 2012 (8)	Peel and col. 2012 (9)
S. aureus	43.5% (18% MRSA)	51.28% (17.61% MRSA)	45% (12% MRSA)	41.7% (7.3% MRSA)	56% (26% MRSA)
S. epidermidis	5.4%	18.72%	5%	15.6%	24%
P. aeruginosa	9.7%	2.12%	8%	6.3%	10%
Acinetobacter spp.	9.6%	-	8%	-	1%
Enterococcus spp.	7.8%	12.35%	9%	4.2%	15%
Enterobacter spp.	5.8%	3.54%	-	6.3%	6%
Klebsiella spp.	4.4%	1.11%	9%	4.2%	3%
Streptococcus spp.	5.2%	1.68%	-	3.1%	4%
Proteus spp.	2.7%	2.12%	5%	2.1%	7%
E.coli	2.6%	4.38%	2%	3.1%	11%

Antibiotic sensitivity testing revealed an increased frequency of MDR strains (40.36%), mainly represented by *S. aureus* (18.11%) and *Acinetobacter* spp. (8.8%). *S. aureus* with a high vancomycin sensitivity, therefore with a low- and high-level of resistance, signaled as early as 1996 and 2001, respectively, was fortunately not identified. Most types of MDR Gram negative bacilli had an increased sensitivity to carbapenem and fluoroquinolones.

The treatment of orthopedic device-related infections most often consists in long term antibiotic therapy and implant ablation. In our study, implant ablation was performed in only 29% of the patients, the remaining patients being treated with intravenous antibiotics and local debridement (71%).

In all patient subgroups, the rate of *S. epidermidis* varied between 2.99 and 6.33%. These microorganisms are part of the commensal skin flora and may be transmitted from the improperly decontaminated surfaces at the level of the soft tissues or bone during intraoperative interventions.

In addition, several intrinsic and extrin-

sic factors could be involved in the pathogenesis of these infections. Intrinsic factors include: age, nutritional status and obesity. In our study, old age was responsible for the infections, although we failed proving it as an independent factor as reported by other studies (10). Old patients frequently associated other risk factors, such as hyperglycemia (6.33%) and other conditions affecting the immune system, such as peripheral venous insufficiency (21.08%), obesity (4.91%) or chronic hepatitis (2.58%).

Obesity is a more common risk factor in knee prosthesis than in hip prosthesis (11). The highest infection rate was noticed in patients with morbid obesity (CMI≥40 kg/m^2)(12). As a rule, some of these patients also have diabetes or peripheral venous insufficiency.

In 2007, Meding et al. identified preoperatively 15% of the patients with diabetes, out of which only 58% were aware of this diagnosis (13).

CONCLUSIONS

Prosthetic infections not only affect patients, but also constitute a complication which increases medical expenses, thus having consequences for the whole medical system through the supplementary expenses they impose.

S. aureus was most frequently found in the subgroup without implant (58.2%) and in the subgroup with subcutaneous implant (45.5%).

P. aeruginosa was more frequently met in the group with endoprosthesis (30.2%), while for the other subgroups the rate was of approximately 7%.

The presence of *Acinetobacter* spp. was significantly higher in the subgroup with deep implant (12.9%), the lowest frequency being recorded in the patients without implant (4.5%).

Antibiotic sensitivity testing showed an increased frequency of MDR strains (40.36%), mainly represented by *S. aureus* (18.11%) and *Acinetobacter* spp. (8.8%).

The management of orthopedic device-related infections requires further studies in order to identify as quickly as possible the microorganisms responsible and their antibiotic sensitivity, as well as the associated risk factors in view of implementing effective prevention measures and a proper treatment and thus reducing the hospitalization costs and, last but not least, improving patient quality of life.

Future multicentre studies might identify all relevant risk factors for such infections, and thus a proper diagnosis and treatment algorithm. We believe that the implementation of a preoperative screening protocol for the identification of healthy *S. aureus* carriers (MSSA/MRSA) and subsequent decolonization would be an efficient way for reducing the incidence of infections caused by this bacterium.

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NEWS

ANGIOTENSIN-CONVERTING ENZYME AND ALZHEIMER'S DISEASE PREVENTION

Alzheimer's disease (AD) is characterized by neuronal loss and cognitive decline caused by cerebral accumulation and aggregation of amyloid β protein (Aβ) associated with an inflammatory reaction determined by the reactive astrocytes and activated microglia. The most commonly form of Aβ in AD is a neurotoxic peptide with 42 amino acids (Aβ1-42). Accumulation of Aβ1-42 is caused by overproduction or by insufficient clearance. The immune system cells and the peptidases are implicated in Aβ1-42 clearance. Angiotensin-converting enzyme (ACE) is a peptidase with multiples functions. ACE is responsible by vasoconstrictor angiotensin II synthesis, but also by Aβ1-42 degradation in the brain. Using a genetically murine model, Bernstein and collaborators, showed that the ACE overexpression in myelomonocytic lineage cells can reduces Aβ1-42, enhances the capacity of immune system to clear Aβ, reduces cerebral inflammation and prevents cognitive decline in mice with AD.These findings prove the role of enhanced immune response in AD prevention (Bernstein KE, Koronyo Y, Salumbides BC et al. Angiotensin-converting enzyme overexpression in myelomonocytes prevents Alzheimer's-like cognitive decline. *J Clin Invest*. 2014. pii: 66541).

Cătălina Luncă

QUANTITATIVE AND QUALITATIVE BONE ASSESSMENT OF THE POSTERIOR MANDIBLE IN PATIENTS WITH DIABETES MELLITUS: A CONE BEAM COMPUTED TOMOGRAPHY STUDY

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QUANTITATIVE AND QUALITATIVE BONE ASSESSMENT OF THE POSTERIOR MANDIBLE IN PATIENTS WITH DIABETES MELLITUS: A CONE BEAM COMPUTED TOMOGRAPHY STUDY (Abstract): Evaluation of mandibular bone features is very important for the diagnosis, treatment planning and management of dental procedures such as osseointegrated implants. **Aim:** To evaluate the bone quantity and quality of the posterior region of the mandible using the CBCT technique for implant planning in patients with diabetes mellitus. **Methods:** Twenty-three patients with diabetes mellitus and 27 control subjects participated in the study. Mandibular bone measurements were performed on the cone beam computed tomography (CBCT) images. One previously calibrated oral radiologist with CBCT experience measured the distance from mandibular canal to alveolar ridge and the distance from bucal to lingual cortical wall. Also, it was assessed the bone quality of each region of interest, by measuring the bone mineral density. The study protocol was approved by the local Ethics Committee. **Results:** The study revealed the presence of significant correlations between bone quality and values of glycosylated hemoglobin and a significant association between patient gender and bone type. There is a significant inverse correlation between the values of cortical and trabecular bone density in the posterior region of the mandible and HbA1c. **Conclusions:** In conclusion the use of CBCT in preoperative bone measurements will help provide valuable information about the quantity and quality of bone before the placement of dental implants. **Keywords:** DIABETES MELLITUS, CONE BEAM COMPUTED TOMOGRAPHY, IMPLANT PLANNING, PREOPERATIVE, QUANTITATIVE ANALYSES, BONE DENSITY, MANDIBLE.

Diabetes mellitus has been associated with the occurrence of a series of complications involving the skeletal system, such as the reduction of bone mineral density, with an increased risk for occurrence of osteopenia and osteoporosis, poor osseous healing characteristics and impaired bone regeneration potential (1).

However, the influence of systemic diseases, such as diabetes mellitus, on the bone quantity and quality of the jaws has been investigated to a minor extent.

Different oral and maxillofacial imaging techniques used for the assessment of bone quantity and quality, as well as localization of the anatomical structures in the

jaws, are indispensable in treatment planning before placement of dental implants.

Cone beam computed tomography (CBCT) become one of the most useful imaging technique for acquiring bone images before performing dental implant surgery and had been increasingly replacing multi slice CT (MSCT), because it provides adequate image quality associated with a lower exposure dose (2 - 4).

Several authors have reported the use of CBCT intensity values as a measurement to assess bone mineral density, which is expressed in Hounsfield units (HU) (5), characteristics that provide important information about the quality of the bone.

The posterior region of mandible may be the implant site requiring the most rigorous assessment of bone quantity and quality because both will directly influence the results of the implant treatment as well as implant stability.

The aim of this study was to evaluate the bone quantity and quality of the posterior region of the mandible using the CBCT tehnique for implant planning in patients with diabetes mellitus.

MATERIAL AND METHODS

The patients were randomly chosen from the Department of Oral Radiology, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy, Iasi, Romania who had received a CBCT examination as part of their dental implant planning. The study protocol was approved by the local Ethics Committee.

Quantitative and qualitative assessment was performed on 50 adult patients (28 male, 22 female) with a mean age of 51.3 years (range 41– 67 years). Of those, 23 patients had diabetes mellitus and 27 were non-diabetic, who served as a control group.

During the preliminary screening, patients had to meet the following entry criteria: (a) absence of second premolars and molars in both sides of the mandible; (b) no history of bone grafting at the implant site; (c) no smoking; (d) absence of systemic disease, infections or illness, except diabetes mellitus for diabetic group and (e) no current use of drugs which could influence the bone quality.

All of the study subjects had blood sampling by venipuncture of the antecubital vein, after a minimum 8 hours of fasting, in order to determine glycated haemoglobin (HbA1c) values. In the study it was used a single standardized commercial laboratory to evaluate the HbA1c levels.

The CBCT device used was the PlanmecaPromax 3D Mid (Planmeca OY, Helsinki, Finland). The scans were carried out under the following exposure conditions: 90 kV, 12 mA, and exposure time of 18.3 seconds. Initial and final reconstructions were carried out by Romexis 2.3.1 software (Planmeca, Helsinki, Finland).

One previously calibrated oral radiologist with CBCT experience measured the distance from mandibular canal to alveolar ridge (bone height) and the distance from bucal to lingual cortical wall (bone width). Quantitative evaluations in each case were made using reconstructed sagital sections of CBCTs images for each potential implant site (fig. 1).

Also, it was assessed the bone quality of each region of interest, by measuring the bone mineral density, which was classified in to four groups: D1, D2, D3 and D4. Qualitative evaluations were made using panoramic reconstructions for each region of interest (ROI) (fig. 2, 3). The measurements were performed bilaterally in the

premolar and molar regions of the mandible.

The evaluation was repeated by the same examiner within an interval of 30 days to ascertain the reproducibility of the HU and the area measurements.

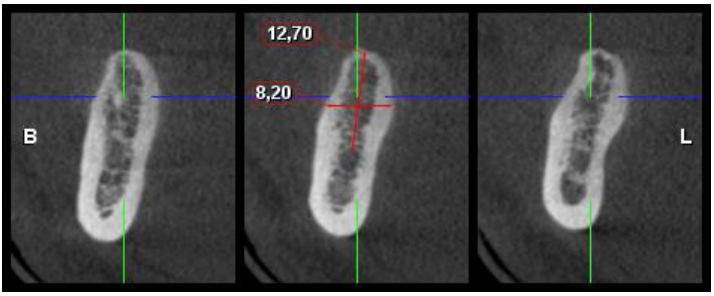

Fig. 1. Measurements of bone quantity (height and width) using sagital images

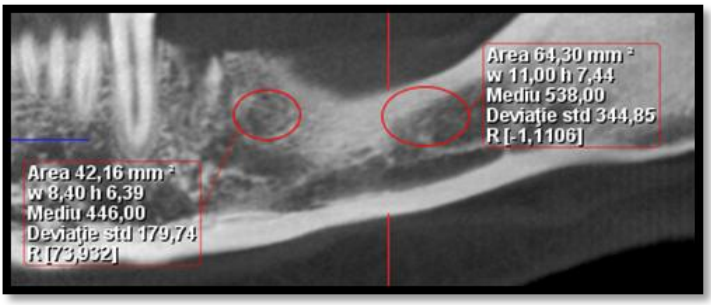

Fig. 2. A reconstructed panoramic CBCT image showing the ROI set in the trabecular bone of the posterior mandible for bone density measurement

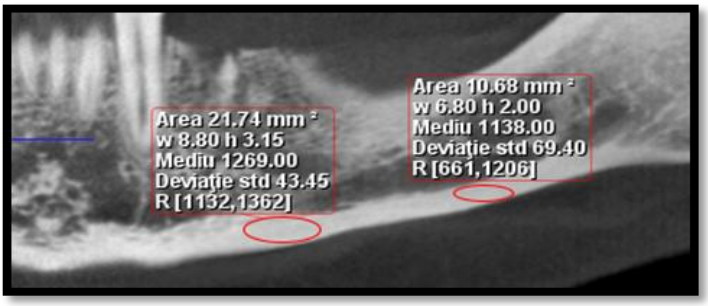

Fig. 3. A reconstructed panoramic CBCT image showing the ROI set in the cortical bone of the posterior mandible for bone density measurement.

The data were analyzed with a statistical software package (*SPSS Version 10.0*; SPSS Inc., Chicago, IL). Mann Whitney U-test was used to measure the differences between bone quantity, quality and those of other parameters (gender, age and HbA1c levels) between diabetes and control groups. The correlation or regression analysis was used

where appropriate. The level of statistical significance was set at P , 0.05.

RESULTS

The patients were stratified on the basis of their HbA1c levels in patients without diabetes (HbA1c level of < 6.0 percent), with well-controlled diabetes mellitus (HbA1c level from 6.0-8.0 percent), with moderately controlled diabetes mellitus (HbA1c level from 8.1-10.0 percent) and with poorly controlled diabetes mellitus (HbA1c level of > 10.0 percent) (tab. I).

The study revealed the presence of significant correlations between bone quality and values of glycosylated hemoglobin (fig. 4).

TABLE I

The distribution of bone quality according to the glycosylated hemoglobin value.

HbA1c (%)	Bone quality				Total
	D1	D2	D3	D4	
≤ 6%	7	20	0	0	**27**
	25.93%	74.07%	0.00%	0.00%	
6.1% - 8%	0	0	8	0	**8**
	0.00%	0.00%	100.00%	0.00%	
8.1% - 10%	0	0	9	0	**9**
	0.00%	0.00%	100.00%	0.00%	
>10.1%	0	0	0	6	**6**
	0.00%	0.00%	0.00%	100.00%	
					50

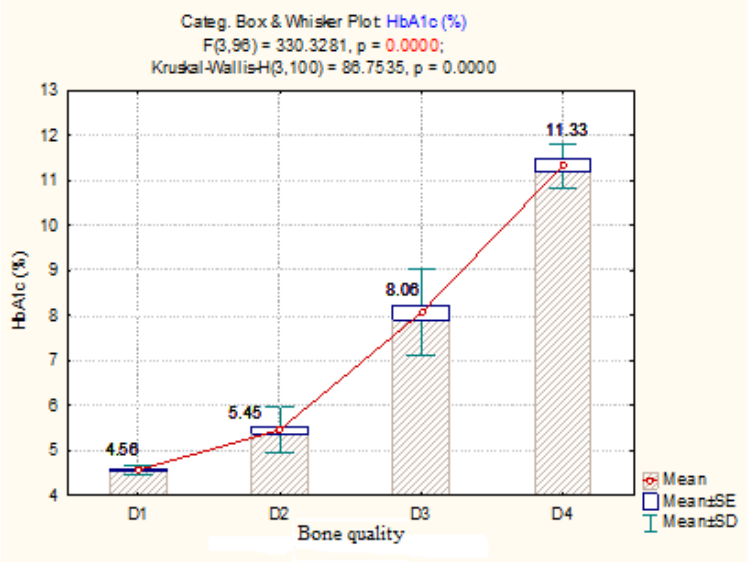

Fig. 4. The mean value for HbA1c (%) levels according to the type of bone

The evaluation of non-parametric correlation showed a significant association between patient gender and bone type. Thus, female patients held a large share of cases from D2 bone type and male patients are found in high numbers in D2 and D3 bone type.

The age showed significantly higher values in patients from D4 bone type compared with the age of patients from D1, D2 or D3 bone type (fig. 5).

There is a significant inverse correlation between the values of cortical and trabecular bone density in the posterior region of the mandible and HbA1c. This result demonstrates that for high levels of HbA1c are found low levels of bone density.

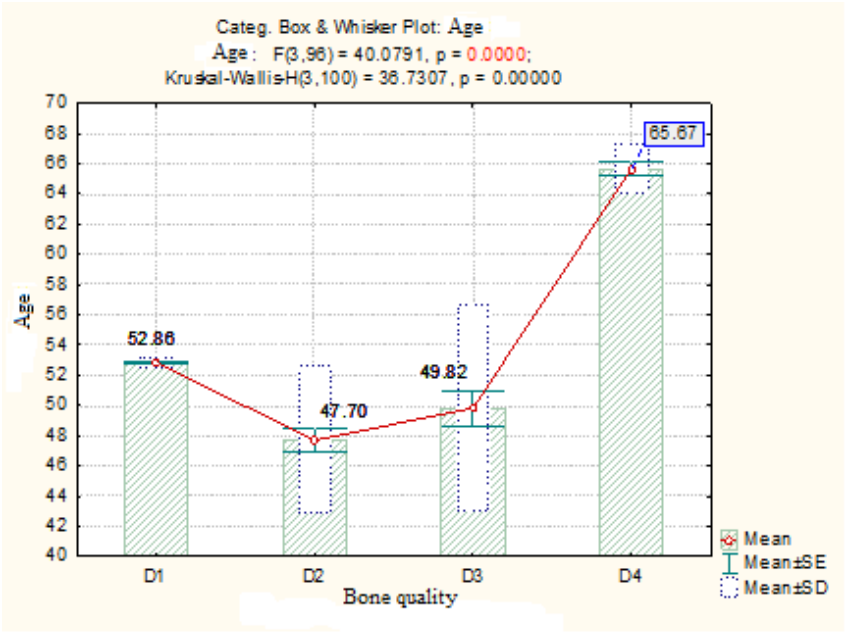

Fig. 5. The mean value of age according to the type of bone.

DISCUSSION

Because of the number of patients with diabetes mellitus, this condition cannot be neglected in the daily routine of dental clinics. The aim of the present study was to assess the mandibular bone quantity and quality in a diabetic patient population, because mandibular bone height, width and density gains special importance in some dental procedures such as osseointegrated implants and grafting.

Well known complications of diabetes mellitus include retinopathy, nephropathy, neuropathy, macrovascular diseases and altered wound healing. Currently it is thought that delayed healing and compromised neutrophil functions are associated with the periodontal diseases, periodontal abscess and implant failures (6, 7).

Mechanisms that might account for an association between diabetes mellitus and bone quantity and quality are not clear. In order to solve the mechanism, several studies have focused on sex hormones, hyperglycaemia, obesity and hyperinsulinaemia, but still the pathogenesis is a subject of

controversy (8). There are few studies evaluating the relationship between diabetes mellitus and mandibular bone quantity and quality. Studies are mostly focused on bone implant problems in diabetic patients. In an experimental study, Nevins et al (9) observed that bone implant contact was significantly reduced for diabetic compared with control animals, but the quantity of bone formation was similar.

Olson et al (10) suggest that the duration of diabetes may be associated with implant failure and their study supports the concept of endosseous dental implant in the mandibular symphysis of type 2 diabetic patients as a predictable procedure.

Recently, the use of CBCT prior to dental implant therapy has contributed to an increasing attention on bone quantity and quality of the jaws.

CBCT has been used in dentistry since 1998 (11). The amount of radiation delivered by this technique is much lower than that used in CT (2 - 4), although the dose of radiation used is at least four times higher than the dose used during panoramic radiography.

Some studies have found that the variable quality of bone is more important to treatment outcome than the quantity of bone in the region of a dental implant. A corrected HU in CBCT data is useful for determining the bone density of ROI prior to implant placement, and this valuable information about bone quality enables better treatment planning by dental practitioners regarding implant positions.

This study clearly demonstrated that patients with diabetes who present poor metabolic control have a low bone density in the posterior region of the mandible.

Bone density of posterior region of the mandible is higher corresponding to premolars are, comparing to molars area. The knowledge of the bone density is very important, the positive outcome for osseointegration depending mainly on it. With this respect, conventional radiographs are not a useful tool.

The mandibular bone quantity and quality clearly reflects the physiological condition of the bone due to systemic diseases of bone degeneration, like diabetes mellitus.

CONCLUSIONS

In conclusion the use of CBCT in preoperative bone measurements will help provide the clinician with a prognostic indicator that not only provides valuable information about the quantity and quality of bone but helps to avoid placement of implants into the very poorest bone, where failure is more likely.

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NEWS

ORAL HEALTH STATUS IN HAEMODIALYSIS PATIENTS

A study realized by a group of indian researchers aimed to assess the oral and dental manifestations in non-diabetic and diabetic uremic patients who were undergoing haemodialysis. The study also aimed to estimate and to compare the salivary pH in the two mentioned groups. Were included ninety seven uremic patients who were undergoing maintenance haemodialysis. Subjective and objective findings were evaluated and recorded. Unstimulated whole salivary pH was recorded by using pH-measuring strips. Dental health assessment consisted of DMFT and CPITN indices. A subjective oral manifestation of dysgeusia was found to be more significant in non-diabetic patients ($p<0.008$). Statistically, a high significance was observed with mucosal petechiae in 31.9% patients of diabetic group .The overall DMFT score was significantly higher in diabetic group. A moderate significance was found with a CPI score of 5 ($p<0.015$). The pH of saliva was significantly higher among diabetic patients. The conclusions of the study showed that the diabetic subjects who were on haemodialysis were at a high risk for developing periodontal disease and they exhibited a potential threat for dental decay and xerostomia. A lower salivary pH and a poor glycaemic control may affect their oral health. (Swapna LA, Reddy RS, Ramesh T, Reddy RL, Vijayalaxmi N, Karmakar P, Pradeep K. Oral health status in haemodialysis patients. *J Clin Diagn Res.* 2013; 7 (9): 2047-2050).

Irina Grădinaru

CLINICAL AND EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF RHEUMATOID ARTHRITIS IN SOME DEMOGRAPHIC STRUCTURES

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CLINICAL AND EPIDEMIOLOGICAL STUDY ON THE PREVALENCE OF RHEUMATOID ARTHRITIS IN SOME DEMOGRAPHIC STRUCTURES (Abstract): The structure of the epidemiological process of rheumatoid arthritis (RA) is integrated in a broader framework of multiple interactions between intrinsic predisposing factors and the external environmental factors that may have a triggering or stimulating effect. The summative phenomenon is the result of the additive effect of risk factors, the individualized response of body structures and functions, depending on the genetic makeup explain the complexity of the epidemiologic study. **Aim**: To evaluate the epidemiological, clinical and biological features of a series of RA patients. **Material and Methods**: The study included 103 RA patients admitted to the Rheumatology Clinic of the Iasi Rehabilitation Hospital in the interval January, 2010 – December, 2012. **Results**: The analysis of epidemiological data provided information on pre-existing infections caused by the living environment conditions, diet, etc., that may cause damage at cellular and molecular level. **Conclusions**: The present clinical and epidemiological study describes the biological mechanisms and phenomena dependent on physical and social environment. These mechanisms favor and stimulate the occurrence, expansion and development of the disease at population level. **Keywords**: EPIDEMIOLOGY, CHRONIC ARTHROPATHY, INFLAMMATION, PREVALENCE.

Rheumatoid arthritis (RA) is the most common chronic arthropathy in the group of rheumatic diseases. RA has a multifactorial etiology and occurs mostly in women. There is as a pronounced progressive course in RA, which is characterized by exuberant destructive inflammation of the membrane lining the joint cavity, namely, the synovial membrane. The clinical features of RA include pain, joint stiffness, swelling, local heat, and relative functional impairment of the limb whose joints are damaged (3, 4, 5). The group of chronic rheumatic diseases includes RA, ankylosing spondylitis and also degenerative diseases, such as osteoarthritis of the hip, gonarthrosis, arthrosis of the foot, shoulder, elbow and hand; cervical, thoracic and lumbar spondylosis (6, 9, 10). Etiological and predisposing factors of the rheumatic process are found in the physical, biological and social environment, in the ecosys-

tem. The research in the complex field of chronic rheumatic diseases encompasses epidemiological population studies, complemented by immunological, pathophysiological, prevention and therapeutic studies that will provide new information on the risk factors, incidence, prevalence, etiopathogenesis and will facilitate the development of prevention, control and treatment programs with great medical, social and economic benefits (13, 14).

The prognosis depends on the early detection of the disease and on the promptness of therapeutic measures, which take into account the intrinsic factors specific to each patient.

This study aims to evaluate the epidemiological, clinical and biological features of RA in a group of patients receiving care for a chronic rheumatic disease.

MATERIAL AND METHODS

The study included 103 patients (75 females, 28 males) diagnosed with RA, with a mean age of 52.7 ± 12.02 years and average duration of disease of 9.10 ± 7.28 years.

The clinical and epidemiological study aimed to gather, process and analyze data on the prevalence, to evaluate the role of intrinsic and extrinsic factors, to assess the effectiveness of treatment programs, and the economic and social impact of the disease. Study methods included the statistical analysis of clinical and epidemiological data.

RESULTS

It is estimated that RA affects about 1% of the Romanian population and up to 3% of the population aged over 65, which shows that there are approximately 200,000 patients at national level.

Patients included in the study received care in the interval January, 2010 – December, 2012 in the Rheumatology Clinic of the Iasi Rehabilitation Hospital and were monitored and assessed according to a common protocol with multiple parameters.

The study group included 75 female patients (72.8%) and 28 male patients (27.2%). Female / male ratio was 75/28 or 2.67/1. Our findings are in agreement with literature data that show a higher frequency of RA in women, suggesting a possible involvement of endocrine factors in the pathogenesis of this disease (tab I).

TABLE I

Sex distribution in the study group

Sex	Patient no.	%
Male	28	72,8
Female	75	27,2
Total	103	100

Analysis of age distribution shows that RA is most frequent is males aged 50-55 years compared with 60-65 in females.

The mean age of patients included in the study group was 52.7 ± 12.02 years, range 26-77 years. The average duration of RA was 9.14 ± 7.28 years, as patients with recent onset of the disease and patients with a RA history of over 10 years were included in the study (tab II, III, fig 1, 2).

Histogram: AGE

Sex:1 AGE =28*5* normal (x,53.6071,11.5449)

Sex :2 AGE =75*5*normal (x52.3733, 12.2516)

The clinical picture showed the presence of myalgia (32%), muscle weakness (25.2%), muscle fatigue (11.7%), and muscle atrophy (2.9%).

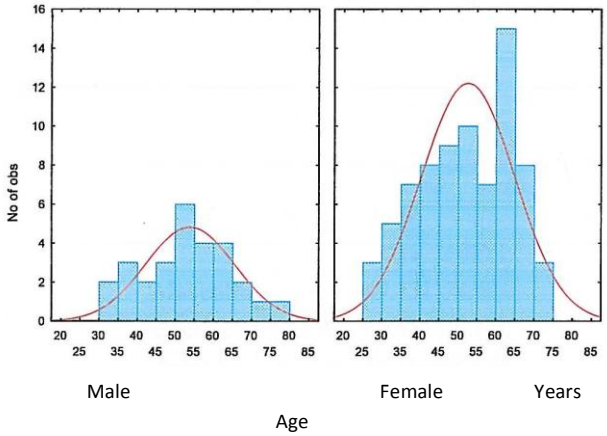

Fig. 1. Age and sex distribution in the study group

TABLE II

Mean age in the study group

SEX	MEAN	AVERAGE AGE – YEARS	
		-95%	+ 95%
MALE	53.607	49.131	58.084
FEMALE	52.373	49.554	55.192

TABLE III

Duration of RA in the study group

SEX	AVERAGE	AVERAGE AGE – YEARS	
		-95%	+ 95%
MALE	6.786	4.549	9.023
FEMALE	10.027	8.274	11.779

Histogram: DURATION Sex

Sex:1 DURATION =28*5* normal (x,6.7857,5.7694) :2 DURATION =75*5*normal (x10.0267, 7.6175)

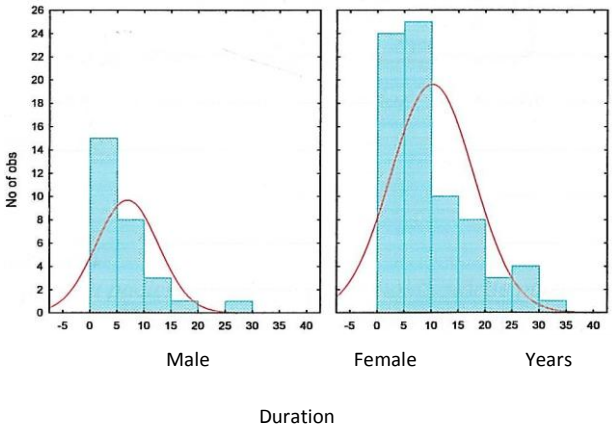

Fig. 2. RA duration in the study group correlated with sex distribution

DISCUSSION

The epidemiology of rheumatic conditions is highly diverse as a result of stimulating and varying climatic, social and economic factors. The disabling nature of the disease and the steady development of RA justify the need for a long clinical and epidemiologic follow-up and for comprehensive measures in order to ensure an optimal quality of life.

The area distribution indicates that the highest prevalence is in the urban area. The urbanization phenomenon seems to bring an additional exposure of the body to the action of rheumatogenic factors (6, 9).

The following occupational and workplace factors were evaluated: humidity, temperature fluctuations, physical exercise, sedentary attitudes, noise, etc. The relationship between RA incidence in different parts of Moldova and the geographic and climatic factors shows that although they may be considered non-specific risk factors, they negatively influence the body resistance to disease and promote rheumatogenic mechanisms. For instance, the onset of RA occurred in October and February in 67% of the total number of 103 RA patients. Environmental factors interact with a susceptible genotype and the damaging role of endogenous agents (collagen and IgG molecules) leads to the maintenance of the disease. The medical history taken at the workplace showed a non-uniform distribution of some environmental factors: cold (22.7%), humidity (23.9%), air flow (16.3%), and noise (13.3%) (fig. 3). The housing conditions favored exposure to environmental factors, such as, cold (36.9%), humidity (30.5%) and mold (13.6%) (fig. 4).

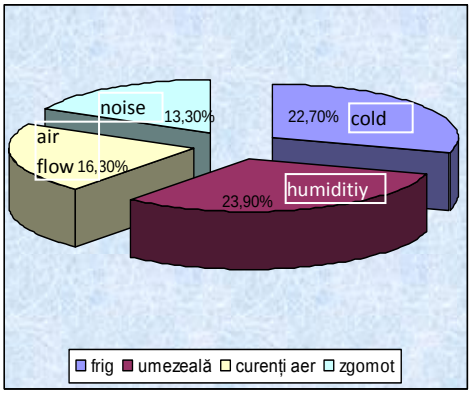

Fig. 3. Medical history showed a non-uniform distribution of some environmental factors

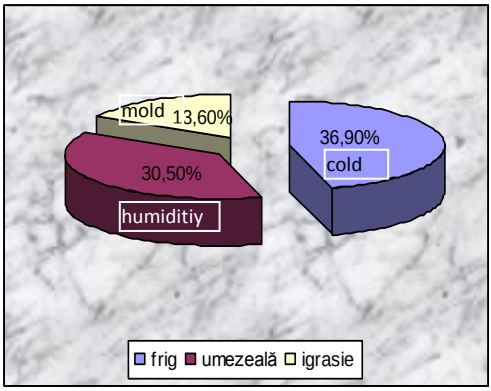

Fig. 4. Housing conditions favored exposure to environmental factors

Chronic joint infections or remote infections have caused RA and no particular infectious agent that could act as a trigger of pathologic processes of the joint was identified. Psychological stress as "a triggering factor" of RA was found in 29.37% of the total number of patients. The psychological stress may be caused by recent stressful events or by a special psychological state. The stress was due to family or employment problems in 23% of the total number of patients. The most common

diseases in the medical history of the patients were viral hepatitis (15.7%), streptococcal angina (24.4%), acute articular rheumatism (3.7%), genitourinary infections (25.2%), and lung disease (7.1%) episodes of acute cholecystitis (10.7%), biliary dyskinesia and gallstone disease (13.2%). Morbidity and mortality rate in RA patients showed a significant decrease following surveillance measures, due to the characteristics of the epidemiological process (9, 10, 15).

Our research has also revealed the insidious onset of RA, with articular manifestations due to the inflammation of the synovial membrane, which is characterized by swelling, pain, local heat, erythema, and limitation of movement.

The distal joints of upper and lower limbs are damaged in RA. Also reported were fatigue, weight loss, myalgia, prolonged morning stiffness, polyarthralgias for weeks or months until the articular manifestations occurred. The articular manifestations are the main feature of RA, namely the symmetry of the damaged joints (13, 14).

The process emerged during a variable period of time and was associated in our study as in the literature with attacks which increase joint pain to the point where disability occurs (3, 9, 11). The mesenchymal structures are painful and many extraarticular manifestations can be seen. The most common RA manifestation was pain of the damaged joints, independent of the intensity of inflammation, and morning stiffness for more than one hour was almost typical.

The damage to the articular structures (bones, ligaments, capsule) and muscle structures induced by the inflammatory immunological process affects to a great extent the functional and professional status of the individual. Complete diagnosis includes the determination of the stage of disease and assessment of deterioration in functional status and ability to work (3,9).

The main complaint of the patients in the study group was pain, which reduces the ability to work due to swelling, deformities and articular ankylosis especially in the upper limb. A proportion of 64.5% of the total number of patients showed symmetrical lesions at onset and they became symmetrical during the first year in 74.5% of RA patients.

Radiological examination findings differed from patient to patient, from osteoporosis to diffuse erosive and destructive phenomena.

Laboratory examinations assessed the inflammatory syndrome, dysimmunitary syndrome, synovial fluid. ESR values were correlated with other tests for inflammation and were a quick evaluation method of patient's state. ESR values ranged between 20 and 50 mm/h, but they have reached 100 mm / h during the inflammatory period or in fast developing forms of RA.

Normochromic or hypochromic anemia was present in most patients and it was characterized by the absence of response to iron therapy, but on the other hand by a favorable response to the use of NSAIDs and blood transfusion. Anemia of chronic inflammatory diseases is part of the syndrome of "hematological stress" induced by activation of macrophages and lymphocytes in response to inflammation. This activation is reflected by excessive iron sequestration in macrophages, increased erythrocyte destruction in the spleen, decreased activity of erythroid stem cells (16). Increased activity of IL-1 stimulates the synthesis of large amounts of apoferritin in the macrophages which sequester the

iron. Lactoferrin in the macrophages, which is synthesized in excess in RA, plays also an important role. Lactoferrin competes with apotransferrin and apoferritin, and it has a high affinity for iron that fixates and blocks iron in macrophages and so the iron becomes virtually unusable during erythropoiesis. Autoantibodies that were investigated are associated with an increased inflammatory process and increased circulating immune complexes (CICs). IgG is significantly increased in all patients and the correlation between the degree of anemia and the increased activity of IgG immune complexes was found in most of the patients (16).

The complex rehabilitation treatment administrated to RA patients was adapted to the clinical and functional stage of RA, psychological characteristics of the patient, social and economic status of the patient, and the results achieved following treatment. The choice of a therapy protocol was made based on tolerance to drugs, co morbid conditions, patient age, which was an important selection criterion because in older people there is a large number of coexisting disorders, acquired at a younger age or later, in old age.

TNF inhibitors (infliximab, etanercept, adalimumab) in monotherapy or in combination with MTX have proved to delay the onset of destruction and deformities in 24.25% of the total number of RA patients.

Patients on long-term glucocorticoid therapy at a dose equivalent to 5 mg prednisone/day or more received calcium and vitamin D. To prevent the decrease in bone mineral density, bisphosphonates (alendronate 70 mg / week), calcitonin (tonocalcin 100 IU / day, 1 vial for 2-3 days), vitamin D analogs (alpha D3 at a dose of 2 tablets / day) were also prescribed (9).

The therapy for degenerative diseases of the locomotor system in the elderly, such as gonarthrosis, cervical spondylosis, coxarthrosis, senile kyphosis, included antiarthritic drugs, namely analgesics and anti-inflammatory drugs. The symptoms were alleviated on a long-term basis by using condroprotective agents.

Kinesiotherapy exercises aimed to preserve or achieve joint suppleness and amplitude, toning of the damaged muscles and to restore the functional synergism, the integration of the joint into the activity of the segment, regional coordination of motility that may lead to social and occupational reintegration (11, 13).

CONCLUSIONS

Our clinical and epidemiological data aimed to identify in a certain demographic sample underlying infectious pathological states typical to RA caused by the living environmental conditions, diet, etc., which may induce disturbances at cellular and molecular level.

RA is a chronic, progressive disease, which in the absence of proper treatment leads to severe joint damage, impaired functional status, physical disabilities and associated visceral lesions that may by life-threatening.

Our study showed the importance of knowing the structural elements of the epidemiological process of RA for the planning of prevention programs to reduce the incidence and prevalence of relapses.

Kinesiotherapy exercises managed to improve physical and mental health of the patients with RA of different stages and severity, achieving a better social and occupational integration or reintegration of these patients by means of a therapeutic educational and rehabilitation process.

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NEWS

CYSTATIN C VERSUS CREATININE TO ASSESS THE RISK OF CHRONIC KIDNEY DISEASE

Estimated glomerular filtration rate based on creatinine level was mostly used for staging of chronic kidney disease. Lately, Cystatin C assessment began to be tested as an alternative marker of glomerular filtration function, the advantage being that it not depends on the muscle mass of the patient. We selected a meta-analysis that evaluated 11 studies population (90 750 participants) and 5 cohort studies in patients with chronic kidney disease (2960 participants), all participants benefit from dosing creatinine and cystatin C. Renal function parameters were correlated with the rate deaths (13202 cases in 15 cohorts), cardiovascular deaths (3471 cases in 12 cohorts) and end-stage renal disease (1654 cases in 7 cohorts). The results of the meta-analysis concluded that the introduction of cystatin C values in the formulas for calculating glomerular filtration rate allowed better prediction of risk of death and cardiovascular complications. Thus, the glomerular filtration rate threshold at which the risk of death increases significantly became 85 ml/min/1,73m^2, compared to the known value of 60 ml/min/1,73m^2 body surface area. (Shlipak MG, Matsushita K, Ärnlöv J, et al., Cystatin C versus Creatinine in determining risk based on kidney function, N Engl J Med 2013; 369: 932-943)

Corina Dima-Cozma

Rad52 PROTEIN FUNCTION STUDY BY FLUORESCENCE TAGGING

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Rad52 PROTEIN FUNCTION STUDY BY FLUORESCENCE TAGGING (Abstract): The Rad52 protein plays a significant role in DNA lesions repair by homologous recombination in eukariotic cells. Human Rad52 function somewhat overlaps with BRCA2 and has a role in cell survival in the absence of BRCA1-BRCA2 mediated recombination. Additional Rad52 function analysis and intracellular localization studies are probably necessary. We present a method for Rad22 protein tagging, a *Schizosaccharomycespombe*Rad52 homologue,byCrerecombinase-mediated cassette exchange (RMCE) using the versatile pAW8 plasmid. Rad22 protein was C-termini yEGFP tagged; the resulting strain was analyzed by fluorescence microscopy. The yEGFP signal was observed (Rad22 foci) for 7.5µM camptothecin, 0.005%methyl methanesulfonate, and 4 mMhydroxyurea treated cells. The RMCE method was efficient, and the presence of tagged Rad22 protein was confirmed by Western-Blot and fluorescence microscopy. **Keywords:** RAD52,EGFP,HOMOLOGOUS RECOMBINATION, CASSETTE EXCHANGE.

Rad52 protein plays a central role for DNA lesion repair by homologous recombination (HR) and single strand annealing (SSA). Yeast experiments have proved its role as a recombination mediator and an annealing facilitator (1); however it may be less important for recombination in humans – the breast cancer type 2 susceptibility protein (BRCA2) protein and some RAD51 paralogs may have a similar function (2-3). BRCA2 plays a recombination mediator role by promoting the formation of the RAD51 nucleofilament; it does not retain the single strand annealing function described for Brh2 (*Ustilagomaydis*) and Rad22 (*S. pombe*) in case of replication protein A (RPA) coated DNA strands (4). Therefore RAD52 seems to play an additional role to BRCA2 – double mutants are lethal (5). On the other hand, BRCA2 has been proved to be involved in early lesion repair, while Rad52 seems to have a late intervention involving a blocked or stalled replicating fork. These data suggest human Rad22 involvement in single strand annealing repair (6).

Recent studies showed Rad52 to play a role in cell survival in the absence of BRCA1-BRCA2-mediated homologous recombination, thus Rad22 may be a thera-

peutic target for BRCA deficient cancers (7). Rad52 function studies required fluorescent tagging - a technique enabling microscopical visualization - and mutant's development for the protein domains study.

This paper presents the method for Schizosaccharomycespombe Rad22 (human Rad52 ortolog) carboxi-terminal tagging with enhanced green fluorescent protein (yEGFP) usingCre-mediated cassette exchange. The exchange involves a chromosomal cassette containing a selection marker and the *rad22* gene flanked by two sequences loxP/loxM3 and a cassette (*rad22*-yEGFP) between lox sites from a Cre expressing pAW8 plasmid (8).

MATERIAL AND METHODS

For Rad22 C-terminal tagging we cloned the *rad22* gene without STOP codon from a wild type *S. pombe* as a SphI/SpeI fragment. The fragment obtained was introduced in pAW8_rad22 plasmid by digestion and ligation. A linker-yEGFP-loxM3 was subcloned as a SpeI/BamHI fragment from pAW8E*Nde*I-cyEGFP plasmid (GenBank: JF340116.1) in the new plasmid. Finally, we obtained pAW8_CyEGFP_ rad22 and CyEGFP-rad22 strain as well.

The cloning of rad22 gene without STOP codon as a SphI/SpeI fragment. We cloned rad22 as a SphI/SpeI; we amplified rad22 from a wild type strain with primers rad22_F aaaa*gcatgc*TAATTACGAAAATCCTTTAATTCAAATAAAATTTTGTTTT and rad22_NOSTOP_R aaaa*actagt*TCCTTTTTTGGCTTTCTTATCCA. The PCR reaction contained 10 ng DNA template, 1 µM primers, 0.2 mMdNTPs, 10x KOD Polymerase buffer, 3 µl MgSO4, and 1 µl KOD HotStart DNA (Novagen). The total volume was 50 µl. The PCR program: 94°C for 3 minutes followed by 30 cycles of 94°C 30 seconds, 55°C 20 seconds, 72°C 60 seconds. The PCR product was run in an agarose gel 1% and the gel purified (Machary Nagel kit). The digested fragment was purified and cloned inpAW8_rad22 plasmid. The new pAW8_rad22_NOSTOP plasmid was checked by sequencing.

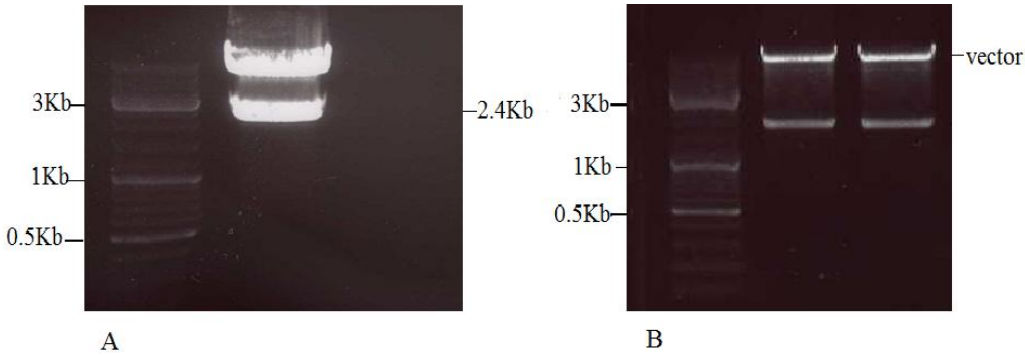

Fig. 1. A. The running of pAW8_NdeI_cyEGFP plamid after digestion with SpeI, BamHI restriction enzymes in order to obtain the insert (linker-yEGFP) for ligation. First line - DNA ladder.Second line - digested DNA.

The construction of pAW8_CyEGFP_ rad22 plasmid. In order to tag *rad22* gene with yEGFP at C-terminus end we have obtained linker-yEGFP-loxM3 as a SpeI/ BamHI fragment from pAW8_NdeI_ cyEGFP (Accession number P30546) (fig. 1A).

A SpeI/BamHI fragment of pAW8_ rad22NOSTOP plasmid (fig. 1B) was changed by the SpeI/BamHI fragment from pAW8_NdeI_cyEGFP. The restriction digestion with SpeI/BamHI enzymes was carried out according to the manufacturer's conditions (New England Biolabs).

B. The running of pAW8_rad22NON-STOP plasmid after digestion with SpeI, BamHI. First line - DNA ladder.Ssecond and third lines - digested DNA.

The vector and linker-yEGFP insert were ligated in a 1:2 ratio using T4 DNA Ligase (New England Biolabs). The resulted plasmid termed pAW8_CyEGFP_ rad22 was transformed into competent *E. coli* DH5alpha cells and the construct confirmed by sequencing (GATC Biotech-Germany).

***S. pombe* transformation and cassette exchange**. The rad22 base strain (loxP-*rad22-ura4*+-loxM3 *ade6-704 leu1-32 ura4-18h*-) was transformed with pAW8_ CyEGFP_rad22 using the lithium acetate procedure as described by Bähler*et al* (1998) (9). The protocol used after transformation was according to Watson (2008) (8).

Western Blot analysis. Protein extraction was performed according to Moreno (1991) (10). After lysis in a Ribolyser (Hybaid), cells were resuspended in sodium dodecyl sulfate (SDS) sample buffer. Proteins were separated in polyacrylamide (10%) by electrophoresis. Western blotting was carried out and the blots were treated with anti-GFP mouse monoclonal antibodies (1/4000) and rabbit anti-mouse IgG-peroxidase antibody (1/5000) (Sigma-Aldrich, USA). The Western-Blot was performed as described by Tanaka (2001) (11).

Visualization of yEGFP signal in Cy-EGFP-rad22 strain. We treated CyEGFP-rad22 cells with 7.5 μM camptothecin (CPT), 0.005%methyl methanesulfonate (MMS) and 4 mMhydroxyurea (HU) for 4 hours. Microscopy was performed using the Delta Vision microscope system (Applied Precision, Issaquah, WA). For fixed cells, 10 Z-axis sections at 0.4μm intervals were combined using the softWoRx quick projection program. Cells were collected by centrifugation and suspended in 100 μl of 1 μg/ml Hoechst 33342 and incubated for 5 min at room temperature in the dark. After incubation, 1 ml methanol was added and the tubes incubated on ice for 10 minutes. The cells were pelleted and washed with 1 ml of acetone and pelleted again. 10 μl of the pellet was spotted onto 0.6% low melting agarose. Each set of images for EGFP and Hoechst 33342 signals were obtained at 25–26°C.

RESULTS

In this paper we have tagged Rad22 protein with yEGFP in a *S. pombe* strain using a Cre-mediated cassette exchange protocol for gene replacement. In a "base strain" - Nt-loxP-rad22-ura4-loxM3 -Ct ade6-704 leu1-32 ura4 D-18 a cassette was inserted instead of the *rad22* gene; this cassette consists of *S. pombe*gene sequence and *ura4*$^+$ selectable marker flanked by a loxP site at one end and by a modified heterospecific lox site (loxM3) at the other. Having the cassette at the chromosomal locus in *S. pombe*, the exchange by homologous recombination was achieved by transformation with a plasmid containing the yEGFP tag with *rad22* gene sequence between loxP/loxM3, Crerecombinase gene and a selection marker (*Saccharomyces cerevisiae* LEU1 gene). Recombinants were selected by uracil prototrophy using the reagent 5-fluoroorotic acid (5-FOA) (8).

The new strain was checked using *rad22* gene frame and EGFP downstream primers 5'-3'(rad22_CHK3-AGCTAAACCAGTGCAGAGAAGC, rad22_M_CHKR-GGCGGAAAGAGGATCAAGGT, rad22_-CHK1-GTCTTGGCTTGAGCGTGATTG and rad22_M_CHKR-GGCGGAAAGAGGATCAAGGT). We obtained the right length of PCR fragments (expected length was 1.6 Kb for rad22_CHK3/ rad22_M_CHKR and 2.2 Kb for rad22_CHK1/rad22_M_CHKR primers) from CyEGFP-rad22 strain (fig. 2A).

These fragments cloned from CyEGFP-rad22 strain were sequenced (GATC Biotech-Germany). The results showed the yEGFP sequence in frame with rad22 gene separated by a linker 5'-ACTGGTTCTACAGGATCAACCGGCTCCACGGGGTCG-3' that is translated in a T G S T G S T G S T G S peptide (T - threonine, G - glycine, S - serine) assuring the flexibility of the yEGFP and Rad22 in the new hybrid protein.

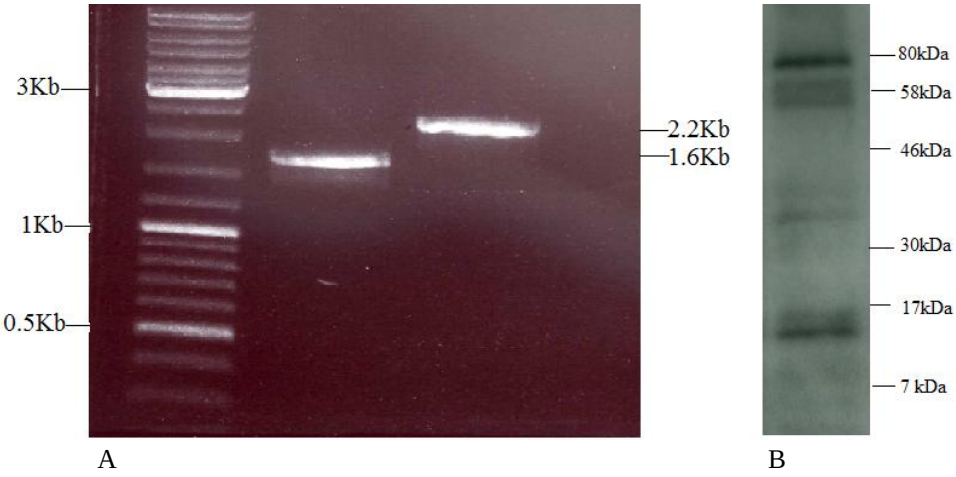

Fig. 2. A. The checking of CyEGFP-rad22 strain by PCR with primers downstream of EGFP sequence and from the gene *rad22*. Line I - DNA ladder, line II - the amplicon obtained with rad22_CHK3 and rad22_M_CHKR primers, line III - the amplicon obtained with rad22_CHK1 and rad22_M_CHKR primers. B. Western Blot of CyEGFP-rad22 *S. pombe* with anti-GFP antibodies.

The Western Blot analysis of CyEGFP-rad22 with primary antibodies (monoclonal anti-GFP from mouse) and secondary antibodies (rabbit IgG anti-mouse – peroxidase**)** revealeda 78.9 kDa protein (the first band) corresponding to the molecular weightof Rad22 and yEGFP (52 kDa + 26.9 kDa) (fig. 2B).

Having the correct sequence of the construct (*rad22-linker-yEGFP*), CyEGFP-rad22 cells were visualized by epifluorescence microscopy. The yEGFP signal was strong allowing us to localize Rad22 protein inside the cell. We could not observe nuclear foci in uninduced cells but distinct yEGFP-Rad22 foci were seen after 4 hours of CPT, MMS and HU exposure (most cells have more than 2 foci) (fig. 3 A,B,C,D).

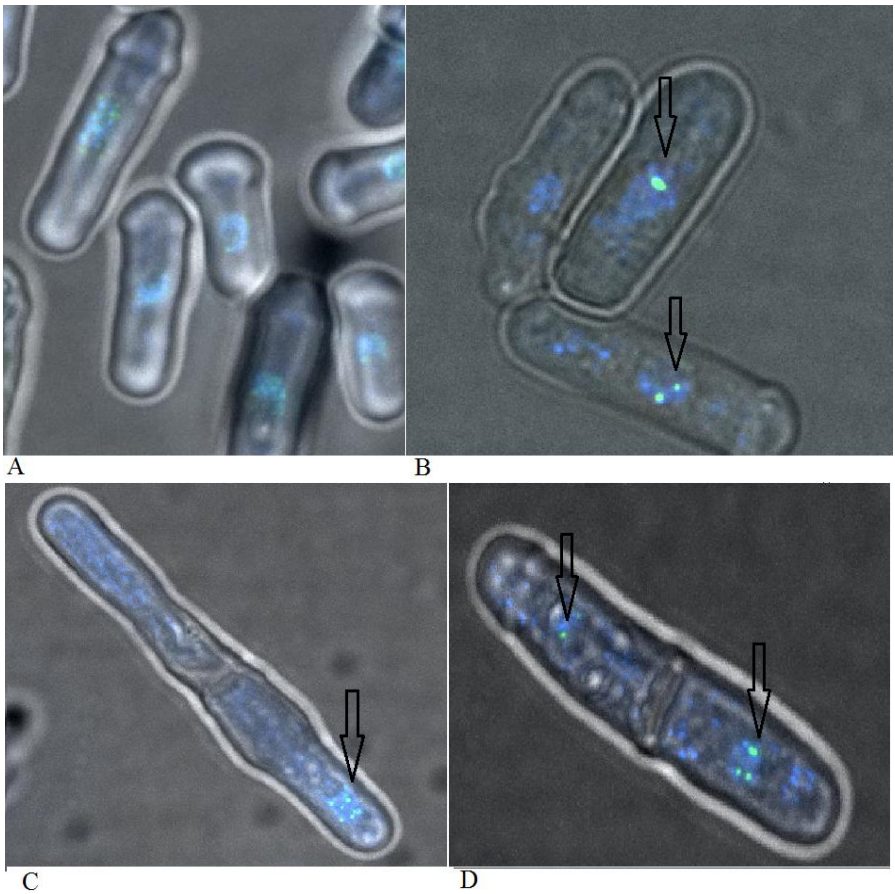

Fig. 3. The yEGFP-rad22 signal of exponentially growing CyEGFP-Rad22 cells observed using a Delta Vision microscope system attached to an inverted Olympus IX70 microscope. Visualization of yEGFP tagged Rad22 in asynchronous, untreated cells (A) and in cells treated 4 hours with 7.5 μM CPT (B), 0.005% MMS (C) and 4 mM HU (D).

DISCUSSION

After obtaining CyEGFP-rad22 strain we treated cells with camptothecin, methyl methanesulphonate and hydroxyurea that affect nucleic acids and alter their function. MMS is an alkylating agent interfering with the DNA replication by modifying the bases of the DNA, HU inhibits the ribonucleotidereductase enzyme reducing the production of deoxyribonucleotides, and comptothecin inhibits the DNA enzyme topoisomerase I. Rad22 protein is involved in DNA repair after genotoxins exposure. We observed CyEGFP-Rad22 nuclear foci after 4 hours of genotoxic (CPT, MMS, HU) exposure reflecting Rad22 centers actively engaged in repair.

CONCLUSIONS

Cre-mediated cassette exchange protocol has proved an efficient and rapid protocol for EGFP Rad22 C-terminal tagging as far as the new hybrid Rad22-EGFP protein retains the repair ability in genotoxins ex-

posed cells. The new strain must be further analyzed comparing it with a wild type strain by other complex methods.

ACKNOWLEDGEMENTS

We would like to thank Prof. A.M. Carr and Dr. A. Watson from Sussex University - Genome Damage and Stability Center for technical support. The first author is the beneficiary of the Financing Contract POSDRU/88/1.5/S/58965 in the project "Doctoral Scholarships for increasing competitiveness in the medical and pharmaceutical field".

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SURGICAL REVASCULARIZATION IN CHRONIC MESENTERIC ISCHEMIA

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SURGICAL REVASCULARIZATION IN CHRONIC MESENTERIC ISCHEMIA. CASE REPORTS (Abstract): Chronic mesenteric ischemia (CMI) is a disease causing death either by starvation or enteromesenteric infarction. Diagnosis is often delayed before the patient is referred to a vascular surgery unit. Atherosclerosis is the main cause of CMI. Arteriography is essential in diagnosing CMI and delineating the atherosclerotic lesions. The revascularization procedure consists in an aortomesenteric bypass reconstructing 1-3 visceral arteries. This paper presents two consecutive CMI cases treated at the Vascular Surgery Unit of the Iaşi "Sf. Spiridon" Hospital during 2010. Both patients had symptoms suggestive of mesenteric and aortoiliac diseases. CT angiography revealed specific lesions both for aortoiliac disease and stenotic or occlusive lesions in the celiac trunk and mesenteric arteries. Both cases benefited from aortobifemural bypass surgery using a synthetic graft associated with aortic-superior mesenteric artery bypass with reversed vein graft (in the first case both mesenteric arteries were revascularized). Immediate and remote results were favorable, with remission of intestinal symptoms and weight gain. Bypass patency was followed-up by CT angiography and Doppler ultrasound. CMI is a diagnostic and therapeutic challenge. Open surgery provides symptom remission in 90% of cases. Permeability at 5 years is 80-90% for open surgery, higher than by endovascular therapy. Average permeability of the two types of intervention is 70% at 5 years, similar to the infraaortic bypasses. **Keywords:** MESENTERIC BYPASS, CHRONIC MESENTERIC ISCHEMIA, AORTOBIFEMORAL BY PASS

Chronic mesenteric ischemia (CMI) is a disease causing death either by starvation or entero-mesenteric infarction. The diagnosis is often delayed prior to patient referral to a vascular surgeon.

The pathophysiological mechanism underlying CMI is the failure to achieve normal postprandial mesenteric hyperemia.

Hemodynamicaly, significant arterial stenosis causes attenuated postprandial response leading to imbalance between required and available O_2 and metabolites causing postprandial pain (mesenteric angina).

Atherosclerosis is the main cause of visceral artery occlusive disease and consequently the cause of CMI. Other occasional causes are: fibromuscular disease, aortic dissection, neurofibromatosis, rheumatoid arthritis, Takayasu disease, postradiotherapy lesions, Burgers Disease, systemic lupus erythematosus and drug use (cocaine, ergot) (1).

The abdominal digestive tract is supplied by three major arterial axes (celiac trunk, superior and inferior mesenteric arteries) interconnected by an extensive collateral network. Usually, a significant occlusion of 2 or 3 visceral vessel is required for the patient to become symptomatic. But isolated occlusion of the celiac trunk or superior mesenteric artery (SMA) alone can give rise to symptoms when the collateral network is inefficient (1).

There are 3 stages of CMI: chronic intermittent ischemia, permanent ischemia and enteromesenteric infarction (2).

The patient complaints are: intestinal angina (30-60 min postprandial), weight loss caused by food phobia (fear of eating), altered bowel function, bloating, or vague abdominal discomfort, diarrhea or constipation (2).

Patients with CMI frequently have concomitant renal or aortic artery occlusive disease.

Arteriography is essential for confirming the diagnosis and delineating the lesions topography (3).

Late studies are controversial with respect to: the type of surgery (open surgery *vs.* endovascular surgery), endarterectomy *vs.* antegrade/retrograde bypass, number of bypassed vessels (1-2-3), the best type of graft (synthetic *vs.* venous - internal saphenous vein / superficial femoral vein) (1).

Studies in the United States revealed that mesenteric bypass represents less than 0.5% of all peripheral vascular bypasses (1).

CASE REPORTS

This paper presents two consecutive cases of CMI treated at the Iaşi, Vascular Surgery Unit, during 2010.

CASE 1

G.A., 51-year-old male presented with the following complaints: postprandial abdominal pain, significant weight loss, frequent loose stools, loss of appetite, significant fatigue, intermittent claudication and decreased walking perimeter (< 50m).

Laboratory tests revealed hypoproteinemia (total proteins = 56 g/dl) and hypoalbuminemia (albumin = 2.53 g/dl).

Preoperatively CT angiography confirmed the suspicion of CMI revealing severe celiac trunk stenosis of about 12 mm from the origin and atherothrombotic occlusion in the initial segment of SMA of about 20mm, occlusion of the left internal iliac artery and its branches, and also the atheromatosis of the abdominal aorta, iliac arteries and abdominal aorta branches (fig. 1).

Fig. 1. Preoperative CT angiography - Patient G.A

Pre-surgical screening was performed and anticoagulant, vasodilator and hypolipemiant treatment was administered.

The surgical intervention consisted in a deep aortic bifemoral bypass with Dacron graft associated with an aortic–inferior mesenteric artery (IMA) and aortic- superior mesenteric artery (SMA) bypass with

autologous venous graft.

Through median laparatomy we entered the left mesenteric–colic space. The abdominal aorta was exposed by dissecting the peritoneum from the ligament of Treitz to the IMA. The left renal vein was identified as a landmark for locating the renal artery and SMA (fig. 2).

The next step was the i.v. administration of 5000 U Heparin. After 5 minutes the infrarenal aorta was clamped proximally and distally. An aorto-bifemoral bypass was performed using a Dacron graft. All anastomoses (syntetic graft to aorta and synthetic graft branches to femoral arteries) were termino-lateral (fig. 3).

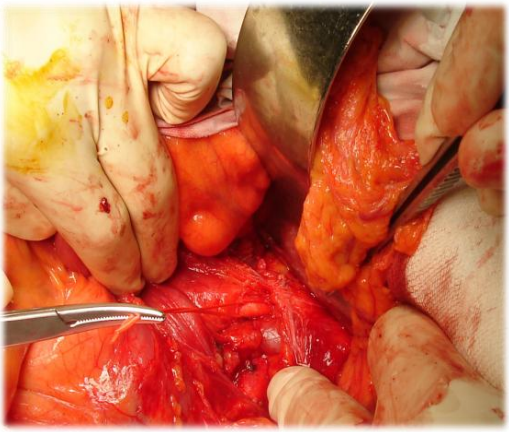

Fig. 2. SMA with vessel loop

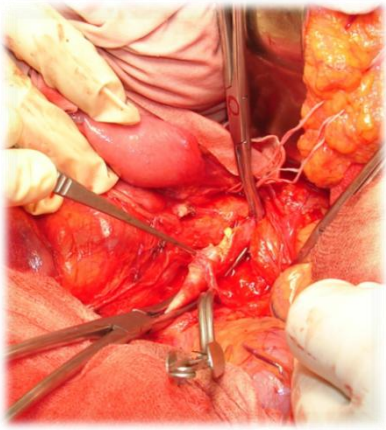

Fig. 3. Clamped aorta. Longitudinal Aortotomy. Significant Atherosclerosis

The next step was to perform the aortic – IMA and SMA bypass with reversed autologous vein graft previously harvested (fig. 4, 5).

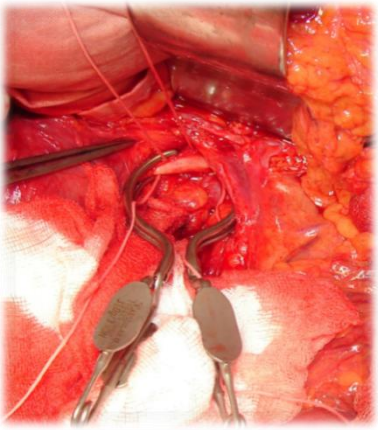

Fig. 4. Clamped AMS

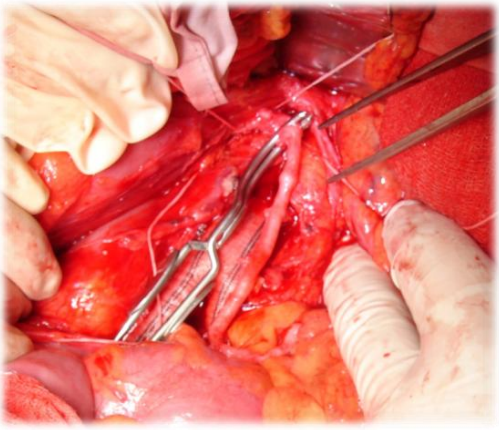

Fig. 5. Proximal T-L aortic-AMS anastomosis

We aimed to adjust the length of the graft to allow its positioning in relaxed “C" position (fig. 6, 7).

The omentum was brought in the aortomesenteric space by coloepiploic detachment. This way the omentum was used as an anchor fringe (fig. 8).

After surgery the patient received the three "A" therapy (antibiotic, anticoagulant, analgesic) associated with vasodilators and hypolipemiant agents.

Immediate and remote results were favorable: warm skin, bilateral popliteal pulse, bilateral Doppler signal along the anterior tibial artery, remission of the abdominal pain, oral feeding after gas transit was resumed, and bowel transit.

A control CT angiography was performed 2 weeks after surgery (fig. 9). It showed that the aortobifemoral and aortomesenteric bypasses were patent. The echo-Doppler follow-up at 1, 2, 3, 6 and 12 months revealed persistent graft patency (fig. 10, 11).

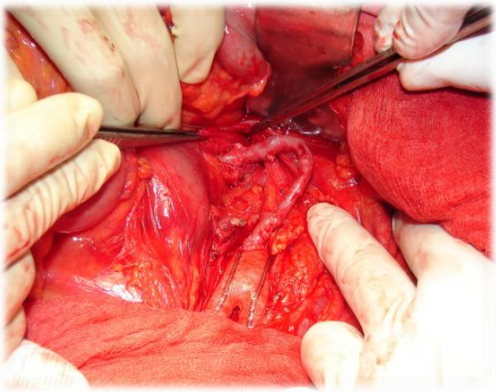

Fig. 6. Aortic-SMA bypass (“relaxed C”)

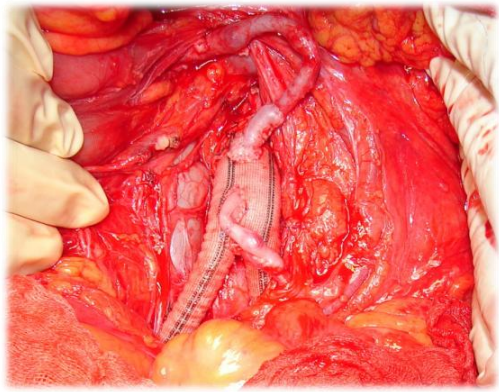

Fig. 7. Aortic-SMA and aortic-IMA bypass

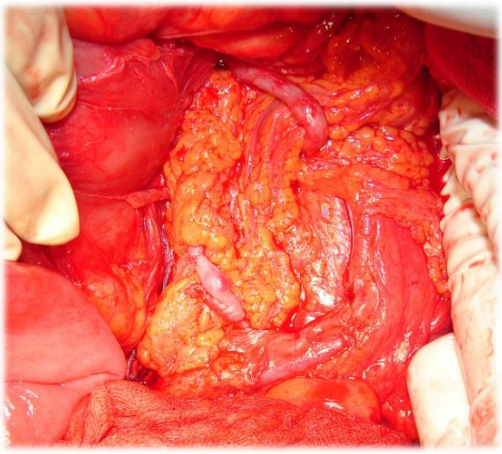

Fig. 8. Epiploic anchor fringe

Fig. 9. Postoperative CT angiography

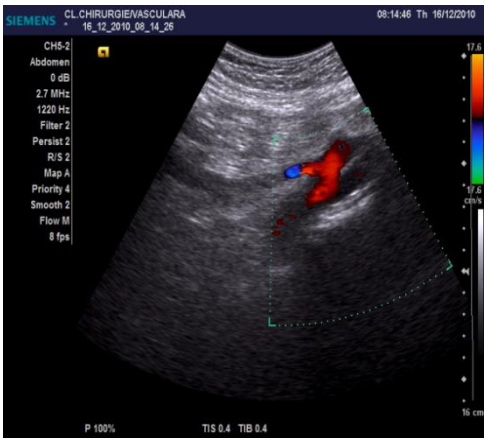

Fig. 10. Color echo-Doppler graft patency

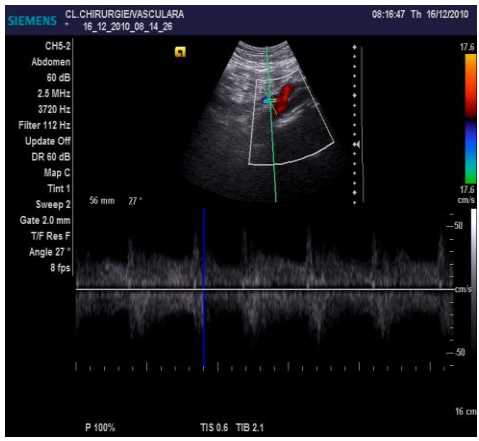

Fig. 11. Superior mesenteric graft blood flow signal

CASE 2

C.G., 60-year-old, male was admitted to the Vascular Surgery Unit complaining of intermittent claudication of both lower limbs (more intense in the left limb), decreased walking perimeter (<50 m) and significant weight loss.

Laboratory findings revealed the absence of the inflammatory syndrome and presence of hypoproteinemia. Doppler signal was detected from the peripheral arteries of the lower limbs.

Preoperative CT angiography revealed abdominal aorta with multiple calcified atherosclerosis plaques and parietal thrombosis, iliac artery with multiple stenosis, thrombosis of the origin of celiac trunk bifurcation (the remaining vessels being patent) and significant SMA stenosis from the origin (fig. 12).

Fig. 12. Preoperative CT angiography. Patient C.G

Fig. 13. Postoperative CT angiography Patient C.G

The surgical intervention consisted in an aortobifemoral bypass with Dacron graft associated with an aortic – SMA bypass with reversed vein graft (right internal saphenous vein) (fig. 13, 14).

The surgical technique was similar to that used in the first case except that only one mesenteric artery was revascularized (fig. 15).

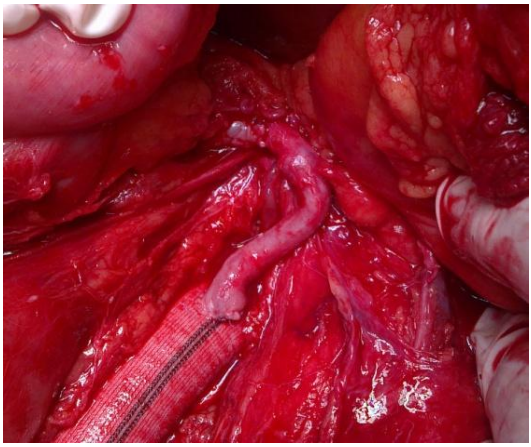

Fig. 14. Aortic-SMA by pass ("relaxed C").

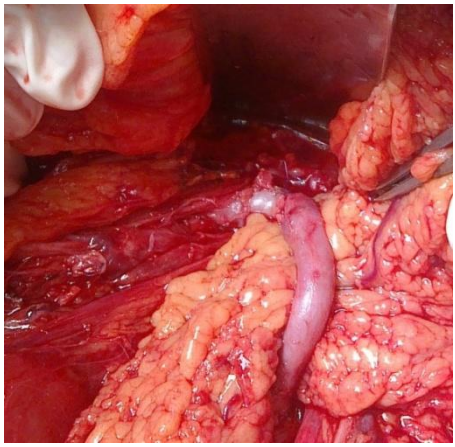

Fig. 15. Epiploic anchor fringe

Immediate and remote results were favorable: presence of pulse in the lower limb peripheral arteries and resumption of physiologic bowel transit.

This case also benefited from CT angiographic control along with Doppler ultrasound follow-up at 1, 2, 3, 6 and 12 months that revealed the patency of the bypass.

DISCUSSION

The diagnosis and treatment of CMI is challenging. Revascularization of the mesenteric artery alone is difficult without replacing the aorta with a synthetic graft and proximal anastomosis rather impossible. Longitudinal aortotomy reveals atherosclerotic material with high probability of embolism.

Present standards are based on the study by Kruger et al. (4) - 12% perioperative morbidity and 2.5% perioperative mortality. Higher values were observed for the simultaneous reconstruction of the aorta, complete revascularization (three branches) and preoperative renal failure.

The remission of symptoms along with weight gain is achieved in 90%-100% of cases (5). The 5-year patency is 80-90% for open surgery (1, 4, 6). Permeability is higher with open surgery than endovascular therapy, with an average for both of 70%, similar to that of bypasses for peripheral revascularization (1, 6).

CONCLUSIONS

Both our cases were successfully treated, no complications being recorded in the immediate postsurgical period and after a 2-year follow-up. The two patients presented for symptoms of aortoiliac disease and CMI, respectively. With appropriate surgery both problems were solved and the patients were reinserted into their families and society.

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NEWS

TREATMENT OF PERI-IMPLANTITIS USING MULTIPLE APPLICATIONS OF CHLORHEXIDINE CHIPS: A DOUBLE-BLIND, RANDOMIZED MULTI-CENTRE CLINICAL TRIAL

Universal strategies for managing peri-implantitis are yet to be adopted. The aim of this study is to examine a protocol of intensive application of chlorhexidine containing chips in sites with peri-implantitis. This multi-centre, randomized, double-blind, parallel, two-arm clinical trial included 60 patients (77 implants) with probing depth (PD) 6-10 mm and bone loss ≥2 mm around 1-2 implants. One to two weeks following scaling and root planing, baseline measurements were made followed by implants' debridement. Patients were randomized to receive matrix chips (MatrixC) or chlorhexidine Chips (PerioC). Measurements and chips placement were repeated at weeks 2, 4, 6, 8, 12 and 18. At 6 months, patients returned for final examination. Probing depth reduction was greater in the PerioC (2.19 ± 0.24 mm) compared with MatrixC (1.59 ± 0.23 mm), p = 0.07. Seventy percentage of the implants in the PerioC and 54% in the MatrixC had PD reduction ≥ 2 mm. Likewise, 40% of the sites (PerioC) and 24% (MatrixC) had PD reduction ≥ 3 mm. Clinical attachment level gains for both groups were significant; however, the changes in the PerioC group were significantly greater than in MatrixC [2.21 ± 0.23 mm. and 1.56 ± 0.25 mm respectively, p = 0.05]. Bleeding on probing was reduced by half in both groups. These results suggest that frequent placement of PerioC and MatrixC together with implants debridement resulted in a substantial improvement in sites with peri-implantitis. Further studies will be required to fully appreciate the mechanism of this treatment (Machtei EE, Frankenthal S, Levi G, Elimelech R, Shoshani E, Rosenfeld O, Tagger-Green N, Shlomi B. Treatment of peri-implantitis using multiple applications of chlorhexidine chips: a double-blind, randomized multi-centre clinical trial. J Clin Periodontol 2012; 39(12): 1198-205).

Gianina Iovan

THE RISK OF LYMPHEDEMA AFTER BREAST CANCER SURGICAL TREATMENT

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THE RISK OF LYMPHEDEMA AFTER BREAST CANCER SURGICAL TREATMENT (Abstract): The **aim** of this study is to emphasize the importance of knowing the predisposing factors of the occurrence of homolateral upper limb lymphedema after breast cancer surgery. **Material and methods:** The study included 1104 patients with breast cancer, who were hospitalized in the IIIrd Surgical Clinic, Iasi, between 2000 and 2010, for surgical treatment followed by oncological adjuvant therapy. The surgical intervention was conservative in 228 cases and modified radical mastectomy – Madden type - in 876 patients. Periodic clinical follow-ups were done every 3 months during the first postoperative year, every 6 months during the second year and annually thereafter. **Results:** Early lymphedema occurring in the first 14 postoperative days or between day 14 and day 21 was found in 8 patients. Late lymphedema, occurring up to 12 months or more after surgery, was diagnosed in 41 patients. Medium and severe lymphedema occurred at 42 patients. We evaluated the preexisting risk factors, the risk factors related to the type of surgery and those related to the cancer staging. **Conclusions:** It's ideal to identify predisposing factors of developing lymphedema related to breast cancer surgery before applying any type of treatment. There are therapeutic methods (general, drug therapy, physiotherapy) and methods related to the surgical act that influences the prophylaxis of lymphedema or have an amazing effect on already occurred lymphedema. **Keywords:** UPPER LIMB LYMPHEDEMA, BREAST CANCER, AXILLAR ADENOPATHY

Multiple statistics consider that lymphedema secondary to surgical treatment of breast cancer has an incidence ranging from 3 to 87% (1). This difference between statistics can be explained by different criteria, the presence of some difficulties in the measurement methodology, the duration of the follow-up of the patients and, not least, through a series of individual particularities of the patients. The risk of developing lymphedema in women treated for breast cancer in The United States of America is expected in more than 2.5 million patients (2). Lymphedema may also occur after conservative surgery of the breast cancer associated with the biopsy of the sentinel lymph node, the percentage reaching up to 23% (3), but with an average of 6%, as 15 studies on 4241 patients show (4,5). The occurrence of lymphedema has multiple consequences: on the upper limb functional status and breast status (for

lymphedema occurring after conservative surgery), the quality of life, the work integration, the relationship with family members (related to lifestyle changes, the self perception in terms of personal independence, the patient considering that she has an invalidity in what her aesthetic appearance is concerned) the risk of erysipelas , sometimes with a relapsing character and not least with important financial consequences (long term medication and therapeutic methods, often expensive). Of course, the studies have a follow-up period ranging from 3 months to 20 years, without a basic value (9). The aim of this study is to find, at a lot of patients treated for breast cancer, the risk factors of the occurrence and progression of lymphedema. Another objective is to analyze the value of preventive methods for upper limb lymphedema in relation to the surgical treatment sequence for breast cancer.

MATERIAL AND METHODS

The study included 1104 patients with breast cancer aged between 26 and 93 years who were hospitalized and operated by the same surgical team between 2000 and 2010. 178 patients were in stage I and II and 926 patients in stage III and IV. The surgical treatment was conservative - quadranectomy with axillar lymphadenectomy in 228 patients and modified radical mastectomy (Madden operation) was performed on 876 patients. All the patients were treated with fractionated heparin and micronized flavonoids (Detralex) or procianidolic oligomers, flavan derivatives (Endotelon), starting with the first day after the surgery for a period of 7 to 21 days. All patients had elastic compression of the chest and arm for 14 postoperative days. The patients began the adjuvant chemotherapy between for 21-30 days after surgery and the radiotherapy –when indicated - up to thirty days after the last infusion of chemotherapy. Our study only took into consideration the lymphedemas from the IInd and IIIrd degree (42 patients) according to the International Society of Lymphology (9).

All the patients were supervised by the same surgical team; the clinical periodic evaluations were performed every 3 months during the first year after surgery, every 6 months during the second year and annually thereafter. Every 6 months we performed an ultrasound examination (bilateral axillary region, bilateral supraclavicular region, bilateral breast region in case of conservative surgery and breast and pectoral region in case of Madden operation). The clinical evaluation also focused on the measurement of the forearm and of the arm circumferences.

RESULTS

From 42 cases of severe and medium lymphedema, in 8 cases (19.04%), we found that an early lymphedema appeared in day 14 after the surgery and between 14 and 21 postoperative days. The late lymphedema that appeared after day 21 after surgery can be divided into 2 categories: lymphedema occurring during the first postoperative 12 months (9 cases) and lymphedema occurring later than 12 postoperative months (25 cases) (tab. I, fig. 1).

Regarding the impact on the patient's clinical condition, we found pain, a feeling of weight in the upper limb and decreased motility of the upper limb (48% of cases). 3 patients with important and persistent lymphedema presented almost a total motor deficit of the affected upper limb. We also evaluated the impact of that lymphedema

of the upper limb had on the state of mind of these patients. Therefore 21 patients had obsessive-phobic concerns related to the absence of the breast, the already installed lymphedema and concerns regarding the fear of recurrence of the disease. 4 of these 21 patients developed depression and were guided to see a psychiatrist.

TABLE I

Risk factors for medium and severe lymphedema after breast surgery related to patient's status

Severe/ medium lymphedema (42 cases)	Age ≤ 45 years	Age > 45 years	BMI- normo-ponderal	BMI I-II obesity	BMI morbid obesity	Same dominant arm	Trauma/burns on same arm	Smoking
No. of cases	8	36	2	38	2	41	6	13

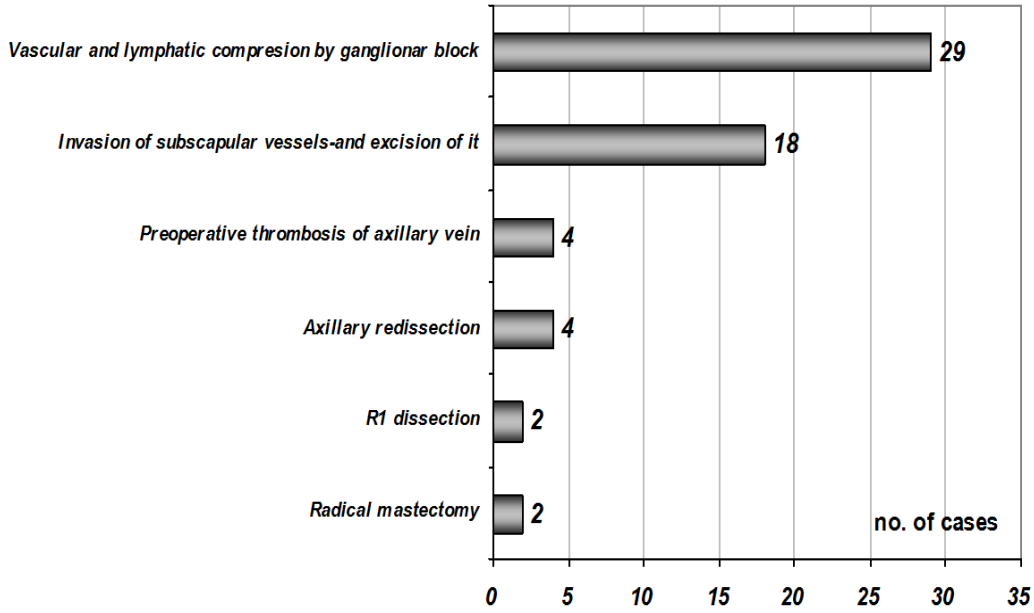

Fig. 1. Risk factors for medium and severe lymphedema after breast surgery related to the extension of the cancer and surgical treatment

DISCUSSION

The current study refers to 42 cases of medium and severe lymphedema, with the following objectives: the assessment of the incidence of postoperative lymphedema in the studied group, the assessment of the risk factors for lymphedema, determining the occurrence interval of postoperative lymphedema, monitoring the degree and the evolution of the lymphedema under different therapeutic methods and estimation of the duration of patient surveillance.

It would be ideal to identify predisposing factors of developing lymphedema before applying any therapeutic methods in order to inform the patient on this possible complication and to apply more precise prevention methods before surgery.

Elder women seem to be more prone to lymphedema – as the majority of the studies show (6, 7, 8) as we also found in our research. We concluded that the extent of mastectomy has no great influence on the occurrence of the lymphedema as other studies also observed (10). Moreover, the results of some studies highlighted that the lymphedema was present in 37% of the cases when axillary lymphadenectomy was performed, 8% of the cases with dissection of the lower third of the axillary lymph nodes and 5% related to the biopsy of an axillary lymph node (10). Other studies observed that women who underwent sentinel lymph node biopsy followed by radiotherapy, had an increased risk of developing lymphedema when chemotherapy regimens were based on anthracyclines (11).

Higher body weight with a BMI over 25kg/m^2 and thorough lymph node dissection were associated with a higher incidence of lymphedema, a fact that is also found in literature.

In the studied group we found that the excision of the axillary lymph node relapse or the redissection of the lymph nodes (in cases of incomplete axillary dissection on a previous surgical intervention) and the dissection of an encapsulated lymphatic collection adjacent to the axillary vein were associated with a medium lymphedema. We also found that the dissection of the fatty tissue around the axillary vein was associated with a higher risk of lymphedema of the homolateral upper limb. This may be due to the removal of some derivative lymphatic pathways traveling close to the vein; there is also, in these cases, a venous stasis and a fibrous tissue organized around the axillary vein, causing its strangulation. Postoperative axillary vein thrombosis that occurred especially after the dissection of the lymph nodes adherent to the vein and preoperative vein thrombosis due to the presence of tumor thrombus are other factors responsible for stasis and edema; in these cases, lymphedema is associated to venous edema. As an argument, we noticed that in those cases when the axillary vein was occluded (by the tumor thrombus or by the lymph nodes), when we managed to spare the subscapular venous package as a derivative pathway, through the intercostals veins, we had no lymphedema, in comparison with those situations in which the subscapular veins were resected together with the axillary ganglionar block.

There are studies showing that postoperative lymphedema is related to the lymph node tumor invasion. On our patients we found that when the lymph node dissection was complete, without residual tumor and with the spearing of the fatty tissue around the axillary vein, the incidence of postoperative lymphedema was 0 and that's because the tumoral invaded lymph nodes did not serve for lymph drainage anymore and there already were derivative pathways. In those cases when we had residual tumor, we usually had postoperative lymphedema.

There is still a controversy regarding the prevalence of lymphedema in the dominant upper limb (13, 14). For our study this element is only relevant in the case of patients declaring the occurrence of lymphedema after physical effort. The occurrence

of lymphedema is related to lymph nodes invasion (12). On the analyzed casuistry we noticed that when in cases when the ganglionar tumor invasion was important, when the limphadenectomy was Ro and when a ganglionar fatty tissue around the axillary vein could be kept, then there was no lymphedema. In the cases where there has remained a ganglionar tumor tissue around the axillary vein as well as at those with detailed dissection around the axillary vein, the lymphedema appeared. The explanation is: in the first case the tumor invaded nodules were not useful at the drainage of the lymph, which found other derivative paths; in the second case the edema that appeared had an important venous component by wrapping the axillary vein with the tumor remains that continued growing. The prophylactic administration of micronized flavonoids and derivatives of flavani for a period of 14 to 21 days starting with the first day after surgery together with the administration of fractionated heparins decreased the incidence of lymphedema. Meanwhile, in patients who developed postoperative lymphedema, we prolonged the administration of this medication associated with elastic compression of the upper limb, gymnastics and maintaining of the upper limb in an elevated position turned severe edema into mild edema. Another factor that led to severe lymphedema remission was the decreasing of the body mass. We also paid attention to the nutrition of the patients by recommending a diet that does not significantly increase the lymphatic flow; the patients are advised to consume foods containing medium chain triglycerides. Another prophylactic method related to the surgical act is to identify the main lymphatic pathways from the upper limb by preoperative infiltration of methylene blue in the forearm, this being able to protect the main lymphatic collectors that transport the lymph from the upper limb to the thoracic duct.

Surgery for lymphedema had no significant long time results. Excisions of subcutaneous fat had been practiced, but postoperative infections and poor cosmetic results were discouraging. Partial excision of subcutaneous fat and ”burying” a skin flap – without epidermis – under the arm muscle aponeurosis was tried by some of us, but without favorable results. More recently, liposuction has been practiced through several small incisions, with relative good results in 12 months of patient surveillance (15). Lympho-venous anastomoses showed good results in 50-77% of patients (16). The results seem to improve when lymph-venous anastomosis was associated with excision of subcutaneous tissue.

CONCLUSIONS

Our study shows that preoperative detection of risk factors for upper limb lymphedema in breast cancer would allow the early and intensive application of targeted therapeutic methods. On the other hand, avoiding aggressive and excessive dissection around axillary vein and maintaining the body fat surrounding axillary vein would limit collateral damage to the lymphatic system and diminish the risk of lymphedema.

Our paper show a relevant factor: micronized flavonoids associated with methods of physical therapy from an early stage provide a good prophylaxis for lymphedema occurring after breast cancer surgery. Patients should avoid extensive physical effort of homolateral upper limb and should

be thought the methods that can sometimes prevent recurrent erysipelas in the upper limb, thus avoiding severe lymphedema installation.

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ANTHROPOMETRIC PARAMETERS – PREDICTIVE FACTORS FOR CARDIO-METABOLIC DISEASES

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ANTHROPOMETRIC PARAMETERS - PREDICTIVE FACTORS FOR CARDIO-METABOLIC DISEASES (Abstract): The **aim** of this study was to evaluate comparatively the predictive power of body mass index (BMI) and waist circumference (WC), two anthropometric parameters used in daily practice, for detecting cardio-metabolic diseases, in a rural community in north-east Romania. **Material and methods:** We evaluated 3248 persons, aged 19 or over, for whom we collected the following data: medical history, anthropometric parameters, blood pressure value and biochemical parameters. **Results and conclusions:** Both WC and BMI help correctly determine the presence of arterial hypertension, diabetes and dislipidemia. However, the cut-off value of each of these parameters differs for each pathology. The predictive value of WC for different cardio-metabolic diseases is maintained even at normal or borderline overweight values of BMI. **Key words:** WAIST CIRCUMFERENCE, BODY MASS INDEX, PREDICTIVE POWER, CARDIO-METABOLIC DISEASES.

The medical community admits as certain the epidemic progression of obesity and acknowledges the risks for morbidity, disability and mortality associated with excess weight (1). Energy imbalance causes various diseases, but those with cardio-metabolic echo warned international fora, causing mobilization of scientists to establish effective programs for prevention and treatment of these people. Data published in the literature have demonstrated the importance of location of excess fat at abdomen level (2), assessed by WC, even for people with BMI within normal limits (parameter recognized by the World Health Organization for diagnosis of obesity) (3). The purpose of this study was to assess comparatively the predictive power for cardio-metabolic diseases of the two anthropometric parameters used in daily practice, BMI and WC.

MATERIAL AND METHODS

We included 3248 persons in our study aged 19 or over, who lived in Deleni, Iaşi County, enrolled in the interval July 2007 – July 2008. We collected the following data for each subject: medical history, anthropometric parameters, blood pressure values and biochemical parameters – fasting glycaemia, total cholesterol, HDL cholesterol, triglycerides. Measuring weight and height

were performed under light clothing and fasting, using calibrated instruments, standardized and calibrated according to international standards. Determination of weight status was based on BMI (BMI = weight/height^2, kg/m^2) and WC was measured midway, between the lower rib margin and the anterior superior iliac spine. Diagnosis of hypertension was set at values >140 mmHg systolic and/or >90 mmHg diastolic and / or antihypertensive treatment. Diabetes was present when fasting blood glucose ≥126 mg/dl and in those with known diagnosis. Diagnosis of dyslipidemia was based on the values of fasting lipid fractions: total cholesterol, HDL cholesterol and triglycerides, and/or specific previous treatment. Diagnostic limits for each were: total cholesterol >200 mg/dl, HDL cholesterol <50 mg/dl in women or <40 mg/dl in men, triglycerides >150mg/dl. Statistical analysis of data was performed using *Statistical Package for Social Sciences program* (*SPSS*) version 16.0.

RESULTS

The study included 3248 people, of which 1341 men (41.28%) and 1907 women (58.71%), aged between 19 and 91 years, mean age 50.58 ± 18.26. We have conducted previous epidemiological studies in this population and the findings were published in literature: the prevalence of obesity (BMI ≥ 30 kg/m^2) 17.5% (n=570) (4), hypertension 31.1% (n=1010) (5), diabetes 5.2% (n=169) (5), hypercholesterolemia 42% (n=1364) (5), decreased HDL cholesterol 13.6% (n=442) (0.3% in men, 13.3% in women) (5), hypertriglyceridaemia 19.6% (n=637) (5).

Based on these data, we compared the predictive power of the two anthropometric parameters (BMI and WC) for the development of one of the co morbidities listed. We used as statistical method the Receiver Operating Characteristic curve (ROC curve), which is formed by representing sensibility (Se - maximum value 1) and specificity (Sp - maximum value 1) for the entire range of variation in the predictor (WC or BMI in our case). We then analyzed, comparatively, the predictive power of WC and BMI for the development of hypertension, diabetes mellitus, hypercholesterolemia, low HDL cholesterol and hypertriglyceridaemia.

The data obtained have statistical significance; hence both WC and BMI help determine the correct presence of hypertension, diabetes and dyslipidemia. There were differences however in the limit value of each of the anthropometric parameters as predictors for each co morbidity. Thus, analyzing the ROC curves we saw the following optimal predictive values: for hypertension, cut-off values were: 25.5 kg/m^2 for BMI, 98 cm for WC (fig. 1); for diabetes, BMI = 27 kg/m^2, WC = 100 cm were cut-off values (fig. 2); for hypercholesterolemia, BMI = 25 kg/m^2, WC = 96 cm were cut-off values (fig. 3); for low HDL cholesterol, BMI = 26.9 kg/m^2, WC = 99 cm were cut-off values (fig. 4); for hypertriglyceridaemia, BMI = 26.2 kg/m^2, WC = 99 cm were cut-off values (fig. 5).

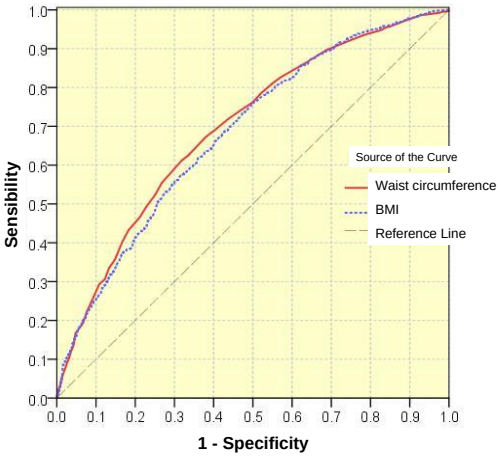

Fig. 1. ROC curve for the prediction of arterial hypertension

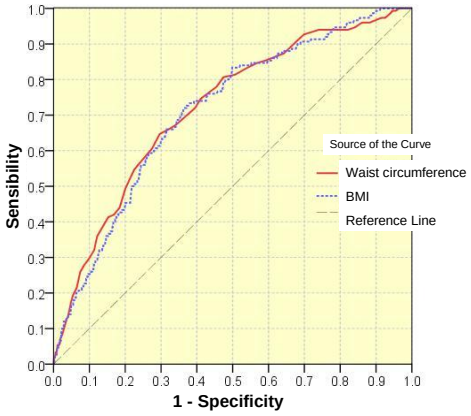

Fig. 2. ROC curve for the prediction of diabetes

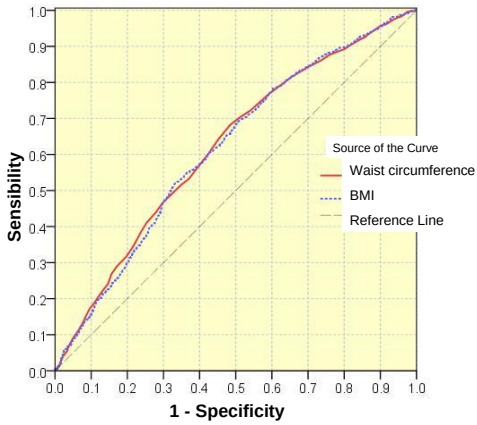

Fig. 3. ROC curve for the prediction of hypercholesterolemia

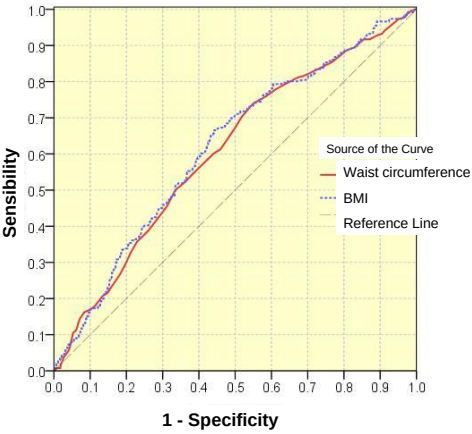

Fig. 4. ROC curve for the prediction of low HDL cholesterol

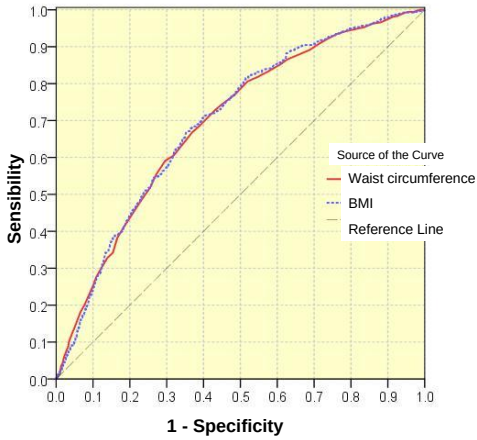

Fig. 5. ROC curve for the prediction of hypertriglyceridemia

DISCUSSION

In modern societies, energy imbalance manifests in various ways in every individual, causing frequent and important co morbidities, among which some life threatening diseases such as coronary or cerebrovascular disease, sleep apnea syndrome. The most evident rapidly modifiable parameter is weight status, often meaning the accumulation of excess fat which is dysfunctional. This leads to excess secretion of proinflammatory adipocitokines which are responsible for the increased cardiometabolic risk of these people. In this context and in the light of the obesity epidemic, global interest in the medical world has turned to finding quick and cheap ways of early identification and assessment of individuals with cardio-metabolic risk. Our previous studies have shown not only a

high prevalence of overweight in this community in northeastern Romania (4), but also of comorbidities with cardio-metabolic risk (5, 6).

The current findings indicate that both WC and BMI, two commonly used anthropometric parameters in daily practice, both in primary care and specialty level, help determine the correct presence of hypertension, diabetes and dyslipidemia and, moreover, have the same predictive power for the conditions specified, in the rural population studied. The predictive value of WC for different cardio-metabolic diseases is maintained even at normal BMI, or borderline overweight values of BMI, which emphasizes the importance of location of excess fat.

The results are even more important as the literature increasingly mentions the need to assess both anthropometric parameters to fully characterize people from a cardio-metabolic perspective. Metabolic syndrome, a constellation which implies the presence of several metabolic disorders, has had WC value for a long time as mandatory criterion for diagnosis, reflecting the importance of evaluation of this parameter in practice. In the literature there are numerous published data on the importance of assessing WC. In this respect, a prospective study that analyzed the effects of change in WC on cardio-metabolic risk factors over a period of nine years was DESIR (Data from Epidemiological Study on the Insulin Resistance Syndrome) (7). The study enrolled over 3800 participants aged 30-64 years and showed that after adjustment for BMI, an increase of ≥ 7 cm of WC significantly increased the risk of co morbidities and metabolic syndrome (odds ratio 7.9 (95% CI: 4.4 to 13.9) in men and 4.7 (95% CI: 2.7 to 8) in women), while decreasing at least 3 cm of WC produced significant reduction in triglycerides, insulinemia, the prevalence of metabolic syndrome in both sexes and decreased blood pressure in women.

A recent analysis of the Dallas Heart Study (8) demonstrated that the number of cardio-metabolic risk factors increased with increasing WC, in both women and men, regardless of race. Risk factors quantified in this study were: triglycerides ≥ 150 mg/dl, HDL cholesterol < 40 mg/dl in men and < 50 mg/dl in women, insulin resistance defined as HOMA value ≥ 1.63, systolic blood pressure ≥ 130 mmHg, diastolic blood pressure ≥ 85 mmHg or antihypertensive treatment, C-reactive protein ≥ 3 mg/l.

National Health and Nutrition Examination Survey (NHANES III study), reference study regarding the health of the population, showed that the value of WC correlates better than BMI with four of the five examined risk factors: LDL cholesterol, HDL cholesterol, hypertension and hyperglycemia (9). Moreover, evidence from this study showed that WC is an independent risk factor for developing type 2 diabetes, being a stronger predictor than other cardio-metabolic risk factors (LDL cholesterol, HDL cholesterol or triglycerides) or than BMI, for the condition (10). Also, several recent studies have shown that visceral obesity and its metabolic complications are not only found in obese individuals, but contribute to increased cardio-vascular risk in normal-weight, apparently healthy people, as well (11, 12). On the other hand, it has been shown that there are obese individuals who are "metabolically healthy", with only a minimal amount of visceral fat (13).

All the evidence suggests that evaluation of visceral fat is a more relevant criterion than the total amount of fat for the assessment of cardio-vascular risk.

A special category is formed by people of normal weight but with phenotypical characteristics of obesity, called "metaboli-

cally obese normal weight". It has been hypothesized that these individuals would be characterized by hyperinsulinemia and / or insulin resistance, hypertriglyceridaemia and hypertension associated with a BMI <25 kg/m^2. Compared with the control group, normal weight metabolically obese subjects have a low insulin sensibility, increased abdominal and visceral adiposity, an atherogenic lipid profile and increased blood pressure (14).

CONCLUSIONS

Our results showed that both WC and BMI had predictive power for the presence of diabetes mellitus, dyslipidemia or hypertension in people from this rural community located in north-eastern Romania. Practically there was no difference between the predictions of WC compared with BMI, both anthropometric indicators being equally valuable in the prediction of cardiometabolic risk factors in the studied population. Therefore, routine measurement of WC, simple and easy to do, can be included in the algorithm for the initial assessment of each individual, in primary care, regardless of BMI.

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STUDIU COMPARATIV – INTERVENȚII CLASICE *VERSUS* TEHNICA LICHTENSTEIN ÎN CURA HERNIARĂ

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COMPARATIVE STUDY BETWEEN CLASSICAL OPERATIONS AND LICHTENSTEIN TECHNIQUE FOR HERNIA REPAIR (Abstract): **The aim** of these study was to compare classical procedures in inguinal hernia repair (Postemski, Kimbarovski, Bassini, McVay) made in the Second Surgery Department of County Clinical Emergency Hospital Oradea with the operations in which was used the Lichtenstein technique in the same department, in a period of two years, with respect to recurrence rate, technical difficulty, convalescence and chronic pain. **Material and methods:** 217 operations were performed in classic way or using the Lichtenstein technique. Follow was performed at 1 month, 3, 6 and 12 months after surgery with a compliance of 80%. **Results:** There was no significant difference in operating time between classic operations and tension free operations. The recurrence rates were significantly lower in the group operated by the Lichtenstein technique. The chronic pain was reported in both groups as mild or moderate. **Conclusions:** Lichtenstein hernia repair was easy to learn took the same time and resulted in fewer recurrences. It is possible to be used successfully in a general surgical unit. **Key words**: INGUINAL HERNIA, LICHTENSTEIN OPERATION, CLASSICAL PROCEDURES

În literatura de specialitate, recurența după chirurgia herniară poate varia între 0,1 şi 20%. Puține alte intervenții chirurgicale prezintă o astfel de variație a rezultatelor (1).

Alterarea metabolismului colagenic are drept urmare slăbirea țesutului fibro-conjunctiv al regiunii inghinale, ducând la apariția herniilor. Utilizarea în intervențiile chirurgicale a acestor țesuturi deficiente, mai ales în condiții de tensiune, va determina o rată crescută a recidivelor. În hernioplastia fără tensiune în locul suturii grosiere a structurilor anatomice, se realizează întărirea peretelui posterior al canalului inghinal prin inserția unei proteze (2, 3, 4, 5, 6).

Scopul acestui studiu este de a compara cunoştințele privind procedurile clasice (Postemski, Kimbarovski, Bassini, McVay) efectuate în Clinica Chirurgie Generală II din Spitalul Clinic Județean de Urgență Oradea, cu operațiile folosind tehnica Lichtenstein efectuate în acelaşi serviciu, pe o durată de doi ani. S-a urmărit evidențierea existenței unor eventuale dificultăți tehnice, rata de recurență, apariția unor particularități ale perioadei de convalescență (prezența şi caracterele durerii cronice postoperatorii) (7, 8).

MATERIAL ŞI METODE

În perioada septembrie 2007 – decembrie 2009, s-au efectuat 217 intervenţii chirurgicale pentru hernie inghinală, dintre care, pentru 73 (33,6%) s-a folosit tehnica Lichtenstein, iar la restul de 144 (66,4%) au fost utilizate procedeele clasice. Vârsta pacienţilor, toţi de sex masculin, a fost cuprinsă între 18 şi 79 de ani (media: 54,5 ± 2 ani). Cazurile studiate au inclus hernii inghinale directe, indirecte, unilaterale şi bilaterale, iar criteriile de excludere au fost reprezentate de herniile femurale, existenţa unor coagulopatii, tratament anticoagulant sau stare biologică improprie. Operaţiile s-au efectuat în anestezie loco-regională sau generală (tab. I, fig. 1, 2).

TABELUL I

Distribuţia cazurilor în relaţie cu vârsta

Vârsta (ani)	Clasic		Lichtenstein	
	Nr.	%	Nr.	%*)
<30	2	1,4	1	1,4
31-40	14	9,7	8	11,0
41-50	33	22,9	19	26,0
51-60	53	36,8	27	37,0
61-70	24	16,7	15	20,5
>70	18	12,5	3	4,1
Total	**144**	**100,0**	**73**	**100,0**
Vârsta medie	**54,5±8,2 ani**		**52,3±7,8 ani**	

*) pentru valorile mai mici de 10, proporţiile sunt orientative

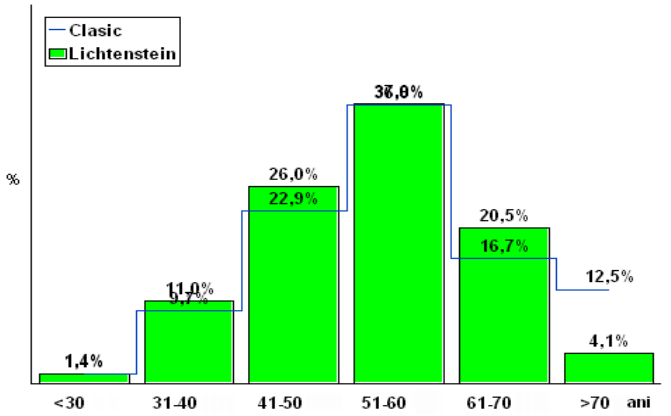

Fig. 1. Distribuţia cazurilor în relaţie cu vârsta

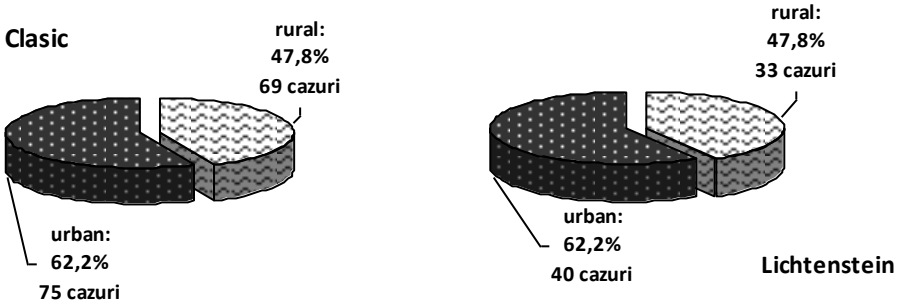

Fig. 2. Distribuţia cazurilor în relaţie cu mediul de proveniență

În herniile indirecte, sacul herniar a fost identificat, disecat de inelul profund şi deschis pentru a permite examinarea conținutului, sacul herniar a fost ligaturat la bază, cu rezecarea porțiunii distale (4).

În cazul aplicării procedurii Lichtenstein, plasa de polipropilenă a fost ajustată pentru a se potrivi dimensiunilor canalului inghinal, fixată la vârf de tuberculul pubic, la ligamentul inghinal şi la tendonul conjunct (5, 6). S-a practicat controlul hemostazei, iar în cazul unor hernii voluminoase, s-a plasat un tub de dren sub aponevroza oblicului extern suturată cu fire separate, urmat de închiderea plăgii.

Îngrijirile postoperatorii au beneficiat de antibioticoprevenție, aproximativ 48-72 de ore, iar la pacienții cu risc crescut s-au administrat heparine cu greutate moleculară mică pentru profilaxia trombozei venoase profunde, cu 12 ore preoperator şi postoperator pe toată durata spitalizării. Analgezia postoperatorie a fost asigurată prin administrarea de antialgice minore. Durata medie de spitalizare a fost de 3 zile.

REZULTATE

Au fost luate în considerație variabilele precum vârsta, tipul de activitate (grea, uşoară sau muncă de birou) şi mărimea testiculului de aceeaşi parte cu hernia. Parametrii preoperatori au fost tipul herniei, anesteziei şi complicațiile postoperatorii. A fost înregistrată durata operațiilor, iar cele de peste 70 minute au fost considerate prelungite (tab. II, fig. 3).

TABELUL II

Distribuția cazurilor în relație cu tipul herniei inghinale

Tipul herniei inghinale	Clasic		Lichtenstein	
	Nr.	%	Nr.	%
indirectă	81	56,3	47	64,4
directă	37	25,7	15	20,5
mixtă	26	18,1	11	15,1

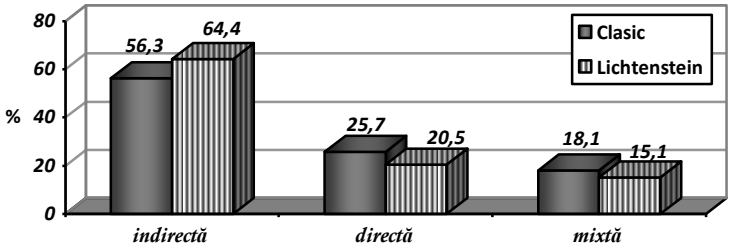

Fig. 3. Distribuția cazurilor în relație cu tipul herniei inghinale

Anestezia regională a fost utilizată în 143 (65,9%) cazuri, cea generală în 61 (28,1%) şi cea regională completată cu generală în 13 cazuri (tab. III, fig. 4).

TABELUL III

Distribuția cazurilor în relație cu tipul anesteziei

Tipul anesteziei	Clasic		Lichtenstein	
	Nr.	%	Nr.	%
Loco-regională	95	66,0	48	65,8
Generală	41	28,5	18	24,7
Loco-regională completată cu generală	8	5,6	2	2,7

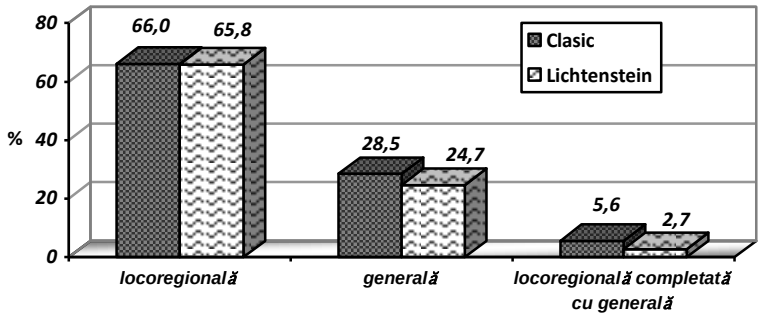

Fig. 4. Distribuția cazurilor în relație cu tipul anesteziei

Durata medie a intervențiilor în care s-a aplicat tehnica Lichtenstein a fost de 55 de minute iar pentru tehnicile clasice a fost de 42 de minute (fig. 5).

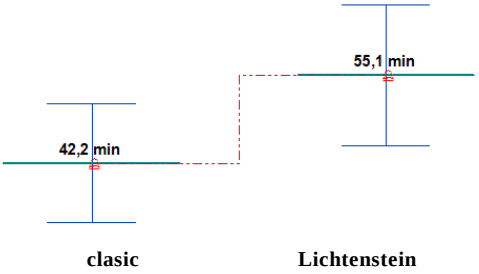

Fig. 5. Durata medie a intervenției chirurgicale

Nu s-au înregistrat complicații majore postoperatorii, dar în perioada imediat postoperatorie, s-au înregistrat 5 cazuri cu complicații la lotul la care s-a practicat operația Lichtenstein (2 hematoame şi 3 seroame); edemul testicular la 3 cazuri cedând ulterior la administrarea de AINS şi drenaj postural. Nu s-au semnalat abcese sau infecții datorate materialului eterogen. Lotul operat prin tehnici clasice a înregistrat 9 hematoame şi 7 seroame. Edemul testicular a apărut la 5 cazuri, atrofia testiculară la 2 pacienți, după 3 şi 6 luni şi 1 caz de orhiepididimită la aproximativ 2 luni postoperator. Dificultăți în urinare care au prelungit perioada de internare s-au înregistrat la 34 de pacienți, dintre care 32 au fost operați sub anestezie regională, iar 17 au necesitat cateterizare uretrală. Durerea postoperatorie a fost măsurată cu o scală vizuală analogă (fig. 6, 7).

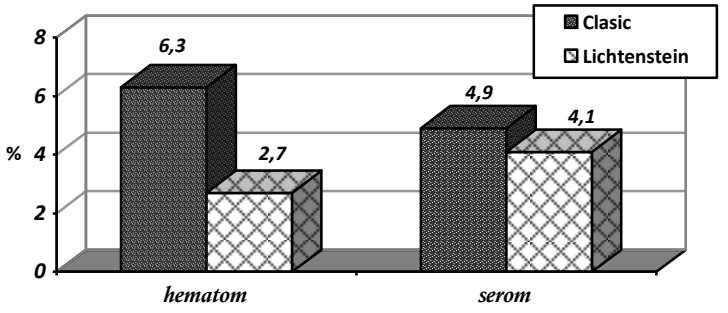

Fig. 6. Distribuția cazurilor în relație cu complicațiile postoperatorii

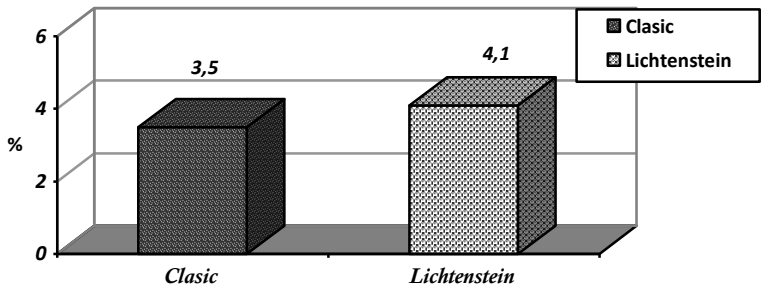

Fig. 7. Prevalența edemului testicular

Urmărirea postoperatorie a fost efectuată la 1 lună, 3, 6 şi 12 luni, cu o complianță de 80%. Pacienții au fost chestionați asupra durerilor persistente, postoperatorii, durata până la recuperarea completă şi numărul de zile de concediu medical. Pacienții care au acuzat dureri persistente de intensitate redusă sau incompletă recuperare au fost reexaminați.

În lotul pacienților operați prin tehnica Lichtenstein s-au înregistrat 5 (6,8%) cazuri cu durere persistentă mai mult de 2 săptămâni, iar în lotul celor operați prin tehnici clasice, 11 (7,6%). Discomfortul persistent a fost caracterizat drept mediu şi a fost înregistrat la 3 (4,1%) cazuri în lotul Lichtenstein şi la 5 (3,5%) pacienți, la lotul cu intervenții clasice. De asemenea, s-a urmărit prezența sau absența atrofiei testiculare de partea operată.

Recurența a fost definită ca prezența protruziei de partea operată sau apariția ei la efectuarea unei manevre Valsalva şi a fost înregistrată în 17 (11,8%) cazuri din lotul pacienților operați prin tehnici clasice şi nici una în lotul operat prin tehnica Lichtenstein. Recurența a apărut la 4 cazuri de hernie directă, 3 cazuri de hernie mixtă şi 10 cazuri de hernie indirectă (fig. 8).

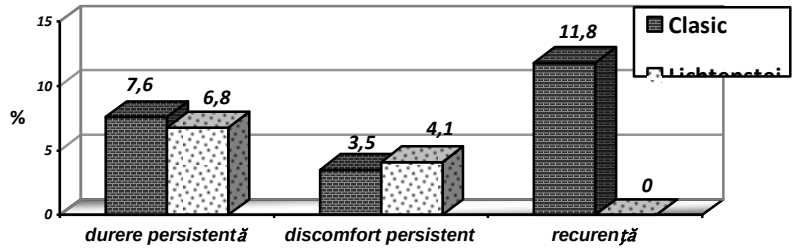

Fig. 8. Distribuția cazurilor în funcție de evoluție

DISCUȚII

Datele din literatura (7, 8) relevă o incidență a recurenței herniare după intervențiile chirurgicale clasice de 12–18%, valori care contrastează cu cele ale operației Lichtenstein de sub 1% (7, 8). Deoarece chirurgia conține şi elemente de manualitate, experiența cu o anumită tehnică poate conduce la rezultate mai bune. Cu toate acestea, tehnica Lichtenstein este o metodă

uşor de reprodus, cu recurenţă a herniei redusă (9, 10). Când o metodă este uşor de învăţat şi reprodus, este atins şi un standard înalt, rezultatele fiind asemănătoare cu cele obţinute într-un centru specializat (10, 11). Spre deosebire de operaţiile tisulare, procedeul Lichtenstein nu are o etapă operatorie de pregătire a canalului inghinal, ci doar una de reconstrucţie (12).

Temerile că materialul prostetic ar favoriza apariţia infecţiilor sau a durerii cronice potoperatorii nu s-au dovedit a fi fondate.

CONCLUZII

Studiul nostru confirmă datele din literatură, care arată că operaţiile tension-free (Licthenstein) sunt uşor reproductibile şi nu necesită o experienţă chirurgicală deosebită. Prin contrast, tehnicile clasice impun o disecţie laborioasă a structurilor anatomice, fiind deseori grevate de complicaţii ca durere cronică persistentă sau recurenţă.

Avantajele tehnicii Lichtenstein sunt simplitatea, reproductibilitatea, eficacitatea, siguranţa şi morbiditatea postoperatorie redusă. De asemenea, metoda prezintă o evoluţie postoperatorie bună, cu control accesibil al durerii şi reinserţie socio-profesională rapidă.

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NEW INSIGHTS INTO THE THERAPEUTIC USE OF NITRIC OXIDE-DONATING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

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NEW INSIGHTS INTO THE THERAPEUTIC USE OF NITRIC OXIDE-DONATING NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (Abstract): Nitric oxide (NO), a radical with increased reactivity, diffusibility and instability plays an important role in the regulation of a wide range of physiological processes, including cellular immunity, angiogenesis, cardiac function, neurotransmission, and platelet aggregation. Numerous studies in recent decades have demonstrated the benefits and cytoprotective role of nitric oxide in different body systems. A modern experimental and clinical research direction is represented by the development of hybrids containing a NO donor coupled with a classical nonsteroidal anti-inflammatory agent, (NSAID) demonstrating an improved efficacy and tolerability compared to the administration of the drug alone in laboratory animals, in various experimental models. These agents are obtained by the ester linkage of a NO-releasing moiety to a standard NSAID, such as acetylsalicylic acid (NO-aspirin), ibuprofen (NO-ibuprofen), flurbiprofen (NO-flurbiprofen), naproxen (NO-naproxen), diclofenac (NO-diclofenac) and indomethacin (NO-indomethacin). Multiple pharmacological strategies regarding the release of NO from these hybrid NO-releasing compounds provide new insights into the pathogenesis and treatment of various pathological states, such as: inflammatory conditions, hypertension, atherosclerosis, neoplastic disease, degenerative diseases, colitis and bronchial asthma. **Keywords:** NITRIC OXIDE DONORS, NON-STEROIDAL ANTI-INFLAMMATORY DRUGS, CARDIOVASCULAR SYSTEM.

Discovered in 1980 (1), nitric oxide (NO) is a signaling molecule, with an essential role in the mediation of various physiological processes in the body and important functions at the central nervous system (CNS), cardiovascular and immune defense level (2).

The importance of NO, also known as the endothelium-derived relaxing factor, has been studied over the years, being recognized in 1998 (3), when a team of researchers who studied the biological effects of the NO was awarded the Nobel Prize for medicine. NO was identified as a messenger in the CNS, being known as an unstable intracellular factor which mediates the increased levels of cyclic guanosine-3',5'-monophosphate (cGMP) (4).

In the last ten years, intense efforts have been made to obtain NO donors, among

which promising to seem to be the diazeniumdiolates, S-nitrosothiols and hybrid NO donors, such as NO-donating non-steroidal anti-inflammatory drugs (NO-NSAIDs) (5).

Despite the obvious potential of NO, notable progress was not recorded regarding the production of NO donors in the context of the scientific enthusiasm generated by the discovery of this molecule, as an important biological messenger (6).

For the realistic evaluation of NO involvement in the mediation of physiological and pathophysiological processes in the body, the endogenous roles, pharmacodynamic effects of this signaling molecule, and the therapeutic use of NO-donors must be clarified and reconsidered.

Hybrid NO-donating NSAIDs in inflammatory & degenerative diseases

Literature data revealed that the use of hybrid NO-donating NSAIDs intensified the traditional anti-inflammatory activity of the agents in different situations of brain inflammation (7). The delivery of NO-aspirin to the T lymphocytes and monocytes derived from the peripheral blood mononuclear cells causes a transient inhibition of the reduction of cellular ATP and cell respiration, which translates in time, the reversible inhibition of cell proliferation and the secretion of the IL-2, IL-4, IL-5 interleukins and interferon gamma. NO-aspirin induces metabolic hypoxia that inhibits lymphocyte reactivity to co-stimulatory molecules, providing a potential counter mechanism to control the immune system activity (8).

Some authors highlighted that the NO-NSAIDs have additional anti-inflammatory and immunomodulatory properties compared to the standard compounds (9). Moreover, it was discovered that compared to the traditional NSAIDs these hybrid NO-donors are more efficient in the treatment of Alzheimer's disease due to their ability to clear the beta amyloid deposits from the brain of affected laboratory animals (10).

The treatment with NO-flurbiprofen is associated with the decrease of mRNA levels of proinflammatory cytokines, caspase-1 and inducible nitric oxide synthase (iNOS) in blood cells, diminution of encephalitogenic T cells proliferation capacity, reduction of T cells number in the CNS, axonal loss and demyelination. NO-flurbiprofen has been proved to be efficient in reducing the beta amyloid deposition in the brain of transgenic mice with experimentally-induced Alzheimer's disease. Also, it has been demonstrated that only one dose of NO-flurbiprofen lead to the prolonged suppression of brain prostaglandin synthesis, without inducing gastric injury (10).

Experimental researches proved that NO-aspirin prevents the suppression of monocyte tissue factor expression, along with cytokine biosynthesis and inhibition of thromboxane activity. The administration of NO-aspirin in mice exhibits a notable increase in the number of tunnel-positive cells and a decrease in CD45-positive inflammatory cells. The released NO is involved in the reduction of vascular inflammation and promotion of apoptosis during vascular remodeling together with neointimal thickening (11).

Various investigations revealed that NO-donating NSAIDs are very effective in the long-term treatment of chronic inflammatory rheumatic and osteoarticular diseases. A new anti-inflammatory mechanism of NO-aspirin, independent of the cyclo-oxygenase involvement is represented by

the inhibition of the caspase-1 activity. It was demonstrated that the use of NO-aspirin induces the release of intracellular NO and decreases IL-1β and IL-18 synthesis, by inhibiting the activity of caspase-11 (11). Also, NO-naproxen showed the reduction in IL-1β and TNFα plasma levels, and the prevention of T-cell proliferation (5).

Hybrid NO-donating NSAIDs in cardiovascular diseases

Today the role of NO in the cardiovascular system is well known. It was discovered that NO induces vasodilatation through different actions, like initiating the cyclic guanosine monophosphate (cGMP) synthesis from guanylate cyclase in vascular smooth muscle cells, but it also has some favorable effects (antiatherogenic, antiplatelet) by inhibiting inflammation, platelet aggregation, cell proliferation and apoptosis (12). Compared to aspirin, NO-aspirin has more valuable effects on the cardiovascular system, being able to inhibit shear stress-induced platelet activation. NO-aspirin can inhibit the formation of superoxide radical and the expression of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase subunits induced by endoxin and cytokines in the vascular wall (13).

Napoli *et al.* (14), in clinical trials, have reported that NO-aspirin could be beneficial for the prevention of restenosis after percutaneous intervention, with significant antihypertensive effects. It was discovered that unlike classic aspirin, NO-aspirin reduces the severe restenosis in mice with experimentally-induced hypercholesterolemia (15). This hybrid agent combines in a single molecule, the classic aspirin, the most used drug in cardiovascular diseases, and the nitric oxide, the most important signaling molecule in human vessels. A comparable effect was also observed in rats with vascular injury and restenosis after NO-flurbiprofen use, its administration significantly reducing the neointimal proliferation after percutaneous coronary angioplasty (16).

NO-aspirin reduces ischemia in the rabbit heart, while the traditional aspirin aggravates the resulting myocardial dysfunction. Some results show that NO-aspirin also has vasodilatory activity in the venous grafts used for coronary artery bypass grafting, this effect not being modified under the action of vascular wall functional abnormalities, as in diabetes mellitus (17). The use of NO-aspirin proved to be beneficial for the prevention of blood clot formation and diminution of cardiovascular side effects (stroke, myocardial infarction), and a possible valuable role in non-alcoholic fatty liver disease (18). Reparative angiogenesis and prevention of apoptosis and oxidative stress are stimulated by the treatment with NO-aspirin, thus reducing the consequences of arterial occlusion.

It is also involved in the diminution of sympathetically-mediated vasoconstriction and lowering of blood pressure in hypertensive rats (19). Moreover, NO-aspirin considerably reduces the plasma LDL levels, decreases the atherosclerotic processes in the arterial wall and the local oxidative stress in the hypercholesterolemic mice (15).

Various other studies revealed the cardioprotective effects of NO-aspirin, probably mediated by NO donation, thus suggesting that this NO-donor agent could be beneficial in the treatment of infarction and myocardial ischemia (20). Similar as other NO-donating NSAIDs, it does not deter-

mine substantial changes in arterial blood pressure and heart rate after intravenous, intraperitoneal or oral administration in rats.

NO-donating NSAIDs are significantly less potent vasorelaxants than the classic nitro vasodilators (5). The inability of NO-NSAIDs to reduce systemic blood pressure in normotensive rats may be explained by the absence of the vasorelaxant effect of these compounds *in vitro*. Other experimental researches demonstrated that NO-naproxen considerably decreases hypertension induced by the obstruction of one renal artery (21).

Aspirin, which exerts its antiplatelet effects by the irreversible inhibition of cyclooxygenase 1 (COX-1), is currently used for the prevention and treatment of cardiovascular diseases. Experimental researches showed that NO-aspirin induces more platelet function inhibition, compared to traditional aspirin, both *in vitro* and *in vivo*, in rats and in humans (22). Also, NO-aspirin was more effective than aspirin in reducing the plasma epinephrine level and the collagen formation. NO-aspirin proved to be efficient against mechanically-induced thrombus formation, and vasodilatation mediated by the release of NO may probably contribute to its protective effect on the vessels (23).

It was demonstrated that NO-aspirin has an improved antiplatelet activity, and, it can protect the gastrointestinal tract exposed to other injurious agents (24). The inhibitory activity of NO-aspirin on leukocyte adherence to the vascular endothelium contributes to its antiplatelet activity. NO-aspirin prevents platelet aggregation mediated by the arachidonic acid and inhibits serum thromboxane biosynthesis. Moreover, it was shown that NO-aspirin does not inhibit the synthesis of platelet thromboxane or gastric prostaglandins, but releases NO and increases the cGMP levels in platelets, when incubated in their presence (2, 25).

CONCLUSIONS

Researching the literature data, we highlighted that NO represents an important molecule that plays a key role in signaling processes with extensive cardiovascular, nervous and immune system functions. In what concerns one new modern pharmacologic research field, we noticed that a valuable therapeutic direction is the development of hybrid NO-donating NSAIDs that have demonstrated efficacy in inflammatory, degenerative and cardiovascular diseases, in both experimental studies and clinical trials.

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ASPECTE PARTICULARE CLINICO-IMAGISTICE ÎNTÂLNITE ÎN TROMBOFLEBITA SINUSULUI LATERAL

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CLINICAL AND IMAGISTIC PARTICULAR ASPECTS IN LATERAL SINUS THROMBOPHLEBITIS (Abstract) Aim: This article focuses on the lateral sinus thrombophlebitis, which is a serious complication of the supurated otopathies**. Material and methods**: It is based on a study of a significant number of both exo- and endocranial complications treated in the the "Sf Spiridon" Hospital- ORL department and at the "Prof. dr. N. Oblu"- Neurosurgery department (57 out of 251 cases admitted in the past 5 years). **Results**: Among the 57 cases, there were 8 lateral sinus thrombophlebitis, as well as 6 cases where meningitis coexisted with other endocranial complications, such as lateral sinus thrombophlebitis or brain abscess. The article contains details about some cases with special problems of diagnostic and treatment. **Conclusions**: The symptoms of the presented cases are completely different from the common symptomatology. However, the diagnostic of such cases is possible due to the modern imaging methods as well. The lateral sinus thrombophlebitis is usually determined by different causes, one of them being the misuse of the antibiotics(class and daily/total dose).When endocranial complications occur the symptoms of the meningitis are masking the symptoms of other complications. Even if the CT scan or MRI exams performed right on the patient's admission show no other complications, the patient should be followed for a period of 15 up to 30 days after the treatment of the meningitis, by repeating imaging tests to identify a possible complication. **Key words**: LATERAL SINUS THROMBOPHLEBITIS, COMPLETE IMAGING EXAM, INFECTION, SURGICAL ABLATION

Complicaţiile extra şi endocraniene ale otitelor medii supurate şi ale otomastoiditelor înregistrează o incidenţă cu valori crescute în România, îndeosebi în Moldova.

În ţările dezvoltate, incidenţa acestor complicaţii este evaluată între 0,5 - 7 %, (1, 2, 3, 4), pentru ca în România, în special în judeţele nord-estice, să reprezinte peste 20% din cazurile de otite medii supurate şi de otomastoidite. În lucrare se încearcă o analiză asupra cauzelor şi explicaţiilor care determină existenţa acestei incidenţe crescute, prezentând în detaliu, unele cazuri cu anumite particularităţi clinice şi o evoluţie diferită faţă de tabloul clasic.

Ne vom referi la cazuri de tromboză a sinusului lateral produsă datorită unei eroziuni osoase de vecinătate prin colesteatom, osteită şi granulaţii care întreţin un abces perisinusal. Abcesul la rândul său a determinat un agregat fibrinos de celule sangvine şi plachete în peretele sinusal, realizând un trombus parietal. Acest trombus se infectează ulterior, creşte în volum

şi determină obliterarea sinusului (1,2,3,4). Clasic, tabloul clinic al tromboflebitei se manifestă prin:

• instalarea unui sindrom infecţios sever, cu creşterea considerabilă a numărului leucocitelor, febră ridicată 39,5 – 40 0 C, frison şi un sindrom de iritaţie meningoencefalică cu tulburări ale vigilenţei dacă coexistă cu o meningită sau un abces cerebral;

• starea generală foarte alterată; lipsa sindromului meningeal franc;

• apariţia frecventă a unor simptome evocatorii: otalgia, cefaleea şi edemul mastoidian (semnul Griessinger) care traduc tromboza septică a venei emisare mastoidiene;

• atingerea frecventă a nervilor VI şi VII sau sindromul de gaură ruptă posterioară (IX,X,XI).

Examenul CT, RMN şi uneori incidenţa clasică Schuller pot evidenţia o opacitate a sinusului lateral şi absenţa opacităţii venoase dupa injectarea substanţei de contrast (semnul Delta). Numai examenul angioRMN poate confirma cu exactitate ocluzia venoasă. EcoDoppler-ul îşi are utilitatea în cazul în care coexistă tromboza venei jugulare, mai ales în urmărirea evolutivă a tromboflebitei sinusului lateral.

Dulli et al. (5), Ram et al. (6) consideră că tromboflebita sinusului lateral s-ar putea datora unei stări pretrombotice, de obicei, congenitale sau acumulate în timpul sarcinii, a unei afecţiuni hematologice sau sistemice postmedicamentoase cum sunt rezistenţa la proteina C reactivă care duce la fenomene de mutaţie a factorului V Leiden. De asemenea, proteina S, un factor antitrombinic III, precum şi sindromul primitiv al anticorpilor antifosfolipidici pot determina o maladie mieloproliferativă cu sindrom nefrotic, hemoglobinurie paroxistică nocturnă, maladia lui Behcet la fel ca şi dozele excesive de corticoizi.

Evoluţia ulterioară a unei tromboflebite a sinusului lateral, netratată la timp, duce la riscuri vitale majore cum este embolia pulmonară septică (sdr. Lemierre) cu Fusobacterium necrophorum (Hughes et al. (7), Ieven et al. (8)), extensia la alte sinusuri venoase din duramater şi ridicarea presiunii intracraniene, septicemie sau septicopioemie.

După 1990, urmare a introducerii pe scară largă a antibioticelor cu spectru larg şi în România, au început să apară forme clinice fruste de otite medii acute, otite medii cronice reacutizate, folosindu-se termenul de mastoidite „decapitate”, urmarea probabilă a utilizării unor antibiotice necorespunzătoare afecţiunii şi a unor doze insuficiente.

În cazul observaţiilor noastre a rezultat modificarea florei microbiene, iar durata tratamentului a fost insuficientă, numeroşi pacienţi au beneficiat de un tratament incomplet pe o durată mai redusă de 7-14 zile şi în absenţa controlului periodic al medicului practician (9,10).

MATERIAL ŞI METODE

În studiu au fost incluse 57 de cazuri de intervenţii chirurgicale la nivel otomastoidian efectuate în ultimii 5 ani în clinica ORL din Spitalul Clinic de Urgenţe „Sf. Spiridon” din Iaşi. Informaţiile au fost acumulate printr-o fişă de anchetă, incluzând datele de caracterizare a lotului de studiu şi cele obţinute din analiza rapoartelor statistice oficiale. Prelucrarea şi interpretarea rezultatelor s-a realizat prin metode clinico-epidemiologice şi statistico-matematice.

REZULTATE ŞI DISCUŢII

Prezentăm cazul unui copil de 10 ani care a beneficiat de un tratament pentru otită medie acută, timp de 7 zile, şi care în

final a acuzat deficit auditiv, hemicranie dreaptă şi discretă otalgie periodică pe o stare discretă de obnubilare fără simptomatologie caracteristică tromboflebitei.

Examenul CT a arătat o îngroşare a peretelui sinusului lateral drept, însă radiografia clasică Schuller şi angioRMN-ul au evidenţiat un posibil proces de tromboflebită. Descoperirea intraoperatorie a sinusului lateral şi puncţionarea sa au demonstrat existenţa unui proces gleros perisinusal şi tromboza parţială a sinusului. La examenele imagistice efectuate nu s-a constatat voal mastoidian şi nici liză a pereţilor celulelor mastoidiene.

Particularitatea acestui caz constă în aceea că nu a prezentat niciunul din simptomele clasice, însă examenele imagistice complementare au putut să inducă posibilitatea existenţei unei tromboflebite a sinusului lateral.

Dacă nu s-ar fi intervenit chirurgical, am fi putut asista ulterior la apariţia altor complicaţii endocraniene cum ar fi abcesul cerebelos, cerebral sau meningita, motiv pentru care se confirmă faptul că focarul infecţios endocranian nu poate fi neglijat în cazul existenţei unei infecţii otomastoidiene.

Un alt caz particular îl constituie şi un bolnav în vârstă de 24 ani cu aticită colesteatomatoasă care s-a prezentat cu o discretă hemicranie, febră (39-40 ^{0}C), frison, 10500 leucocite, cu predominanţa neutrofilelor, iar palparea venei jugulare a relevat o discretă sensibilitate în jumătatea superioară a regiunii cervicale.

Practicarea intervenţiei chirurgicale, cu descoperirea sinusului lateral, a relevat prezenţa unei tromboflebite cu obstrucţia completă a sinusului. După secţionarea sinusului lateral şi eliminarea trombusului s-a reluat circulaţia venoasă în amonte şi în aval.

În evoluţia ulterioară, nivelul febrei a scăzut, dar frisonul a continuat să persiste, iar febra a devenit de tip supurativ (38,5 ^{0} C seara cu 37 ^{0} C dimineaţa).

Cu asemenea evoluţie, s-a suspicionat persistenţa unui proces tromboembolic la nivelul venei jugulare încât s-a decis intervenţia chirurgicală pe vena jugulară în vederea ligaturării acesteia şi a afluenţilor pentru a preîntâmpina o invazie a trombilor în alte teritorii cu apariţia unei embolii pulmonare, septicemii sau septicopioemii.

După ligatura venei jugulare interne a persistat febra de tip supurativ şi după încă 3 zile s-a decis excluderea venei jugulare interne şi a golfului jugularei (intervenţia Grunert), după care evoluţia pacientului a fost favorabilă.

În perioada 2006-2010 au fost asistate în clinica ORL 251 de cazuri de otomastoidite dintre care 57 (22,7%) cu complicaţii (fig. 1).

Din totalul de 251 de cazuri, 9 (3,6%) cazuri au prezentat meningită, iar 6 (2,4%) cazuri au prezentat concomitent tromboflebită de sinus lateral, abces cerebral sau cerebelos. Din aceste 6 cazuri, 3 au fost diagnosticate imagistic după vindecarea meningitei încât, dacă pacienţii ar fi fost externaţi, am fi putut asista ulterior la un aşa zis „deces de cauză necunoscută”.

Este posibil ca la examenul imagistic iniţial când s-a prezentat bolnavul să nu fie descoperită complicaţia endocraniană şi ea să fie decelată ulterior la un interval de 10 - 20 zile datorită faptului că aceasta era în evoluţie şi nu evidenţia prezenţa unor semne certe cum ar fi opacitatea sinusului lateral sau capsula de abces cerebral sau cerebelos.

Al treilea caz este al unui copil de 6 ani care s-a prezentat la internare prin transfer de la Spitalul de Neurochirurgie unde i se evacuase un abces extradural. În clinica O.R.L. a Spitalului „Sf. Spiridon” s-a prac-

ticat evacuarea unui alt abces extradural, existent în preajma focarului infecțios otomastoidian şi eradicarea acestui focar. După practicarea intervenției chirurgicale asupra blocului otomastoidian cu eliminarea focarului infecțios şi drenarea abcesului extradural, bolnavul a prezentat o stare generală bună.

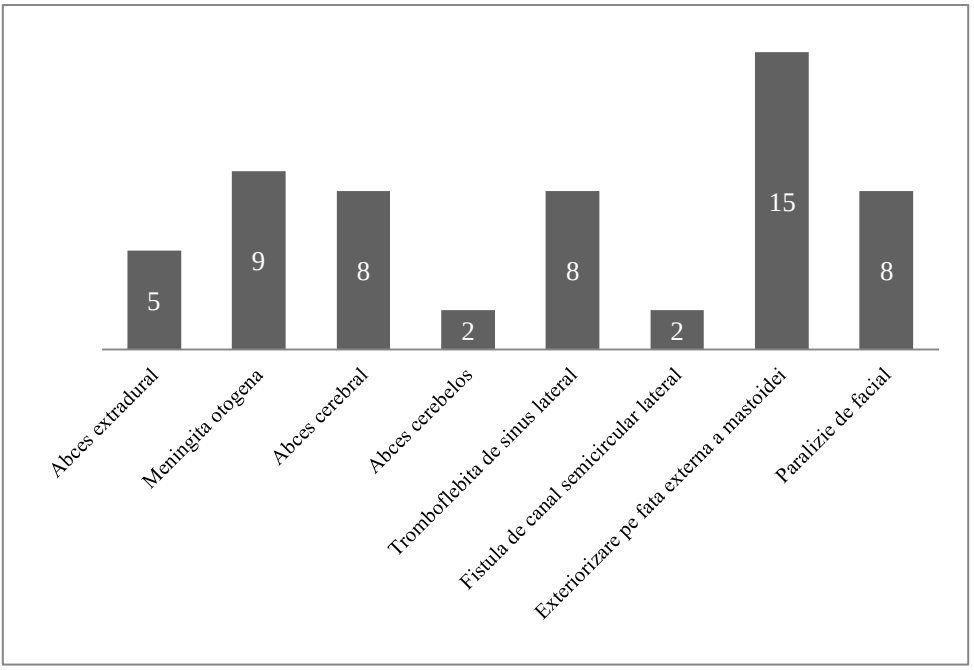

Fig. 1. Repartiția pe tipuri de complicații a lotului de 57 de pacienți

După aproximativ 8-10 zile de la ultima intervenție chirurgicală, pacientul a prezentat o stare de afectare intelectuală cu diminuarea atenției, un grad de dislalie, fapt ce a sugerat posibila existență a unui abces cerebral în evoluție.

Examenul CT a relevat prezența unui abces cerebral, cu dimensiuni de 5-6 cm, care a fost drenat chirurgical la Spitalul de Neurochirurgie şi urmat de o evoluție favorabilă.

Evaluarea evoluției cazurilor discutate a permis a se aprecia că tratamentul tromboflebitei sinusului lateral include pe lângă eradicarea chirurgicală a focarului infecțios otomastoidian şi terapia cu antibiotice asociată cu antiinflamatorii, anticoagulante (11,12).

CONCLUZII

Tromboflebita de sinus lateral rămâne o complicație cu o evoluție posibil severă a otitei medii supurate şi otomastoiditei, cu implicații deosebite în cazul formelor clinice cu simptomatologie frustă.

Examenele imagistice incluzând CT, IRM, angioRMN sunt de un real folos în stabilirea diagnosticului de tromboflebită, mai ales în cazurile cu simptomatologie clinică frustă.

În cazul existenței unui focar supurativ al substanței cerebrale sau cerebeloase, după drenarea acesteia va fi obligatorie şi eliminarea focarului infecțios otomastoidian.

În cazul meningitei otogene rebele la

tratament, coexistente cu alte procese supurative endocraniene, intervenția asupra blocului otomastoidian devine obligatorie pentru a înlătura focarul infecțios otomastoidian care favorizează apariția şi persistența complicațiilor. Concomitent, se impune reevaluarea eficienței antibioterapiei în relație cu rezistența florei microbiene implicate pentru a asigura eficiența tratamentului.

În unele cazuri se impune ligaturarea venei jugulare interne pentru a preveni apariția complicațiilor de tip septic.

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REINTERVENȚIILE CHIRURGICALE PENTRU PERITONITELE POSTOPERATORII

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SURGICAL REOPERATIONS FOR POSTOPERATIVE PERITONITIS (Abstract): In our clinical study postoperative peritonitis represent 17.01% by operations for neglected peritonitis. The other abdominal complications are eviscerations, postoperative oclusions and residual abscesses. **Material and method:** We have to analize the incidence, etiology, diagnosis and surgical management of 25 cases by postoperative peritonitis which appears after operations of 147 cases by neglected peritonitis. The semiology of a postoperative peritonitis it is easy to recognize in young pacients with reactions which appear in a few days after operations : pain localised to the abdominal area well localised in an area in which palpation show contraction. In a few hours the pain became flow. It appears fever,nausea,vommiting and all the clinical signs of peritonitis.**Results:** The highest prevalence of postoperative peritonitis for which we perform reoperations it appears after appendectomy in 18 cases with fatality 11.11%; next are coming reoperations on stomac and duodenum 4 cases with rate of fatality by 50%, reoperations on small bowell in 2 cases with fatality by 50% and reoperations of big bowell (1 case). On our series of postoperative peritonitis we have a global rate of fatality by 20% from all cases. We have considered this level well in comparison with the dates from another similar experience which show us a rate of fatality which are gone until 90%. Our media of reoperations were about three operations on each cases. If we compare with first operation for neglected peritonitis we have observe that in reoperations we change our plans in the way of making faster minimum necesary because of high risk to dead of this cases. **Conclusions**: Clinical diagnosis by postoperative peritonitis is difficult to establish and only folow up very care clinical signs of this cases beneath laboratory findings (leucogram, uree, ionogram) and with radiological examination (CT) can tell us about postoperative peritonitis.**Key words**: REOPERATIONS, POSTOPERATIVE PERITONITIS, NEGLECTED PERITONITIS.

Semiologia unei peritonite postoperatorii poate fi uneori edificatoare în special la pacienții tineri care au o bună reactivitate: la câteva zile după intervenție bolnavul acuză o durere violentă în abdomen, inițial bine localizată într-o zonă în care palparea constată apărare sau contractură. În câteva ore durerea şi contractura difuzează, apar febra, frisonul, vărsăturile şi toate semnele clinice şi biologice care întregesc tabloul clinic obişnuit al peritonitelor.

De cele mai multe ori aspectul clinic al peritonitelor postoperatorii diferă însă în mod esențial de cel cunoscut, datorită caracterului atenuat atipic sau chiar prin lipsa semnelor obişnuite care devin inaparente

datorită şi tratamentului la care sunt supuşi bolnavii după operaţie.

Peritonita postoperatorie este o formă clinică specială nu numai prin cauză dar şi prin aspectele semiologice deosebite care pot ridica probleme delicate de diagnostic. Etiologic contaminarea se poate realiza în timpul operaţiei prin diseminarea unei colecţii purulente preexistente sau prin răspândirea intraperitoneală a conţinutului septic al unui organ cavitar. Mai des însă momentul infecţiei este postoperator prin dehiscenţa mică sau largă a unei suturi sau anastomoze de organ cavitar care lasă să se scurgă un conţinut septic în peritoneu.

MATERIAL ŞI METODĂ

Pe cazuistica studiată peritonitele postoperatorii reprezintă 17,01% din totalul cazurilor operate pentru peritonite neglijate. Celelalte complicaţii abdominale sunt reprezentate de eviscerația postoperatorie (3,40%), ocluzia intestinală postoperatorie (6,80%) şi abcesele reziduale (10,88%).

Vom analiza mai detaliat incidenţa, etiologia, diagnosticul şi atitudinea terapeutică chirurgicală în cele 25 de cazuri de peritonite postoperatorii survenite după operarea unui număr de 147 de cazuri de peritonite neglijate. Semiologia unei peritonite postoperatorii poate fi uneori clară în special la pacienţii tineri care au o bună reactivitate: la câteva zile după operaţie bolnavul acuză o durere violentă în abdomen, iniţial bine localizată într-o zonă în care palparea constată apărare sau contractură. În câteva ore durerea şi contractura difuzează, apar febra, frisonul, vărsăturile şi toate semnele clinice şi biologice care întregesc tabloul clinic obişnuit al peritonitelor. Pe toată cazuistica studiată am înregistrat următoarele complicaţii survenite postoperator (tab. I).

Se observă cum complicaţiile generale survin în 23,80% din cazuri şi sunt reprezentate de complicaţiile cardiovasculare, pulmonare şi renale. În 40,82% din cazuri apar complicaţiile locale reprezentate de supuraţia plăgii.

TABELUL I

Complicaţii survenite postoperator

	Complicatii survenite	Nr. cazuri	%
1	Supuraţia plagii	60	40,82
2	Eviscerația postoperatorie	5	3,40
3	Abcese reziduale	16	10,88
4	Ocluzie intestinală postoperatorie	10	6,80
5	Peritonita postoperatorie	25	17,01
6	Complicaţii pulmonare	12	8,16
7	Complicaţii cardiovasculare	8	5,44
8	Complicaţii renale	15	10,20

Peritonita postoperatorie este o formă clinică specială nu numai prin cauză dar şi prin aspectele semiologice deosebite care pot ridica probleme delicate de diagnostic (1). Etiologic contaminarea se poate realiza în timpul operaţiei prin diseminarea largă a unei colecţii purulente preexistente sau prin răspândirea intraperitoneală a conţinutului septic al unui organ cavitar. Mai des însă momentul infecţiei este postoperator prin dehiscenţa mică sau largă a unei suturi sau anastomoze de organ cavitar care lasă să se

scurgă în peritoneu un conținut septic (2). Cazuistica noastră cuprinde un număr de 7 cazuri de peritonite produse prin dehiscențe ale suturilor sau anastomozelor efectuate (tab. II) în care am practicat exteriorizarea de anastomoză dezunită în 3 cazuri şi sutură secundară cu colostomie de protecție în 4 cazuri.

TABELUL II

Tratament chirurgical – Intervenția chirurgicală principală în peritonita neglijată datorată dehiscențelor anastomotice

Tip de intervenție chirurgicală	Nr. cazuri	%
Exteriorizare de anastomoză dezunită	3	42,86
Sutura secundară cu colostomie de protecție	4	57,14
Drenaj	7	100,00
Refacerea peretelui abdominal în plan total	7	100,00

TABELUL III

Semne clinice în peritonitele postoperatorii

Semne clinice	Nr. cazuri	%
Dureri abdominale	19	76
Meteorism abdominal	12	48
Apărare musculară	10	40
Contractură abdominală	6	24
Oprirea tranzitului abdominal	7	28
Fund de sac Douglas destins	14	56
Vărsături	11	44
Lichid fetid exteriorizat prin drenuri şi plagă	6	24
Sughiț	2	8
Limba uscată	18	72
Facies peritoneal	7	28
Oligoanurie	16	64
Tahicardie	18	72
Frisoane	19	76
Agitație psihomotorie	10	40
Febră	21	84

Cazurile cele mai frecvente au fost cele ale peritonitelor postoperatorii care au survenit fără a avea legătură cu motivul şi sediul primei operații fiind consecința unor leziuni determinate de alți factori.

Durerea abdominală apărută după un interval liber postoperator a fost primul semn revelator, dar numai în 19 cazuri, adică 76% din numărul total de cazuri. Deoarece peritonita postoperatorie presupune o infecție, febra a fost un semn constant, apărând în 21 de cazuri, adică 84% din numărul total de cazuri. La fel, tahicardia a fost un semn constant, apărând în 18 cazuri, adică 72% din numărul total de cazuri, iar frisoanele au fost prezente în 19 cazuri, adică

76% din numărul total de cazuri.

Dar ceea ce a atras de la început atenția la toți bolnavii, determinând recurgerea la investigații clinice, radiologice şi de laborator, a fost alterarea bruscă în postoperator a stării generale (3).

Controlul plăgii pe unde se scurg lichide septice şi repermeabilizarea tuburilor de dren a înlesnit diagnosticul în 24% din cazuri. Un foarte important semn clinic a fost bombarea şi „țipătul Douglasului"(4), prezent în 14 cazuri, adică 56% din numărul total de cazuri.

Din punct de vedere al grupelor de vârstă (tab. IV), s-a constatat o frecvența ridicată a peritonitelor postoperatorii la grupa de vârstă 50-59 ani.

Cea mai mică frecvență a peritonitelor postoperatorii s-a înregistrat la grupa de vârstă 70 ani şi peste (un caz, respectiv 4%).

TABELUL IV

Repartiția peritonitelor postoperatorii pe grupe de vârstă

	Nr. cazuri	**%**
10 - 19 ani	2	8
20 - 29 ani	3	12
30 - 39 ani	4	16
40 - 49 ani	3	12
50 - 59 ani	8	32
60 - 69 ani	4	16
70 ani şi peste	1	4
TOTAL	**25**	**100**

Prevalența cea mai mare a peritonitelor postoperatorii am înregistrat-o după operațiile pe apendice, 18 cazuri, cu o rată a fatalității de 11,11%; urmează intervențiile pe stomac şi duoden, 4 cazuri, cu o rată de fatalitate de 50%, intervențiile pe intestinul subțire, 2 cazuri, cu o rată a fatalitate tot de 50% şi intervenția pe colon (un caz), cu evoluție favorabilă.

Pe lotul nostru de peritonite postoperatorii am înregistrat o rată globală de fatalitate de 20% din totalul cazurilor de îmbolnăvire. Acest indice este considerat bun, în comparație cu datele din literatura de specialitate, care indică o fatalitate de până la 90% (5). În aceste cazuri de peritonite postoperatorii am practicat următoarele reintervenții chirurgicale (tab. V).

TABELUL V

Reintervenții chirurgicale în peritonitele postoperatorii

Pe stomac şi duoden - sutura secundară
- excizie - sutură
- rezecție gastrică cu anastomoză tip Pèan la cazurile cu sutură nereuşită prima dată
- drenaj simplu în dreptul dehiscenței
Pe intestinul subțire
- enterectomie complementară cu anastomoză termino-terminală
- exteriorizarea anastomozei dezunite
Pe colon - exteriorizare de anastomoză dezunită
- sutură secundară cu colostomie de protecție
Eviscerații - refacerea peretelui abdominal cu inele Georgescu

La 4 pacienţi din totalul de 25 s-a reintervenit de mai multe ori. Un număr de doi bolnavi dintre aceştia (50%) au decedat. Media reintervenţiilor a fost de trei. Comparativ cu prima intervenţie efectuată pentru peritonită neglijată se observă la reintervenţii o schimbare uşoară a conduitei chirurgicale în sensul simplificării operaţiei datorită gravităţii cazului (6). Astfel se observă că unul din principiul de bază al reintervenţiilor chirurgicale este acela de a efectua un act chirurgical minim,cât mai simplu pentru a uşura şi a face suportabil un act chirurgical repetat, cu o nouă anestezie generală pe un pacient suferind. Principiul de bază al reintervenţiilor este simplitatea gestului chirurgical de rezolvare a cauzei care a produs peritonita postoperatorie (7). Existenţa de peritonite postapendicectomie atrage atenţia că şi această „operaţie banală" trebuie executată de chirurgi experimentaţi, care ştiu să prevadă şi să ocolească cauzele acestei complicaţii. Pacienţii care au dezvoltat insuficienţă renală şi şoc toxicoseptic au decedat (8). Media reintervenţiilor în aceste cazuri a fost de trei.

Pe stomac şi duoden reintervenţiile au constat în suturi secundare, excizii şi sutură, rezecţie gastroduodenală cu gastroduodenoanastomoză la sutură nereuşită, conversie gastrojejunală după dezunire de anastomoză, sau simplu drenaj lângă dehiscenţă (9).

Pe intestinul subţire reintervenţiile au constat în enterectomie complementară cu rezecţia porţiunii compromise cu anastomoză entero-enterală termino-terminală şi numai într-un caz o exteriorizare de anastomoză dezunită (10). Am constatat că cel mai bun procedeu de refacere a circuitului digestiv după o peritonită postoperatorie datorată unei dehiscenţe de anastomoză termino-terminală de intestin subţire este rezecţia până în ţesut sănătos cu enteroanastomoză terminoterminală şi numai la nevoie se recurge la ileostomie de protecţie (11).

Pe colon reintervenţiile au constat în exteriorizare de anastomoză dehiscentă sau sutură secundară cu colostomie în amonte. De asemenea în literatura de specialitate se menţionează o reducere a dehiscenţelor suturilor colice dacă se practică în amonte o colostomie de protecţie (12).

Nu am practicat cecostomie.

Întotdeauna după o incizie tip MacBurney am ales calea de abord mediană ombilicopubiană şi nu o prelungire a inciziei iniţiale. După laparotomie urmează descoperirea şi evaluarea colecţiilor peritoneale.

În urma desfiinţării cavităţilor formate prin secţionarea bridelor am toaletat cavitatea peritoneală cu ser fiziologic cald şi betadină şi cu îndepărtarea falselor membrane de pe seroase (13).

După realizarea drenajului decliv am terminat intervenţia chirurgicală prin refacerea peretelui abdominal în plan total. În câteva cazuri am utilizat inelele Georgescu cu bune rezultate. La fel am procedat şi în cazurile de eviscerație postoperatorie prin închiderea peretelui abdominal cu aceste inele. Pe lângă simplitatea gesturilor am căutat ca timpul operaţiei de reintervenţie să fie cât mai mic (14). Am combinat simplitatea cu eficienţa.

Nu am folosit niciodată metoda deschisă, a laparostomiei şi nici a lavajului drenaj postoperator cu diferite soluţii deoarece consider că o toaletă bună efectuată intraoperator şi un drenaj eficient ajută mai bine peritoneul să se apere. În toate aceste cazuri antibioterapia a fost condusă conform rezultatelor antibiogramei. În perioada postoperatorie am tratat aceşti pacienţi ca fiind multişocaţi (şoc operator, toxicosep-

tic) şi cu multiple disfuncţii ale organelor (MSOF).

DISCUȚII

Prevalența peritonitelor postoperatorii este dificil de apreciat datorită condițiilor de apariție la un bolnav deja operat. Peritonitele postoperatorii au o evoluție gravă şi imprevizibilă (11,14).

Dificultatea stabilirii diagnosticului este datorată şi modificărilor tabloului clinic prin folosirea antibioticelor, analgeticelor şi existența aspirației nasogastrice; tabloul clinic poate simula alte afecțiuni precum infarctul de miocard, embolia pulmonară sau septicemia.

Creşterea duratei spitalizării preoperatorii creşte riscul colonizării bolnavului cu germeni rezistenți ceea ce face ca antibioterapia să fie ineficace. Alteori, pregătirea preoperatorie a bolnavului este insuficientă sau se referă la pregătirea preoperatorie mecanică şi chimică a colonului sau la lipsa eradicării unui focar infecțios ce nu are legătură cu boala pentru care se intervine chirurgical.

Un alt factor de gravitate a bolii este durata actului operator, riscul de deces dublându-se cu trecerea fiecărei ore (12, 14).

Cauzele peritonitelor postoperatorii sunt diverse: dezunirea anastomozelor digestive efectuate pe un segment intestinal inflamat, prost vascularizat sau deperitonizat.

După anastomozele colorectale prevalența variază între 1-10% fără diferențe semnificative între anastomozele efectuate prin sutură sau mecanice (13, 14).

Anastomozele gastrojejunale sau esojejunale se pot complica cu peritonite postoperatorii cu o frecvență mai mare în chirurgia oncologică.

Toaleta peritoneală incompletă sau drenajul ineficient pot cauza peritonite postoperatorii.

O altă cauză o constituie contaminarea directă a peritoneului în cursul laparotomiei fie din afară când nu se respectă regulile de asepsie, dar mai frecvent din interior prin manevrele efectuate pe organe inflamate şi cu conținut septic.

Peritonitele postoperatorii se întâlnesc la 4-6% dintre apendicectomizați, iar 43% dintre aceştia nu au prezentat inițial o apendicită complicată cu peritonită neglijată (14). În cazuistica noastră am înregistrat o prevalență similară.

De asemenea peritonitele postoperatorii pot apărea datorită unor leziuni ignorate în timpul primei intervenții sau incorect apreciate ca potențial evolutiv sau pot fi datorate unor leziuni provocate şi nerecunoscute cum se întâmplă în timpul eliberării aderențelor, sau pot fi datorate infectării secundare a revărsatelor peritoneale de la alte infecții localizate.

Alte cauze de producerea peritonitelor postoperatorii pot fi reprezentate de deraparea ligaturilor cu hemoragie secundară şi suprainfecție, lezări accidentale nerecunoscute cum sunt cele întâlnite în chirurgia laparoscopică.

CONCLUZII

Peritonitele postoperatorii prezintă o gravitate deosebită cu toate progresele înregistrate atât în diagnostic cât şi ca tratament.

Diagnosticul afecțiunii este dificil de stabilit fiind pus cu întârziere ceea ce atrage după sine pierderea momentului operator optim.

Peritonita postoperatorie necesită un tratament complex medico-chirurgical.

Baza tratamentului este reintervenția chirurgicală. Se impune toaletizarea cavității peritoneale cu îndepărtarea colecțiilor

purulente, a falselor membrane şi un lavaj intraoperator bun al cavității.

Tratamentul chirurgical în reintervenţii trebuie să fie simplu şi adecvat leziunilor găsite intraoperator, completat cu un tratament de reechilibrare hidro-electrolitică, de corijare a dezechilibrelor şi de combatere a infecției.

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NEUTROPENIA FEBRILĂ DE CAUZĂ TOXICĂ

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TOXIC CAUSE FEBRILE NEUTROPENIA (Abstract): Agranulocytosis is an acute disease with severe leucopenia, especially with low neutrophils, elements with an essential role in the organism's defense. It is more frequent at adult age (between 30 to 70 years of age) especially in women. We present the case of a 84 years old patient that, following passive exposure to pesticides, develops a severe neutropenia with a fast response to the administration of Methylprednisolone and granulocyte colony-stimulating factor (G-CSF – Filgrastim 48MIU/0.8mL/day, for three days). The diagnostic certainty was set by the bone marrow puncture which excluded a possible aleukemic acute leukemia or a myelodysplastic syndrome. **Key words:** TOXIC AGRANULOCYTOSIS, PASSIVE EXPOSURE TO PESTICIDES, FAST RESPONSE TO FILGRASTIM

Leucocitele sunt elemente nucleate, diferite morfologic şi funcţional care circulă împreună în sângele periferic şi participă la procesul de apărare a organismului.

Granulocitopoeza are loc în măduva osoasă din celulele reticulare imature, celule "stem", trecând prin următoarele etape: mieloblast, promielocit, mielocit, metamielocit nesegmentat, ajungându-se la celule mature, segmentate cu nucleu polilobat şi granulaţie în citoplasmă.

Agranulocitoza desemnează o stare de scădere critică a leucocitelor, în special a neutrofilelor, ce poate apărea ca reacţie adversă la diverse clase de medicamente, de la banalele analgezice până la chimioterapice şi medicaţia SNC. Aceasta se poate instala prin mecanism farmacodinamic secundar, mecanism toxic sau imunoalergic, se manifestă dramatic şi poate pune viaţa în pericol.

Agranulocitoza este o stare acută, ce presupune o leucopenie severă, în special de scădere a neutrofilelor, o clasă importantă a celulelor cu rol esenţial în apărarea organismului. În agranulocitoză, concentraţia granulocitelor (neutrofile, bazofile şi eozinofile) scade sub 100 celule/mm³, ceea ce reprezintă doar 5% din valoarea normală.

Diagnosticarea agranulocitozei se face după numărarea elementelor figurate sangvine, atunci când numărul neutrofilelor este sub 500/mm³ putând ajunge chiar şi la 0 celule/mm³. Celelalte elemente figurate sunt, în general, în parametri normali.

Pentru a pune diagnosticul de agranulocitoză trebuie însă înlăturate alte posibile afecţiuni, precum mielodisplazia sau leucemia acută forma aleucemică.

PREZENTARE DE CAZ

Prezentăm cazul unui pacient de 84 ani care se adresează iniţial Spitalului de Neurologie Iaşi unde se află în evidenţă datorită unui accident ischemic tranzitor suferit în martie 2009.

Motivele internării: disfagie pentru lichide şi solide, cefalee importantă, febră (39ºC), astenie fizică marcată.

Datorită decelării unui număr scăzut de globule albe (GA= 1000/mm³), fără a se obiectiva o acutizare a simptomelor neurologice este orientat către Clinica Medicală a Spitalului "Dr. C. I. Parhon" Iaşi pentru continuarea investigaţiilor şi conduită terapeutică.

Din antecedentele patologice personale menţionăm hipertensiune arterială aterosclerotică şi cardiopatie ischemică cronică din 2008 şi un accident ischemic tranzitor în martie 2009. Antecedentele heredo-colaterale sunt nesemnificative.

La domiciliu se afla sub tratament cu Rosuvastatină 5 mg x 1 cp/zi, Clopidogrel 75 mg (1 cp/zi), Detralex 500 mg (2 cp/zi), Milgamma (2 cp/zi), Diurex 50 mg (1 cp/zi), Pentoxifilin Retard 400 mg (2 cp/zi), Betaserc 24 mg (2 cp/zi). Neagă consumul de analgezice de tipul metamizol sodic sau acetaminofen.

De menţionat expunerea pasivă la pesticide (substanţe de stropit copacii în urmă cu două luni).

Examenul clinic: febră (la internare 39ºC), tegumente uscate cu pliu cutanat persistent, limbă saburală, congestie orofaringiană cu tumefacţie şi congestie amigdaliană, tensiunea arterială TA=110/70 mmHg, frecvenţa cardiacă FC= 86 b/min.

Prezentăm cazul unui bărbat de 84 ani care este adresat Clinicii Medicale de către Spitalul de Neurologie unde se află în evidenţă pentru investigarea unei leucopenii febrile însoţite de deshidratare importantă în absenţa oricărei medicaţii medulotoxice. Menţionăm că pacientul a fost expus pasiv la pesticide (substanţe de stropit copacii) în urmă cu două luni.

Ipoteze de diagnostic clinic a sindromului febril:

1. Leucopenie febrilă de cauză leucemică (leucemie acută formă aleucemică). Argumente pro: numărul scăzut de leucocite plus febră înaltă.

2. Stare septică de etiologie neprecizată. Argumente pro: febra prelungită, înaltă asociată cu leucopenia ce poate sugera sepsis sever.

3. Deshidratare secundară febrei. Argumente pro: febră înaltă si tegumente uscate cu pliu cutanat persistent.

4. Angina eritematoasă. Argumente pro: febră, congestie orofaringiană şi congestie şi tumefacţie amigdaliană, disfagie pentru lichide şi solide.

5. Endocardită bacteriană subacută. Argumente pro: stare febrilă, pacient vârstnic; argumente contra: absenţa suflurilor cardiace şi a semnelor clinice de decompensare cardiacă dreaptă sau stângă.

Din explorările paraclinice observăm:

10.10.2010

GA = 1000 /mm³, Hb = 13,5g/dL, Ht = 39,2%, Tr = 281.000/mm³, VSH = 95mm/1h

11.10.2010

GA = 660/mm³, GR = 3.710.000/mm³, Hb = 11,6g/dL, Ht = 35,3%, VEM = 95,1/fL, HEM = 31,3/pg, CHEM = 32,9g/dL, Tr = 278.000/mm³, VSH = 103mm/1h

13.10.2010

GA = 490/mm³, Ly% = 93,9%, Ly = 460/mm³, Neutrofile% = 4,1%, Neutrofile = 20/mm³, Monocite% = 2%, Monocite = 10/mm³, Basofile = 0/mm³, Eosinofile = 0 mm³, GR = 3.210.000/mm³, Hb = 9,9g/dL, Ht = 30,3%, VEM = 94,4/fL, HEM =

30,8/pg, CHEM = 32,7g/dL, VSH = 124 mm/h. Hemoculturi negative; urocultură negativă; exudat faringian negativ.

Datorită agravării leucopeniei cu neutropenie chiar de la prima determinare şi faţă de cea de la Spitalul de Neurologie se decide efectuarea puncţiei medulare din creasta iliacă şi a frotiului din sânge periferic.

Rezultatele puncţiei medulare din creasta iliacă infirmă suspiciunea clinică de leucemie acută forma aleucemică, cu absenţa blaştilor pe toate liniile celulare şi deviere discretă la stânga a curbei de maturaţie a neutrofilelor.

Rezultatele frotiului de sânge periferic: anizocitoză cu normocromie, leucopenie severă cu limfocitoză relativă, limfocite de aspect normal.

Transaminazele crescute în dinamică (TGP = 74UI/L până la 193 UI/L; TGO = 80 UI/L până la 151 UI/L) au impus efectuarea serologiei virale hepatice care a fost negativă atât pentru hepatita virală B cât şi hepatita virală C.

Funcţia renală este în limite normale (clearance-ul de creatinină (MDRD) = 140 ml/minut/1,73m² s. c.).

Electrocardiograma (EKG) de repaus evidenţiază la internare (Fig.1): extrasistole ventriculare şi supraventriculare fără tendinţă la sistematizare, supradenivelare de segment ST de 2,5mm cu undă T bifazică din V2 până în V4; iar la externare ritm sinusal, segment ST izoelectric şi undă T bifazică din V2 până în V4.

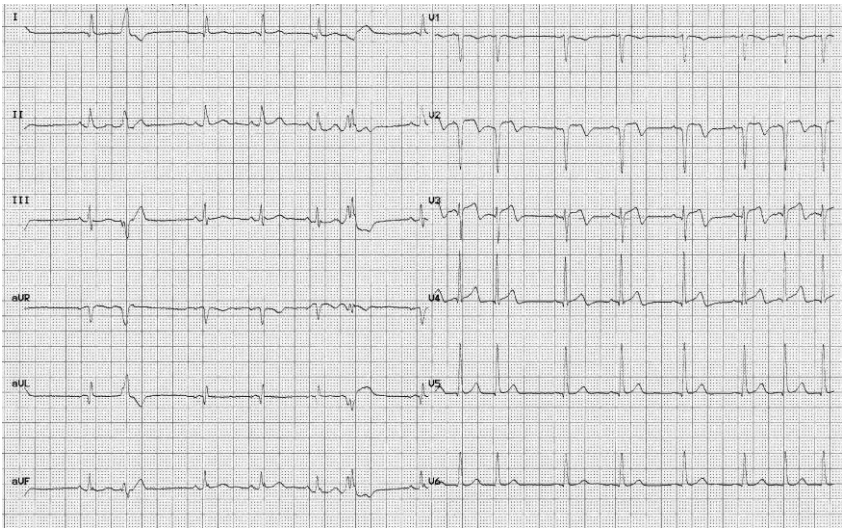

Fig. 1. EKG la internare: extrasistolie supraventriculară şi ventriculară şi modificări ischemo-lezionale în teritoriul antero-septal

Ecocardiografic: valvă aortică cu închidere şi deschidere corectă, valvă mitrală cu deschidere corectă şi închidere incompletă, cu mic prolaps. Insuficienţă mitrală gradul I, insuficienţă tricuspidiană gradul I/II prin dilatarea inelului tricuspidian. Vena cavă inferioară nedilatată (16mm), colaps inspirator complet, presiunea sistolică a arterei pulmonare 38 mmHg - hipertensiune pulmonară uşoară. Atriul stâng cu dimensiuni normale, crosa aortei, aorta abdominală şi aorta ascendentă de dimensiuni normale.

Absenţa vegetaţiilor.

Ecografia abdominală evidenţiază colecist cu multiple imagini hiperreflectogene, cea mai mare peste 11mm şi alte imagini de adiţie aderente de perete- polipi colesterolotici. Prostata global mărită, asimetrică cu întinse zone de necroză.

Consultul urologic decelează adenom periuretral.

Examenul clinic coroborat cu examenele paraclinice orientează către următoarele diagnostice:

1. Neutropenie febrilă severă de cauză toxică (expunere pasivă la pesticide).
2. Hepatită toxica post-expunere la pesticide.
3. Angină eritematoasă.
4. Angină pectorală agravată.
5. Hipertensiune arterială aterosclerotică.
6. Status post-accident ischemic tranzitor.
7. Adenom periuretral.
8. Polipi colesterolotici vezicula biliară.

DISCUŢII

Datorită vârstei înaintate şi a severităţii neutropeniei prognosticul a fost rezervat încă de la internare. Reducerea progresivă şi semnificativă a globulelor albe şi a neutrofilelor a impus introducerea imediată (după recoltarea uroculturii, hemoculturii şi a exudatului faringian) a antibioterapiei în triplă asociere şi cu spectru larg vizând şi anaerobi (Metronidazol 250mg/zi, Cefort 1gx2/zi şi Tazocin 4,5gx3flacoane/zi).

De asemenea, severitatea afectării liniei granulocitare a necesitat consult hematologic opiniându-se pentru stimulare medulară cu Metilprednisolon i.v. şi factor de stimulare a granulocitelor (Neupogen 48 MUI/0,8ml/zi timp de trei zile). Pacientul a fost izolat restricţionându-se accesul vizitatorilor.

Pentru afecţiunea cardiacă a primit tratament anticoagulant (Fraxiparină), tratament coronarodilatator (ISDN), α şi β-blocant (Carvedilol) cu evoluţie favorabilă a aspectului EKG, menţionând că pacientul a fost permanent asimptomatic.

Pentru deshidratarea secundară febrei a fost hidratat zilnic pe toată durata spitalizării.

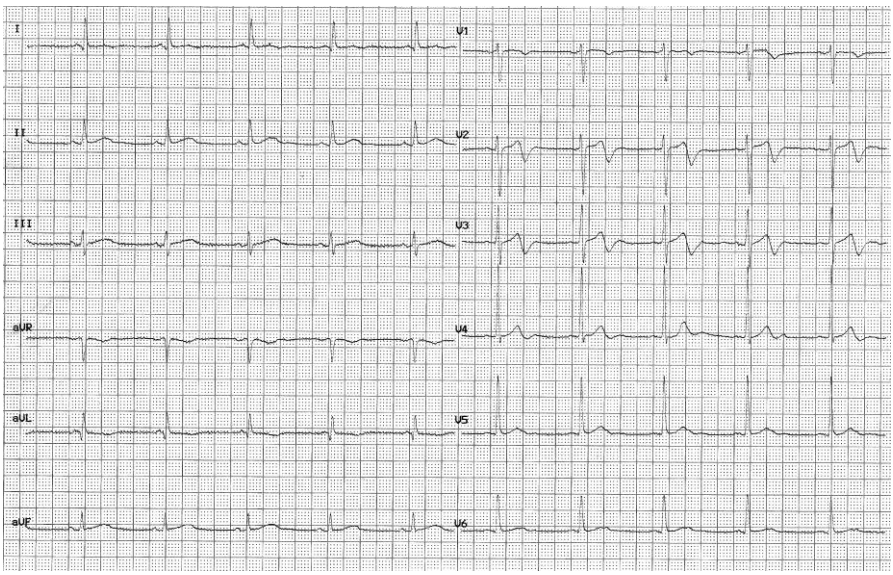

Fig. 2. EKG la externare: unde T ischemice

Evoluţia a fost lent favorabilă cu obţinerea afebrilităţii sub tratament antibiotic şi creşterea progresivă a numărului de globule albe după stimularea cu Metilprednisolon şi factor de stimulare a granulocitelor (GA= 6980/mm³, Neutrofile= 49%).

Probele hepatice s-au normalizat la controlul efectuat la externare considerând că afecţiunea hepatică a fost secundară expunerii pasive la pesticide.

Pacientul a fost externat după 17 zile de la internare fiind afebril, cu hemoleucograma, funcţia hepatică, renală şi electrocardiograma (Fig.2) în limite normale, apetit îmbunătăţit şi autonomie păstrată. La trei şi la şase luni de la externare controlul hematologic a fost în limite normale.

PARTICULARITATEA CAZULUI

Constă în vârsta înaintată, sexul masculin (ştiind că afecţiunea apare mai frecvent la adultul cu vârsta cuprinsă între 30-70 ani şi îndeosebi la femei) şi răspunsul prompt la administrarea de factor de stimulare a granulocitelor timp de trei zile, cu menţinerea normală a numărului de leucocite (pe toate liniile) la trei şi la şase luni de la administrare.

De notat reacţia particulară după expunerea pasivă la pesticide deşi nu sunt citate în literatură cazuri de medulotoxicitate secundară.

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TUMORILE OVARIENE CU POTENȚIAL MALIGN SCĂZUT (TUMORILE OVARIENE BORDERLINE)

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OVARIAN TUMORS OF LOW MALIGNANT POTENTIAL (BORDERLINE OVARIAN TUMORS) (Abstract): Ovarian borderline tumors are rare, their good prognosis depending on their stage at the time of diagnosis, and the presence of invasive implants. There is little information on tumor type identified intra-surgically, as well as on the most favorable treatment in borderline ovarian tumors. **Aim:** To determine the criteria of identification and pre-surgery and intra-surgery differentiation of the ovarian borderline tumors from the invasive carcinoma and benign ovarian tumors. **Material and methods:** This study included 54 patients with TPMS (ovarian borderline tumors) surgically treated in the past 22 years (January 1988-December 2009) at the 4th Gynecological Clinic of the Iasi "Gr. T. Popa" University of Medicine and Pharmacy. In this interval 1,107 ovarian tumors: 575 benign, 478 malignant, and 54 TPMS (4,87%) were recorded. The age of the patients with borderline tumors ranged between 20 and 78 years, mean age 46 years, and the histological types were: mucinous (27 cases), serous (18 cases), mixed (8 cases), and Brenner tumor (1 case). **Results:** We have analyzed the distribution of these cases according to the time when surgical treatment was performed. The frequency of borderline ovarian tumors in our study is 4,87%, lower than in the literature. We believe that this low percentage in our study is due to missing the microscopic data in some macroscopic benign tumors. **Conclusions:** By this research we aimed at elaborating a therapeutic strategy for each case using with discernment the modern treatment (surgery, chemotherapy, radiotherapy), as well as new chemical drugs with the goal of obtaining better results and longer survival. There are no tumor markers which could predict the progression of a borderline ovarian tumor to invasive tumors, but the invasive course is only 0,7%. **Key words:** BORDERLINE TUMOR, INVASIVE AND NON-INVASIVE TUMOR

Tumorile ovariene cu potențial malign scăzut (TPMS) sau tumorile ovariene borderline au fost descrise inițial de H.C. Taylor (1929) şi clasificate de F.I.G.O. în 1973(1). Caracteristicile acestui tip tumoral sunt: a) proliferare epitelială, formare de papile, pseudo-stratificare; b) prezența unor elemente de morfologie malignă ca atipii nucleare, activitate mitotică intensă; c) absența invaziei stromale adiacente (imagine strict caracteristică). TPMS sunt entități rare, cu un prognostic bun, dependent de stadiul tumoral în momentul diagnosticului (2). Frecvența lor variază între 9% şi 20% din toate tumorile epiteliale ovariene. Diagnosticul se pune frecvent în stadii inci-

piente, supraviețuirea mai mult de 5 ani ajungând până la 100%(3).

MATERIAL ŞI METODĂ

Sunt prezentate 54 de cazuri cu TPMS înregistrate în Clinica a-IV-a Ginecologie a U.M.F. "Gr. T. Popa" Iaşi, în perioada ianuarie 1988-decembrie 2009. Au fost analizați următorii factori: vârsta, antecedentele, stadiul tumoral, localizarea, dimensiunile tumorale, starea capsule, tipul histologic, microinvazia, tratamentul, prognosticul. În intervalul precizat, în Clinica a-IV-a au fost operate 1107 tumori ovariene astfel: 575 benigne, 478 maligne, 54 TPMS (4,87%). Vârstele limită ale tumorilor borderline au fost 20, respectiv 78 ani, iar vârsta medie 46 de ani (tab. I şi tab. II).

TABELUL I

Distribuția pacientelor din studiu după criteriul vârstei, pe intervale de vârstă şi după vârstă principală, medie, minimă şi maximă la Clinica a-IV-a Iaşi

Nr. crt.	Vârsta (ani)	Paciente (număr)	Paciente (procent)	Vârsta minimă	Vârsta medie	Vârsta maximă
1	15 - 25	7	12.9%	20		
2	25 – 35	6	11.11%			
3	35 – 45	12	22.22%			
4	45 - 55	12	22.22%		46	
5	55 – 65	11	20.37%			
6	65 – 75	4	7.4%			
7	75 - 85	2	3.78%			78
8	TOTAL	54	100%			
9	Vârsta principală	55				

TABELUL II

Prevalența cazurilor de TPMS în relație cu numărul de tumori de ovar maligne, respectiv benigne operate la Clinica a-IV-a Iaşi în perioada ianuarie 1988-decembrie 2009

Nr. crt.	Anul tratamentului chirurgical	Total cazuri TPMS	Nr. crt.	Anul tratamentului chirurgical	Total cazuri TPMS
1	1988	--	12	1999	2
2	1989	2	13	2000	2
3	1990	5	14	2001	3
4	1991	--	15	2002	--
5	1992	2	16	2003	1
6	1993	2	17	2004	7
7	1994	2	18	2005	1
8	1995	1	19	2006	2
9	1996	--	20	2007	2
10	1997	1	21	2008	3
11	1998	3	22	2009	13

În privința mediului de proveniență: 21 de cazuri (39%) din mediul rural. Principalele tipuri histologice erau: seros (18 cazuri) Fig.1., mucinos (27 cazuri) fig.1., mixte (8 cazuri) şi o tumoră Brenner (1 caz) fig. 3. (4).

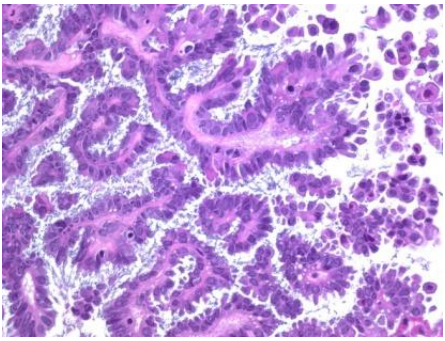

Fig. 1. TPMS seroasă (x10)

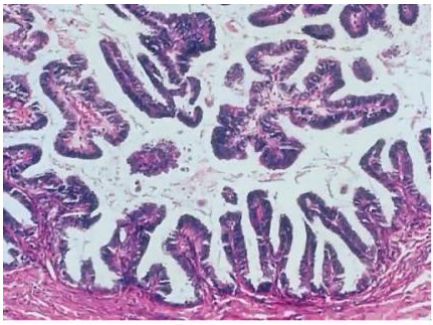

Fig. 2. TPMS mucinoasă (x10)

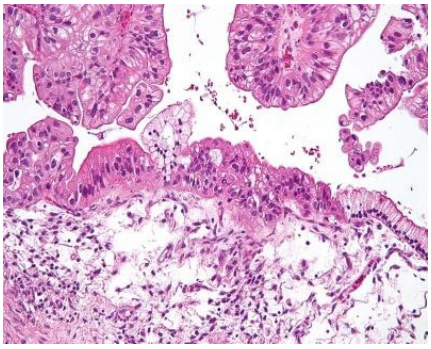

Fig. 3. TPMS Brenner (x10)

REZULTATE

Datele obținute sunt prezentate în Tabelul III şi prelucrate grafic şi în histogramă în fig. 4 şi fig. 5 pentru TPMS în stadiul I, fig. 6, fig. 7 pentru TPMS în stadiul II/III/IV, fig. 8 şi fig. 9 pentru număr şi procent total de TPMS şi fig. 10, fig. 11, fig. 12 pentru alte tipuri de TPMS în stadiul I, stadiul II/III/IV. Tratamentul a fost exclusiv chirurgical, stadiul în momentul diagnosticului permițând această conduită. A fost practicat examenul citologic peritoneal (din fluid sau după lavaj) (5) la toate cazurile.

Am analizat distribuția numărului de cazuri de TPMS în funcție de perioada de timp în care s-a fost efectuat tratamentul chirurgical şi am prezentat rezultatele în tabelul IV. Am reprezentat apoi grafic numărul total de cazuri cu TPMS în funcție de anul corespunzător în fig. 14, în histogramă tridimensională în fig. 15 şi în distribuție procentuală în fig. 16.

Proprietățile TPMS intraoperator sunt descrise în Tabelul V şi în fig. 16, fig. 17, şi fig. 18.

Tipurile de intervenție chirurgicală practicate (6) au fost: histerectomia totală (subtotală în 5 cazuri la cererea pacientelor), anexectomia bilaterală (3 cazuri), omentectomia (16 cazuri), limfadenectomia pelvină (6 cazuri). Tratamentul standard este histerectomia totală cu anexectomie bilaterală. Chirurgia conservatoare (aplicată în 3 cazuri) s-a aplicat în scopul prezervării capacității fertile. Urmărirea evoluției postoperatorii în cazurile tratate conservator este esențială (7).

TABELUL III

Comparație între rezultatele obținute în prezentul studiu cu principalele studii recente referitor la cazurile cu TPMS operate în funcție de valorile CA125

Nr. Crt	Referințe studiu pe tipuri histologice de TPMS	Nr. Pacienți	Stadiul I Peste 35U/ml	Stadiul I Peste 35U/ml	Stadiul II/III/IV Peste 35U/ml	Stadiul II/III/IV Peste 35U/ml	Total Peste 35U/ml	Total Peste 35U/ml
1.	Tamakoshi et al.(1996)	93	41/73	56%	14/20	70%	55/93	59%
	TPMS seroase	41	18/27	67%	10/14	71%	28/41	68%
	TPMS mucinoase	52	23/46	50%	4/6	67%	27/52	52%
2.	Gotlieb et al. (2000)	54	19/36	53%	11/13	85%	30/49	61%
	TPMS seroase	39	15/26	58%	13/16	81,25%	29/39	74,35%
	TPMS mucinoase	10	3/10	33%	0	0%	3/10	33%
	Alte tipuri TPMS	5	1/5	20%	1/5	20%	2/5	40%
3.	Ayhan et al. (2007)	60	20/50	40%	9/10	90%	29/60	48%
	TPMS seroase	38	14/30	45%	8/8	100%	22/38	58%
	TPMS mucinoase	22	6/20	30%	1/2	50%	7/22	32%
4.	Johan Bulten, Eva Kolwijck et al. (nov.2009)	123	46/112	41%	11/11	100%	57/123	46%
	TPMS seroase	43	20/33	61%	10/10	100%	30/43	70%
	TPMS mucinoase	80	26/79	33%	1/1	100%	27/80	34%
5.	Analiza Pooled	325	126/271	47%	45/54	83%	171/325	53%
	TPMS seroase	161	68/116	59%	39/45	87%	107/161	67%
	TPMS mucinoase	164	58/155	37%	6/9	67%	64/164	39%
6.	Prezentul studiu la Clinica IV (aprilie 2010)	54	14/54	26%	17/54	31,48%	31/54	57,4%
	TPMS seroase	18	7/18	38,89%	7/18	38,88%	14/18	77,78%
	TPMS mucinoase	27	7/27	26%	8/27	29,63%	15/27	55,56%
	Alte tipuri TPMS	9	1/9	11,12%	2/9	22,22%	3/9	33,33%

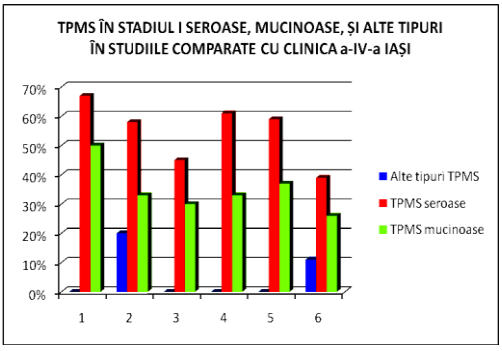

Fig. 4. Valorile procentuale TPMS st.I Clinica a-IV-a și în studiile comparate

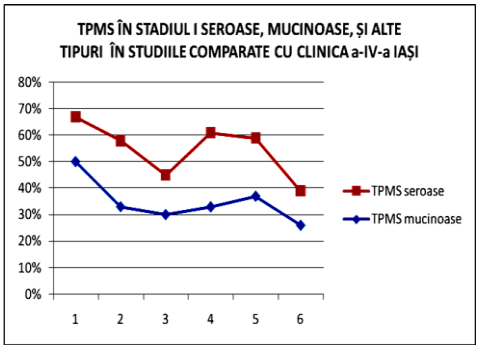

Fig. 5. Evoluția indicelui procentual TPMS stadiul I în studiile analizate

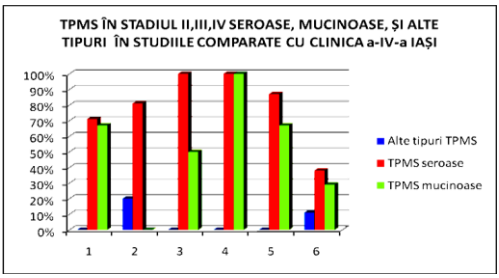

Fig. 6. Valorile procentuale TPMS st.II, III, IV Clinica a-IV-a şi studiile comparate

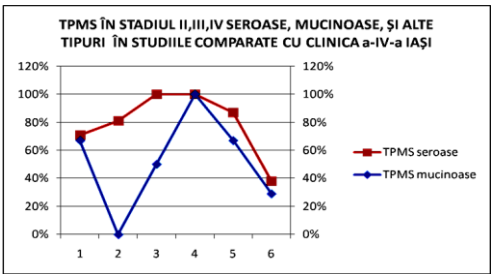

Fig. 7. Evoluţia indicelui procentual TPMS stadiul II, III, IV în studiile analizate

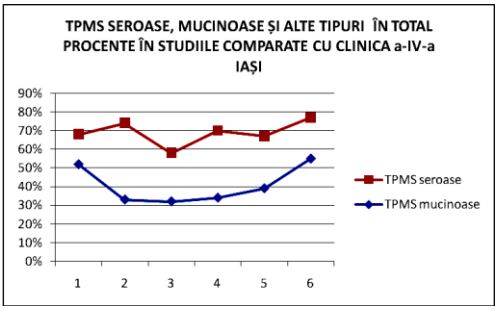

Fig. 8. Indice procentual total TPMS seroase şi mucinoase Clinica a-IV-a şi studiile comparate

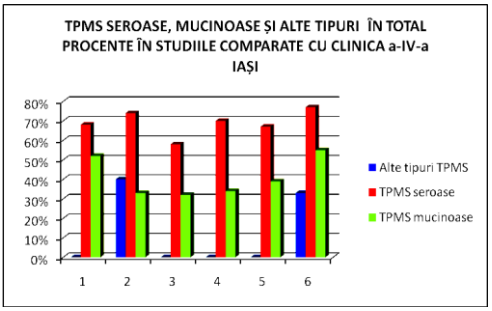

Fig. 9. Valorile procentuale totale TPMS Clinica a-IV-a şi în studiile comparate

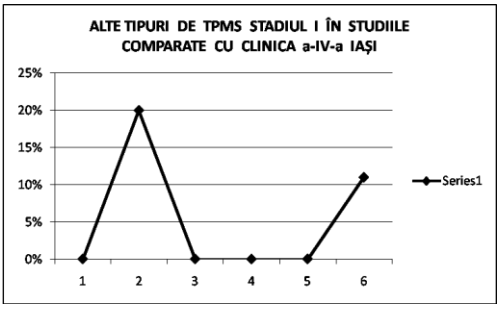

Fig. 10. Evoluţia indicelui procentual TPMS stadiul I alte tipuri în studiile analizate

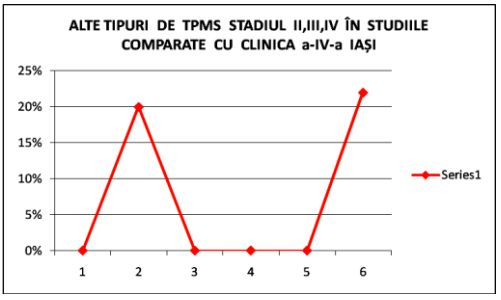

Fig. 11. Evoluţia indicelui procentual TPMS alte tipuri stadiul II, III, IV în studiile analizate

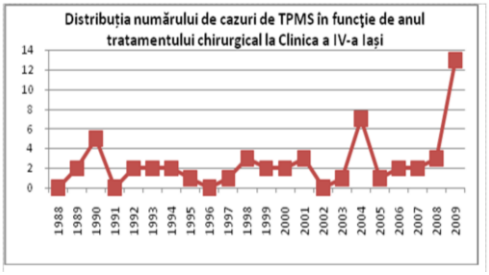

Fig. 12. Indice procentual total TPMS alte tipuri Clinica a-IV-a

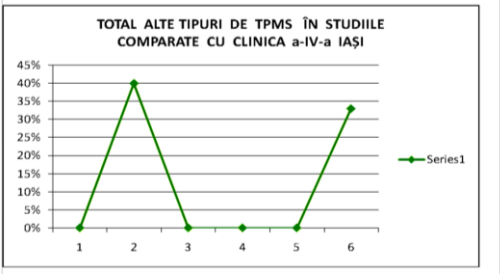

Fig. 13. Distribuţia numărului de cazuri comparate TPMS în funcţie de ani Clinica a-IV-a Iaşi

TABELUL IV

Repartiția cazurilor TPMS pe anii tratamentului chirurgical la Clinica a-IV-a Iași

Nr. Crt.	Tumori ovariene tratatela Clinica a-IV-a Ia și	Paciente Număr	Paciente Procent
1	Borderline (TPMS)	54	4.87%
2	Maligne (Neoplasm)	478	43.18%
3	Benigne	575	51.95%
4	TOTAL	1107	100%

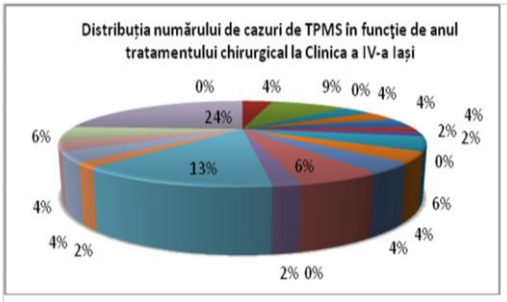

Fig. 14. Distribuția cazurilor TPMS în funcție de anul tratamentului chirurgical Clinica a-IV-a

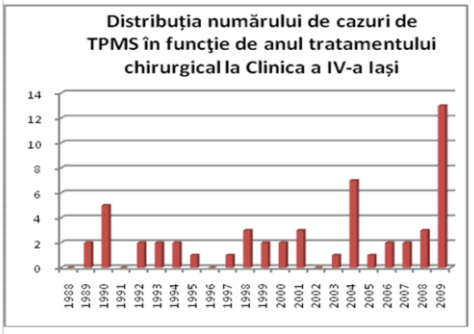

Fig. 15. Evaluarea procentuală a cazurilor TPMS în funcție de anul tratamentului

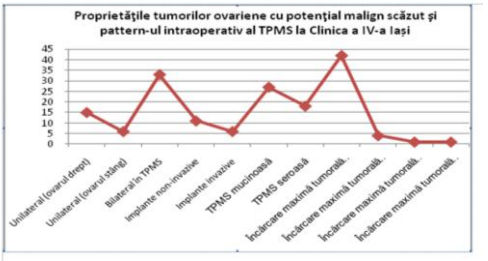

Fig. 16. Evoluția indicelui de diseminare intraoperator a pattern-ului tumoral Clinica a-IV-a

TABELUL V

Rezultatele intra-operator cu proprietățile și tipurile de TPMS la Clinica a-IV-a Iași în perioada 1988-2009

Nr. crt.	Proprietățile și tipurile de TPMS intra-operator	Paciente (n=54)	Paciente Procent %
1	Unilateral (ovarul drept)	15	27,57%
2	Unilateral (ovarul stâng)	6	11,76%
3	Bilateral în TPMS	33	61,12%
4	Implante non-invazive	11	20,37%
5	Implante invazive	6	11,76%
6	TPMS mucinoasă	27	50,00%
7	TPMS seroasă	18	33,33%
8	Încărcare maximă tumorală în câmpul B1	42	82,35%
9	Încărcare maximă tumorală în câmpul B1 și B2	4	7,8%
10	Încărcare maximă tumorală în câmpul B3	1	1,96%
11	Încărcare maximă tumorală în câmpul A3	1	1,96%

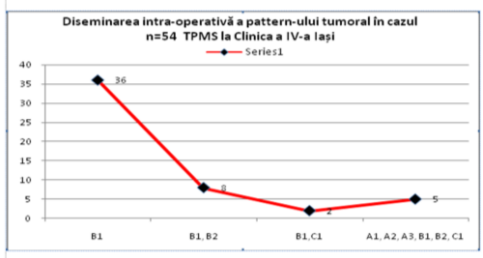

Fig. 17. Graficul de variație a proprietăților TPMS în funcție de pattern-ul tumoral

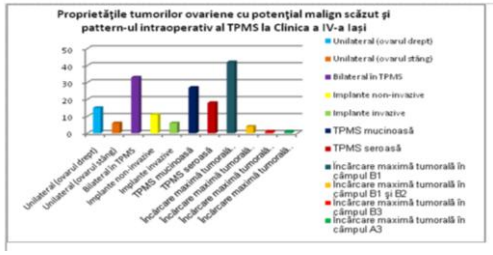

Fig.18. Distribuția cazurilor TPMS la Clinica a-IV-a Ia și în funcție de proprietăți și pattern-ul tumoral în perioada 1988-2009

DISCUȚII

TPMS formează o entitate distinctă. Termenul „borderline” poate crea o senzație de falsă securitate, motiv pentru care se impune precizarea că aceste cazuri trebuie privite drept cazuri cu cancer ovarian. Stadializarea trebuie făcută cu ocazia laparotomiei inițiale (8). Acest principiu este greu de respectat pentru că tumorile se descoperă în majoritatea cazurilor în stadiul I. Tumorile au aspect de benignitate, apartenența la categoria TPMS fiind stabilită ulterior microscopic. Preoperator, diagnosticul clinic este la fel de improbabil (9). Sonografia este utilă, iar rolul markerilor tumorali nu este precizat (10). Evaluarea chirurgicală este necesară pentru a exclude prezența leziunilor extrapelvine şi pentru realizarea diferențierii între implantele invazive şi a celor non-invazive (11). În observația noastră doar 6% din cazuri au prezentat implante invazive. Aceste implante pot influența ratele de supraviețuire şi pot indica un tratament chimic. Citologia peritoneală nu are o mare fiabilitate în estimarea prezenței implantelor (12). Semnificația implantelor peritoneale ramâne neclară, iar răspunsul la terapia adjuvantă pare a fi minim sau absent, prognosticul nefiind în mod evident influențat. Tumorile seroase sunt cele mai comune, dar în statistica noastră cel mai frecvent a fost tipul mucinos. Putem afirma că titrurile CA125 mijlocii sau ridicate au fost mai frecvente la tumorile seroase şi la cele diagnosticate în stadii mai avansate. Considerăm că diferențierea preoperatorie bazată pe nivelele CA125 este dificilă. Frecvența TPMS de 4,87% observată în studiul nostru este mai mică decât procentajele afi şate în literatură. Presupunem că această incidență redusă poate fi explicată de lipsa catalogării microscopice a cazurilor considerate macroscopic benigne. Vârsta medie a cazurilor (46 ani) este mai ridicată decât cea de 40 de ani citată în alte studii (13). Această constatare este corelată atitudinii terapeutice (14)(după vârsta de 40 de ani am recomandat histerectomia totală cu anexectomie bilaterală) (15). Tratamentul conservator a fost recomandat cazurilor ce au dorit să- şi păstreze capacitatea fertilă, în cuno ştință de cauză şi cu obligativitatea controlului post-operator. Într-un studiu publicat în Germania (G. Schumacher et al. 2009)se arată că în țările din vestul Europei se diagnostichează anual cca 4000 cazuri de TPMS. Frecvența cea mai mare ar fi la grupa de vârstă 15-29 ani (16) (diferit de ceea ce am constatat noi). Prognosticul TPMS este favorabil. Principalii factori de prognostic sunt: stadiul chirurgical, uni sau bilateralitatea, tipul histologic. Nu există markeri cu valoare predictivă pentru TPMS care evoluează spre invaziv. O analiză a 22 de serii de cazuri (total 953) a ajuns la concluzia unei rate de supraviețuire de 92%. În stadiile inițiale, transformarea invazivă se observă rar (0,7%).

CONCLUZII

Criteriile microscopice necesare încadrării tumorilor ovariene în categoria de TPMS sunt: a) hiperplazia epitelială (stratificare, aspect cribriform, micropapilar); b) atipie u şoară sau moderată; c) activitate mitotică minimă; d) absența invaziei stromale. Existența focarelor de carcinom intraepitelial nu modifică prognosticul, dar este importantă pentru tratament.Tipul micropapilar influențează negativ prognosticul. Procentajele supraviețuirilor postterapeutice la 5 ani sunt în stadiul I până la 100%. Prognosticul nu diferă semnificativ în raport cu terapia utilizată (chirurgie – metodă unică, chirurgie şi tratament adjuvant). Supravegherea post-terapeutică îndelungată este obligatorie.

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CANCER GENETIC COUNSELING. PART I - WESTERN EUROPEAN COUNTRIES

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CANCER GENETIC COUNSELING. PART I-WESTERN EUROPEAN COUNTRIES (Abstract): Genetic counseling services represent a relatively new concept aimed to identify patients or relatives with hereditary predisposition for cancer susceptibility and to act accordingly for early diagnosis, cure and prevention. It has become an accepted part of oncology care in many European countries. Unfortunately, despite the same state of knowledge, the patients from different European countries would experience different care. This situation is not in accordance with the European Union directives, which are enacted to implement and protect the idea of free movement of goods, services, people and capital. This study aimed to examining the current practice of genetic counseling for cancer in different European countries, with special accent on familial breast and ovarian cancer management as the most studied and managed hereditary cancer. We also sought to ascertain the needs for the unitary politics in cancer genetics services, to decrease the difference in cancer genetic counseling opportunity and facility. As a first step we compared the national guidelines regarding genetic counseling for familial/hereditary breast and ovarian cancer in some countries from Western Europe. All guidelines recommend embedding genetic testing within a framework of genetic counseling, and all agree to perform genetic testing first in an affected person. However, we found some differences regarding the thresholds, detailed description of selection criteria, the risk calculation methods, the age of diagnosis, the relationship with counselor and physician etc. There are also many open questions that are not covered by the guidelines, for instance: how to deal with phenocopies, unclassified variants, and newly identified breast cancer susceptibility genes or with family that not fitting the criteria, that need to be discussed. New evidence is usually slowly integrated into the guidelines. An exchange process towards the harmonization of the guidelines will ensure high quality health care across Europe. **Keywords:** HEREDITARY BREAST AND OVARIAN CANCER; BRCA, GENETIC COUNSELING.

Cancer genetic counseling represents one of the most innovative new concepts in healthcare. Worldwide, the number of cancer cases is 18.1 million of which 2.1 million new cases of breast cancer, 1.8 million colorectal cancers and 295,414 ovarian cancers. Worldwide, cancer is the second leading cause of death with 9.5 million deaths reported for the year 2018 by *International Agency for Research on Cancer* (IARC). Epidemiological data shows that in Europe, 4.2 million new cases of cancer were reported with 1.9 million new cases in women and 2.3 million new cases in men, according to IARC. Of the total cases recorded in Europe, ovarian cancer encoun-

tered for 67,771 new cases, being responsible for 44,576 deaths. Breast cancer is registered with a total of 522,513 new cases, with a number of 137,707 deaths (1).

Each day, worldwide, millions of people fight a battle against cancer, undergoing devastating therapies that hugely impact on their *Quality of Life*. Over the next two decades, the number of new cases is expected to rise by about 70%. Oncogenetics can contribute to change this future scenario, addressing those people for which prevention is still possible and the disease's onset avoidable. This medical discipline, in progressive expansion, is focused on understanding and monitoring the genetic predispositions of persons at risk, due to mutations or family histories of cancer. The implementation of evidence-based prevention strategies will lead to considerable improvements in clinical decisions and outcomes. In order to move towards this predictive system, it is essential to raise awareness of those vulnerable people and to empower them. On this basis, it will be possible to facilitate an appropriate checking and an early detection of prodromes, as well as the modification of harmful habits and behaviors (2).

The objective of oncogenetics is to understand genetic predisposition to cancers and care for persons at risk. Genetic predispositions are often associated with a family history of cancer. However, cancers are very frequent and family histories exist, so a family history of cancer is not synonymous with a genetic predisposition. The purpose of a genetic consultation is to determine the share of family history and possible predisposition. Genetic tests, stills seldom practiced, sometimes confirm a hereditary origin. If an alteration is identified in a family, it can be sought in its relations. This makes it possible to reassure those having no predisposition and following up those at risk (3).

However, the realization of the full potential of oncogenetic counseling requires close collaboration between diverse kind of specialists and institutions. These include researchers, clinicians, research institutions, healthcare providers, research and technological development funding agencies, public health agencies, policy makers, industry, regulatory authorities, health insurers and, crucially, the citizen. Genetic testing is already finding its way into several specific clinical applications. Breast cancer, for example, is one of disease in which enormous progress has been made in this regard (3, 4).

However, as things presently stand, the full potential of cancer genetic cancelling cannot be realized in all European countries for several reasons. These include fragmentation of European efforts, nationally and regionally restricted activities, and a lack of concerted approaches in the different areas of ontogenetical services. In addition, In Europe, despite efforts in harmonizing standards for genetic testing and further patients care management problems arise mostly due to differences in countries' health care systems and service delivery settings. Therefore, to advance ontogenetical services, it is likely to achieve strategic interaction between key European players. These include decision-makers in diverse scientific disciplines, research policy and funding, patient interest groups, different national healthcare systems, regulatory and governmental bodies and private enterprise. Only then can a well-balanced and successful development of cancer genetic services be achieved (2).

This review aimed to examining the current practice of cancer genetic counseling in different European countries with special accent on familial breast and ovarian cancer management as the most studied

and managed hereditary cancer disease. We also sought to ascertain the needs for the unitary politics in cancer genetics services.

Cancer care and genetic counseling development in Western Europe

Cancer care has always been part of the health care provided within national health systems across the EU, but only in the last 20 to 25 years, more systematic approaches formulating and applying the comprehensive national policies or plans specifically directed at improving the organization of services for people with cancer have been adopted in some countries. The rationale is that outcomes can be greatly improved by more effective clinical organization and better operational delivery of cancer services (3).

The first explicit attempt to prepare a comprehensive national cancer policy was published by the Chief Medical Officers of England and Wales in 1995 (2). The Calman-Hine report, named "Responding to the challenge of cancer in Europe after the Chief Medical Officers involved" was accepted by government as the basis for the future provision of cancer services in the United Kingdom. The first comprehensive national cancer plans followed a few years later in Denmark (National Board of Health, 2000), England (Department of Health, 2000) and France (French National Cancer Institute, 2003). All began around 2000 (3).

The next logical step was to create Cancer Centers because it could offer more expertise to treat different types of cancer at high standards and would provide sustainability to use expensive equipment for treatment and research. In this respect it can be mentioned National Institute for Health and Clinical Excellence (NICE), in UK, French National Cancer Institute (Institute National du Cancer (INCa) in France, German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC include 12 cancer centers in Germany) - there are a few examples of the oldest Cancer Institutions in Western Europe that provide both research and clinical services (3).

Since 2000, in the most of western European countries testing for hereditary cancer susceptibility has become an integral part of medical management. At-risk individuals can be provided with regular surveillance to identify cancer at an early stage. Prophylactic surgery aims to prevent the development of cancer, and moving in the direction of personalized medicine, targeted therapies for affected mutation carriers become available. The essential step towards a rational approach is how to identify individuals who will benefit from testing, without straining the financial budget of the national health system, although recently established techniques like next generation sequencing may significantly reduce the costs (4).

Meanwhile, guidelines have been established on how to identify individuals at risk for familial breast cancer. In UK the National Institute for Health and Clinical Excellence (NICE), develop guidelines for different issues. The NICE clinical guideline (CG) 41 was created in 2004 and focuses on "Familial breast cancer: the classification and care of women at risk of familial breast cancer in primary, secondary and tertiary care". In France, access to cancer genetic services and testing is covered by the National Health Care System and since 2003 through the initiative of the Cancer Plan. "The Cancer Plan 2009-2013" has been developed by the French Ministry of Health in coordination with the French National Cancer Institute (Institut National du Cancer (INCa)). It incorporates the knowledge on hereditary predisposition to cancer within the rubric "Oncogénétique"

http://www.e-cancer.fr/soins/prises-en-charge-specifiques/oncogenetique/ (5, 6).

In the Netherlands "Erfelijke Tumoren: Richtlijnen voor Diagnostiek en Preventie" 2010 was published by the Netherlands Foundation for the Detection of Hereditary Tumors (STOET) and the Dutch Society of Clinical Geneticists (VKGN) (2). In Germany, the German Consortium for Hereditary Breast and Ovarian Cancer (GC-HBOC) was established in 1996 as a cooperative research project supported by the German Cancer Aid (Deutsche Krebshilfe). Twelve university-based interdisciplinary centers developed a standardized approach for the management of families with hereditary breast and ovarian cancer. This concept, including the genetic counselling, the molecular genetic diagnostics and the clinical surveillance, became part of the regular health care system in 2005 (3, 6).

In all these countries *genetic diagnostics* is available to search for mutations in the breast cancer genes *BRCA1* and *BRCA2* (3, 4, 5). Although a similar frequency of mutation is assumed each country has established its own genetic testing including criteria. The individual guidelines and recommendations are roughly similar for most selection criteria. All guidelines recommend embedding genetic testing within a framework of genetic counseling, and all agree to perform genetic testing first in an affected person. The access to genetic counseling differs within the countries. While in the UK and the Netherlands for referral from primary care, a general practitioner or secondary care, a specialist is required, in France and Germany referral is usual, but not obligatory. An individual also has direct access to genetic counselling. In this case, difficulties can arise for reimbursement (3).

According to French guidelines, in order to increase the percentage of detected *BRCA1/2* mutation carriers, the including criteria for genetic counselling have been extended and include isolated ovarian cancers diagnosed before the age of 70 and even at an older age (7, 8).

Regarding the approach towards genetic testing, it is recommended first to test the index patient. After a mutation is identified in index patient in all Western European countries, a predictive testing can be offered to healthy relatives from the beginning of a young adult age. The health insurance covers all genetic counseling and testing costs while the threshold for testing a family is quite different between the countries (6, 7).

Risk prediction models are known and used in each country; however, the purpose or reason for their use differs. For example, Tyrer-Cuzick or BOADICEA can be used as prediction tool in UK (9) but should not totally replace careful manual assessment of family trees by a genetic specialist. The Manchester scoring system was developed in 2003 and updated in 2009 to facilitate a simpler and more accurate selection of families for *BRCA1/2* testing (10). Although it is known that all available risk calculation models show considerable weaknesses (11, 12), Cyrillic 2.1 is a helpful tool and allows uniform decision-making across Germany. Thresholds of a risk of heterozygosity of ≥20% or a residual lifetime risk for breast cancer of ≥30% qualify a woman for management in the high-risk group (3).

Recommendations for the clinical management have also been developed. Intensified surveillance strategies including MRI screening and prophylactic surgical options are offered for people who caring a mutation in all western European countries.

There are some specific accents in different county guidelines. In the current French guidelines, the prenatal or preim-

plantation diagnostics could be allowed for people with cancer syndromes after a favorable assessment by a specific advisory board. The request for opinions of French cancer geneticists and prenatal center professionals can differ according to the type of hereditary cancer involved (2, 4).

Furthermore, the Dutch guidelines for clinical geneticists take into consideration the occurrence of phenocopies among breast tumors and deal with unclassified variants (UV) as genetic test results. It is suggested to perform segregation analysis within the family to clarify the effect of the UV (13).

In the German Act on Genetic Diagnostics (14) informed consent is defined as the main purpose of the law, which regulates the conditions for genetic analysis and utilization of genetic data to avoid disadvantages and discrimination. The right "not-to-know" is deeply anchored in the law and has the same value as the right "to-know". Before a genetic test can be initiated, the test person must be informed about the prospects and limitations of the test at-issue as well as about the consequences of the possible results and must agree to the test in written form. In the case of predictive genetic testing, pre- and post-test genetic counseling is mandatory, and refusal must be documented (3, 15).

CONCLUSIONS

Despite a real progress on genetic testing a further data management at the European level, there was a lack of consensus about the best way to carry out genetic counselling, which meant that there were many different approaches to this subject. Counseling has been an area that has had a widespread investigation, leading to each country coming up with its own legislations, guidelines and definitions. Due to this, at a European level, the EuroGenTest (www.eurogentest.org) was established to "*harmonize genetic testing across Europe.*" This was a tool developed to aid professionals of genetic counseling and allowing the creation of a network that would help in the sharing of experiences and information between health professionals also aids patients to understand their rights and to guide them through genetic testing and genetic counseling. A reasonable approach to harmonize the thresholds for inclusion criteria and other guidelines details would be beneficial. It could be a compromise between the strict family history-orientated NICE guidelines from the UK and the less constrained French criteria. The Dutch and German guidelines already represent something in between, so it could be a good start for guidelines harmonization. Only the Dutch guidelines take into consideration the phenomenon of phenocopies, and triple-negative tumors within *BRCA1*-associated breast cancer. There are still a lot of open questions as families with male relative or the situation that no index patient is available for testing that are not covered in any guidelines. A simple scoring method to classify the family, like the Manchester scoring system, seems to be a reasonable and an easy to use tool and suitable to assert throughout different European countries. Guidelines must be orientated on different levels and disciplines, and the access for every player involved should be thereby facilitated. General practitioners, clinical specialists, geneticists and researchers need different information and different support for decisions. An open communication process including all these topics seems most promising to react adequately to the current level of knowledge and to improve the quality of genetic counselling and testing for familial/ hereditary breast and ovarian cancer across Europe.

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SINDROMUL VESTIBULAR – ASPECTE PATOGENICE INTRICATE. PREZENTARE DE CAZ

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PERIPHERAL VESTIBULAR SYNDROME INTRICATED PATHOGENIC ISSUES. A CASE REPORT (Abstract): **Background**: Ménière's syndrome appears to be the final common pathway of the mechanisms the inner ear responds to almost any injury. Although the autoimmune disease seems to play a major role, exposure to intense noise can also be a trigger in the appearance and/or aggravation of the disease. **Material and methods**: The case of 41 years old musician with a history of ankylosing spondylitis, diagnosed with Ménière's syndrome 5 years ago, is presented. Recently the symptoms became more polymorphic, revealing the association between benign paroxysmal positional vertigo (BPPV), Ménière's syndrome and high frequency hearing loss in an autoimmune background. Besides general, neurological and ENT examination, the diagnostic workup comprised of tonal audiogram, brain stem auditory evoked potentials, computerized dynamic posturography and videonistagmography. **Results**: The final diagnosis was acute noise trauma, Ménière's syndrome, left horizontal semicircular canal BPPV, bilateral sensorineural sudden aggravated hearing loss in high frequencies (above 9 kHz) and allegedly autoimmune inner ear disease. Treatment with an association of corticosteroids, vasodilators and vitamins combined with Vannucchi's maneuver were followed by a significant relief of the symptoms. **Conclusion**: Although no causal relationship was found between acoustic trauma and increased endolymphatic pressure, both literature data and the case presented show that intense and prolonged noise exposure may aggravate peripheral vestibular syndrome. **Keywords**: MÉNIÈRE´S DISEASE, BENIGN PAROXYSMAL POSITIONAL VERTIGO, AUTO-IMMUNE DISEASE

Prosper Ménière a dat numele său unei afecţiuni a urechii interne ce se manifestă prin episoade recurente de vertij rotator, hipoacuzie fluctuantă şi acufene. Această afecţiune poate fi: idiopatică (boala Ménière) sau secundară unor variate afecţiuni ale urechii interne care au ca rezultat tulburarea mecanismului de producere sau resorbţie a endolimfei (sindrom Ménière).

Majoritatea autorilor consideră că boala Ménière se asociază în mod obligatoriu hidropsului endolimfatic (1). Studii recente au arătat că această asociere patogenică nu este obligatorie: unii pacienţi cu boala Ménière nu au hidrops endolimfatic, iar hidropsul endolimfatic fără asocierea simptomelor caracteristice bolii Ménière este mult mai frecvent identificat (6%), comparativ cu prevalenţa bolii Ménière de numai 0,2% (2).

Sindromul Ménière pare a fi modalitatea comună prin care urechea internă răspunde la aproape orice agresiune (sistemică sau locală). Există o relaţie de cauzalitate între afecţiunile autoimune şi sindromul Mé-

nière, cercetări recente evidenţiind rolul imunologic al sacului endolimfatic al urechii interne (3). Terenul autoimun poate cauza sau se poate asocia cu boala autoimună a urechii interne (hipoacuzie bilaterală lent progresivă, uneori fluctuantă, acufene, crize vertiginoase). Deşi incidenţa bolii autoimune a urechii interne este redusă, sub 1% din toate cazurile cu hipoacuzie şi vertij, la 16 % dintre pacienţii diagnosticaţi cu boală Ménière această afecţiune este secundară disfuncţiei autoimune (4).

Spondilita anchilopoetică face parte din categoria bolilor reumatice autoimune. În 28% din cazuri s-a observat asocierea acesteia cu apariţia hipoacuziei neurosenzoriale bilaterale (3).

Pe de altă parte, expunerea la sunete/ zgomote intense poate constitui un trigger în apariţia simptomelor caracteristice bolii Ménière. Astfel, în plan fiziopatologic, creşterea presiunii endolimfei, poate fi implicată în apariţia vertijului paroxistic poziţional benign (VPPB) prin dislocarea fragmentelor otoconiale şi deplasarea lor la nivelul unuia sau a mai multor canale semicirculare. Există pacienţi la care, în evoluţie, se pot asocia mai mulţi factori etiologici, în aceste cazuri diagnosticul fiind mai dificil.

PREZENTAREA CAZULUI

Prezentăm cazul unui pacient în vârstă de 41 ani, muzician. Acesta a prezentat, începând din anul 2005, crize de vertij rotator însoţit de greţuri şi vărsături, instabilitate ortostatică cu tendinţă de deplasare spre partea stângă. Crizele au avut următoarele caracteristici : debut brusc, durata 24-48 de ore, remisiunea manifestărilor acute fiind urmată de persistenţa unei senzaţii de ameţeală, epuizare fizică şi psihică. Consultul neurologic şi rezonanţa magnetică nucleară cerebrală, efectuate după prima criză, nu identifică existenţa unor elemente patologice. Simptomatologia s-a reprodus cu o frecvenţă de 1-2 crize/an până în septembrie 2009 când devine polimorfă prin apariţia în cadrul tabloului clinic a hipoacuziei şi acufenelor la nivelul urechii stângi, precum şi a senzaţiei de plenitudine aurală stângă. Pacientul s-a adresat serviciului nostru, unde a beneficiat de investigaţiile clinice şi paraclinice disponibile în momentul respectiv din care rezulta prezenţa unei funcţii auditive şi vestibulare normale. Şi de această dată explorările imagistice cerebrale (computer tomografie şi rezonanţă magnetică) nu au identificat elemente patologice. Din luna mai 2010 crizele pacientului au devenit mai frecvente, motiv pentru care revine în clinică. Dispozitivele achiziţionate recent permit efectuarea audiogramei pe frecvenţele înalte (care identifică o pierdere de auz bilaterală pe frecvenţele mai mari de 9000 Hz, simetrică, de 40-50dB) precum şi evaluarea funcţiei vestibulare cu ajutorul posturografiei dinamice computerizate (explorarea fiind efectuată înafara crizei nu obiectivează prezenţa unui deficit vestibular). După ultima criză, în august 2010, pacientul relatează, alături de simptomatologia descrisă mai sus, accentuarea bruscă a hipoacuziei şi a acufenelor la nivelul urechii stângi împreună cu evitarea decubitului lateral pe partea stângă, această poziţie agravând senzaţia vertiginoasă şi simptomatologia vegetativă.

Condiţiile de viaţă şi muncă prezintă o mare importanţă la acest pacient. Trebuie remarcat faptul că pacientul este muzician activând într-o orchestră, lucru important deoarece în mare parte crizele de vertij au debutat în timpul concertelor sau al repetiţiilor.

Dintre antecedentele personale patologice reţinem diagnosticul de spondilită anchilopoetică (în anul 2000) pentru care a urmat tratament cu salazopirină (timp de 6 luni) şi ulterior cu antiinflamatorii nesteroidiene.

Examenul clinic pe aparate şi sisteme împreună cu cel ORL au fost în limite normale. Evaluarea pacientului a inclus bilanţul

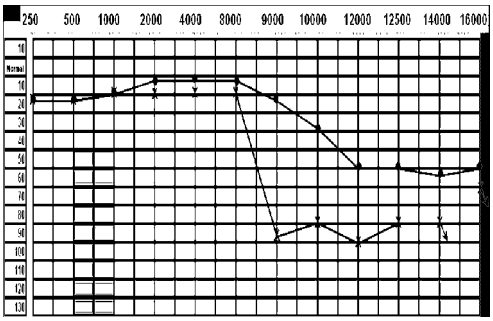

Fig. 1. Audiogramă extinsă – august 2010 (hipoacuzie brusc agravată pe urechea stângă)

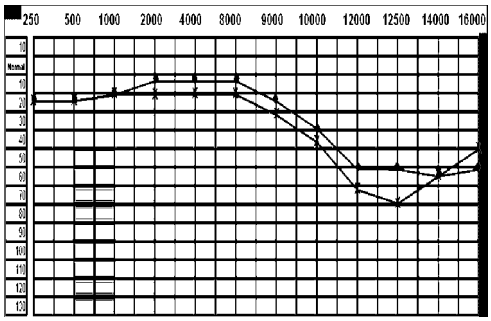

Fig. 2. Audiograma extinsă – mai 2010 (hipoacuzie simetrică bilaterală)

audiologic şi al funcţiei vestibulare. Astfel, la evaluarea audiologică s-au obţinut următoarele rezultate : timpanogramă tip A bilateral, audiogramă tonală liminară extinsă accentuarea deficitului auditiv la nivelul urechii stângi (fig.1) comparativ cu pragurile auditive identificate la prima audiogramă de acest tip efectuată în mai 2010 când pierderea auditivă avea un aspect simetric bilateral (fig.2).

S-au efectuat potenţiale evocate auditive de trunchi cerebral cu parametri în limite normale, fără a identifica elemente de suferinţă retrocohleară.

Evaluarea funcţiei vestibulare s-a realizat şi de această dată, înafara crizei. Nu s-a constatat prezenţa unui deficit vestibular în cadrul examenului clinic sau prin posturografia dinamică computerizată (PDC).

Videonistagmografia (VNG) aduce elemente noi prin studiul nistagmusului şi prin proba calorică. Se observă prezenţa nistagmusului poziţional declanşat de manevra de provocare McClure (rotirea capului aflat în poziţie orizontală spre părţile laterale). După efectuarea manevrei pentru partea stângă, se înregistrează, cu o scurtă perioadă de latenţă, nistagmus orizontal ageotrop ce se epuizează în 30 secunde (pe plan subiectiv pacientul relatează apariţia vertijului şi a senzaţiei de greaţă). Se formulează astfel diagnosticul de VPPB de canal semicircular orizontal (CSO) stâng. Prin proba calorică se identifică hipovalenţa vestibulului stâng.

Astfel, în urma analizării datelor anamnestice şi a rezultatelor investigaţiilor prezentate se formulează diagnosticul pozitiv de : traumă sonoră acută, sindrom Ménière, VPPB de canal semicircular orizontal stâng, hipoacuzie neurosenzorială bilaterală pe frecvenţe înalte (peste 9 kHz) şi observaţie de boală autoimună a urechii interne.

În acest context s-a considerat util tratamentul corticosteroid sub protecţie gastrică, vasodilatator şi vitaminic, alături de manevra de repoziţionare prelungită descrisă de Vannucchi (decubit lateral pe partea sănătoasă timp de 12 ore).

Evoluţia la finalul tratamentului a fost favorabilă : audiograma de control arată ameliorarea uşoară a hipoacuziei (10 dB), reducerea intensităţii acufenelor la nivelul urechii stângi, dispariţia nistagmusului poziţional la videonistagmografie.

DISCUŢII

La cazul descris mai sus putem scoate în evidenţă patru particularităţi care pretează la discuţii :

- formularea diagnosticului de sindrom Ménière şi a posibilei etiologii a acestuia
- rolul pe care mediul profesional îl are în declanşarea crizelor din sindromul Ménière
- dacă morfologia curbei audiometrice (cu afectarea frecvenţelor înalte, iniţial

simetrică şi bilaterală) se datorează terenului autoimun prezentat de pacient sau unei traume sonore cronice la care a fost expus prin natura profesiei sale

- intricarea patogenică în cadrul tabloului clinic de sindrom vestibular periferic între VPPB, sindromul Ménière şi boala autoimună a urechii interne

Este bine documentată în literatura de specialitate dificultatea stabilirii diagnosticului de boala/sindrom Ménière deoarece majoritatea pacienţilor nu prezintă la debut întreaga triadă simptomatică caracteristică acestei afecţiuni.

Putem include în această categorie şi cazul clinic prezentat : pacient cu crize de vertij de tip periferic dar fără acuzele cohleare caracteristice bolii Ménière (hipoacuzie neurosenzorială fluctuantă sau stabilă cu afectarea frecvenţelor grave). La acest pacient diagnosticul de sindrom Ménière a fost formulat respectând criteriile Academiei Americane de Otorinolaringologie pentru diagnosticul acestei afecţiuni (4). Conform acesteia, există o categorie aparte de pacienţi care prezintă numai simptome vestibulare (vertij asemănător celui din Ménière) fără asocierea hipoacuziei neurosenzoriale caracteristice. La aceştia se poate formula diagnosticul de posibilă boală Ménière monitorizarea evoluţiei urmând să constate întregirea tabloului clinic într-o perioadă variabilă de timp. Într-un studiu retrospectiv Jeffrey şi colaboratorii au observat că în grupul cu posibil Ménière vestibular existenţa hipoacuziei nu este exclusă, însă aceasta este atipică (5).

Obiectivarea prin teste specifice a bolii autoimune a urechii interne (dozări de anticorpi anticohleari, antiendoteliali, antisulfoglucuronozil) nu este posibilă datorită faptului că nu dispunem de metode fiabile cu un grad înalt de reproductibilitate care să fie utilizate pe scară largă. Suspiciunea de boală autoimună a urechii interne a fost ridicată pe baza anamnezei (pacient diagnosticat cu o afecţiune autoimună), aspectului audiogramei (obiectivarea unei hipoacuzii bilaterale cu afectarea frecvenţelor înalte, simetrice, lent progresive) şi a probei terapeutice.

Stabilirea unei relaţii de cauzalitate între expunerea ocupaţională la zgomot şi dezvoltarea unei disfuncţii vestibulare (vertij/sindrom Ménière) constituie o preocupare activă. Deşi studiile experimentale pe animale au arătat că în 35% cazuri trauma sonoră sau expunerea cronică la sunete de mare intensitate produce modificări histologice la nivelul labirintului vestibular asemănătoare hidropsului endolimfatic la om acesta relaţie nu a fost demonstrată (6). Totuşi prin expunerea pacienţilor diagnosticaţi cu boală Ménière la sunete grave de înaltă intensitate s-a observat o sensibilitate ridicată a acestora la aceasta categorie de sunete consecinţa expunerii fiind declanşarea simptomatologiei vestibulare posibil prin efect Tullio (energia sonoră activează receptorii vestibulari) (7). În cazul clinic prezentat expunerea profesională la zgomot este posibil să fi contribuit la creşterea frecvenţei de apariţie a crizelor bolii Ménière, fapt susţinut de anamneză şi de evoluţia favorabilă sub protecţie fonică.

Intricarea simptomelor vertijului paroxistic poziţional benign în tabloul clinic al acestui pacient este susţinută de datele din literatura de specialitate. Se arată că boala Ménière poate predispune pacienţii la apariţia episoadelor de VPPB. Repercusiunile histopatologice ale hiperpresiunii endolimfei asupra maculei utriculare şi saculare reprezintă, probabil, mecanismul care explică coexistenţa bolii Ménière şi a VPPB (8).

În general, la pacienţii cu sindrom Ménière unilateral, vertijul paroxistic poziţional benign a apărut pe aceeaşi parte. Doar rareori VPPB a afectat ambele părţi în cazul pacienţilor cu boala Ménière unilaterală (8). Explicaţia se regăseşte probabil în modificările histopatologice ce apar şi la nivelul urechii interne aparent indemne în

cazul pacienţilor cu boala Ménière unilaterală, modificări identificate prin studii histopatologice realizate pe oase temporale umane ale unor pacienţi diagnosticaţi cu această afecţiune (9).

CONCLUZII

Există pacienţi la care, în evoluţie, se pot asocia mai mulţi factori etiologici ai sindromului vestibular periferic, în aceste cazuri diagnosticul fiind mai dificil.

Cazul prezentat este particular atât datorită asocierii unor afecţiuni între care poate exista relaţie de cauzalitate (spondilita anchilopoetică, hipoacuzie bilaterală, sindrom Ménière, vertij paroxistic poziţional benign), cât şi prin activitatea sa (muzician) prin care se expune la o agresiune suplimentară asupra urechii interne, accentuând asocierea patogenică prezentată.

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HISTOLOGIC AND IMMUNOHISTOCHEMICAL ASSESSMENT OF GINGIVAL TISSUE'S CHANGES INDUCED BY PERIODONTAL DISEASE IN ASSOCIATION WITH CHRONIC HEPATITIS C

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HISTOLOGIC AND IMMUNOHISTOCHEMICAL ASSESSMENT OF GINGIVAL TISSUE'S CHANGES INDUCED BY PERIODONTAL DISEASE IN ASSOCIATION WITH CHRONIC HEPATITIS C (Abstract): Periodontal disease and chronic hepatitis C are two types of chronic inflammatory reactions that target the supporting tissues of the teeth and the liver tissue, respectively. Common pathogenic pathways may be shared by the two types of disease, which the current study **aims** to histologically assess within the gingival tissue. **Material and methods:** The study comprised of gingival tissue samples, originating from periodontal patients with asymptomatic chronic hepatitis C and from periodontal patients with no systemic illness (collected during periodontal surgery), as well as from healthy control subjects (collected during extraction of maxillary premolars for orthodontic reasons). Upon standard histological preparation, part the samples were stained with hematoxylin-eosin technique and part were subjected to immunohistochemistry reaction for matrix-metalloproteinase-9 (MMP9) detection. **Results** revealed inflammatory morphopathological changes, which were significantly more intense and more frequent within the tissue samples of periodontal patients with chronic hepatitis C, than in those of systemically healthy ones with periodontal disease. The coloring reaction, used to evaluate the existence of MMP9 within the tissue samples, was significantly more positive for the group of periodontal and chronic hepatitis C patients than for the group of periodontal patients with no systemic illness. **Conclusions:** the inflammatory reaction is more intensive in the gingival tissue of periodontal patients with chronic hepatitis C than in that of periodontal patients without systemic conditions, proving the additional negative impact that the viral hepatic disease has on the inflammatory status, which periodontal disease involves.
Keywords: HISTOLOGY, GINGIVAL TISSUE, MATRIX-METALLOPROTEINASE 9, PERIODONTAL DISEASE, CHRONIC HEPATITS C.

Periodontal disease consists of a chain of pathological events triggered by the interactions occurring between the bacterial plaque and the host immune response, which are mediated by certain local and systemic additional risk factors (1, 2). Usually, the inflammatory reaction is firstly manifested within the marginal periodontium, the gingiva, afterwards gradually progressing towards the deeper structures which support the teeth, as the periodontal ligament and alveolar bone (3).

From a histopathological standpoint, the inflammatory events which occur during periodontal disease have been divided into four types of morphopathological lesions, named after their moment of manifestation: the initial, the early, the established and the advanced lesions (4). Each of these types of lesions present their own morphopathological characteristics, generated by the events that unveil during periodontal inflammation at a specific moment. From a clinical standpoint, these stages of inflammation cannot be always divided and observed, as they unveil with speed and at the same time in different areas of the periodontium (5).

The severity and extent of periodontal inflammation is closely influenced by the efficiency of the immune cells' chemotactism and phagocytosis that enable them to pursue and attack bacteria (6). Some periodontal pathogens such as *Porphyromonas gingivalis* and *Fusobacterium nucleatum* can inhibit cellular phagocytosis and have a cytotoxic effect on the immune cells (7). Therefore, periodontal disease's pathogeny is not fixable by the usual defensive mechanisms of the human organism and will develop into a chronic, prolonged inflammatory reaction that causes the destruction of the supporting tissues of the teeth.

Collagen is an important component of the periodontium and has a major role in keeping the right structure and function of the periodontal tissues (8). During periodontal inflammation, collagen fibers are damaged by the decreased pH and the collagenolytic enzymes secreted by bacteria. In addition, the production of new collagen is also perturbed, and immune cells can cause the dissolution of collagen by releasing matrix-metalloproteinase (MMPs) enzymes (9, 10, 11). As a result, the periodontal tissues lose their flexibility and are gradually destroyed, causing the teeth to become mobile.

MMP9, also called gelatinase-B, is an important member of the MMP enzyme family and it is found in increased levels in inflamed gingival tissues (12, 13). The main action of MMP9 is to disintegrate collagen fibers contained by gingival tissue, periodontal ligament and alveolar bone, as early as the first week of periodontal inflammation (14). Secreted by immune cells such as fibroblasts and macrophages, MMP9 can alter the chemotactic patterns of neutrophil and keratinocyte cells and can disrupt the healing process occurring inside the gingival epithelium, thus extending the inflammatory reaction (15, 16). The enzyme also stimulates the activation of some pro-inflammatory markers like interleukin 1β, further enhancing the development of periodontal inflammation (17, 18).

Chronic hepatitis C is the prolonged inflammation of the liver caused by the infection with hepatitis C virus (19). Metabolic and immune changes occur during the condition, as the liver plays an important role in the modulating of these systems (20). These changes also manifest orally, making hepatitis C patients more prone to other inflammatory disease such as periodontal

disease (21).

The aim of our study was to histologically assess the changes that occur within gingival tissues during periodontal inflammation in patients with chronic hepatitis C and to evaluate if the intensity of the inflammatory reaction is higher in such patients than in periodontal patients without systemic conditions

MATERIAL AND METHODS

The study included patients who addressed the Periodontology Department of the University of Medicine and Pharmacy of Craiova, for various periodontal complaints and was approved by the university's ethics committee. Upon signing the informed consent of participation, the patients were periodontally examined.

Non-surgical periodontal therapy (scaling and root planning) was conducted. After four weeks, the results of the initial periodontal treatment were reassessed, and patients were given indication for surgical periodontal therapy in connection with their periodontal status. Of these patients, only those with maximum periodontal pocket depth of 5mms, were further included in the study.

Regarding systemic illness, only patients who declared to suffer from chronic hepatitis C were included in the study. Other systemic conditions such as diabetes mellitus, cardiovascular disease or rheumatoid arthritis were excluded from the study.

The study also included control patients, consisting of patients who were addressed to the department for periodontal examination prior to orthodontic treatment initiation. These patients were not diagnosed with any form of periodontal disease but required maxillary premolar extraction for orthodontic purposes. They were referred to the department of Oral Surgery for tooth extraction and sampling of gingival tissue. Only systemically healthy patients were included in the control group.

After implementing all of the above inclusion and exclusion criteria, three groups of patients were set for the study:

1. P group – 5 patients (3 males, 2 females, age 40-48 years) who were diagnosed with periodontal disease and required periodontal surgery, systemically healthy;

2. PH group – 5 patients (2 males, 3 females, age 36-47 years) who were diagnosed with periodontal disease and required periodontal surgery, chronic hepatitis C;

3. H group – 3 patients (1 male, 2 females, age 32-38 years), who required tooth extraction for orthodontic purpose, periodontally and systemically healthy.

The gingival tissue samples were collected during periodontal surgery (for P and PH groups) or during the extraction of teeth for orthodontic purpose (for H group), from the same area for all patients of the three groups: vestibular marginal gingival of the maxillary premolars. Upon collection, the samples were set in 10% formalin solution for preservation and refrigerated at 8°C. After the collection of the samples was completed, they were transferred to the Department of Histology of the University for Specific Preparation.

The samples were subjected to standard paraffin inclusion and hematoxylin eosin staining. For the immunohistochemical assessment of MMP9, LSAB technique was used with rabbit antibodies (Abcam ab38898, Cambridge, Great Britain). The immunohistochemistry reaction was detected with the Dako EnVision system (DAKO, Glostrup, Denmark). The staining agents

used were 3.3'-Diaminobenzidine (Sigma Chemical Co., Saint Louis, USA) and Mayer hematoxylin. The results of the histological and immunohistochemical processes were observed via optical microscope (Nikon Eclipse 90i, Tokyo, Japan) and relevant pictures of the samples were taken (NIS Elements, Nikon, Tokyo, Japan). A visual quantitative evaluation of the immunohistochemistry reaction was performed and the most important inflammatory morphopathological changes were described. Afterwards, the pictures were assessed with Integrated Optical Density (IOD) analysis, which measured the total color intensity of the immunohistochemistry images, using absolute white and black as reference points. Average IOD values were calculated for each patient group.

RESULTS

The histological assessment of the gingival tissue samples originating from periodontal patients, with/without chronic hepatitis C, exhibited inflammatory changes (tab. I).

TABLE I

Prevalence of histopathological changes

	Group		
Histopathological change	**PH**	**P**	**H**
Acantholysis	80%	80%	33.3%
Orthokeratosis	100%	0%	0%
Papillomatosis	80%	60%	33.33%
Inflammatory infiltrate	100%	100%	0%

All the samples of group PH showed signs of an intense inflammatory reaction. Orthokeratosis was observed within the gingival epithelium, the component cells having elongated nuclei and highly colored chromatin. In addition, acantholysis was observed between the superficial epithelial cellular layers in 80% of the samples, together with an important inflammatory infiltrate. The junction between the epithelial and the connective part of the gingival tissue was affected by papillomatosis, giving a typical wave-like appearance of this area.

Inside the gingival connective tissue, the inflammatory infiltrate, which included granulocytes, lymphocytes and macrophages caused the destructuring of collagen fibers. In some areas, the connective tissue appeared to be fibrotic, showing a damaged extracellular matrix and injured cells with agglutinated chromatin. Furthermore, some blood vessels inside the connective tissue were congested and expressed microhemorrhages (fig. 1, fig. 2).

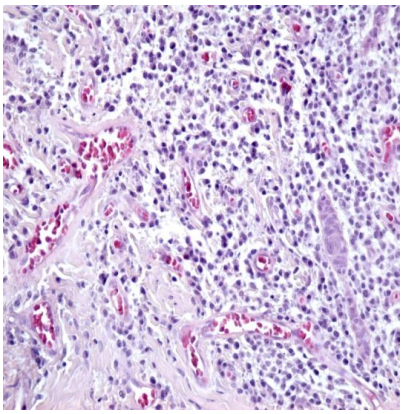

Fig. 1. Gingival tissue sample of PH group (HE staining, x100)

The gingival tissue samples originating from the P group expressed changes in the

normal architecture of the epithelial cellular layers, as a result of the chronic inflammation of the tissues. Acantholysis was observed within the squamous cellular layer due to intense cellular multiplication and immune cell infiltration (80% of the samples in P group).

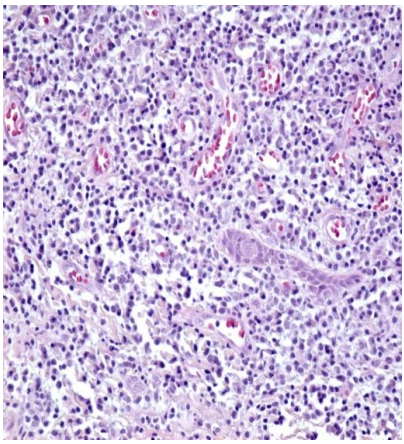

Fig. 2. Gingival tissue sample of PH group (HE staining, x100)

The junction between the epithelium and the connective tissue had a waved shape due to the elongation of the rete pegs, as a sign of papillomatosis (60% of the samples in P group). The inflammatory infiltrate in the connective tissue was moderate, while the blood vessels expressed venous stasis due to the increased osmotic pressure (fig. 3). The gingival samples of the H control group showed few or no signs of histopathological changes, only one of them expressing faint epithelial acantholysis and junctional papillomatosis.

The immunohistochemical assessment of the presence of MMP9 within the gingival samples was positive for the samples of the PH and P groups (tab. II). The PH samples expressed a 50-60% degree of the cellular nuclei coloration, while that of the P samples was lower, around 20-30%. The histological areas which usually expressed a positive reaction were mainly the gingival epithelium and its junction with the underlying corium (fig. 4, fig. 5). The reaction was more positive in the superficial gingival corium for the PH samples and in the gingival epithelium for the P ones. The immunohistochemistry assessment was lowest for samples of the H group.

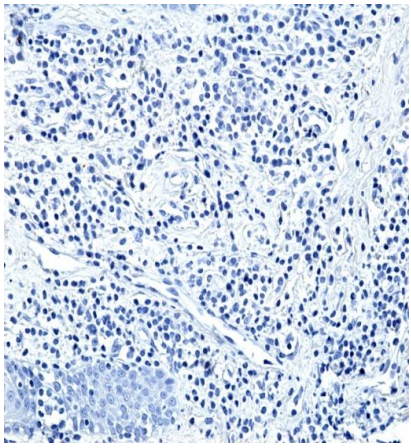

Fig. 3. Gingival tissue sample of P group (HE staining, x100)

The lowest average IOD value was that of the H group, while of the P group was 8.1 times higher and of the PH group 13.6 times more elevated. The average IOD value for the PH group was 1.6 times higher than that of the P group (tab. II).

TABLE II

Prevalence of positive immunohistochemistry reactions for MMP-9 detection and IOD average values

Group	Percentage of positive reaction	Average IOD value (± standard deviation)
PH	50-60%	293 117 ± 112 336
P	20-30%	174 761 ± 84 214
H	< 5%	21 448 ± 7 486

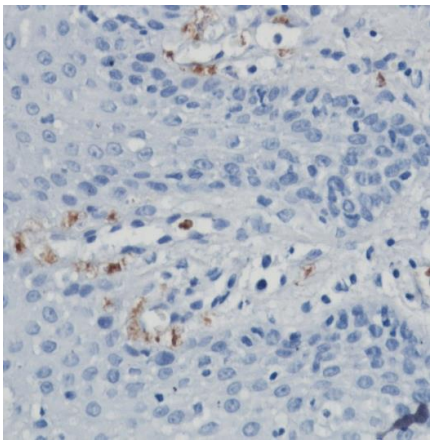

Fig. 4. MMP9 immunohistochemistry positive reaction, PH group sample (x200)

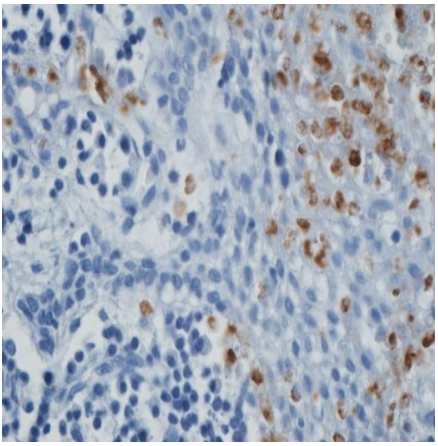

Fig. 5. MMP9 immunohistochemistry positive reaction, P group sample (x200)

DISCUSSION

The histological assessment of the gingival tissue samples revealed the existence of important structural changes caused by the chronic inflammatory reaction in periodontal patients both with chronic hepatitis C and without this systemic disease.

The inflammatory infiltrate affecting the gingival tissue is directly proportional in quantity with the amount of time passed since the debut of the periodontal disease and the systemic conditions of the patient and their type of immune response (5).

In our study, the inflammatory infiltrate was significantly increased in the PH group samples rather than in those of the P group, suggesting that a more intensive inflammatory reaction occurs in such patients.

In the gingival epithelium immune cells like granulocytes and lymphocytes were observed, suggesting an increase in vascular permeability and a decrease in epithelial cell density, due to the dismantling of the hemidesmosome connections (22). Consequently, epithelial acantholysis can occur, as it has been observed in the gingival samples mainly originating from the PH group. This alteration of the normal cellular architecture leads to an epithelial cellular proliferation towards the underlying corium, causing an elongation of the rete pegs (23) The elongated rete pegs, an element of papillomatosis (24), give a characteristic waved shape of the epithelial-corium junction, as it was observed predominantly in the samples of the PH group.

As a result of the inflammatory reaction, the epithelial gingival cells will react by intensifying the keratinization process (25), a type of process which was also observed within the squamous cellular layer of the studied samples. The level of orthokeratosis was higher for the PH group samples than the rest, suggesting a more intensive inflammatory reaction for this group of patients. In addition, the types of cells encountered in more advanced and severe gingival inflammatory reactions as macrophages, lymphocytes and plasmocytes (26) were predominantly observed within the PH group samples, as opposed to the predominantly granulocytes observed within the P group sam-

ples, which are usually found in early, less-severe gingival inflammatory reactions (27).

The proteolytic enzyme, MMP9, especially targets the dissolution of type IV collagen, which is predominantly a component of the basal lamina propria (28). The study delivered similar results, as the immunohistochemistry reaction was particularly positive along the epithelio-connective junction within the P group samples. In patients with chronic periodontitis, MMP9 can also be found in a dispersed manner in both gingival epithelium and corium, in a direct relation with the severity of the disease, as diagnosed by clinical means (29, 30). The samples of the PH group expressed a positive anti-MMP9 reaction in a more increased percentage than the P group samples, suggesting that the inflammatory reaction was more intense for periodontal patients who also suffered from chronic hepatitis C.

MMP9 was found in significantly increased quantities in the blood serum of chronic hepatitis C (31) and it is believed to be involved in the angioneogenesis process that is closely linked to the development of hepatocellular carcinoma, a frequent complication of chronic hepatitis C (32, 33). The prolonged existence of the hepatitis C virus in the hepatic tissues is believed to increase MMP9 production (31), making chronic hepatitis C patients prone to the onset of tissue damaging conditions like periodontal disease.

The immunohistochemistry reaction for MMP9 detection was positive in a significant percentage in the gingival tissue samples of periodontal patients with chronic hepatitis C, in accordance with the enzyme's frequent use as an indicator of hepatic inflammatory status (34, 35, 36). As serum concentration of MMP9 has been noticed to decrease after interferon hepatitis C treatment (37), further studies could address the impact of such therapies on the inflammatory status of periodontal patients with chronic hepatitis C.

CONCLUSIONS

In all, given the limitations of this current study caused by the small number of participating patients, the histological assessment of the gingival samples has revealed that in what concerns the structural pathological changes of the tissue, the periodontal inflammatory reaction is more intensive in patients with chronic hepatitis C than in systemically-healthy periodontal patients. Together with the enhanced expression of MMP9 in such patients' gingival tissue samples, it can be concluded that chronic hepatitis C might have an additional negative impact on the inflammatory status of periodontal patients.

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DIAGNOSTICUL ŞI TRATAMENTUL CHISTULUI HIDATIC CU LOCALIZARE ABDOMINALĂ LA COPIL – STUDIU MULTICENTRIC

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CHILDREN ABDOMINAL HYDATID CYSTS DIAGNOSIS AND TREATMENT. A MULTICENTRIC STUDY (Abstract) : Echinococcosis is one of the most frequent parasitosis and is endemic in Romania. **Material and method** : The data on 111 children with abdominal localization of hydatidosis who underwent exploration and treatment in 3 pediatric surgical clinics were reviewed. The study evaluated 59 patients who were treated at „Sf. Maria" pediatric hospital Iasi during 2004 and 2009, 47 at „Marie Curie" pediatric hospital from Bucharest during 2000 - 2009 and 5 children at Bacău General Hospital 2004-2009. The medical records were reviewed for : demographics, age, gender, location of the cyst, operative procedure, medical treatment, outcome, length of hospital stay and postoperative follow up. **Results** : Age ranged between 2 and 17 years and male/female ratio was 1,1 / 1. 67% of patients lived in rural areas. The liver was the most common organ involved : in 69 cases it was the only organ involved and there were 2 kidney cyst cases, 8 spleens involved, 2 cases in broad ligament, 13 peritoneal cases and 26 cases of cyst located in great omentum. The preferred surgical procedure was Lagrot partial pericystectomy. For medical antihelminthic treatment it was used Albendazole. As complications there were : biliary fistulas, postoperative cholangitis, infection of the residual cavity, anaphylactic reactions. The mortality rate was 0%. **Conclusions** : Most of the patients were from rural area. The liver was the major organ involved. The treatment includes both surgical and medical means. There is a relatively high rate of postoperative complications (although some of them being minor) in 31 patients. **Key words :** ABDOMINAL HYDATID DISEASE, SPLENECTOMY, PERY-CYSTECTOMY, LAPAROSCOPY

Echinococoza este o boală parazitară cronică şi complexa cu distribuţie cosmopolita cauzata de larvele de *Echinococcus granulosus* şi care cel mai frecvent afectează ficatul şi plămânii, chiştii hidatici cu alte localizări se întâlnesc relativ mai rar totuşi ei putând fi întâlniţi în orice organ şi ţesut (6,7). Prezentul studiu vine sa confirme aceste lucruri fiind totodată în concordanţa cu datele din literatură ficatul fiind cel mai afectat organ urmat de epiplon, peritoneu, splina, rinichi, ligamentul larg. Scopul acestui studiu este de a documenta evoluţia clinică, datele epidemiologice, modalităţile de diagnostic şi tratament, evoluţia postoperatorie a cazurilor tratate chirurgical şi evoluţia celor tratate medical.

MATERIAL ŞI METODĂ

Locaţia studiului

Pacienţii cuprinşi în lotul de studiu provin din 3 servicii de chirurgie pediatrică :

- Clinica de Chirurgie pediatrică a Spitalului de copii „Sf. Maria" Iaşi, unde au fost diagnosticaţi şi trataţi un număr de 59 de pacienţi distribuiţi pe un interval temporal de 5 ani, respectiv 2004 - 2009, ceea ce a permis realizarea unui studiu prospectiv ;
- Clinica de Chirurgie pediatrică „Marie Curie" Bucureşti, unde au fost diagnosticaţi şi trataţi un număr de 47 de pacienţi distribuiţi în două perioade temporale :
- 2000 - 2004 interval temporal care a permis efectuarea unui studiu retrospectiv ;
- 2004-2009 interval temporal care a permis efectuarea unui studiu prospectiv.
- Serviciu de Chirurgie pediatrică al Spitalului Judeţean Bacău unde au fost diagnosticaţi şi trataţi un număr de 5 pacienţi distribuiţi într-o perioadă temporală cuprinsă pe parcursul a 4 ani 2004 - 2009, ceea ce a permis elaborarea unui studiu prospectiv.

REZULTATE ŞI DISCUŢII

Analiza distribuţiei pacienţilor în funcţie de vârstă şi de apartenenţa la centrele în care s-a efectuat cercetarea este redată în tabelul I

După localizare am identificat următoarea repartizare a cazurilor :

Parametrii sanguini

Hipereozinofilia a fost prezentă la 47 de pacienţi, iar leucocitoza la 31,53% din cazuri.

Un număr redus de pacienţi a prezentat leucopenie (5 cazuri).

VSH a fost crescut în majoritatea cazurilor, dar am identificat pacienţi la care constantele biochimice se încadrau în limite normale.

Dintre pacienţii cu chist hidatic hepatic (101 cazuri), 8 cazuri au prezentat hiperbilirubinemie, iar în 5 cazuri s-au identificat alterări ale funcţiei hepatice (valori anormale ale TGO şi TGP).

TABELUL I

Vârsta pacienţilor funcţie de centrul în care s-a efectuat studiul

	Vârsta minimă (ani)	Vârsta maximă (ani)	Vârsta medie (ani)
Iaşi	2	17	12,18
Bucureşti	3	16	10,72
Bacău	5	14	9,35
Nr. total	2	17	11,51

Parametrii serologici

Testele serologice (ELISA) au fost efectuate în 25 de cazuri, cu o rată a rezultatelor pozitive de 96%.

Toate cazurile cu pozitivitatea testelor serologice au fost confirmate prin rezultatele intervenţiei chirurgicale.

Examenul ecografic

Încadrarea chisturilor hidatice hepatice s-a făcut conform clasificării Gharbi (7) :

- tip I colecţie lichidiana pura - 45 chisturi
- tip II colecţie lichidiana cu perete dehiscent - 71 chisturi
- tip III colecţie lichidiana cu septuri - 68 chisturi
- tip IV chist cu ecouri heterogene - 14 chisturi
- tip V chist cu perete gros reflectogen - 7 chisturi

Examenul tomografic computerizat - a confirmat prezenta celor 205 chisturi cu localizare hepatica si in plus a permis o mai buna precizare a raporturilor chisturilor cu structurile vasculare biliare si organele de

TABELUL II

Cazurile de chist cu localizare hepatică şi pluriorganică

Chisturi hepatice	69
Hepatice + splenice	4
Hepatice + renale	1
Hepatice + epiplonice	15
Hepatice + peritoneale	4
Hepatice + ligamentul larg	1
Hepatice + splenice+ peritoneale+ epiplonice	2
Hepatice +peritoneale +epiplonice	5

vecinătate.

Tratamentul medical – din cei 111 pacienţi cuprinşi in studiu 71 au urmat tratament cu albendazol anterior intervenţiei chirurgicale (63,96%) 10 mg/kg/zi iar 102 pacienţi (91,89%) au fost supuşi acestui tratament după intervenţia chirurgicala timp de 2 săptămâni până la 2 luni. In 5 cazuri diagnosticul ecografic a specificat prezenta de chisturi hepatice localizate central care au necesitat administrare de Albendazol. Din acestea 4 au prezentat in cursul urmăririi ecografice semne de degenerare arătând eficiente tratamentului in timp ce unul s-a mărit exteriorizându-se si care a fost operat.

Tratamentul chirurgical

Pacienţii cuprinşi în lotul de studiu au fost împărţiţi în funcţie de metoda terapeutică, chirurgicală, în:

- pacienţi care au urmat tratament chirurgical clasic (95 cazuri);
- pacienţi care au urmat tratament laparoscopic (16 cazuri);
- pacienţi care au urmat tratament laparoscopic convertit în chirurgie deschisă (8 cazuri).

Tratamentul chirurgical clasic – a fost metoda terapeutica utilizata in cazul a 95 de pacienţi ca prima metoda si in 8 cazuri fiind vorba de conversia intervenţiei laparoscopice. Ca modalitate de abord s-au folosit:

- laparotomia mediana
  - supra şi subombilicală - 50 cazuri
  - supraombilicală - 19 cazuri
  - subombilicală - 5 cazuri
- abord subcostal drept – 20 cazuri
- toraco-freno-laparotomie - 9 cazuri

Obiectivele tratamentului chirurgical în cazul chistului hidatic indiferent de metoda folosita sunt aceleaşi : inactivarea şi indepartarea parazitului în condiţii de izolare perfecta a cavitaţii peritoneale, tratarea cavitaţii reziduale hepatice, tratarea complicaţiilor biliare.

Pentru inactivarea parazitului s-au folosit:

TABELUL III

Cazurile de chist cu localizare unică abdominală extrahepatică

Chisturi renale	1
Chisturi splenice	2
Chisturi epiplonice	4
Chisturi peritoneale	2
Chisturi in ligamentul larg	1

TABELUL IV

Complicaţiile capitonajului

	Nr. cazuri
Abces	1
Colangită	0
Fistulă biliară	3
Recurenţă	1

TABELUL V

Complicaţiile omentoplastiei

	Nr. cazuri
Abces	3
Colangită	1
Fistulă biliară	4
Recurenţă	3

- ser hiperton Na – Cl 20% - 53 cazuri
- alcool absolut 70-95% - 19 cazuri
- apă oxigenată - 13 cazuri
- metronidazol - 11 cazuri
- cetrimide - 5 cazuri

Ca interventii chirurgicale s-au practicat:

- perichistectomie parţiala Lagrot - 89 pacienţi cu chist hidatic hepatic
- perichistectomie ideala (totala, în cazul chisturilor marginale) - 9 pacienţi
- segmentectomie (în cazul lobului hepatic stâng complet distrus) -3 cazuri
- splenectomie - 6 cazuri, restul de 2 chişti splenici fiind trataţi prin perichistectomie parţială
- în cazul chisturilor hidatice renale s-a practicat perichistectomie parţiala într-un caz şi nefrectomie în celalalt
- chisturile localizate în epiplon şi peritoneu au fost extirpate complet.

În ceea ce priveşte cavitatea restanta s-au practicat următoarele metode:

- capitonajul - 36 cazuri
- marsupializarea cu drenaj extern pe sonda Foley - 11 cazuri

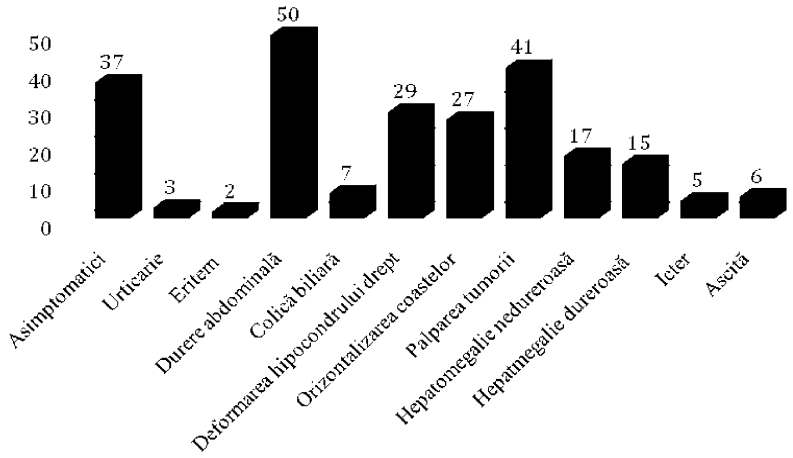

Fig. 1. Simptomele clinice ale pacienţilor care au prezentat chisturi hidatice hepatice cu localizare mono şi pluriorganică

- omentoplastia - 25 cazuri
- drenaj extern - 76 cazuri
- drenaj intern chisto-digestiv - 2 cazuri

Metoda laparoscopică nu a fost utilizata decât la Bucureşti studiul pe acest lot începând în anul 2004. Conversia în intervenţie clasica a fost necesara în 8 cazuri din următoarele motive : localizarea chistului (în domul hepatic) - 3 cazuri, complexitatea leziunii – 2 cazuri, incidente intraoperatorii (hemoragie) 2 cazuri, examen imagistic irelevant - un caz. Deşi pare mare aceasta rata de conversie ea reflecta debutul ca modalitate de tratament a chistului hidatic a laparoscopiei în chirurgia pediatrica.

Intervenţia chirurgicală clasică a durat în medie 75 minute, cu valori cuprinse între 35 şi 165 de minute.

Intervenţia laparoscopică a durat în medie 115 minute, cu valori cuprinse între 65 -160 de minute.

Se constată că durata medie a intervenţiei clasice a fost mai mică decât a celei laparoscopice. Acest lucru poate fi explicat prin faptul că în momentul efectuării studiului laparoscopia era o metodă nou introdusă în România, motiv pentru care chirurgii se aflau în perioada curbei ascendente de învăţare ceea ce a crescut semnificativ durata intervenţiei.

Complicaţii postoperatorii pot fi împărţite în complicaţii ale intervenţiei chirurgicale propriu-zise care apar în cursul intervenţiei, şi complicaţii postoperatorii la distanţă.

Complicaţiile în cazurile tratate prin metoda Lagrot au asociat :

- Abces în cavitatea reziduală – 2 cazuri
- Colangită – 2 cazuri
- Fistulă biliară – 9 cazuri care au avut evoluţii lente, pe o perioadă cuprinsă între 4-8 săptămâni,

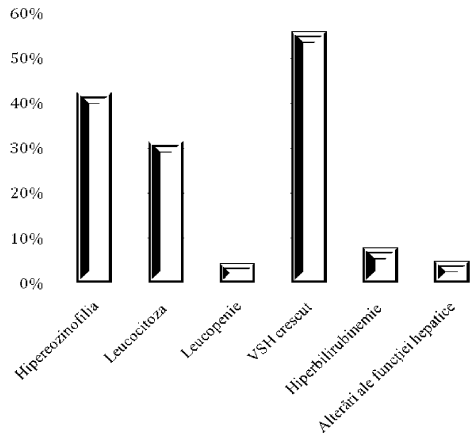

Fig. 2. Repartiţia pacienţilor funcţie de rezultatele investigaţiilor de laborator

- Recurenţă – 6 cazuri

Prezenţa acestor complicaţii a crescut durata de spitalizare la 14,7 zile modificând implicit costurile aferente.

În ceea ce priveşte complicaţiile capitonajului acestea sunt redate în tabelul IV.

Durata media a zilelor de spitalizare în cazul pacienţilor care au prezentat complicaţii ale capitonajului a fost de 12,2.

Durata media a zilelor de spitalizare în cazul pacienţilor care au prezentat complicaţii ale omentoplastiei a fost de 12,2. Se constată prin analiza comparativă a complicaţiilor capitonajului *versus* omentoplastie că numărul de abcese respectiv recurenţa afecţiunii a fost mai mare în cazul omentoplastiei.

CONCLUZII

Tratamentul bolii hidatice hepatice încă nu este codificat şi aceasta pentru că nu există o tehnică perfectă, aplicabilă într-o anumită situaţie şi ale cărei rezultate să fie întotdeauna aceleaşi, iar boala hidatică este deosebit de poliformă atât ca localizare cât şi ca modificări anatomo-patologice.

Între argumente şi contraargumente, chirurgul trebuie să aibă capacitatea de a adapta fiecărui caz varianta tehnică cea mai potrivită, urmărind evitarea complicaţiilor postoperatorii, fără a pune inutil în pericol viaţa pacientului.

Experienţa ne demonstrează că fiecare metodă terapeutică, chirurgicală şi/sau medicală trebuie adaptată stării generale a pacienţilor, activităţii şi etapei evolutive a infecţiei parazitare, poziţiei şi extensiei chisturilor hidatice şi nu în ultimul rând experienţei chirurgului terapeut.

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THE INFORMED CONSENT IN PEDIATRICS – A CHILD'S RIGHT

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THE INFORMED CONSENT IN PEDIATRICS-A CHILD'S RIGHT (Abstract): Consent signifies the patient's knowingly authorization of a medical intervention. Revealing the truth about the patient's condition refers to the provision of information by the physician regarding the diagnosis and the treatment, as well as their understanding by the patient. His or her decision-making capacity refers to the ability of understanding the data that have been provided. The independent decision refers to the patient's right to make a free decision regarding his or her treatment, without constraint or manipulation. Informed consent is one of the most sensitive issues of the physician-patient, researcher-subject relationship. Minors do not have the capacity of independent decision; therefore, the decision by substitution given by parents or legal representatives is required. The minor child's consent to the medical treatment and pediatric scientific research expresses itself under the form of assent, which is valid starting with the age of 14. Doctors should ask for parental consent before medical interventions (except in cases of emergency, when parents cannot be contacted). From a practical standpoint, when it comes to obtaining a valid consent, it is useful to make a distinction between the problem-solving process and the decision-making process. The permission given by parents includes all standard elements of informed consent. In each case, the doctor has to make sure that he or she has a valid consent because, in legal terms, treating a patient without his or her consent is a crime, while treatment based on inadequate consent constitutes negligence. **Keywords:** INFORMED CONSENT, DECISION MAKING, PEDIATRIC MEDICAL CARE.

THE HISTORICAL ASPECTS

The consent represents the informed authorization regarding the medical intervention made by a patient (1). Revealing the truth about the patient's condition refers to the provision of information by the physician regarding the diagnosis and the treatment, as well as their understanding by the patient. His or her decision-making capacity refers to the ability of understanding the data that have been provided (2). The independent decision refers to the patient's right to make a free decision regarding his or her treatment, without constraint or manipulation. The first "Save the Children" organization was founded in London in 1919 by Englatyne Jebb, who made the decision to try to save and also to improve the lives of children around the world; thus, her aim was to establish an international organization

capable of intervening in areas where child protection was not applied, and where children's rights were not respected (1). Promoting children's rights was a movement that began in 1924 at the time of the adoption of the Declaration of the Rights of the Child by the League of United Nations, which refers to the fact that the child must be protected against any form of exploitation, that he or she has the right to the means necessary for his or her material as well as spiritual normal development, that orphans and widows should be protected (2, 3).

Unfortunately, the 1924 Geneva Declaration on the Rights of the Child was not enforceable under international law; instead, it constituted a guide for the member countries. Thus, fighting hunger and providing care to children were the main concerns. Englantyne Jebb designed and facilitated the adoption of the Declaration of the Rights of the Child by the League of Nations (now the United Nations), which represents the first international document where "the child's rights" are mentioned and promoted in a unitary manner, and where the child's protection is implicit (3, 4). This Declaration, with its extended form in 1959, is the source of inspiration used in drafting the United Nations' Convention on the Rights of the Child adopted by the United Nations General Assembly on November 20th, 1989 (4, 5). The Convention consists in 54 articles that address the basic needs of children anywhere in the world: the right to live, the right to develop to their full potential, the right to be protected from harmful influences, the right to be protected from abuse and exploitation and the right to fully participate in the familial, cultural and social life (5, 6). The four core principles of the Convention are non-discrimination, devotion to the child's best interests, the right to life, survival and development and the respect for the child's points of view (5, 7). According to the Convention, a child is defined as *"[...] every human being below the age of 18 years unless, under the law applicable to the child, majority is attained earlier*". Any State which signs the Convention acknowledges its content, undertaking to respect and apply it properly. So far, the Convention has been adopted by 193 countries, members of the United Nations, except the USA and Somalia. In the late 60s, the focus was on the participation rights of the child, as several opinion leaders claimed that underage youngsters were able to make decisions on important issues in their lives and that they should be allowed to participate in making these decisions (6, 8).

The Law in British Columbia and New Brunswick increase pediatricians' accountability and state that a child can consent to health care. The consent of the minor is sufficient and effective, and physicians do not need the written consent of the parent or of the legal guardian of the child, if the pediatrician is sure that the underage patient understands the benefits and the risks associated with the treatment (4, 9). The pediatrician should consider the medical interests of the child, which includes the lack of distress, the restoration of the body functions and the physical health (8, 10). Children aged over 10 years old are held responsible in court, as the "doli incapax" principle was abolished in 1998. However, minors do not have the rights the adults benefit from; therefore, what is actually targeted is the autonomy that should be the same for everyone, because it is a rule about how everyone

around should be treated (11). Eekelaar proposed a periodic assessment of children, in order to understand exactly the child's interests and desires, and to reconcile them with their autonomy. Unfortunately, there are many constraints such as parents' divorce, the custody, communication difficulties, abuse, neglect, various diseases; they all decrease children's decision-making ability and distort their real desires. The "Rule of seven"' defines a child as incompetent until he is 7 years of age; the child is generally incompetent, with some exceptions, before the age of 14 years old; the child is considered to be competent, with some exceptions, between 14 to 21 years old (8, 12). In the State of Ontario, it is considered that a sane person, of any age, can decide on their own medical treatment. Health Care Consent Act does not allow that the treatment should be administered to any person able to make their own decisions, regardless of age, without their consent. The consent of the parent or of the guardian is required if the minor is unable to make decisions because of the nature of their illness. The Law in Manitoba, Newfoundland and Labrador state that minors under 16 are legally incapable of decision making. New Brunswick, Quebec and British Columbia allow capable minors to decide on their own treatment. The Constitution acknowledges that each person, adult or mature minor has the right to accept or to reject the suggested investigations and / or treatment (11, 13).

The Convention on the Rights of the Child

In 1978, a year before the International Year of the Child, the Polish Government proposed elaborating a *Convention on the Rights of the Child*. A special working group was founded that had to draw up a draft of the Convention. On September 2nd, 1990, the Convention became international law; it consists of 54 articles that address the basic needs of children everywhere, such as the right to live, the right to develop to their full potential, the right to be protected from abuse, exploitation and harmful influences, and the right to fully participate in the familial, cultural and social life (5, 14). The four core principles of the Convention are non-discrimination, devotion to the child's best interests, the right to life, survival and development and the respect for the child's points of view. According to the Convention, children should be treated with respect, and the state is obliged to ensure the best conditions for their development, to respect their rights and protection norms; the Rights of the Child need to apply to all minors without discrimination. Thus, every child has the right to life, education and harmonious development (9, 15).

The United Nations, in the Universal Declaration of Human Rights and in the International Covenants on the same subject, proclaimed and agreed that everyone is entitled to all the rights and freedoms set forth therein, without distinction of any kind, such as race, color, sex, language, religion, political or other opinion, national or social origin, property, birth or other status. In the same Declaration, the United Nations recognized the right of children to receive special care and assistance, with the conviction that the family, as the fundamental unit of society and the natural environment for the growth and the well-being of all its members (especially children),

should benefit from the necessary protection so that it can fully assume its responsibilities within the society. For the full and harmonious development of his or her personality, the child should grow up in a family environment, in an atmosphere of stability, love and understanding (6, 13). A child should be fully prepared to live an individual life in society and be brought up in the spirit of the ideals proclaimed in the Charter of the United Nations, with an emphasis on dignity, freedom, tolerance, equality and solidarity. The need to extend the particular care given to the child has been stated in the Geneva Declaration on the Rights of the Child of 1924 and in the Declaration of the Rights of the Child adopted by the General Assembly on November 20th, 1959 (12, 15). It has also been recognized in the Universal Declaration of Human Rights, in the International Covenant on Civil and Political Rights (articles 23 and 24), in the International Covenant on Economic, Social and Cultural Rights (article 10) and in the statutes and applicable instruments of specialized agencies and of international organizations concerned with the child's welfare ("Given his or her lack of physical and mental immaturity, the child needs special safeguards and care, including appropriate legal protection, before as well as after birth" – the Declaration of the Rights of the Child) (3, 9, 16). The Convention on the Rights of the Child incorporates, more than any other document, the full spectrum of human rights (civil, political, economic, social and cultural) in order to ensure the full development potential of children, in an atmosphere of freedom, dignity and justice. The Convention defines children's rights in a complex manner.

Aspects from Romania

Romania ratified the Convention on September 28th, 1990 by Law No. 18/1990 (published in the *Monitorul Oficial* No. 314 of June 13th, 2001) and was inspired by it when drafting the Law No. 272/2004 on the protection and the promotion of the child's rights. The first Romanian law on child's protection was passed in 1970 (Law No. 3/1970) (11, 16). The National Authority for the Protection of the Child and the Ministry of Education, the Ministry of Labor, Social Solidarity and Family, the Ministry of Health as well as the other related public and private institutions will provide initial and ongoing training of the staff who, while performing their duties, come into contact with the protection and the promotion of the child's rights (article. 143 paragraph 3 of Law No. 272/2004 on the protection and the promotion of the child's rights). Before 1989, the informed consent did not apply to the Romanian medicine.

During the communist period, the paternalism was promoted in the doctor-patient relationship (the former used to decide on the therapy which suited the patient best, while research did not involve informing the patient included in the study) (6, 11, 13). The small number of specialists in the field of bioethics, together with the lack of clear norms regarding the implementation of the patient's informed consent go hand in hand with the lack of education of healthcare professionals in this field, of researchers performing clinical studies and, last but not least, of the population requiring medical attention. If the patient's decision-making capacity or consciousness is temporarily or permanently affected or if the patient is underage, the decision-making responsibility is transferred to the patient's legal representa-

tive or to a panel of arbitrators (8, 13, 17).

A particular issue – the vulnerable patients

The thirteenth principle of the *International Ethical Guidelines for Biomedical Research involving human subjects 2002* defines vulnerable individuals as being "relatively or totally incapable of protecting their own interests. Furthermore, these individuals might have insufficient strength, intelligence, education, resources and other attributes necessary to protect their own interest" (2, 4, 10). The Informed Consent targets both patients and healthy subjects (experiments-research). In February 1931, the government in Weimar (Germany) prohibits "Any experiment if consent is not obtained after all the explanations have been provided" (3, 10).

Mikhail Bulgakov, Russian physician and writer recalls in his book, "Stories of a young doctor", several events which took place from 1917 to 1918 and in which the question of consent arose, including the case of a young girl suffering from diphtheria croup, when tracheotomy was not done before the mother gave her consent (5, 17). A study conducted in Denmark in the period 1960-1994 highlights the reasons why there is no such thing as true informed consent: people are suffering, so they cannot think as they normally would in other situation, the patient is afraid, physicians and / or researchers do not provide information about side effects, they minimize them intentionally or unintentionally or they do not give explanations in a way that patients can understand them (9, 18).

In 1964, "The World Medical Association" adopted the Declaration of Helsinki - "Helsinki I". This includes ethical rules of experimentation on humans and principles of informed consent; thus, the document states the purposes of the study, the methodology, the expected results, the possible side effects, the risks for the patient (7, 12). The patient must be informed orally and in writing. In 1975, the "Helsinki II" Declaration was adopted in Tokyo; this document states that, in certain circumstances, it is possible to waive informed consent (10, 17).

After 15 years from the adoption of the Declaration of Helsinki II, the Danish Ethics Committee decided that everyone should have the right to informed consent. In 1994, the Amsterdam Declaration is issued, which, among others, also comprises information on the informed consent of vulnerable individuals (children, elderly people, mentally ill people). At the International Conference of Medical Orders 1987 the following is stated: "Apart from emergencies, the physician must explain the effects and the consequences of a treatment to the patient". The informed consent can be achieved in three ways: a) orally - in the presence of the medical personnel, b) in writing, c) by means of video recording, with witnesses from outside the medical facility (11, 12, 13). When the patient is unable to express their will and an emergency medical intervention is required, the medical personnel is entitled to infer the patient's consent from a previous expression of his or her will (6, 16). If the patient requires an emergency medical intervention, their legal representative's consent is not required; when their legal representative's consent is required, the patient must be involved in decision-making process as far as their ability to understand allows them to. If health care providers believe that the intervention is in the patient's best

interest and the legal representative refuses to give his or her consent, the decision becomes the responsibility of a specialized panel of arbitrators, which is made of three doctors for the patients admitted to hospital and of two doctors for outpatients. In addition, the patient's consent is required for his or her participation in clinical medical education and scientific research. The individuals who are not able to express their will cannot be subjects in scientific research unless consent is obtained from their legal representative and if the research is done in the interest of the patient. It is not allowed to use photos or films of the patients in a medical facility without their consent or the consent of their legal guardians unless the images are necessary to establish a diagnosis or a treatment and to avoid suspicion of malpractice (14, 16).

The Declaration for promoting Patients' Rights in Europe, issued at the European Consultation on the Rights of Patients (15, 17) refers to the informed consent in detail, with clear information on vulnerable people. Patients should be given explanations using plain language about the steps to be taken, about diagnosis, treatment approaches and about the existence and the nature of the implied risks (9, 13). Thus, the European countries have developed their own documents. In Romania, these issues were enacted in 2003 when law 46 was published – the Patient's Rights Law (*Law No. 46/2003 – Monitorul Oficial 2003*). Under this law, any medical act must be carried out with the informed consent of the patient or of his or her legal representative; some believe that parents or guardians have the right to give consent "by substitution" for underage patients. By the early twentieth century, children had been regarded as the property of parents, the latter having the decision-making right on behalf of the underage individual. The child is an independent being, not a miniature adult (16, 18). A child differs from an adult by anatomical, physiological and psychological characteristics and is defined as any human being below the age of 18 years unless, under the law applicable to the child, majority is attained earlier. American Laws designate two cases in which underage individuals have the authority to make decisions regarding their health (8, 17): they are self-sufficient, they are married, they have children, they belong to the military or, on their own initiative, require medical treatment for certain diseases or conditions (sexually transmitted diseases, pregnancy, abuse / dependence on alcohol and drugs (emancipated minor) (9, 18). Competence and mental capacity in the medical context acquire distinct meanings. Competence is a legal term that signifies the legal authority in the management of particular situations, in a particular area of life, while mental capacity refers to the cognitive, perceptual and communicative ability to perform a particular task (3, 11, 19).

The decision-making ability can be determined by reference to the ability to make a choice, to understand relevant information, to appreciate the consequences of decisions and to the ability to manage information rationally. Patients with decision-making capacity should be included in the informed consent process, regardless of their level of competence (5, 19). The understanding capacity refers to the process by which the patient engages constantly in understanding the diagnosis and the therapeutic alternatives.

The American Academy of Pediatrics argued that "the whole doctrine" of the informed consent has a particular applica-

tion in pediatrics (5, 11). Research involving children is permissible only if it is done with therapeutic purposes to benefit the subjects and with the consent of parents or of legal guardians; it should be accompanied by the older child's approval, thus respecting the principle of patient autonomy and having the approval of competent ethics committees (when the child is able to understand correctly and fully, his or her consent is necessary, together with that of his or her legal representative) (13, 18). Non-therapeutic experiments on children are not accepted, except for situations where the use of an experimental drug is the last chance to save the life of that particular underage patient. Research of any kind on institutionalized subjects is not advisable and it requires special regulations. Most institutionalized subjects integrate in the concept of paternalism, rejecting autonomy (11, 13, 19).

There is a provision in Article 4 of the Charter of Hospitalized Children developed by UNESCO which stipulates their right to be informed, in a manner that should be appropriate to his age. Both the physician and the parents must be involved in the informing process (4, 7, 11); this attitude explains itself psychologically by the fact that the anguish of silence can induce discomfort to the child. Until the age of 4, the child is scared of the medical act; between 4-8 years of age, curiosity leads the child to accept medical procedures; after 8 years of age, the child will participate actively in performing therapeutic procedures (9). The UN Convention on children's rights-New York,1991-expressly provides that "Member States shall ensure the child who is capable of discerning the right to express their views freely on all matters which affect them, and their opinion will be taken into consideration according to their age and maturity".

CONCLUSIONS

The capacity to consent always requires the ability to understand the implications of the decisions as well as to evaluate in a rational manner the consequences of those decisions. Frequently, the doctor struggles alone with a medical ethical decision-making pressure, without any legal protection and with important professional, psychological and social implications.

The society has the ability to provide consent through its institutions for those particular cases where the consent cannot be given by the parents or the legal guardians, except for the emergency situations when the doctor is authorized to decide the most appropriate approach in order to preserve life.

Until 10 years ago in Romania there was no legislation that specifically dealt with child protection in the process of scientific research. In the case of underage individuals, there is no mention about a specific age beyond which consent is mandatory and the doctors should ask for parental consent before medical. The permission given by the parents includes all standard elements of informed consent. The authorization granted by the underage individual may be revoked at any time by another competent authority on the child best interest.

From a practical standpoint, a valid consent requires to make a distinction between the problem-solving process and the decision-making process. The doctor has to make sure that the subject consent is valid because, in legal terms, treating a patient without his or her consent is a crime, while treatment based on inadequate consent is negligence.

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RELATIONSHIP BETWEEN IDIOPATHIC SCOLIOSIS AND FACIAL ASYMMETRY IN YOUNG PATIENTS

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RELATIONSHIP BETWEEN IDIOPATHIC SCOLIOSIS AND FACIAL ASYMMETRY IN YOUNG PATIENTS (Abstract): This study aims to evaluate the relationship between idiopathic scoliosis and facial asymmetry, on a sample of young patients. **Material and methods:** 17 orthopedic patients (1 boy and 16 girls) aged 15-28 years (mean age 20.06±4.575 years) admitted to Medical Rehabilitation Centers in the northeastern region of Romania (Iasi and Bacau cities) between January 2014 and April 2015 were assessed. Orthopedic data were extracted from medical records and radiological examinations, and orthodontic data were obtained from physical and photostatic examinations. Statistical evaluation was performed using *SPSS 16.0* software. **Results:** All patients were diagnosed with idiopathic scoliosis at puberty. Cobb angle of the main spinal curvature was ≤20° in 35.3% of patients, 20-40°(45°) in 17.6% of patients and ≥40°(45°) in 47.1% of patients. Convexity of the major curve was to the right in 88.2% of patients and to the left in 11.8% of patients. 58.8% patients underwent orthopedic treatments and 41.2% surgical treatments. All orthopedic patients presented facial asymmetry (right/left and overall/lower face) and lateral deviation of the head (right/left and mild/moderate). Only 52.9% of patients had malocclusions, patients who underwent orthopedic treatments. We found statistically significant correlations between scoliosis severity and head deviation, malocclusions, Angle classes and anomalies types; between the direction of convexity of the major curve and the types of facial asymmetries; and between types of scoliosis treatments and types of facial asymmetries, malocclusions, Angle classes and types of anomalies ($p<0.05$). **Conclusions:** Our study found in young patients a relationship between idiopathic scoliosis and facial asymmetry. **Keywords:** IDIOPATHIC SCOLIOSIS, FACIAL ASYMMETRY, MALOCCLUSIONS, BODY POSTURE.

Scoliosis is a three-dimensional deformation of the spinal column, defined as a lateral deviation greater than 10 degrees in frontal plane, associated with axial rotation, located at the cervical, thoracic and/or lumbar level (1, 2). There are several different types of scoliosis: congenital, neuromuscular and idiopathic. Idiopathic scoliosis is the most common type of scoliosis and has a multifactorial etiology (genetic predisposition, metabolic, hormonal and biomechanical factors). It can appear at any age and is more common in teenage girls, 90% of cases presenting a right-sided thoracic curve. The prevalence of adolescent idiopathic scoliosis is between 0.47% and 5.2% (3).

The diagnosis of idiopathic scoliosis is made by history, physical and radiographic examination. Postero-anterior X-ray view of the spinal column determines the degree of

severity of spinal curvature by the Cobb method (4). Idiopathic scoliosis is a progressive disease with aesthetic (important asymmetry) and functional consequences (cardiac and respiratory dysfunctions). It requires clinical observation over time to assess the risk of curve progression. Orthopedic treatment (kineto-physiotherapy, braces) does not correct scoliosis, but prevents its progression during the growth period of the patient. The goal of surgical treatment is to restore and maintain alignment, being indicated when the curve exceeds 40 degrees (3, 5).

The perfect bilateral symmetry of the face and the body is rare due to biological variation, being a theoretical concept. Right-left differences are due to functional and morphological asymmetries (6, 7). Slight, invisible, subclinical facial asymmetry is relatively common, being defined as the difference in size between the right and left sides of the face, observed only by the method of composite face photo (8, 9). Severe facial asymmetry occurs in craniofacial syndromes. Asymmetry affects the lower face (lips, chin) more frequently than the middle (nasal pyramid, orbits) and upper face (12). According to transverse discrepancies, facial asymmetry is classified as dental, skeletal, functional and muscular (10). Clinical features are polymorphic, depending on etiopathogenesis. Clinical and radiological diagnostic procedures aim to identify the causal factor, location and affected tissue. However, clinical examination remains the most important diagnostic tool for facial asymmetry (11).

The interest in finding the relationship between idiopathic scoliosis and facial asymmetry is 50-year-old, being based on the hypothesis of the anatomical and functional relation between the dento-maxillo-facial complex and the spinal column. The results of observational and case-control studies and case reports were inconclusive, and a cause-effect relationship was not found. Some cephalometric studies investigating relationships with posture have mentioned a correlation between facial morphology and head inclination in relation to the spinal column and vertical plane (12).

Our study aimed to investigate the relationship between idiopathic scoliosis and facial asymmetry in a sample of young patients treated in rehabilitation centers, to find the correlations between the characteristics of idiopathic scoliosis and facial asymmetry, and the coexistence of malocclusions in young orthopedic patients.

MATERIAL AND METHODS

We conducted a review of patients with idiopathic scoliosis admitted and treated in Medical Rehabilitation Centers in the Iasi and Bacau cities in northeastern Romania from January 2014 to April 2015. A total of 17 patients, 1 (5.9%) male and 16 (94.1%) females, aged 15-28 years (mean age 20.06 ± 4.575 years), with idiopathic scoliosis were identified. Informed consent was obtained from all patients. Included were only young patients (adolescents and young adults) who had clinical and radiological evidence of lateral spinal curvature and were diagnosed with idiopathic scoliosis. Patients with other spinal conditions, genetic or endocrine syndromes, as well as adults over 30 years of age or those who refused orthodontic examination were excluded from the study.

The following data from the medical records and spinal radiographs of the patients with idiopathic scoliosis included in the study were collected and analyzed: medical history, clinical signs, scoliosis localization, convexity of the spine, Cobb angle, type of treatment and post-treatment out-

comes. The patients were examined clinically and photo statically by the orthodontist in order to establish facial asymmetry by extra-oral examination of the face and head posture; presence/absence of malocclusions (Angle classes, malocclusion and sagittal, transverse or vertical inter-arches asymmetry) by examining the dental arches and occlusion.

Statistical analysis was performed using *SPSS 16.0* software (Chicago, Illinois, USA) for Windows. The data were characterized by descriptive statistics and contingency tables, using the *Pearson Chi-square test* (χ^2). Any *p* value less than 0.05 was interpreted as statistically significant.

RESULTS

According to their medical history, all patients were diagnosed with idiopathic scoliosis at puberty, at the age of 13 to 15 years old. Patients had clinical signs of scoliosis: lateral deviation of the head, shoulder asymmetry, winged scapula (right/left), waist angle deformity (difference between right/left waist angles), hip asymmetry, lateral deviation in frontal plane of the spinal column when the trunk is bent forwards with the occurrence of gibbosity on the side of the convexity of the curve.

The lateral curve of the spinal column was unique ("C" shaped) at cervical level in 2 (11.8%) patients, thoracic level in 9 (52.9%) patients and lumbar level in 1 (5.9%) patient or double ("S" shaped) at the cervicothoracic level in 1 (5.9%) patient and thoracolumbar level in 4 (23.5%) patients. The Cobb angle measured on the postero-anterior spine radiograph was ≤20° in 6 (35.3%) patients, between 20-40°(45°) in 3 (17.6%) patients and ≥40°(45°) in 8 (47.1%) patients. Convexity of the major spinal curve was on the right side in 15 (88.2%) patients and left side in 2 (11.8%) patients. Progression of scoliosis was stopped by orthopedic treatments(kineto-physiotherapy, Milwaukee, Cheneau, Boston braces) in 10 (58.8%) patients and surgical treatments in 7 (41.2%) patients. Post-treatment outcomes were favorable.

Asymmetry of the two hemifaces was established according to the facial midsagittal plane, right hemiface being more developed in 9 (52.9%) patients and the left hemiface in 8 (47.1%) patients. Also, 11 (64.7%) patients had overall facial asymmetry and 6 (35.3%) patients lower face asymmetry. Head inclination was to the right side in 10 (58.8%) patients and to the left side in 7 (41.2%) patients. Also, 10 (58.8%) patients had mild head deviation and 7 (41.2%) patients moderate head deviation. Only 9 (52.9%) patients had malocclusions: 4 (23.5%) patients Angle Class I, 1 (5.9%) patient Angle Class II and 4 (23.5%) patients Angle Class III, associated with dental crowding in 3 (17.6%) patients, inter-arches asymmetry in 3 (17.6%) patients or both in 3 (17.6%) patients.

We found statistically significant differences between: the values of Cobb angle of the major spinal curvature and head deviation (p=0.013), malocclusions (p=0.004), Angle classes (p=0.007) and of anomaly types (p=0.025); between the convexity of the major spinal curvature and the facial asymmetry types (p=0.041); between the scoliosis treatment types of and the facial asymmetry types (p=0.037), malocclusions (p=0.001), Angle classes (p=0.011) and anomaly types (p=0.008). We did not find statistically significant results between the other variables studied: scoliosis localization, right/left facial asymmetry, and head inclination to posture the right/left (p>0.050) (tab. I, II, III).

TABLE I

Statistically significant correlations between Cobb angle and statistical variables

Variables	Angle Cobb						Total		χ^2 test	p value
	≤20°		20-40°(45°)		≥40°(45°)					
	n	%	n	%	n	%	n	%		
Lateral deviations of head									8.743	0.013*
Mild	6	35.3	0	0.00	4	23.5	10	58.8		
Moderate	0	0.00	3	17.7	4	23.5	7	41.2		
Malocclusions									10.812	0.004*
Absent	0	0.00	1	5.9	7	41.2	8	47.1		
Present	6	35.3	2	11.7	1	5.9	9	52.9		
Angle Class malocclusions									17.797	0.007*
Class I	4	23.5	0	0.00	0	0.00	4	23.5		
Class II	1	5.9	0	0.00	0	0.00	1	5.9		
Class III	1	5.9	2	11.7	1	5.9	4	23.5		
Anomalies type									14.432	0.025*
Crowding	3	17.7	0	0.00	0	0.00	3	17.7		
Asymmetry	2	11.8	1	5.9	0	0.00	3	17.7		
Both	1	5.9	1	5.9	1	5.9	3	17.7		
*statistically significant differences when p<0.05										

TABLE II

Statistically significant correlation between convexity of the curve and facial asymmetry

Variables	Convexity of spinal column				Total		χ^2 test	p value
	right		left					
	n	%	n	%	n	%		
Facial asymmetry type							4.156	0.041*
Overall face	11	64.7	0	0.00	11	64.7		
Lower face	4	23.5	2	11.8	6	35.3		
*statistically significant differences when p<0.05								

TABLE III

Statistically significant correlations between scoliosis treatment and statistical variables

Variables	Treatment of scoliosis						Total		χ^2 test	p value
	kinesitherapy		corset		surgical					
	n	%	n	%	n	%	n	%		
Facial asymmetry type									6.600	0.037*
Overall face	3	17.6	1	5.9	7	41.2	11	64.7		
Lower face	5	29.4	1	5.9	0	0.00	6	35.3		
Malocclusions									14.993	0.001*
Absent	0	0.00	1	5.9	7	41.2	8	47.1		
Present	8	47.0	1	5.9	0	0.00	9	52.9		
Angle Class malocclusions									16.469	0.011*
Class I	4	23.5	0	0.0	0	0.00	4	23.5		
Class II	1	5.9	0	0.0	0	0.00	1	5.9		
Class III	3	17.6	1	5.9	0	0.00	4	23.5		
Anomalies type									17.345	0.008*
Crowding	3	17.7	0	0.0	0	0.00	3	17.7		
Asymmetry	3	17.7	0	0.0	0	0.00	3	17.7		
Both	2	11.8	1	5.9	0	0.00	3	17.7		
*statistically significant differences when p<0.05										

DISCUSSION

In our study we evaluated clinically and photo statically a group of Romanian patients diagnosed with idiopathic scoliosis to establish the relationships between the features of scoliosis (location, convexity of the major curve, Cobb angle and treatment) and the features of facial asymmetry (right/left, overall/lower face, head deviation) and the presence/absence of malocclusions (Angle class, crowding and/or sagittal, transverse or vertical inter-archers asymmetry), presenting some new aspects regarding the inclusion and analysis of these statistical variables.

Most of the study patients were women (94.1%) diagnosed with idiopathic scoliosis at puberty, as it was the case with other studies (12). Patients were assessed orthodontically by clinical and photostatic examinations as they were admitted and treated for their orthopedic condition. Kim *et al.* (4) were conducted their study on frontal and lateral cephalograms, because patients with scoliosis presented to orthodontic clinic. In our study, all our patients had facial asymmetry (right/left and overall/lower face), as other studies have reported (12, 13). Also, all our patients showed right/left and mild/moderate head inclination. In scoliosis, the head changes its inclination in relation to the spine, so that it can compensate for the spine deviation and maintain the orthostatic position. Scoliotic curves in frontal plane and lateral head inclination in posture contribute to the development of various dentofacial asymmetries (14). Body posture and cervical muscles are closely associated with the function of the head and mandible support system (15).

In the patients studied, not all of them had malocclusions. Most common were Angle Class I and III malocclusions (23.5%), dental crowding and occlusal asymmetries (sagittal molar and cuspid, mandibular lateral deviation, unilateral crossbite, canted occlusal plane, and infraocclusions). From our observations, these malocclusions were present only in the orthopedically treated patients (58.8%) and not in the surgically treated ones. In the literature, there are not recent study about the relationship between scoliosis and facial asymmetry, and our data cannot be compared.

Regarding head deviation, presence/ absence of malocclusions, Angle classes and types of anomalies differed depending on the severity of scoliosis. Also, the types of facial asymmetry correlated with the direction of convexity of the major spinal curve. Differences were also found in the types of facial asymmetries, presence/absence of malocclusions, Angle classes and types of anomalies depending on the treatment of scoliosis. All these correlations are original results that are not found in previous literature studies.

The spinal column provides the main support for all segments of human body (head, mandible, shoulders, trunk, hips, legs and feet). When the spinal column is misaligned, whole-body posture is influenced, and all body segments become misaligned as they are taking up unequal muscle loads (16). After surgical correction of spinal deviation, the adaptation of subjacent muscles is slow, with the persistence of functional asymmetries through neuromuscular mechanisms, thus explaining the presence of facial asymmetry and head inclination in posture even after the treatment of scoliosis.

This research may improve the study of young patients with idiopathic scoliosis, because unlike previous studies it identified a relationship with facial asymmetry and the presence/absence of malocclusions. Similar future studies may also find other ways to investigate these patients, so that our data to be comparable.

CONCLUSIONS

In our study, all orthopedic patients presented facial asymmetry (right/left and overall/lower face) and lateral head deviation in posture (right/left and mild/moderate) and only those who received orthopedic treatments (kineto-physiotherapy, braces) presented malocclusions (more commonly Angle Class I and III, dental crowding and interarches asymmetry). The value of Cobb angle correlated statistically with head deviation in posture, presence/absence of malocclusions, Angle diagnostic classes and types of anomalies; the direction of convexity of the major spinal curve was statistically correlated with the types of facial asymmetry; the orthopedic/surgical treatment of scoliosis correlated statistically with the types of facial asymmetry, presence/absence of malocclusions, Angle classes and types of anomaly. These aspects suggest that the interdisciplinary concepts of young people diagnosed with idiopathic scoliosis should be extended, both for the complete and complex skeletal treatment, as well as for the neuromuscular rehabilitation body segment posture.

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MODERN ANCHORAGE DEVICES IN ORTHODONTIC THERAPY: A LITERATURE REVIEW

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MODERN ANCHORAGE DEVICES IN ORTHODONTIC THERAPY (Abstract): TADS were one of the most discussed topics of the last decade. It started in the 90' and experienced in the last 15 years an extreme development. The review analyzes the use of mini-implants and mini-plates as orthodontic anchorage, to understand the factors that seem to affect the success rate. The main topics addressed the mini-screw and mini plates studies are as follows: success, failure stability and survival, damage caused and root proximity, dental movements, optical microscopy, torque and strength, placement and location of the mini-screw. **Keywords:** ORTHODONTICS, MINI SCREWS, MINI PLATES.

Anchoring is a determining factor in the success of orthodontic treatment. It allows the application of forces, using a fixed point (anchoring), inducing the displacement of dental units to a given location (1, 2). Designed for orthodontics, bone anchors allow a more conservative treatment, avoid orthodontic surgery and patient cooperation is no longer necessary. They provide an interesting option to bypass conventional anchors such as intermaxillary elastics and extra-oral appliances (3). Considering the treatment plan and the choice of anchor solutions, the orthodontist needs to evaluate pain prevention, restoration of the oral functions, maintenance of the patient's physiognomy, hygiene and responsibility (4).

Prevalence of use

The search was done from the database, Science Direct, by using following key words in French and English according to the current nomenclature: Mini-screws, Mini-vis, Mini-implants, Micro-implants, Mini plates, Skeletal Anchorage System. The selection criteria and filters were as follows: the year of publication 2010 to 2016, from the countries: France, United States, Germany, Taiwan, and Japan, published in French or English; *in vivo* studies, longitudinal, transversal and cohort studies, no isolated clinical cases. The research on *"Science Direct"* revealed 418 articles, only 44 articles were chosen, according to the searched items, of which on mini-screws (36 articles) and the other on mini-plates (8 articles) which includes the following criteria: Title of the author, Year reference, Sample size, Duration of follow-up if mentioned, Themes treated, Conclusion findings.

77% of the articles were on the topic of mini-screws, and 23% on mini-plates.

The main topics addressed in the mini-screw studies were success, failure stability and survival: 47%; Damage caused and root proximity: 13%; Dental movements:13%; Studies under optical microscopy: 7%; Torque and strength: 7%; Placement and location of the mini-screw: 7% (fig. 1).

The topics for the mini-plates took in consideration were survival, success and stability, 34% of studies; Maxillary traction: 22%; Resistance: 11%; Pain: 11%; Recidivism: 11%; Bone stress: 11% of studies on mini-plates (fig. 2).

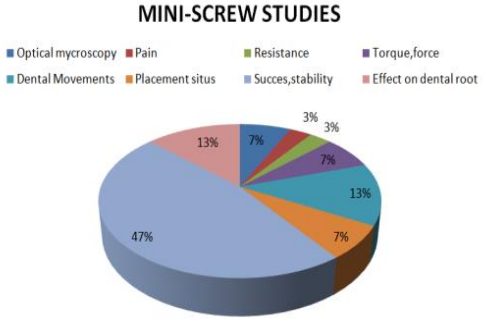

Fig. 1.The main topics for the mini-screw studies

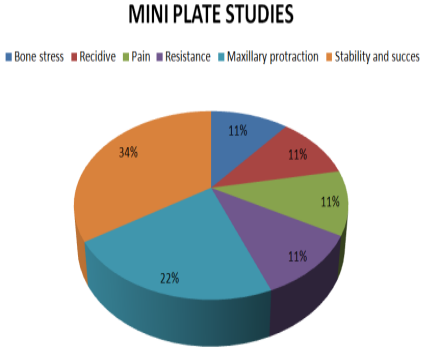

Fig. 2. The main topics for the mini-plates studies

Factors that influence mini-implant therapeutical success

The success of the orthodontic treatment is dependent by three factors: a correct therapeutical plan, parental consent and support and patient cooperation (5).

With regard to success, failures, stability and survival of the mini-screws, the studies showed a very high success rate, for 3162 mini-screws studied on 1548 patients, success was 94.3% (tab. I). Age and sex do not influence the success rate, which remains very satisfactory and similar: 91.2% for men, 92% for women, 86.5% for under 20 years and 89.5% for over 20 years old (tab. II). The success rate of maxillary mini-screws was significantly higher than for the mandible, so we found a success rate of 90% for the maxillary and 79.5% for the mandible (tab. III). The dimensions of the mini-screws are also a factor to consider because the success rate varies in function of the diameter and length chosen. Thus, to obtain a satisfactory success rate, the authors recommend a length of at least 7 mm and a diameter of at least 1.3 mm.

Regarding the pre-drilling or not option, Iwai *et al.* (6) achieved 91.5% success without pre-drilling, and 94.4% with pre-drilling, the main instability and failure is due to contact with the root. Son *et al.* (7) achieved 96% success for both techniques. However, care should be taken with root contact for mini-screws without pre-drilling which has decreased stability. Mini-screws therefore offer a secure alternative with a satisfactory success rate. Regarding damage and root proximity: Shinohara *et al.* (8) showed that root proximity increased the risk of failure of a mini-implant and could affect its stability. In addition, the root can be damaged during a

bad evaluation and placement of the wrong Mini-screw. Ahmed *et al.* (2) found that 41.7% of patients had moderate cement repair, 50% of patients a good repair and 8.3% of patients an excellent repair. No root resorption was observed, nor ankylosis. Nevertheless, other research done by Renjen *et al.* (9) indicates that ankylosis was observed only when the wound root canal was accompanied by a displacement. In addition, according to Lee *et al.* (10) state that prolonged contact of the mini-screw with the root may cause root resorption.

TABLE I

Studies regarding the success, failures, stability and survival of the mini-screws

N	Authors	Mini-screws	Patients	Manufacturer	Success
6	Iwai *et al.* (2015)	142	80	ISA, Biodent, Tokyo, Japan	91.5%
7	Son *et al.* (2014)	140	70	ISA Self drill type anchor screw, Biodent, Tokyo, Japan	96.0%
8	Shinohara *et al.* (2013)	147	50	Isa orthodontic implant, Biodent, Tokyo, Japan	94.6%
10	Lee *et al.* (2010)	260	141	C-implant, Dentium, Seoul Korea	91.5%
11	Heravi *et al.* (2011)	210	128	KLS Martin, Jacksonville, Fla et Orthoplant, Biomaterials, Seoul, Korea	90.8%
13	Park et Kwon (2011)	78	23	Dentos, Daegu, Korea et Osteomed, Dallas,Texas	89.5%
17	Suzuki *et al.* (2011)	280	95	Systema nacionales Brazil et ACR Biomaterial, Seoul Korea	95.0%
18	Suzuki *et al.* (2013)	186	105	AbsoAnchor, Dentos, Daegu, Korea	81.9%
19	Motoyoshi *et al.* (2010)	148	57	ISA orthodontic implant, Biodent, Tokyo, Japan	90.5%
27	Maino *et al*. (2012)	324	144	Spider Screw HDC, Sarcedo Venezia, Italy	91.4%
29	Janson *et al.* (2013)	40	19	AbsoAnchor, Dentos, Daegu, Korea	90.0%
30	Jung *et al*. (2013)	228	130	-	87.7%
31	Karagkiolidou *et al.* (2013)	384	196	Ortho Easy, Pforzheim, Forestadent, Germany	97.9%
32	Lai TT *et al.* (2014)	266	129	AbsoAnchor, Dentos, Daegu, Korea	97.0%
33	Lee *et al.* (2016)	127	71	AbsoAnchor, Dentos, Daegu, Korea	85.0%
35	Motoyoshi *et al*. (2016)	202	110	Biodent, Tokyo, Japan	95.5%
	TOTAL	3162	1548		94.3%

TABLE II

Studies regarding the age and gender of patients benefiting of mini-screws

N	Authors	Male	Female	< 20 years	> 20 years
10	Lee *et al.* (2010)	89.8%	92.0%	82.4%	84.4%
11	Heravi *et al.* (2011)	88.2%	91.8%	85.5%	94.2%
30	Jung *et al.* (2013)	85.7%	88.4%	84.7%	92.3%
31	Karagkiolidou *et al.* (2013)	96.6%	99.1%	-	-
32	Lai TT *et al.* (2014)	94.4%	93.3%	96.1%	90.6%
33	Lee *et al.* (2016)	87.1%	84.4%	83.6%	87.0%
35	Motoyoshi *et al.* (2016)	96.3%	95.3%	-	-
	TOTAL	91.2%	92.0%	86.5%	89.7%

TABLE III

Studies regarding the success rate of mini-screws placed in the maxillary *versus* the mandible

N	Authors	Maxilla		Mandible	
		Mini-screw	Success	Mini-screw	Success
8	Shinohara *et al.* (2013)	68	95.6%	79	93.7%
10	Lee *et al.* (2010)	220	91.5%	-	-
11	Heravi *et al.* (2011)	210	90.8%	-	-
17	Suzuki *et al.* (2011)	233	94.8%	47	85.1%
18	Suzuki *et al.* (2013)	122	93.3%	64	70.3%
29	Janson *et al.* (2013)	40	90.0%	-	-
30	Jung *et al.* (2013)	228	84.7%	-	-
31	Karagkiolidou *et al.* (2013)	384	97.9%	-	-
32	Lai TT*et al.* (2014)	250	97.2%	16	68.8%
35	Motoyoshi *et al.* (2016)	202	95.5%	-	-
	TOTAL	1957	90.0%	206	79.5%

Concerning the dental movements: Heravi *et al.* (11) have studied the effects of intrusion of a molar into supra eruption. The intrusion of the molar was made using 2 mini-screws, one in mesio-buccal and one in mesio-palatine, with a spring in TMA (titanium alloy and molybdenum) which was ligated from the mini- screw to the molar bracket maxillary molars were successfully intruded. Root resorption was observed, 0.3 mm palatal, 0.4 mm mesio-buccal and distovestibular, a slight de-

crease in the peak was observed. The intrusion was 1.5 to 4.5 mm. The use of mini-screws thus makes it possible to avoid, with respect to conventional techniques, the extrusion of adjacent teeth. Senisik *et al.* (12) compared the intrusion of maxillary incisors to reduce a deep-bite in Class II-2 malocclusion, Park and Kwon (13) studied the distalization of posterior teeth in patients using mini-screws. The results were satisfactory they obtained less tilting and rotation of the distal molars. Success was reported by Cernei *et al.* (14) in a case with second molar up righting and space closing after first molar extraction. Thus, we can deduce that the Mini-screws offer an alternative to traditional treatments and avoid stray movements and loss of anchorage.

Studies under ***optical microscopy*** revealed several anomalies: Sebbar *et al.* (15) studied the surface condition of mini-screws after 30 months of use. It has been observed "corrosions in the form of punctures or crevices". Indeed, the corrosion can change the properties of the Mini-screw and mainly its resistance. Corrosion can also release substances inducing and promoting inflammations and other local and general reactions. Sebbar *et al.* (15) also histologically studied peri-implant soft tissues after Mini-screw implantation. In this study there was an inflammatory infiltrate in the tissues, independent of the location of Mini-screw or periodontal disease. Other studies, Petcu *et al.* (16) have shown the correlation between the general status of the patient, implant site and micro-structural details in the biomechanical properties, emphasizing that diabetes can decrease the integration of implant and such the biomechanical proprieties.

Torques and forces: Suzuki *et al.* (17) compared and measured the insertion and withdrawal torque of Mini-screws with and without pre-drilling. The insertion torque of the Mini-screws without pre-drilling was logically greater 14.5 Ncm than when placing the Mini-screws with pre-drilling 9.2 Ncm. This contrast with the value of the torque when removing Mini-screws, in fact the opposite was found with 22 Ncm for mini-screws with pre-drilling and 16.6 Ncm on average for Mini-screws without predrilling. This assumes greater osseointegration for mini-screws placed with pre-drilling, which is an undesired effect because the Mini-screws are not supposed to stay in the mouth and must be easily removed. Suzuki *et al.* (18) studied the ideal insertion torque of Mini-screws with a diameter of 1.3 mm and lengths of 5, 6 and 7 mm. It has been deduced that the ideal torque should be between 5 and 10 N to avoid the failure rate, however this value may differ depending on the type of Mini-screw used and the location used. Motoyoshi *et al.* (19) investigated whether damping and placement torque could provide indications of root proximity to the Mini-screw. In this study 18% of Mini-Screws were in contact with the root. It was found that the placement torque of the Mini-Screws (8.9 Ncm) that were in contact with the root was greater than the Mini-Screws without contact with the root of about (7.5 Ncm). Nevertheless, further studies on this subject are needed to prove this relationship and confirm this study.

Regarding placement and location of Mini-screws: Kau *et al.* (20) showed that 3D imaging is necessary for optimal placement of Mini-screws, indeed in this study imaging showed that 65.2% of placed Mini-Screws were in contact with

the periodontal ligament. Park, Hwang Bo and Kwon (13) have shown in their study that it is preferable to place the mini-screws distal to the point of contact from 0.5 to 2.7 mm with an inclination of 10 to 20° distally.

Factors that influence mini-plate therapeutical success

Discusses studies on mini-plates regarding the survival, success and stability: studies have shown a very high success rate, it is 96% in our case which is a higher success rate than for Mini-screws (tab. IV). Lee *et al.* (10) has shown that several factors can influence the success of Mini-Plates, the first important factor is hygiene, and then comes the second factor which is the experience of the operator who proceeds to surgery. Hygiene is however an important factor for patients who receive dental treatments, more than half of the subjects in a study made by Barlean *et al.* (21) considering appropriate to involve in infection control in the dental office.

TABLE IV

Studies regarding the survival, success and stability on mini-plates

N	Authors	Mini-plates	Patients	Manufacturer	Success
28	Hibi *et al.* (2010)	61	32	Compact lock 2.0 ; Synthes Maxillofacial, Paoli, PA	93.3%
34	Lee *et al.* (2013)	341	217	C tube ; Jin Biomed, Bucheon,Korea	96%
38	Eroglu *et al.* (2010)	74	37	-	98.6%
	TOTAL	476	286		96%

Concerning studies on maxillary protraction: Cagla Sar (22) studied the efficacy of maxillary pro- traction with and without bone anchorage in the case of Class III with maxillary developmental deficit; 2 mini plates were placed at the maxillary level in the lateral of the piriform orifice, and a facial mask was placed at its level. The treatment was 6.78 months on average for the group with mini-plates, and 9.45 months on average for the group with a conventional treatment. The maxillary advanced 2.3mm for the group with mini-plate against 1.83 mm for the group with conventional treatment. The protraction rate was 0.48 mm per month for the group with mini-plates against 0.24 mm per month for the group with conventional treatment. The posterior rotation of the mandible increased the facial height in both groups, but this was much more marked in the group with conventional treatment. Protrusion and mesialization of maxillary teeth found in the healthy group with conventional treatment was eliminated in the group with mini-plates. Elnagar *et al.* (23) compared maxillary protraction in the case of a class 3 with maxillary developmental deficiency using mini-plates according to 2 techniques. The first technique was as previously seen infrazygomatic mini-plates with facial mask. The second technique consists in placing 4 mini-plates, 2 in maxillary infrazygomatic, as well as 2 in the mandibula at the level of the mental symphysis, and the application of elastics at this level. The results obtained according

to the two methods were satisfactory, and class III malocclusion was corrected in both cases. Nevertheless, the method with 4 mini-plates gave better results, because it allowed a better control of the retro inclination and the vertical changes of the mandibular incisors. Pei Chen Lu *et al.* (24) studied the resistance of mini-plates, applying several forces, in different directions; the vertical resistance was 106.8 Ncm and the horizontal resistance 233 Ncm. The mini-plates are thus perfectly suited to the application of orthodontic and orthopedic forces without risk of breakage of the material. Concerning recidivism, Marzouk *et al.* (25) studied the recurrence rate after 4 years following the use of Zygomatic mini-plate for the intrusion of the first molar as well as for the treatment of an open bite. Recurrence for molar intrusion was 10.2% in the first year and 13.4% after 4 years. The recidivism of the open bite was 8.19% in the first year and 11.8% after 4 years. Thus, we can say that 4 years after treatment, the recurrence rate is very low for Zygomatic intravenous mini-plate treatments as well as for open-bite treatments. Regarding bone stress: Yen Wen Huang *et al.* (26) studied bone stress caused by different forms of mini plates using 3D simulation. As a result, the bone stress was greater on the mini-plates in I then in L then in Y and finally in T. The bone stress also decreased when the number of fixing screws was more numerous.

CONCLUSIONS

The mini-screws have a high success rate: they open the possibility to several dental movements such as intrusion, retrusion of the frontal incisors, and other distalisations by avoiding parasitic movements and loss of anchorage. It is advisable to take precautions during placement, to avoid placement in the periodontal ligament or even in extreme cases a root contact. The choice of material to use is also essential in practice because it will determine the operating mode. It is desirable to use mini-screws without pre-drilling due to their low osteointegration, which allows them to be removed easily. Mini-plates have also a very high success rate (96%), this success is influenced mainly by the hygiene of the patient by the experience of the practitioner. To avoid bone stress, it is best to use Y or T mini-plates. The resistance of mini-plates is important and allows the application of orthopedic forces. The maxillary protraction can also be done using 4 mini plates placed on each arch in bilateral with elastics at their levels. Patient cooperation is no longer necessary, and results are better. The recidivism rate is low. Their numerous indications make it possible to replace the conventional anchorage and open the way to new therapies in the future.

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NEWS

SIMILAR EFFECTS OF RADIOSURGERY ON BOTH CYSTIC BRAIN METASTASYS AND SOLID TUMORS

A study regarding 426 patients with brain metastases who undergone radiosurgery treatment at the Tianjin Cancer Hospital and Institute over a 4-year period shows that although considered a treatment mode especially used for solid tumors, radiosurgery is also beneficial metastasis of the cystic brain, where this procedure is less used due to poor prognosis. although the rate of tumor shrinkage is less accelerated, the final results are almost identical (Wang H, Liu X, Jiang X, Song Y, Wang X, Wang J, Dong Y, Li F, Wu Z, Zhang Y, Yuan Z. Cystic brain metastases had slower speed of tumor shrinkage but similar prognosis compared with solid tumors that underwent radiosurgery treatment. *Cancer Manag Res* 2019; 11: 1753-1763).

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CORELAŢII CLINICO-RADIOLOGICE ÎN PARALIZIA CEREBRALĂ

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CLINICO-RADIOLOGIC CORRELATIONS IN CEREBRAL PALSY (Abstract). The aim of this study was to determine clinico-radiologic correlations, using cranial magnetic resonance imaging (IRM)/computed tomographic exam (CT), in cerebral palsy (CP). **Material and method**: We studied 129 children (86 males, 43 females; aged 2-18 years) diagnosed with different types of CP. Computed tomographic examinations was performed in 100 (77,52%) of children and IRM in 29 (22,48%). **Results**: In 19 (14.72%) children the neuroimaging exam was normal. At 110 (85.28%) patients the IRM/CT showed different types of abnormalities. The most common neuroimaging findings was ventriculomegaly (38 cases), followed by focal infarct (28 cases), periventricular leukomalacia (17 cases), cortical and cortical/subcortical atrophy (13 cases), basal ganglia lesions (12 cases) and cortical migration and organization problems (12 cases). The CT/IRM abnormalities was associated in 65.45% of cases with mental retardation and 44,54% presented epileptic seizures. In CP neuroimaging findings are common, but variable and IRM/CT change correlates with neurological assessment. **Conclusion**: Patients with CP and mental retardation or/and epilepsy were more likely to have obvious CT/IRM changes. **Key words:** CEREBRAL PALSY, NEUROIMAGING FINDINGS, CHILD

Paralizia cerebrală (PC) este o afecţiune neprogresivă a mişcării şi posturii, care determină un deficit motor permanent şi reprezintă în multe cazuri prototipul tulburărilor de dezvoltare, fiind cea mai frecventă dizabilitate ,,fizică" a copilăriei care afectează sever dezvoltarea copilului (1). Diagnosticul de PC se bazează pe un istoric de dezvoltare neuromotorie anormală datorată unei leziuni cerebrale neprogresive, pe examene clinice multiple şi explorare neuroimagistică (2).

Examenele neuroimagistice moderne sunt utile atât pentru stabilirea diagnosticului pozitiv, a prognosticului cât şi pentru diagnosticul diferenţial al PC, deoarece consecinţele clinice depind de topografia leziunii (3). De asemenea, explorarea neuroradiologică realizată precoce oferă posibilitatea stabilirii unei legături între tipul agresiunii şi prezenţa leziunilor cerebrale (4). La copiii cu PC cele mai frecvente anomalii neuroimagistice sunt reprezentate de infarcte arteriale focale (22%), malformaţii cerebrale (14%) şi modificări la nivelul substanţei albe periventriculare (12%) (5).

Scopul studiului a fost de a determina la pacienţii de vârstă pediatrică cu PC existenţa unei corelaţii între aspectul clinic şi modificările depistate la examenul neuroradiologic.

MATERIAL ŞI METODĂ

Lotul de studiu a inclus 129 de copii (43 fete şi 86 băieţi) cu vârsta cuprinsă între 2-18 ani, diagnosticaţi cu diferite forme de PC: 114 (89,37%) cu forme spastice, 8 (6,2%) cu PC diskinetică, 5 (3,87%) cu PC

TABELUL I
Aspecte neuroimagistice la pacienţii cu paralizie cerebrală studiaţi

Aspecte neuroimagistice (CT/IRM cerebral)	Nr. pacienţi
Normal	19
Porencefalie	28
Agenezie/hipoplazie de corp calos	2
Chist arahnoidian	3
Hipoplazie/ atrofie cerebeloasă	5
Dilatare ventriculară posthemoragică/hidrocefalie	38
Atrofie corticală/ cortico-subcorticală	13
Hemiatrofie	2
Calcificări intracerebrale	9
Leziuni la nivelul ganglionilor bazali	12
Leziuni de leucomalacie periventriculară	17
Tulburări de dezvoltare corticală	
- displazie corticală	2
- polimicrogrie	3
- schizencefalie	4
- pahigirie/lisencefalie	3

ataxică şi 2 (0,56%) cu PC mixtă.

La 100 (77,52%) dintre pacienţi s-a efectuat CT-scan cranio-cerebral, 29 (22,48%) au beneficiat de IRM cerebral, iar 87 (67,44%) aveau efectuată din perioada neonatală ecografie transfontanelară.

Leziunile relevate de examenul CT şi/ sau IRM au fost clasificate în 4 grupe: leziuni corticale, leziuni centrale sau periventriculare, tablouri mixte şi anomalii de dezvoltare.

REZULTATE

La 19 (14,72%) copii aspectul neuroimagistic a fost normal, 4 (21,05%) dintre aceştia fiind cu forme severe de PC, iar 15 (78,95%) cu forme uşoare sau medii de paralizie cerebrală.

La 110 (85,28%) pacienţi s-au evidenţiat neuro-radiologic leziuni localizate la diferite nivele (tabelul I). Dintre aceştia 47 (42,73%) aveau forme severe de PC, 24 (21,82%) forme medii şi 39 (35,45%) forme uşoare de PC. De asemenea, 72 (65,45%) dintre bolnavii la care s-au observat modificări la CT/ IRM cerebral aveau întârziere mintală, iar 49 (44,54%) prezentau crize epileptice.

Leziunile porencefalice au fost evidenţiate la 28 (21,70%) pacienţi (fig. 1A, 1B), dintre care 27 erau clinic diagnosticaţi cu hemiplegie spastică şi unul cu tetraplegie spastică.

La 13 (10,07%) copii, toţi având forme spastice sau mixte de PC, s-a observat la examenul neuroimagistic atrofie corticală/ cortico-subcorticală, probabil consecinţa hipoxiei şi ischemiei perinatale (fig. 2A, 2B, 2C). Hemiatrofia cerebrală a fost prezentă în 2 cazuri de hemiplegie spastică.

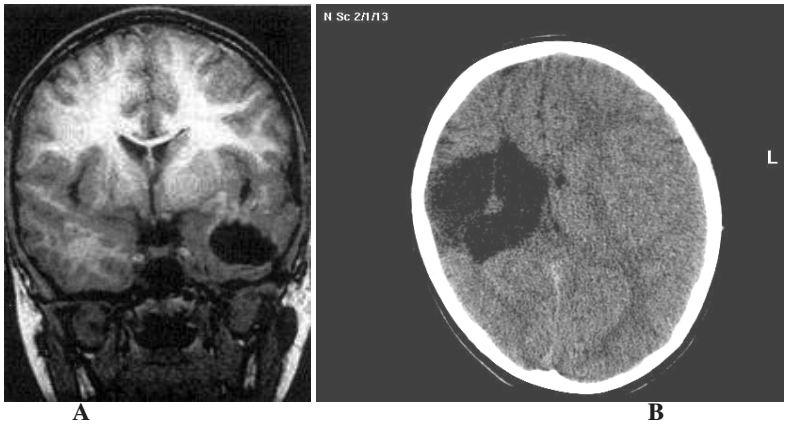
La un număr de 38 pacienţi s-a remarcat dilatare ventriculară de cauză posthemoragică sau infecţioasă din perioada neonatală, dintre aceştia 11 (28,94%) au prezentat în evoluţie hidrocefalie (fig. 3A, 3B).

Leziunile localizate la nivelul ganglionilor bazali şi a talamusului au fost prezente la 12 copii (9,3%), dintre care 8 (66,66%) erau diagnosticaţi cu PC diskinetică, 3 (25%) cu tetraplegie şi unul (8,34%) cu diplegie spastică.

Calcificările cerebrale au fost secundare infecţiilor intrauterine, în special cu citomegalovirus şi *Toxoplasma gondi*, fiind prezente la examenul neuroimagistic în 9 cazuri.

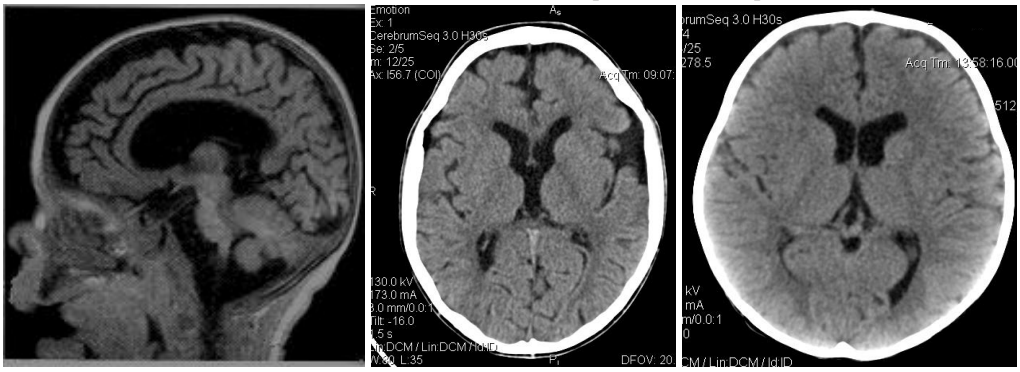
În cazurile cu PC ataxică modificările neuroimagistice au fost limitate la fosa posterioară la 4 copii; la un pacient anomaliile au fost localizate atât la nivelul cerebelului cât şi a lobilor parietali.

Leziunile de leucomalacie periventri-



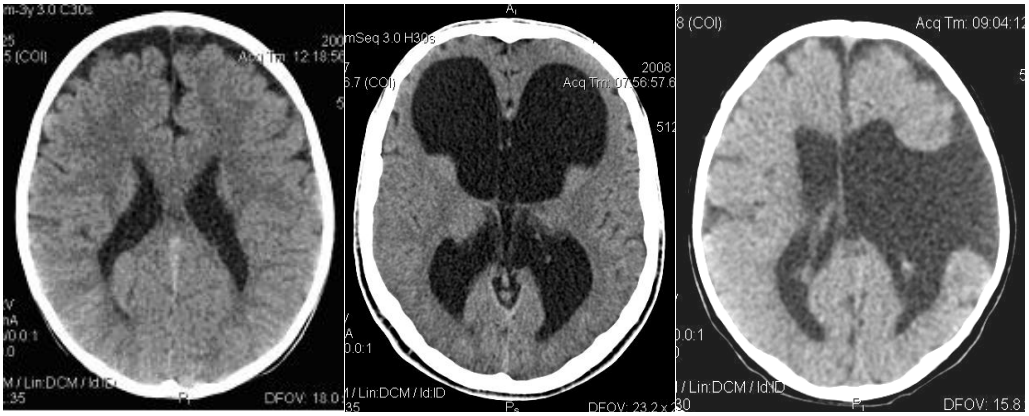
A **B**

Fig.1. A. IRM cerebral secţiune coronală : chist porencefalic temporal stâng.
B. CT-scan cranio-cerebral : chist porencefalic drept



A **B** **C**

Fig. 2. **A** - IRM cerebral secţiune sagitală : atrofie cortico-subcorticală difuză, dilatarea cisternei prepontine, prebulbare şi cisternei magna. **B** - CT cerebral : atrofie corticală bilaterală, chist arahnoidian stâng. **C** - CT cerebral : atrofie cortico-subcorticală bilaterală



A **B** **C**

Fig. 3. A. CT cerebral hidrocefalie neprogresivă, atrofie corticală bilaterală. **B.** CT cerebral hidrocefalie internă stabilizată. **C.** CT cerebral schizencefalie

culară (LPV) au fost remarcate la 17 pacienţi (13,17%), toţi diagnosticaţi cu forme spastice de PC: 10 (58,82%) cu diplegie spastică, 4 (23,52%) cu tetraplegie spastică şi 3 (17,66%) cu hemiplegie spastică. Tulburările de migrare şi organizare corticală au fost înregistrate la 9,30% din copiii cu PC luaţi în evidenţă şi au fost reprezentate de displazie corticală (2 cazuri), polimicrogirie (3 cazuri), schizencefalie (4 cazuri), pahigirie/lisencefalie (3 cazuri) (fig. 3C). Displaziile corticale au fost prezente la copiii cu forme spastice de PC.

DISCUŢII

Examenele neuroimagistice sunt importante la copiii cu PC deoarece în majoritatea cazurilor pot explica etiologia deficitului motor sau a unor patologii asociate. Candy (1993) a stabilit cu ajutorul IRM cerebral etiologia la 23% din pacienţii cu PC investigaţi, iar Cohen (1981) a remarcat o corelaţie între modificările prezente la CT cranio-cerebral şi existenţa crizelor epileptice sau a retardului mintal la copiii hemiplegici. Pe de altă parte, Molteni (1987) apreciază că, la pacienţii cu PC, nu există o legătură clară între aspectele morfologice şi cele clinice care s-ar putea explicata prin complexitatea modificărilor structurale şi prin proprietăţile de remodelare a SNC după injurii pre/postnatale. În lotul studiat 65,45% dintre bolnavii la care s-au observat modificări neuroimagistice aveau întârziere mintală, iar 44,54% prezentau crize epileptice.

Ashwal (2004) apreciază eficienţa CT cranio-cerebral în medie de 77% în depistarea anomaliilor cerebrale şi a IRM cerebral de 89%. Aspectul normal al examenului neuroimagistic apare la 11,7-31% dintre copiii cu PC (5, 10). În studiul nostru la 14,72% dintre pacienţi nu s-au identificat anomalii neuroradiologice.

Analizându-se modificările înregistrate la CT/IRM cerebral la ceilalţi copii cu PC urmăriţi s-a încercat realizarea unei corelaţii clinico-radiologice. Astfel, s-a remarcat că toţi pacienţii cu PC diskinetică aveau leziuni localizate la nivelul ganglionilor bazali şi a talamusului. Aceste date sunt în concordanţă cu cele prezentate de Bax (2006) care a observat anomalii în aceste regiuni la 75,6% dintre copiii cu forme extrapiramidale de PC.

De asemenea, în lotul studiat toţi copiii cu PC ataxică au prezentat anomaliile neuroimagistice care în 4 cazuri au fost limitate la fosa posterioară, iar într-un caz modificările au fost localizate atât la nivelul cerebelului cât şi a lobilor parietali. Aceste observaţii se regăsesc şi în studiul lui Miller (1989) care utilizând CT cranio-cerebral la 29 de bolnavi cu PC ataxică, a evidenţiat anomalii de fosă posterioară la 28% din pacienţi, leziuni şi la nivelul lobilor parietali la 55% şi aspect normal la 38% dintre cazuri.

La pacienţii cu PC spastică se observă frecvent leziuni cortico-subcorticale, malformaţii cerebrale şi anomalii ale substanţei albe. Leziunile corticale focale au fost prezente în special la copiii hemiplegici, date asemănătoare cu cele raportate şi de alţi autori. Wu (2006) a observat anomalii focale la 45% din pacienţii cu hemiplegie spastică investigaţi. De asemenea, autorul remarcă frecvenţa crescută a displaziilor corticale (14%). La rândul său, Bax (2006) a observat la IRM cerebral malformaţii la 9,1% din pacienţii de vârstă pediatrică urmăriţi şi anomalii ale substanţei albe la 71,3% dintre cei cu diplegie spastică, la 34,1% cu hemiplegie şi la 35,1% cu tetraplegie. În studiul nostru, tulburările de migrare şi organizare corticală au fost prezente la 12 (9,30%) copii cu PC spastică, iar leziunile de LPV au fost observate la 17 pacienţi, dintre care 58,82% erau diagnosticaţi cu diplegie spastică, 23,52% cu tetraplegie şi 17,66% cu hemiplegie.

CONCLUZII

Explorarea neuroimagistică este utilă la copiii cu PC deoarece informaţiile furnizate sunt importante în înţelegerea etiologiei şi aspectelor clinice. Pacienţii cu PC care prezintă anomalii neuroimagistice au un risc crescut pentru crize epileptice şi întârziere mintală. Evaluarea morfologiei cerebrale prin examen neuroradiologic trebuie inclusă în protocolul de evaluare a fiecărui copil cu paralizie cerebrală.

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NOUTĂŢI

DISECŢIA DE AORTĂ ŞI ABUZUL DE AMFETAMINE

Incidenţa disecţiei de aortă (DA) este estimată la aproximativ 2,9 cazuri pentru 100000 de adulţi vârstnici, fiind rară la adulţii tineri (doar 7% dintre subiecţii cu DA având vârsta sub 40 de ani). Un studiu recent pune în discuţie o posibilă legătură între consumul de anfetamine şi riscul de DA la adulţii tineri : printre subiecţii cu vârsta cuprinsă între 18 şi 40 de ani spitalizaţi pentru diverse maladii între anii 1995-2007 au fost identificate 3116 de DA toracice sau toraco-abdominale. După ajustările efectuate asupra factorilor de risc cunoscuţi (vârstă, HTA, consum de cocaină, tabagism, dislipidemie, boli cardiovasculare ereditare, vascularite inflamatorii, traumatisme, sindrom Turner), consumul şi/sau dependenţa de amfetamine s-a dovedit statistic legate de un risc major pentru DA (odds ratio : 3,33 ; interval de încredere la 95% : 2,37-4,69 ; $p < 0,0001$). Stupefiant ! (Westover J et al. Aortic dissection in young adults who abuse amphetamines. *Am Heart J* 2010, 160 : 315-321).

Al.D. Ciochină

MORPHOLOGICAL DIAGNOSIS OF MYOCARDIAL INFARCTION - IN THE LIGHT OF THE CURRENT CLINICAL CLASSIFICATION

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MORPHOLOGICAL DIAGNOSIS OF MYOCARDIAL INFARCTION-IN THE LIGHT OF THE CURRENT CLINICAL CLASSIFICATION (Abstract): Ischemic heart disease is one of the leading causes of morbidity and mortality worldwide. Diagnosis of myocardial infarction (MI) can be a challenge, especially in the absence of acute coronary occlusion. Accurate histopathological identification and timing of myocardial infarction in humans are of particular importance, especially for clinical practice or for legal medicine. The post-mortem diagnosis requires not only up-to-date knowledge of coronary and myocardial ischemic pathology, but also a correct interpretation of these features in relation to the clinical scenario of the disease. For these reasons, it is important for pathologists to know the different clinical types of MI and to differentiate myocardial infarction from other forms of myocardial injury. **Keywords**: ISCHEMIA, MYOCARDIAL INFARCTION, SMALL VESSEL DISEASE.

Acute ischemic cardiac syndromes, which are acute myocardial infarction (MI), unstable angina and sudden coronary death, are acute life-threating diseases with high mortality rates (1). Coronary artery disease (CAD), which is the basis of most cases of MI, and also the ischemic myocardial pathology in various stages of injury and repair, is extensively studied to improve post-mortem diagnosis (2). Auxiliary techniques for highlighting ischemic injury have been developed or are now under investigation for improving diagnosis (3). Recent acquisitions, such as non- or minimally invasive post-mortem imaging techniques used to detect coronary occlusion and ischemic injuries, nowadays they attract much interest, as autopsy rates tend to decrease in many countries (4, 5). However, in some cases, these diagnostic modalities alone may prove inadequate or insufficient to explain a clinical suspicion of myocardial ischemia. Examples are sudden coronary death without thrombi, cases of peri-procedural myocardial ischemia after therapeutic coronary interventions or non-coronary causes of ischemia (6). Last but not least, there are certain types of myocardial injury, other than ischemic, that need to be considered. This is reflected in the current clinical classification of myocardial infarction, which differentiates various types of pathological mechanisms and evolving treatment strategies (7). Our paper proposed to review the previous MI appearances and to highlight the new concept

in the light of the current clinical MI classification.

1. Definition. The 4th universal definition of myocardial infarction (2018), agreed by the members of the European Society of Cardiology, American Heart Association and World Heart Association Federation, etc., describes five distinct MI subtypes based on large variety in the pathophysiology aspects of MI encountered in clinical practice. The term acute myocardial injury should be used when there is evidence of increased cardiac troponin (cTn) values, what many doctors label as "troponin leakage", "troponinemia" (8).

The known clinical diagnosis of MI is based on the presence of increased levels of cardiac troponin, in combination with prolonged chest pain, ECG records or regional wall anomalies indicative of recent onset ischemia or angiographic detection of a coronary thrombus, while the new concept proposes the need for differentiation of myocardial infarction from myocardial injury by highlighting the peri-procedural myocardial injury after cardiac and non-cardiac procedures and to use of cardiovascular magnetic resonance and computerized coronary tomographic angiography in diagnosis of suspected myocardial infarction (4, 5).

2. The new MI classification. In 2018, a group of pathologist experts differentiate five distinct MI subtypes (Table I) according with various pathophysiology MI appearances (9).

TABLE I

The new Myocardial Infarction classification

MI Types	ESC/AHA/ACC/WHF classification of MI 2018
Type 1	Acute atherothrombotic occlusion or mural thrombus with critical flow reduction initiated by plaque rupture or erosion
Type 2	Ischemic injury due to a myocardial oxygen supply-demand mismatch, which is not caused by coronary atherothrombosis (tab. II)
Type 3	Cardiac death in a clinical setting suggestive of ischemic injury (chest pain, ECG changes) but without definitive cardiac biomarker evidence
Type 4	PCI-related ischemic injury < 48 hrs. after procedure. Includes also cases of MI due to late stent thrombosis or restenosis
Type 5	CABG-related ischemic injury < 48 hrs. after the procedure

Type I of MI is the result of acute coronary atherothrombosis. In clinical studies, coronary thrombotic events can be observed in approximately 80% of patients with acute coronary syndromes (ACS). They are still classified based on ECG findings as either ST elevation types of MI (STEMI) or non-ST elevation types of MI (non-STEMI).

The most common cause of acute myocardial ischemia is atherothrombotic occlusion of a coronary artery (10). This implies that, in cases of sudden death, acute coronary occlusion may explain the arrhythmic death. The underlying pathology of mural or occlusive coronary thrombosis may be due to rupture or erosion of the plaque, or less frequently, to protruding calcified nodules. Also, intraplaque bleeding contributes to the acute flow reduction in these

cases (11).

Irreversible damage of myocardial cells is the hallmark of MI also known as ischemic necrosis, which is histologically a "coagulative" type necrosis. It should be differentiated from other types of necrosis, which can be found at autopsy, the type of contraction band necrosis, which is a feature of many other forms of myocardial injury (12).

The onset of ischemic necrosis is not immediate. The acute ischemic lesion develops in the "area at risk" (area of perfusion of the obstructed artery). Ischemic necrosis of myocardial cells can be detected with the current diagnostic methods after about 2-4 hours, but the onset depends on many variables, such as collateral circulation and microvascular pathology. After the onset of occlusion, myocardial necrosis evolves in a wave front-like pattern over several hours from the endocardium to the epicardium in the "risk area", eventually causing a transmural MI. During the wave evolution of the myocardial necrosis, ventricular arrhythmias may occur in the sub endocardium, even in the earliest stage of still reversible damaged myocardium, but also several hours after occlusion, when the wave of necrosis has evolved substantially. This is also important for pathologist to realize that early onset arrhythmia and SCD is an early sign of myocardial injury in the absence of any other changes, prior to the development of myocardial necrosis wave. It should be noted that later stages of myocardial necrosis, including fibrous scars may serve as a substrate for life-threatening arrhythmias (13).

Topographic distribution of MI in the heart. According to the involved myocardial region, MI is classified as regional when it involves the area of perfusion of one epicardial artery or circumferential when it affects a large part of the circumference of the ventricular wall. Regional MI can be either transmural, usually associated with ST segment elevations on ECG (STEMI), or only subendocardial (non-STEMI). Circumferential MI is mostly due to the general decrease in coronary perfusion pressure that interests the three major coronary artery branches and is localized in the subendocardial region only (fig. 1).

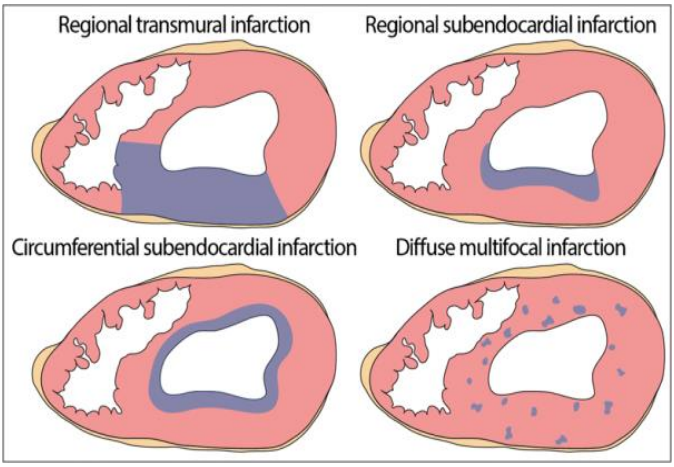

Fig. 1. Topographic distribution of MI in the heart.

The location of the MI is related by the perfusion territory of the three large epicardial arteries, such as: (a) obstruction of the anterior descending left coronary artery determines an antero septal AMI located in the anterior wall of the LV, 2/3 anterior IVS and the apex of the heart (40%; (b) obstruction of the posterior descending coronary artery causes a postero-inferior AMI, located in the posterior or inferior wall of the LV and 1/3 posterior of the IVS (30%); (c) obstruction of the circumflex branch of the left coronary artery gives a lateral AMI, located in the lateral wall of the LV, except the heart apex (20%); (d) obstruction of the left coronary artery before artery branching causes a massive antero-lateral AMI (10%) (14).

Other MI locations. Atrial infarctions occur in relation with ventricular infarctions and have a variable incidence in patients with MI ranging from 0.7 to 42%. Isolated atrial infarctions are rare (15). The main cause of atrial MI is coronary atherosclerosis. The pathological significance is related by the two potential complications of atrial MI: the first is the mural thrombus formation followed by thromboembolization, especially in patients with atrial fibrillation (16); the second is the rare atrial rupture that can result in cardiac tamponade (15).

Postmortem diagnosis of MI is based on gross and histological changes. Clinical-pathological correlations can be made by differentiating four evolutive stages of the lesions, which are related to the important clinical scenarios of ischemic morbidity and mortality (9).

a. **The earliest stage of cell death (the first hours).** Under experimental conditions, transmission electron microscopy (TEM) reveals ischemic changes (mitochondrial swelling and cardiomyocyte interruptions already occurring 10 minutes after onset). This method is not useful for detecting early human ischemia due to similarities between early ischemic changes and autolysis. The earliest light microscopic changes are regional wavy pattern of myocytes as a result of stretching of dead non-contractile myocytes due to adjacent functional myocardium during the cardiac cycle (8). In the first hours, the interstitial edema appears, and early onset of coagulative necrosis shown by cytoplasmic hypereosinophilia also develops, representing the early onset of coagulative necrosis (fig. 2). Enzymatic detection of early necrosis in fresh myocardium is made by using NBT reaction which is reported to be positive from 3 hours after the onset of ischemia. NBT stains only in the presence of intracellular lactate dehydrogenase (LDH). Nitro blue tetrazolium (NBT) staining of a fresh myocardial slice demonstrates early ischemic necrosis (due to leakage of enzymes from irreversibly damaged myocardium) while the vital myocardium is stained in deep purple (9, 14).

b. **Inflammatory stage (first week).** Neutrophil infiltration begins at MI border. Neutrophils can also invade the central area of a small MI, but this occurs much later depending on the size of the infarction. Coagulation necrosis continues by revealing the nuclear changes and myocyte disintegration. This is associated with heavy interstitial infiltrates of intact neutrophils and karyorrhexis of the neutrophils. Early phagocytosis of dead cells by macrophages, infiltration of other mononuclear cells, such as lymphocytes, and the onset of marginal fibrovascular response, show a further continuation of the healing process (fig. 3).

However, in large MIs, residual necrotic areas can still be detected even after many

weeks. Macroscopically, MI acquires a gray-yellowish color. At this stage, the necrotic myocardium is weak and vulnerable to septal, papillary muscles or free wall ruptures in case of transmural infarction, all with high mortality (9, 14).

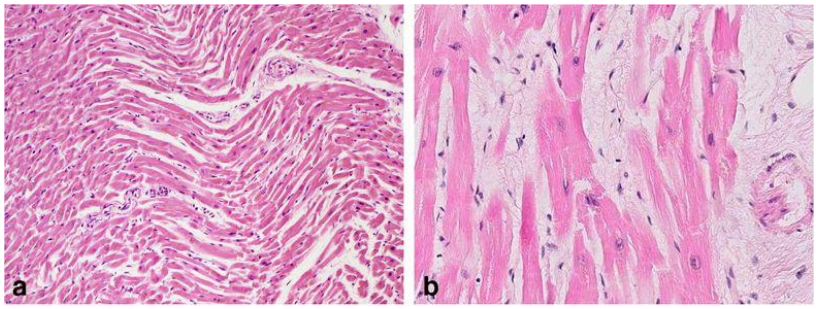

Fig. 2. a- myofiber waviness; b-interstitial edema.

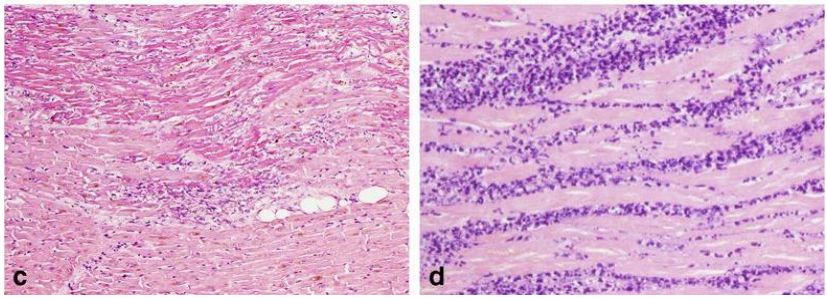

Fig. 3. c-hyper eosinophilia and coagulative necrosis of cardiomyocytes; d- heavy granulocyte infiltration.

c. **Granulation tissue stage (one week to several weeks).** This stage is characterized by capillary and fibroblasts proliferation with initial deposition of loose arranged collagen fibers and a persistent inflammatory infiltrate of lymphocytes, rare plasma cells, macrophages including siderophages, while the number of neutrophils decreases. Granulation tissue is most abundant at 2-3 weeks (fig. 4). Grossly, this tissue can be seen as a gelatinous hyperemic border around the necrosis (9, 14).

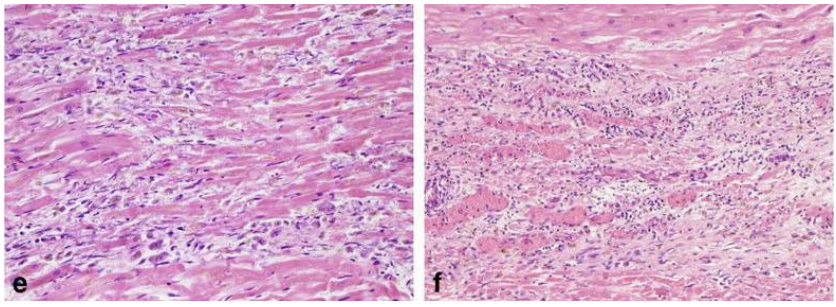

Fig. 4. e-macrophages infiltration; f - granulation tissue with formation of micro vessels.

d. **Late fibrotic stage (scarring) (over several weeks).** The granulation tissue gradually disappears and is replaced by dense collagen leading to fibrotic scars, which usually contain dilated vessels with thin walls (fig. 5). The rate of granulation tissue disappearance depends on the size of MI but is completed by the second month in most cases, leaving a hypocellular scar (9, 14).

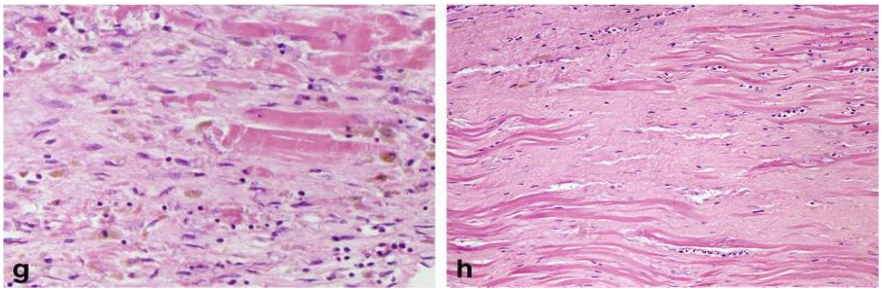

Fig. 5. g-fibroblast proliferation; h-dense fibrous scar replacing myocyte loss.

Type 2 MIs are heart attacks that result from an imbalance between myocardial oxygen demand and oxygen supply and are not due to acute disruption of coronary plaque and thrombosis (tab. II). In general, the frequencies reported by type 2 MI ranges from 10 to 30% of all patients with MI (17).

For example, MI with normal or low-grade angiographic stenosis (stenosis ≤50%) is a clinically recognized syndrome (called MI with non-obstructive coronary arteries, MINOCA), caused by a variety of pathology in and outside the heart. In a case of a fixed stenotic stable plaque of > 75% stenosis, exercise or spasm, can cause irreversible myocardial injury (18). Stenosis of 90% or more causes myocardial ischemia, even at rest, can be observed as a point-like/pinpoint lumen, less than 1 mm in diameter. Many of these stenoses are caused by fibro-calcified plaques but without thrombus. In these cases, microscopy of the malperfused territories of the myocardium can be a helpful adjunct in diagnosis.

TABLE II.

Causes of Myocardial Infarction of 2nd type

	Causes of MI without atherothrombotic coronary artery disease
Fixed coronary atherosclerotic plaques	In combination with non-coronary causes of oxygen demand-supply imbalance
Non-atherosclerotic coronary artery disease	Spasm/SVD, vasculitis, dissection, embolism, congenital anomalies, fibromuscular dysplasia, iatrogenic (stent or graft restenosis), PCI-related no-reflow
Non-coronary cardiac oxygen demand-supply imbalance	Sustained tachyarrhythmias, bradyarrhythmia, LV hypertrophy and/or dilatation
Extra cardiac oxygen demand-supply imbalance	Respiratory failure, severe anemia, hypovolemic shock

Morphologically, there are no distinctive gross or histologic hallmarks of coronary artery spasm (CAS). Drugs (androgenic anabolic steroids), physical and mental

stress are considered as precipitating factors of spasm (18). The presence of an area of regional myocardial infarction could indicate spasm in the artery when no other explanation for infarction is provided. It can affect either the normal or atherosclerotic epicardial arteries or the microcirculation, or both. It is the result of vascular smooth muscle cell hyper-reactivity as a pathophysiological substrate for spasm.

Microvascular dysfunction (MVD) is a functional impairment of flow in intramyocardial vessels and may cause MI. It is especially seen in hypertrophic and dilated hearts, preferentially in the subendocardial areas of the myocardium. Moreover, microvascular embolization can occur in patients with atrial fibrillation (19).

Type 3 MI. The patients presenting with type 3 MI have new ECG changes or are in ventricular fibrillation (VF), but who die before cardiac biomarkers of ischemia can be identified. A frequency of 3-4% of total MI has been reported. HP study of the myocardium reveals coagulative myocytolysis as a hallmark for VF (20).

Type 4 MI is diagnosed by a significant increase in cardiac biomarkers related to percutaneous coronary revascularization procedure. It can be temporally linked to the procedure (within 48 hours) leading to critical reduction of myocardial blood flow; it may be also due to acute complications of a device, such as in-stent thrombosis, coronary dissection or the late stent complications, such as restenosis and late-onset thrombosis (21).

Prompt restoration of coronary blood flow preserves the myocardium. Histological changes include hemorrhage, contraction band necrosis, endothelial swelling, and vascular plugs.

Type 5 MI is due to ischemic injury associated with coronary artery bypass grafting (CABG) that occurs within 48 hours of the procedure. It can be related to the procedure or to low-flow or reperfusion injury (9).

3. Ischemia and reperfusion and other forms of injury. After coronary intervention, PCI or CABG, the myocardial and microvascular pathology is characterized by peri-procedural ischemia, myocardial reperfusion injury and no-reflow phenomena, in association with pre-existent ischemic pathology of the myocardial risk area at (22).

Contraction band necrosis (CBN) of myocytes is the earliest sign of myocardial injury, arising at 10 min after onset of ischemia and reperfusion, and is characterized by the occurrence of thick eosinophilic bands in the cytoplasm of cardiomyocytes due to clustering of hypercontracted contractile proteins (fig. 6). After flow restoration (reperfusion), dying cardiomyocytes with ischemic damaged cell membranes are exposed to high serum Ca^{2+}concentrations, leading to massive Ca^{2+} influx followed by irreversible hypercontraction (CBN). CBN occur frequently in the periphery (the 'border zones') of ischemic infarctions, which is due to microvascular collateral perfusion from adjacent vital myocardium (23).

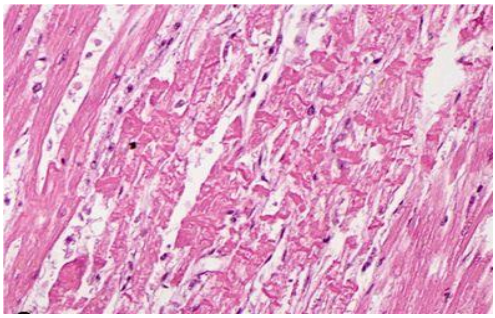

Fig. 6. Contraction band necrosis

Contraction bands are not only a feature

of ischemic infarction alone. They can also be observed after CPR including defibrillation and other trauma. CNB are related to PCI, but they also are observed in situations of catecholamine excess, free radical injuries, etc. CBN is accompanied by microvascular injury leading to endothelial swelling, microvascular obstruction and interstitial hemorrhages (9, 24).

4. Experts appreciation. The 2018 New Concepts in differentiation of myocardial infarction from myocardial injury consists (7) in: (a) Highlighting peri-procedural myocardial injury after cardiac and noncardiac procedures; (b) Use of cardiovascular magnetic resonance in suspected myocardial infarction; (c) Use of computed tomographic coronary angiography to define the etiology of myocardial injury.

In both cases of MI associated with PCI or CABG (clinical types MI 4 and 5), the application of post-mortem coronary angiography should be considered. This method allows to localize stents, to visualize the patency of stents and grafts and to evaluate the distal arterial bed and presence of collateral vascularization. Histological sampling of the involved myocardium is crucial for investigating the presence of myocardial injury.

CONCLUSIONS

Histopathological exam represents the first and last opportunity to make an accurate diagnosis in AMI and SCD. The methods of investigation has been updated including not only a protocol for examination of the heart and histological sampling, but, when necessary, toxicology, microbiology, biochemistry, genetic, molecular investigation and imagistic methods.

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PRELIMINARY STUDIES ON THE INFLUENCE OF PEPTIDE POPULATIONS ON THE SURFACTANT PROPERTIES OF A HYDROLYZATE

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PRELIMINARY STUDIES ON THE INFLUENCE OF PEPTIDE POPULATIONS ON THE SURFACTANT PROPERTIES OF A HYDROLYZATE (Abstract): **Aim:** To highlight the involvement of the peptide population in the variation of surface tension of a hydrolysate obtained from bovine hemoglobin. **Material and methods:** Hydrolysis was performed using a 1% (w/v) hemoglobin solution. The hydrolysis reaction was ended after 2.5 minutes, 10 and 120 hours using a 5M NaOH solution to adjust the pH value to 9.5 in order to inactivate pepsin. The HPLC system used included a Waters TM 600 pump controller and a Waters 996 diode bar detector. The surface tension was measured using a KRUSS DSA 10Mk2 tensiometer. **Results:** The analysis of the chromatographic profiles obtained for the peptic hydrolysates revealed the existence of many bioactive peptides. Large peptides were present in the 2.5-minute hydrolysate and hem could be detected (38 minute-retention time). For the 10- and 120- hour hydrolysates, hem could be detected with a retention time of 35 minutes. Surface tension varied at lower concentration, while at higher concentrations it was almost constant. The 1% (w/v) unhydrolyzed hemoglobin solution had a 40 $mN \cdot m^{-1}$ surface tension. **Conclusions:** The process of hydrolysis influenced the surface tension of protein hydrolysate solutions. It was found that the hydrolysates exhibited reduced surface tension when compared with unhydrolyzed hemoglobin. A preliminary characterization of hydrolyzers proved they had similar stability. **Keywords:** PEPTIDIC POPULATION, SURFACE TENSION, BOVINE HEMOGLOBIN.

The nutritional properties of food proteins have been investigated for many years, and it was found to depend on their amino acid content (1, 2). The possibility of releasing the biologically active peptides from food proteins has gained a lot of interest recently due to their effects on numerous physiological functions. Bioactive peptides are short sequences of amino acids, that are inactive within the sequence of the parent protein, but once released, they have positive effects on various body systems including the circulatory, gastrointestinal, and nervous sys-

tem. Such peptides can be released through chemical or enzymatic hydrolysis (3, 4).

Enzymatic protein hydrolysis is a reaction that involves the breakdown of peptide bonds under the action of an enzyme, resulting in the release of longer or shorter fragments, called peptides. The use of enzymatic hydrolysis to produce bioactive peptides is preferred to microbial fermentation due to the short reaction time and predictability (4, 5). As a result of enzyme hydrolysis, an improvement of organoleptic properties of proteins is observed and very complex peptide populations are obtained. However, numerous tests performed on these hydrolysates reveal low concentrations of several peptides of multiple interests, the so-called bioactive peptides (5). The physical stability of emulsions containing bioactive ingredients is an important aspect for the functional development of foods. There are a few studies on the foaming properties of peptide solutions, which are of interest for the study of selective peptide extraction systems due to the connection between their surfactant properties and their physicochemical state (6, 7). Larre *et al*. (8) compared the foaming properties of several hydrolysates obtained from rapeseeds at different pH levels. The hydrolysates obtained by basic chemical hydrolysis and pepsic hydrolysis showed different peptide populations, but their foaming properties were optimal at 3% hydrolysis degree in both cases. Perez A.A. (9) investigated the foaming properties of several hydrolysates with various hydrolysis degrees (5.6, 23.5, 45.3). Increasing the hydrolysate concentration leads to an increase in foaming capacity and to a more stable foam.

Our research investigated the effects of various peptide populations with different hydrophobic characteristics on the surface tension of hydrolysates obtained from bovine hemoglobin. Bovine hemoglobin was selected because it is inexpensive, easily available, well-characterized, and has been used in numerous studies (10-15).

MATERIAL AND METHODS

Solution preparations

The 14.36% hemoglobin solution was prepared by dissolving the corresponding amount of bovine hemoglobin powder (Sigma, ref. H2625) in 100 mL distilled water, which was then centrifuged at 4000 rpm to remove the insoluble portion.

The pepsin stock solution was prepared by dissolving 100 mg pepsin in 20 mL distilled water.

A 5M sodium hydroxide solution was also used.

Peptic hemoglobin hydrolysis

Hydrolysis was performed on a 1% (w/v) hemoglobin solution obtained by diluting the 14.36% hemoglobin solution.

50 mL of 1% (w/v) hemoglobin solution (adjusted to pH = 3.0 with 1M HCl) was mixed with 10 mL pepsin solution (3200 AU/mg) corresponding to a 2.5% (w/v) enzyme/substrate ratio. Hydrolysis reaction was stopped after 2.5 min, using the 5M NaOH solution, in order to adjust the pH at 9.5 that inactivates pepsin. 10 h- and 120 h- hydrolysates were obtained under the same conditions. All hydrolysates were filtered through 0.45 μm cellulose acetate filters, and then analyzed by reverse phase high performance liquid chromatography (9, 10).

The HPLC system used included a Wa-

ters TM 600 pump controller and a Waters 996 diode bar detector. A Vydak C4 column (0.46×25 cm) was used for separation. The mobile phase consisted in an A eluent containing water and trifluoroacetic acid (TFA) in a 1000:1 (v/v) ratio, and a B eluent composed of acetonitrile, water, and TFA in a 600:400:1 (v/v) ratio at a flow rate of 1 mL/min. The gradient applied was 0-67% (v/v) B over 30 minutes, then 67–87% (v/v) B over 35 minutes (11). UV absorbance scans were performed between 200 and 300 nm.

Surface tension measurement

Surface tension between the aqueous phase containing the peptides and air was measured in order to determine the surfactant properties of various hydrolysates. A KRUSS DSA 10Mk2 tensiometer was used. The first drop of solution that was formed was neglected and the measurements were made on the second drop.

After droplet suspension, surface tension measurements were done after 5 minutes, time required for peptides that have surfactant properties to position themselves at the water/air interface in a stable manner. In order to obtain a stable and reproducible measurement there was a 30-minute waiting time. The tensiometer was connected to a data station that recorded the data and calculated the surface tension depending on the droplet shape. Surface tension of the unhydrolyzed hemoglobin solution and 2.5 min-, 10 h-, and 120 h-hydrolysates was measured.

RESULTS AND DISSCUSION

Hydrolysis was performed on bovine hemoglobin in native state. Figure 1 shows the chromatographic profiles of hemoglobin used to obtain the hydrolysates, where hem molecules were revealed at a 38-minute retention time, while the alpha and beta molecules were detected at retention times between 40 and 50 minutes.

Once the hydrolysates were obtained, the reaction was stopped after 2.5 min, 10 h and 120 h respectively, using the 5M NaOH solution. The chromatographic profiles of the hydrolysates (figs. 2-4) revealed a wide variety of peptides. The 2.5 min-hydrolysate showed a peptide population with retention time between 20 and 36 minutes. There were large peptides with highly hydrophobic surfaces. The hem was detected through chromatography at 38 minute- retention time. Those peptide fractions were not found in the other two hydrolysates. In the 10- and 120 h-hydrolysates, the peptides were smaller and more hydrophilic. The hem was detected in the chromatogram at a 35 minute-retention time. Those results showed that by increasing the hydrolysis time, smaller peptides were produced.

We have observed that the hem in all analyzed sample was not degraded by pig pepsin. Hemoglobin chains were hydrolyzed in order to obtain peptide hydrolysates containing various peptides from the initial protein based on their molecular weight, number of ionizable sites, and surface hydrophobicity.

Figure 5 shows the comparative results obtained during the measurements of surface tension as well as the evolution of surface tension for nonhydrolyzed hemoglobin and hydrolysates.

The process of hydrolysis influenced the surface tension of the solutions. It was found that hydrolysates exhibited lower surface tension compared with nonhydro-

lyzed hemoglobin. Also, the 10- and 120 h-hydrolysates had a distinct profile compared to the 2.5 min-hydrolysate, which led to the conclusion that hydrophobicity and peptide size were important parameters that influenced surface tension.

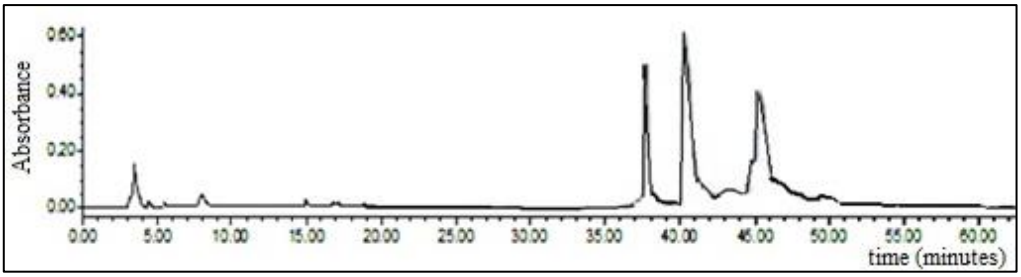

Fig. 1. Chromatographic profiles of hemoglobin

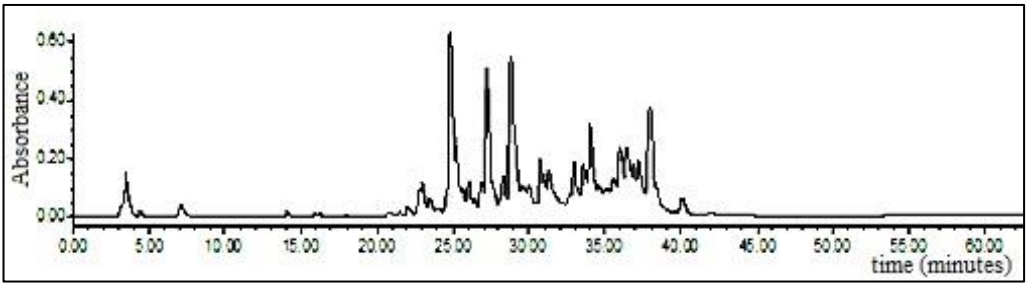

Fig. 2. Chromatographic profiles of 2.5 min-peptide hydrolysate

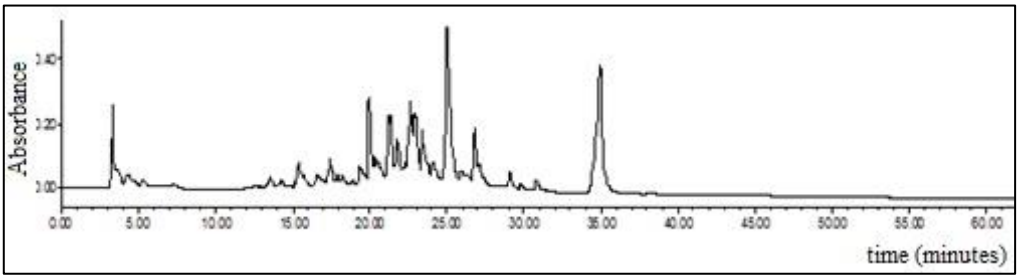

Fig. 3. Chromatographic profiles of 10 h-peptide hydrolysate

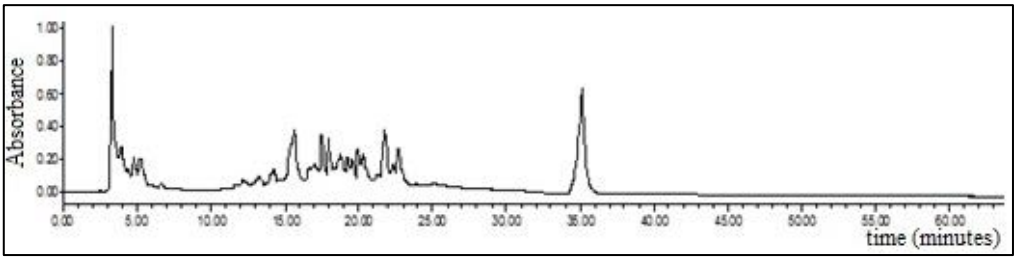

Fig. 4. Chromatographic profiles of 120 h-peptide hydrolysate

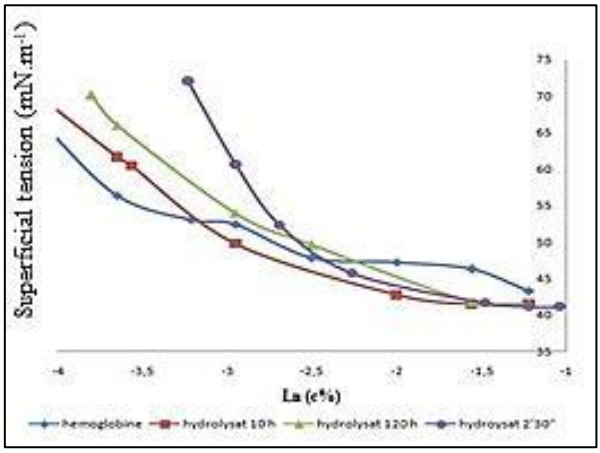

Fig. 5. Evolution of surface tension depending on the concentration of nonhydrolyzed and hydrolyzed bovine hemoglobin

The correlations between chromatographic profiles and stress curves showed that high hydrophilicity resulted in lower values of surface tension that translated into a better affinity of peptide populations to the air/liquid interface. For the hydrolysates, it was noticed that at low concentrations the surface tension values were distinct, while at higher concentrations they tended towards the same value. The surface tension of the nonhydrolyzed hemoglobin solution stabilized around 40 $mN.m^{-1}$, which was similar to other studies (11, 15).

CONCLUSIONS

In the present study, we have obtained peptide hydrolysates through bovine hemoglobin hydrolysis using pepsin. We have used droplet tensiometry to investigate the surface tension between air and the aqueous phase containing the peptides. The hydrolysates presented lowersurface tension values than nonhydrolyzed hemoglobin, meaning that the presence of peptide population reduces the surface tension. Therefore, further studies are needed to determine the critical micelle concentration or hydrophilic-lipophilic balance value indicating the surfactant properties of the peptides. These studies will also allow us to determine the optimal concentration of a hydrolysate required to achieve stable surfactant properties.

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NEWS

CARDIAC AMYLOIDOSIS: WHAT'S NEW, WHAT'S OLD

AND WHAT SHOULD WE EXPECT

New perspectives for diagnosing cardiac amyloidosis using especially the imagistic part of this illness are under research. From a symptomatic point of view, till present time there are no new discoveries. The same symptoms that were previously discovered are also present today. Advancements in the imagistic part offers perspectives by using cardiac MRI, bone scintigraphy and PET. Imagistic presents an easy path for diagnosing cardiac amyloidosis. MRI presents new perspectives for understanding pathophysiological processes. Furthermore, MRI can detect and diagnose cardiac amyloidosis but cannot differentiate the subtype involved. Bone scan has almost completely reduced the need for cardiac biopsy, by detecting, diagnosing and even differentiating the subtype of cardiac amyloidosis involved. PET can quantify cardiac and extracardiac amyloid burden, but this method is still being studied (L. Chacko, R. Martone, F. Cappelli, M. Fontana. Cardiac Amyloidosis: Updates in Imaging. *Curr Cardiol Rep* 2019; 21: 108).

STUDY ON THE BENEFITS OF USING DIAGNOSTIC WAX-UP IN DENTAL TREATMENT

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STUDY ON THE BENEFITS OF USING DIAGNOSTIC WAX-UP IN DENTAL TREATMENT (Abstract) The diagnostic wax-up is a representation of the future prosthesis, allowing the patient's checking and adaptation with the shape, color and position of the future prosthetic appliance, prior to the treatment being executed. It offers the possibility of the patient's active intervention in order to perform the prosthetic work and, with its help, the functional recovery and the correct evaluation of the occlusal rapports can be verified. **Material and methods**: This paper is a prospective study on a batch of 103 patients, who came to the dental practice and who were questioned, in order to evaluate the necessity to introduce the diagnostic wax-up into the prosthesis algorithm. The modality of obtaining the data was by the accounting of the information obtained after the answers to a questionnaire; the data obtained from the questionnaires were statistically interpreted by using *SPSS version 14* program. **Results**: the results show that most of the patients (96%) consider the diagnostic wax-up to be useful as a communication tool with the dentist. However, there are patients who are reluctant (8%); the patients believe that the communication with the dentist can be improved which would lead to the increase in the satisfaction degree regarding the dental prosthesis in the oral cavity. **Conclusions**: Following this analysis, it can be concluded that diagnostic wax-up will become an indispensable tool in the dental practice and its advantages recommend it as a compulsory stage in the complex oral rehabilitation. **Keywords**: DIAGNOSTIC WAX-UP, PROSTHETIC APPLIANCES, FUNCTIONAL REHABILITATION.

The prosthetic rehabilitation involves the restoration of the functions of the dental-maxillary system and the re-establishment of a new dental-somato-facial balance.

In order to improve the dentist-technician-patient communication, in order to obtain appropriate results, it has been imposed a technological stage included in the first phase of the clinical-technological algorithm: the diagnostic wax-up (1). The assessment of the treatment needs for every patient is a routine procedure in the dental practice. The estimation may vary from one practitioner to another and represents a complex aspect and sometimes even inconsistent between the clinical reality and the patients' expectations, who are often unable to estimate the limitations imposed by the features of the prosthetic field and who do not have the ability to predict the final re-

sult. The diagnostic wax-up allows, besides the radiological exploration and the preliminary footprint, heading towards a treatment plan and offering the patient a clear and correct perception upon the real situation.

Used for decades, the diagnostic wax-up is nowadays able to assure a prosthetic rehabilitation at the highest aesthetic, morpho-functional and biological standards due to the versatility of its uses. The achievement of the diagnostic wax-up involves the prefiguration of the final results from the very first phase, as well as the adjustment of the treatment plan and the patient's active intervention in achieving the prosthetic work. With its help, one can evaluate the aesthetics, phonetics and functionality of an oral rehabilitation or to evaluate and correct the occlusion in the case of a mandibular cranial mal-relation. This diagnostic wax-up is successfully used in prostheses by direct and indirect techniques or in temporary prosthesis and it can function as an operating pattern in the preparation of organic substructures or even in implantology. A strong advantage is the fact that it allows the optimization of the cervical adaptation of prosthetic restorations obtained by computerized technologies. Dentists agree that the success of the dental treatment consists not only of perfect technological execution, but also requires a detailed planning of the treatment. Thus, it is found that the analysis of the study layout is not enough in obtaining a successful final result, which is why the intermediate pieces have been imposed - the diagnosis models. More and more often, trial layouts are sent to the dental technique laboratory so that the technician will perform diagnosis layouts in order to visualize the finality of the proposed treatment plan. Sometimes, these layouts are used for emergency prosthesis performed by the indirect technique (2). Unlike the temporary prosthetic pieces obtained by the direct technique in the dental practice, the prostheses achieved in the laboratory have an increased resistance to the pressure and traction forces and have a superior functional rehabilitation.

The purpose of using the diagnostic wax-up is for the patient to visualize the future prosthetic solution, a better communication with the dental technician, a clinical examination upon the adaptation and oral integration of the prosthetic construction. (3,4). The layout process from diagnosis wax is not a frequently used clinical-technological stage but it should be taken into consideration at the onset of every dental treatment, as it involves improving the patient-physician communication by actively involving the patient in establishing, modifying, or adjusting the therapeutic solution, improving the dentist-technician communication, the evaluation and correction of occlusion, the composing of an operator guide in order to correctly prepare the organic structures, the evaluation of aesthetics, functionality, operator guide for applying implants, the optimization of cervical adaptation in CAD CAM technology (5). According to the desired type of diagnostic wax-up of the dental technique laboratory, special waxes or white monochrome waxes can be used. This wax has various properties of common wax used in the layout process, whether parchment wax or for crowning. The analysis of the advantages and disadvantages is upon the dentist's discretion, who will establish the material used in the therapeutic strategy (6).

MATERIAL AND METHODS

In this paper, we performed a prospective study on a batch of 103 patients, aged between 19 and 80, who came to the dental practice with functional disorders. After the

clinical examination, the establishment of the diagnosis and the therapeutic strategy, we explained the utility of introducing in achievement algorithm of the dental prosthesis belonging to layout process stage diagnosed in the dental treatment. In the study protocol, we specified the aim and the field of research (the evaluation of the necessity to modify the clinical-technological algorithm for performing the prosthetic works) having as an objective the study of the correlations between different parameters. For this purpose, the patients filled in a questionnaire including the following: full name, age, sex, job, regularity of the visits made to the dentist, food preferences, the awareness of the importance to maintain the natural teeth on the arcade, knowing the limits of prosthetic restorations, the communication with the dentist, informing on the diagnostic wax-up, interest in getting involved into the knowledge of the treatment stages and the utility of the diagnostic wax-up, the desire to integrate the diagnostic wax-up in the current dental treatment.

The patients were informed that the aim of this questionnaire is to assess the necessity to introduce a new stage – the diagnosis layout – within the dental treatment and were assured that the information is confidential.

The data obtained after filling in the questionnaires were accounted and statistically interpreted by the SPSS program 14. According to the defined variables, we introduced every person's data who filled in the questionnaire and we obtained a table with 103 lines and 13 columns.

In order to express the centralized data with the aid of questionnaires, we used frequency tables expressed as graphics. Thus, we can create an overall image upon the information that will be used to observe correlations.

RESULTS

A first analyzed aspect was whether patients had any knowledge about this treatment stage and about its utility. Thus, we found that almost 55% of patients had information about the diagnostic wax-up (fig. 1) and 95% considered it extremely useful for the therapeutical success (fig. 2).

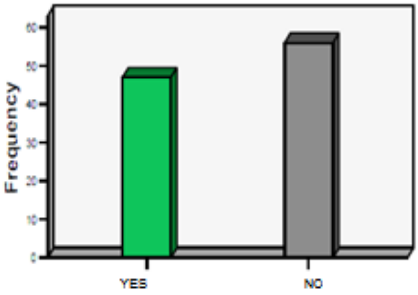

Fig. 1. The comparative diagram with patients who had information about the diagnostic wax-up previous to the filling in of the questionnaire

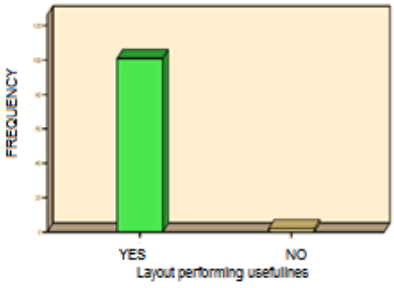

Fig. 2. The comparative diagram with patients who considered the layout performing useful in the dental treatment

A second aspect that we looked for was whether patients thought that diagnostic wax-up improve the communication with their physician and whether they want to

introduce this stage into the working algorithm. 93% of the respondents stated that in the diagnostic wax-up stage they had a clear image of the future prosthetic work and were able to intervene with suggestions on making morphological or color changes, which definitely improved the doctor-patient collaboration (fig. 3). Also, 96% of respondents agree to make the diagnostic wax-up before the onset of the prosthetic treatment.

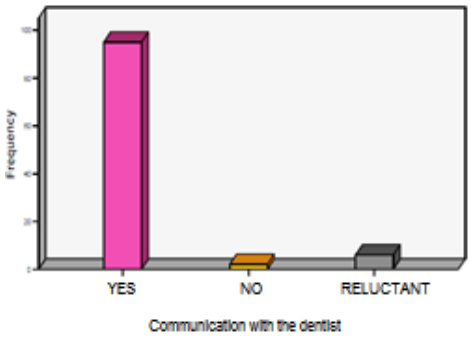

Fig. 3. The comparative diagram with patients who consider useful making the diagnostic wax-up for improving the communication with the dentist

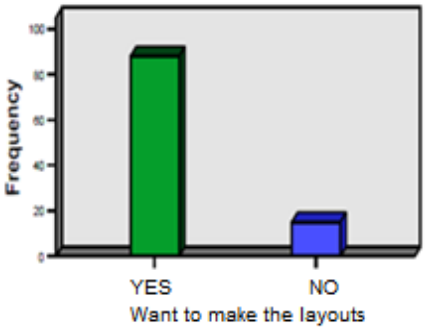

Fig. 4. The comparative diagram with patients who want to make the layouts before the onset of the prosthetic treatment

DISCUSSION

Besides the awareness effect upon the population regarding the existence of diagnostic wax-up, the comparative study of the variables included has proven that: most patients find the diagnostic wax-up useful as a communication tool with the dentist, however, there are reluctant patients (8%); patients believe that the communication with the dentist can be improved, which would lead to the increase in the satisfaction degree on the dental prostheses applied in the oral cavity (7).

In the study batch, the major aesthetic requests showed around the age of 24, and the disinterest in the aesthetic aspect of the dental rehabilitation is seen between the age of 60-80. The highest numerical values of those satisfied/dissatisfied with the dental treatment are around the age of 25; the major interest in maintaining the natural teeth on the arcade is noticed at the age of 22, progressively decreasing with the age.

There is a comparable percentage (59 41%) about the achievement of a diagnostic wax-up before the onset of the prosthetic treatment for dental prostheses and the achievement of diagnostic wax-up to determine the possibilities of aesthetic and functional improvement in the case of people without prostheses (fig. 5).

Although there are many patients who find useful the diagnostic wax-ups and want them to be done, there are also patients who find them useful but do not want their dental technician to create them (fig. 6)

The better the collaboration with the dentist and the dental technician, the more functional and aesthetic the prosthetic reconstruction will be, the patient will adapt much better to the artificial substitute and the social integration is faster (8) (fig. 7).

Most patients who have prostheses and

have been satisfied with the applied treatment agreed with the utility of performing the diagnostic layouts as intermediate therapeutic stage (fig. 8).

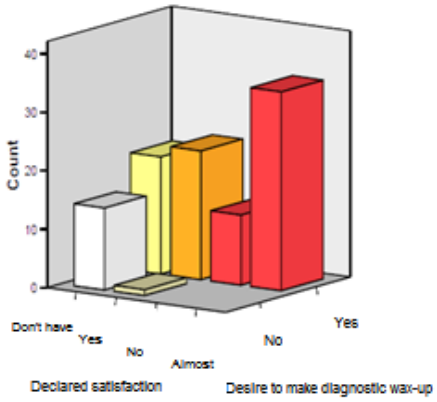

Fig. 5. The correlation between the satisfaction declared by patients about the dental prostheses and the desire to make diagnostic wax-up

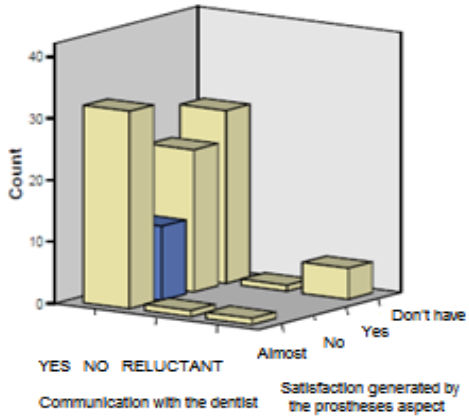

Fig. 6. The correlation between the communication with the dentist and the satisfaction generated by the prostheses aspect

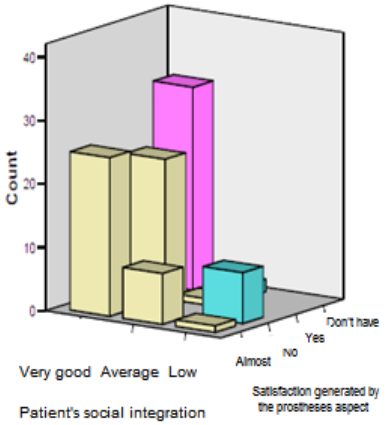

Fig. 7. The correlation between the patient's social integration and the satisfaction generated by the prosthetic piece in the oral cavity

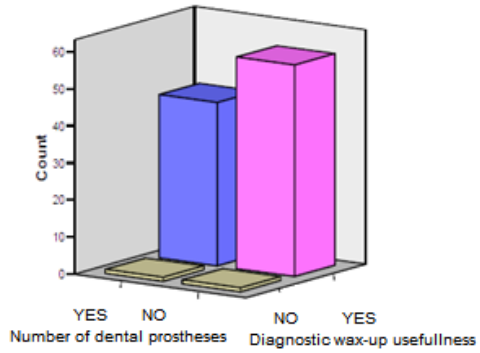

Fig. 8. The correlation between the number of patients who find the diagnostic wax-up useful and the number of dental prostheses

CONCLUSIONS

After the completion of the building stage of diagnostic wax-up, the following aspects could be specified in detail: the necessity to adjust the treatment plan, the modification of the occlusal relief in relation to the particularities of the clinical case, the modification of the chromatic or morphological parameters in relation to the patient's desires.

Supported by the statistical study, whose results prove that most patients de-

sire the creation of the diagnostic wax-up, this clinical-technological stage is very useful in implementing a therapeutic plan because it represents a communication method that allows the patient, the dental technician and the dentist to manage the complex prosthetic cases.

The diagnostic wax-up will increase the predictability degree of the final result, reducing the time that the patient spends in the dental practice while avoiding disagreements at the end of the treatment.

It also allows the creation of oral changes before the onset of the treatment plan, thus increasing the functionality of the finished prosthetic piece and guaranteeing a satisfying result of the treatment.

This intermediate piece predicts the therapeutic result and allows the patient to have a clearer explanation regarding the possible limits of the rehabilitation therapy.

The technology of creating the diagnostic wax-up is relatively easy to learn by dental technicians, and the appearance of digital layout technologies on the market substantially reduces the time allocated to this diagnostic wax-up provides the possibility of adjustments to restore the aesthetic function, under visual control, in the oral cavity both during rest and during exercising functions. With their help, temporary dental prostheses can be created, which increase the patient's compliance with the prosthetic treatment (9).

The patients will always accept more easily a prosthetic construction that they have seen in progress.

The questionnaires allowed us to obtain the patient's informed acceptance and his/her active involvement in dental prosthesis, with a better communication, an increased level of confidence in the dentist and an increased satisfaction at the end.

Taking into consideration the characteristics of the diagnostic wax-up, it can be stated that this will become an indispensable tool in the dental practice, its advantages indicating it as a usual stage in the clinical-technological algorithm of creating the complex oral rehabilitation.

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VALOAREA RĂSPUNSULUI CARDIOVASCULAR CA MARKER DE STRES ÎN TERAPIA CHIRURGICALĂ

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CARDIAC RESPONSE AS STRESS MARKER IN SURGERY (Abstract): Surgical area - as a precursor of negative emotions, is the threshold between the two areas of anxiety and anxiety among the general and specific stimuli. By dominant color, noise, odors and environmental factors in complex anxiety these elements define the specific treatment of dental medicine better. The emotional stress on the patient child which undergoes a surgical dento-alveolar therapy is negative and consists of the combination of three important elements, in close relationship: score of dental anxiety, the anticipation and experience of pain and phobic stimuli which is implicitly true precursor of pain. **Material and method**: Our study was performed on 448 patients. Different methods of preanesthesia were made per oral, intravenous, intramuscular, according with DAS score: Midazolam, Diazepam + Algocalmin, Xanax. **Results**: 58% of our patients declare after surgery that their satisfaction score is 100% positive after one of our method of preanesthesia. **Conclusions**: The purpose of premedication should be unconditionally determined by the effect of the analgesic and anxiolytic drugs to improve cardiac response and avoid medical emergency. Doses administered must be correlated with the anxiety score DAS, road use and the probable duration of surgery. **Key words**: PAIN, ANXIETY CONTROL, PREANESTHETICS DRUGS, CARDIAC RESPONSE

CHEMICAL PERSPECTIVE ON ANTI-AGING PROPERTIES OF SOME PHENOLICS FROM PLANTS

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CHEMICAL PERSPECTIVE ON ANTI-AGING PROPERTIES OF SOME PHENOLICS FROM PLANTS (Abstract): Nowadays it is well-accepted that topical polyphenol-rich products can reverse partially the clinical and histological changes in the epidermis and dermis induced by the combination of sunlight exposure and chronologic aging. Natural compounds used against photoaging comprise phenolic compounds, including flavonoids, phenolic acids (benzoic, gallic, cinnamic acids) and stilbenes derived from plants such as tea, grape, bergamot, rooibos, grapefruit, red orange. It was reported that many polyphenols from plants exhibit inhibitory activity against collagenases and elastases. Phenolic derivatives are efficient protectors through reduction of oxidative stress, inflammation and immunosuppression and may be important components in cosmetic formulations. **Keywords**: POLYPHENOLS, SKIN, ANTI-AGING.

Skin is a structure subject to an unpreventable intrinsic aging process. Skin aging is a biological process, complex, progressive and inevitable characterized by atrophy of the skin with loss of elasticity and slowed metabolic activity. Skin aging is characterized by signs as: wrinkling, dryness, laxity, scaling, roughness, hyperpigmentation etc (1, 2).

One of the major features of skin aging is the progressive proteolytic degradation of cutaneous elastic fibers that cannot be suitable replaced by adult dermal fibroblasts (3).

An overproduction of ROS depletes physiological levels of ROS-scavenging enzymes (such as superoxide dismutase (SOD) and catalase), resulting in damage to proteins, lipids and even DNA, and premature photoaging and skin diseases (4, 5).

Reactive oxygen species (ROS) are normally produced in cell metabolism but, when the balance between free radicals and antioxidants is unbalanced, these chemical species can participate to a pathological process known as oxidative stress.

In fact, the oxidative stress is involved in skin aging, the lipid peroxides level being a good parameter to determine the oxidative stress. Superoxide dismutase (SOD) and catalase increases with chronologic aging and they could have been considered as important markers of oxidative stress.

The main process in the skin aging are represented by the intracellular and extra-

cellular oxidative stress initiated by two main events: the formation of ROS and the induction of matrix metalloproteinases (6).

The formation of free radicals which can react with DNA, proteins, sugars and fatty acids can causes oxidative damage and it is a widely accepted that this mechanism causing skin aging by inducing wrinkling, elastosis, drying, roughness, appearance of fine lines, lack of elasticity and hyperpigmentation marks.

The induction of matrix metalloproteinases leads to the accumulation of fragmented collagen fibrils, which prevents neocollagenesis.

Aging is accelerated to sunlight exposure (ultraviolet radiation), a process known as photoageing. It is called photoageing because of a combination of short wavelength (UVB) injury to the outer layers of the skin (epidermis) and long wavelength (UVA) injury to the middle layers (dermis).

UV radiation is absorbed by skin molecules and generates ROS that determine "oxidative damage" for cellular components like cell walls, lipid membranes, mitochondria and DNA. Each UV exposure induces a wound response with subsequent imperfect repair, leaving an invisible "solar scar" repetitive UV exposure eventually lead to development of a visible "solar scar" manifesting as a visible wrinkle over a lifetime. Exposure to UV irradiation induces photochronical generation of ROS that activates cell surface growth factors, cytokine receptors and NADPH oxidase (7, 8). This process induces signal propagation within the cell through phosphorylation of tyrosine residues on the receptors and their associated adaptor proteins. Two nuclear transcription factors are activated: activator protein 1 (AP-1) and nuclear factor-κB (NF-κB), involved in transcription of genes for matrix-degrading enzymes and proinflammatory cytokines (9,10). These transcription factors are associated with skin dryness, pigmentation, laxity, deep wrinkling (11,12).

CHEMICAL CLASSIFICATION OF PHENOLIC COMPOUNDS

Plants are a good source of important products with therapeutic value. There is emerging evidence that topical application or oral intake of some polyphenol-rich plant extracts can reduce the skin aging process.

Phenolic compounds are characterized by the presence in their chemical structure an aromatic ring linked to a hydroxyl group, which has a great ability to donate electron and hydrogen, structural features important for their exceptional antioxidant activities.

Polyphenolic compounds have been extensively studied and are found in several plants including tea leaves (*Camellia sinensis*), grape seeds (*Vitis vinifera*), blueberries (*Vaccinium myrtillus*), almond seeds (*Prunus amygdalus*), pomegranate extract (*Punicagranatum*).

Phenolic compounds comprise one or more aromatic rings and have one or more hydroxyl groups. They are usually found as esters or glycosides rather than as free compounds, and mostly are present as conjugates with mono- and polysaccharides, linked to one or more of the phenolic groups. The term "*polyphenol"* includes a large group of substances, which have more than one phenolic hydroxyl group, bounded to one or more benzene ring systems (13).

The classification and structure of the phenolic compounds are presented in first figure (14, 15).

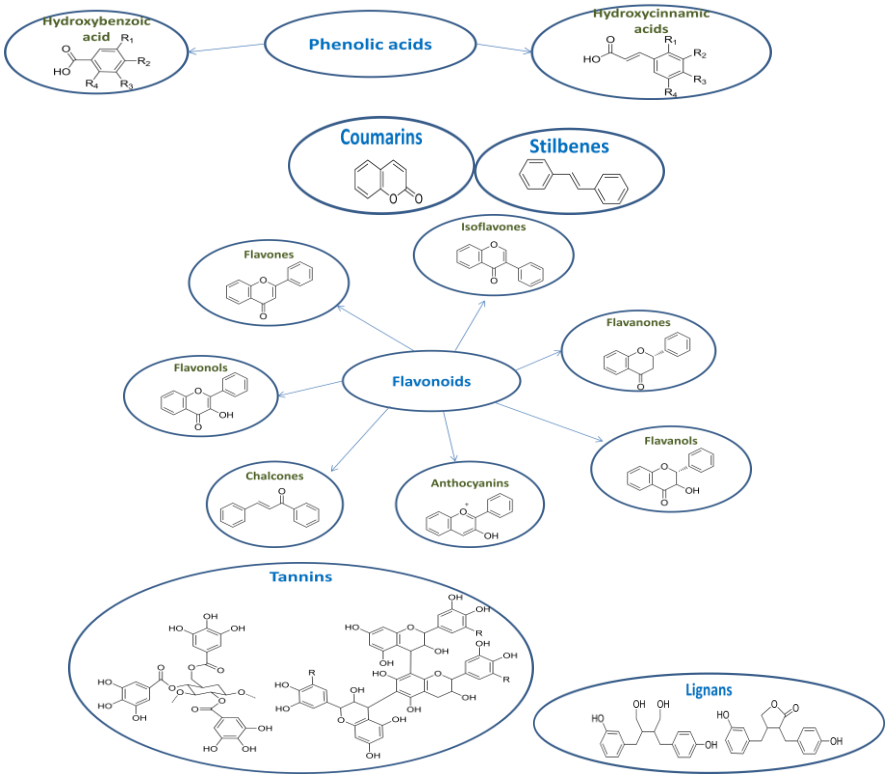

Fig. 1. Classification of the main phenolic compounds

NATURAL PHENOLS IN SKIN AGING PROCESS

The beneficial effects of polyphenols as functional ingredients have attracted considerable attention of the pharmaceutical and cosmetic industry in the last period of time. As a consequence, many skin care products have been developed based on polyphenol-enriched plant extracts. To exert their designated biological activities, topically applied substances have to be able to be released from the formulation, to reach the skin and finally to overcome the stratum corneum barrier and penetrate into the epidermis and dermis. The release of active substances and further skin permeation depends on the molecule properties such as molecular weight and lipophilicity but also on the vehicle formulation (16).

Consumers prefer to use more natural compounds on their skin. Thus, plant phenolic compounds are a promising target for new dermal cosmetics that possess the ability to maintain the skin homogeneity and a proper, healthy look due to effective skin cell renewal, elastin and collagen stimulation and inhibition of excessive melanin synthesis.

A lot of topical treatments for skin aging incorporate antioxidants. They scavenge free radicals and are known to contribute to the physiological effect on skin health, having a anti-wrinkle effect.

Topical application of antioxidant ac-

tive substances can support the skin's own antioxidant system against oxidative stress and could protect the skin against photoaging.

Most of the natural polyphenols can absorb UV radiation. Therefore, when applied topically, they can prevent penetration of the radiation into the skin. This ability of natural polyphenols to act as sunscreens can reduce inflammation, oxidative stress and DNA damaging effects of UV radiation in the skin and, thus, on topical application the photoprotective effects of polyphenols are due in part to this sunscreen effect.

The chemical structure of polyphenolic compounds is correlated with their reducing properties, which allow them to act as antioxidants and free radical scavengers. The antioxidant activity of flavonoids is essentially determined due to substitutions on rings B and C, for example the number and positions of hydroxyl groups in the B-ring as well as double bonds and substitutions in the ring C.

Phenolic extracts are found to inhibit the activity of proteinases, which catalyze the degradation of skin proteins, such as collagen and elastin. Collagen in the dermis is responsible for the firmness, elastin fibers lend the elasticity. An excessive ROS formation provokes the expression of collagenase (MMP-1) and elastase, leading to an accelerated degradation of corresponding proteins.

One of the best sources of phenolic antioxidants is tea (*Camellia sinensis* L.), especially green tea but also black tea, oolong tea and white tea, which possess great antioxidant properties.

The majority of the green tea polyphenols are monomeric flavanols known as catechins. The major catechins found in green tea are: catechin (C), epicatechin (EC), epigallocatechin (EGC), epicatechin-3-gallate (ECG), epigallocatechin-3-gallate (EGCG), gallocatechin-3-gallate (GCG). Green tea catechins are characterized by dihydroxy or trihydroxy groups on the B ring and the meta- 5,7-dihydroxy groups on the A ring, with the B ring appearing to be the principal site of antioxidant reactions. Such antioxidant activity is further enhanced by the presence of a trihydroxy structure in the D ring (gallate) in ECG, EGCG and GCG (17).

Besides tea, grape (*Vitis vinifera* L.), one of the largest fruit crops in the world, is an important source of polyphenols. The antioxidant activity of grapes seems to be depended on the color of the berries; dark fruits have higher antioxidant capacity than the white ones, because the content of anthocyanins and flavonoids increases with increasing color intensity of grapes. Apart from catechin, epigallocatechin and epigallocatechin-3-gallate, resveratrol was also found in grape skin. Antioxidant activity of plant extracts often correlates with the total phenolic content (18). However, the polyphenol profile also determines the effectiveness of extracts, as the activity of individuals substances varies strongly, caused by the differences in the chemical structure.

The high antioxidative activity of grape or tea extracts is derived from a combination of several active substances, which results in an increased activity due to some synergistic effects.

It was reported that many polyphenols from plants exhibit inhibitory activity against collagenases and elastases. During aging, the extracellular matrix proteins are susceptible to excessive activity of proteolytic enzymes-matrix metalloproteinases (MMPs).

The scientific literature highlights the efficacy of polyphenols as topical anti-

aging agents (resveratrol, EGCG, genistein).

Epigallocatechin-3-gallate (EGCG), a compound from green tea is effective in improving the skin condition by the reactivation of the damaged or old cells due to the DNA protection and production of more energy in the cells.

Topical treatment of the mouse and human skin with EGCG prior to UV exposure significantly reduced UVB-induced nitric oxide and hydrogen peroxide production.

Green tea polyphenols can be considered as inhibitors of both tyrosinase activity and expression in melanocytes, thereby inhibiting melanin production induced by UV irradiation. These compounds exert antiwrinkle effects through collagenase by affecting both direct enzyme activity and physical volume.

The steric structure of the 3-galloyl radical of catechin is very important for the inhibition of collagenase activity (19). EGCG has an additional galloyl unit and hydroxyl groups compared to catechin and, thus, can engage in better hydrogen bonding and hydrophobic interactions with collagenase, inducing significant changes in its conformation that result in potent inhibition (20). EGCG and ECG bind to collagen through extensive hydrogen bonding augmented by hydrophobic interactions, preventing the free access of collagenase to active sites on the collagen chains (21).

EGCG is a potent scavenger of ROS with exceptionally strong antioxidant activity. Similarly, Roh *et al.* (22), suggested that the ortho-trihydroxyl group in the B ring and the galloyl moiety at the 3-position of the flavan-3-ol skeleton of ECG and GCG are the most important structural requirements for the free radical scavenging effects. ECG also acts as a free radical scavenger on photodamaged keratinocytes.

EGCG or green tea polyphenols added to hydrophilic ointments inhibit UVB-induced oxidative stress through MAP kinase signaling pathways (22).

Green tea polyphenols act as antiaging compounds. EGCG and epicatechin-gallate are the major polyphenolic components capable to act as antioxidants *in vitro* by scavenging reactive oxygen and nitrogen species and chelating redox - active transition metal ions.

According to Katiyar's studies, EGCG reduces UV-induced erythema and sun burn cell formation and green tea polyphenols have an important role in reduction of UVB-induced pyrimidine dimmer formation in epidermis and dermis (23).

It is well known that excessive and chronic exposure of the skin to UV radiation can overwhelm the cutaneous antioxidant capacity, leading to a premature aging of the skin. Polyphenols from green tea have been shown to inhibit photo-enhanced lipid peroxidation. Topical treatment of the mouse and human skin with epigallocatechin-3-gallate (EGCG) prior to UV exposure significantly reduced UVB-induced nitric oxide and hydrogen peroxide production. It is well established that the infiltrating leukocytes is the major source of nitric oxide and hydrogen peroxide production. EGCG has been shown to have the ability to block UVB-induced leukocyte infiltration in mouse as well as in human skin, and thus may be able to inhibit UVB-induced production of reactive oxygen species by these infiltrating leukocytes. Wei *et al.* (24) have demonstrated that aqueous extracts of green tea have potent scavenging effects on reactive oxygen species.

Resveratrol (3, 4', 5-trihydroxy-trans-stilbene is a polyphenol that exists in two isomeric forms, 3, 4', 5-trihydroxy-cis-stilbene and 3, 4', 5- trihydroxy-trans-

stilbene, the *trans* form being biologically active.

Resveratrol functions as an antioxidant in several ways. It serves as a free radical scavenger that effectively quenches reactive oxygen species such as hydroxyl, superoxide and metal-induced radicals. Topical application of resveratrol has been proven to protect against UVB-mediated skin damage (25).

Specific polyphenol receptor sites have been identified in the skin that bind resveratrol, which then exert protection against the nitric oxide free radical donor sodium nitroprusside (26).

Resveratrol is reported to stimulate healthy cell proliferation as well as collagen synthesis by protecting the dermal matrix from harmful enzymes and improving the function of fibroblasts that produce healthy collagen.

Resveratrol has been shown to be active in neutralizing and inhibiting the formation of reactive oxygen species but *in vitro* conditions, is an effective neutralizer of synthetic DPPH radicals (8).

In vitro studies using the oxygen radical absorbance capacity (ORAC) test showed that resveratrol has 17 times higher antioxidant activity (4,845 μmols vitamin E/g) compared to the synthetic coenzyme Q10-idobenone (279 μmols of vitamin E/g) (9). In contrast, the antiradical activity of resveratrol relative against to peroxide radicals was higher than catechins, gallic acid and ellagic acid (10).

Resveratrol can also protect skin from photo-aging. In *in vivo* studies on hairless mice, it was observed that the single application of resveratrol before UVB exposure significantly reduced skin edema and reduced the UVB induced H_2O_2 production and also could limit lipid peroxidation and reduce leukocyte infiltration.

Resveratrol can successfully affect the protection of human keratinocytes by limiting the expression of NF-κB transcription factor and phosphorylation and degradation of IκB triggered by UVB radiation (27, 28).

In addition, *in vitro* studies using HaCaT cell lines have demonstrated that resveratrol protects keratinocytes against UVA radiation. Under the influence of UVA radiation and other stressors, free radicals and reactive oxygen species, especially superoxide anion and hydrogen peroxide, lead to oxidative stress (25).

The antiaging effect of resveratrol was also observed in clinical studies. During a 12-week clinical trial of 55 women aged 40-60 years old, it was observed that the use of night cream containing resveratrol (1%), baicalin (0.5%) and vitamin E (1%) could improve the skin condition, by limiting the changes caused by the photoaging process. Clinical evaluation showed statistically significant improvement in skin firmness and elasticity, smoothing fine wrinkles and reducing discoloration after 12 weeks (29).

It was found that resveratrol increased dermal and epidermal thickness through impact on greater collagen production, which may increase skin firmness and elasticity and lead to skin rejuvenation as well as wrinkle reduction (30).

Resveratrol cannot be considered as an inhibitor of tyrosinase as a substrate. Moreover, this substrate seems to be converted by tyrosinase into an inhibitor and even if oxyresveratrolacts as a non-competitive inhibitor of tyrosinase with L-Dopa, resveratrol is nevertheless able to occupy the L-dopa site. Resveratrol is a "suicide substrate" for tyrosinase.

One of functions of antiaging products is to prevent the formation of senile lentigines and solar lentigo. Hence, the possi-

bility of influencing tyrosinase activity by resveratrol is of great importance and practical application in the field of antiaging preparations whose function is to prevent the hyperpigmentation (25, 31).

In vitro studies showed that the percentage of resveratrol found in the stratum corneum after permeation was however very low, about 5% of the total applied, but still enough to cause a significant impact on the epidermal antioxidant capacity (32).

Quercetin is a flavonoid that has antioxidant effects, being able to influence cellular viability, lifespan and presenting rejuvenating effect on culture of senescent fibroblasts. Also, this polyphenol has the ability to block ultraviolet irradiation induced inflammation viaNF-κB inactivation on primary keratinocytes (33). Quercetin provided inhibition of matrix metalloproteinase activity and it can be considered an agent with a role in protection of skin collagen from destruction during inflammatory response to extrinsic aging factors (34).

Quercetin shows water solubility and it has a partition coefficient of 1.82 ± 0.32 due to the presence of nonpolar groups in its structure. But despite this log P value, quercetin polar hydroxyl groups hinder its skin penetration capacity (35).Due to its poor solubility, quercetin has limited skin penetration ability and various formulation approaches were taken to increase its dermal penetration.

CONCLUSIONS

Natural polyphenols possess scavenging properties towards radical oxygen species that make them interesting for antiaging purposes in cosmetics formulations skin care.

Polyphenols structures are active ingredients capable of counteracting symptoms of epidermal aging, can improve solar radiation protection and epidermal antioxidant activity and they have been considered important ingredients for anti-aging cosmetic formulations.

Topical antiaging products formulated with polyphenolic components make extensive claims to reduce wrinkles, fine lines and sun damage and their sales are expected to increase, based on the consumer interest in health and enhance the growth of this segment of the market due to the demand of the population opting for less invasive, non-surgical alternatives to slow the effects of aging on the skin.

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NEWS

MICROENVIRONMENT IN COLORECTAL CANCER

Transforming growth factor-beta (TGF-β) signaling is one of the important cellular pathways that play key roles for tissue maintenance. In particular, it is important in the context of inflammation and tumorigenesis by modulating cell growth, differentiation, apoptosis, and homeostasis. TGF-β receptor type 2 (*TGFBR2*) mutations affected by a mismatch repair deficiency causes colorectal cancers (CRCs) with microsatellite instability, which is, however, associated with relatively better survival rates. On the other hand, loss of *SMAD4*, a transcription factor in the TGF-β superfamily signaling, promotes tumor progression. Loss of heterozygosity on chromosome 18 can case SMAD4-deficient CRC, which results in poorer patients' survival. Such bidirectional phenomenon driven by TGF-β signaling insufficiency reflects the complexity of this signaling pathway in CRC. Moreover, recent understanding of CRC at the molecular level (consensus molecular subtype classification) provides deep insight into the important roles of TGF-β signaling in the tumor microenvironment. Here we focus on the TGF-β signaling in CRC and its interaction with the tumor microenvironment. We summarize the molecular mechanisms of CRC tumorigenesis and progression caused by disruption of TGF-β signaling by cancer epithelial cells and host stromal cells (Itatani Y, Kawada K, Sakai Y. Transforming Growth Factor-β Signaling pathway in colorectal cancer and its tumor microenvironment. *Int J Mol Sci*. 2019;20(23):5822).

STYLUS-BASED TACTILE PROFILOMETER CAN PRODUCE RELIABLE RESULTS OF GLASS IONOMERS' SURFACE ROUGHNESS MEASUREMENT: INTER- AND INTRA-OPERATOR RELIABILITY STUDY

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STYLUS-BASED TACTILE PROFILOMETER CAN PRODUCE RELIABLE RESULTS OF GLASS IONOMERS' SURFACE ROUGHNESS MEASUREMENT: INTER- AND INTRA-OPERATOR RELIABILITY STUDY (Abstract): The roughness measuring instruments, which analyze the roughness degree of sample surface using a stylus, can give limited information about surface alterations. The alternative three-dimensional surface mapping methods have better performance, but their spread is limited. The aim of this study was to determine the intra- and inter-operator reliability of surface roughness measurements by three independent operators with a tactile stylus-based profilometer on glass ionomer specimens. **Material and methods:** Ten light-cured glass ionomer specimens, prepared according to the manufacturer's instructions from premeasured unit dose capsules of GC Fuji II LC CAPSULE, have been selected. Three previously trained operators measured individually in four diagonals the surface roughness of samples with MarSurf XR1 roughness measuring instrument. After ten days the measurements were repeated using the same protocol. The Average Roughness was used to characterize the surface texture. Intra-class correlation coefficients were calculated for both individual and group measurements to check the level of reliability of the used technique. **Results:** The inter- and intra-operator reliability of the measurements showed high degrees, the coefficient values being in the good (0.75-0.90) and excellent (>0.90) categories of reliability. **Conclusions:** Linear surface roughness measurements of dental materials using a tactile roughness measuring device can be performed with high reliability by single or multiple users. This procedure has its limitations and further studies, with more materials, are warranted in this topic. **Keywords:** GLASS IONOMER, SURFACE ROUGHNESS, PROFILOMETER, RELIABILITY.

There is a huge number of in vitro studies based on methodologies which uses different devices to measure microtopography on surfaces of dental materials and teeth. Among various available techniques, two different methods can be distinguished: the quantitative approach, based on surface profile analysis and the qualitative method, involving optical and scanning microscopy (1).

The profilometer uses a fixed stylus on an arm, which is constantly in contact with the surface, to show the micro-topography. As the tip of the stylus moves linear, the two-dimensional profile of surface is detected and a software allows computing different parameters of roughness profile and topography (2). The obtained results provide quantitative information about the linear route taken by the tip (3).

The non-contact approach is based on optical phenomena. The modern interferometers are applying white light to detect the roughness, in combination with microscopy (optical profilometer) a good resolution and significant vertical range can be obtained. Other optical devices used are: scanning confocal microscope, scanning electron microscope (SEM), scanning tunneling microscope (STM) and atomic force microscope (AFM) (2, 3).

In study designs based on repeated measurements of specimens, reliable roughness measurement protocols are necessary. A possible source of measurement errors arises from technical failure made by the same operator or multiple operators on the same specimen. The high degree of reliability, which refers to reproducibility and repeatability, is provided by identical or similar measurement results (4, 5).

The results of studies, aimed to quantify the accuracy of tactile profilometers are varied as are the used study designs and statistical methods, too (6-8). An eligible parameter of reliability is the intra-class correlation coefficient (ICC). ICC is designed to assess consistency or conformity between two or more quantitative measurements (9). This method is suitable to evaluate the inter- and intra-operator reliability of a procedure or a measurement (10). Reliability studies can be fully standardized only under laboratory conditions (11).

The aim of this study was to determine the intra- and inter-operator reliability of surface roughness measurements produced by three independent operators with a tactile profilometer on glass ionomer specimens. The null hypotheses tested were that the results obtained with the same methodology and repeated after 10 days, can produce high values (> 0.75) of both *i)* intra- and *ii)* inter-operator intra-class correlation coefficients.

MATERIAL AND METHODS

Ten resin reinforced glass ionomer specimens were prepared in accordance with the manufacturer's instructions by a single operator using GC Fuji II LC (GC EUROPE, Belgium) premeasured unit dose capsules. The standardized, uniform size of the disc-shaped samples were achieved by the use of a silicone mold having a plastic sheet attached at the bottom to obtain smooth surfaces. The activated and mixed capsules were dispensed with an applier into the 6 mm diagonal cavities of mold followed by 30 seconds of light curing with Demetron LC (Kerr Corporation, USA) halogen unit. The glass ionomer specimens were stored in distilled water between measurements.

Four diagonals of each specimen were measured using a calibrated contact profilometer (MarSurf XR1, Mahr Gmbh, Germany). After ten days the measurements were repeated. A diamond stylus traversed every diagonal in a length of 1,74 mm and with a cut-off length of 2,50 µm at a 0,10 mm/s speed (fig. 1). The cone shaped diamond tip with 90° angle had a radius of 2.00 µm. The used Average Roughness (R_a) surface texture parameter

represents the arithmetic average of all deviations of the profile from the centerline (12). The measuring process was performed by three different, previously trained operators. The operators worked alone at both measurements.

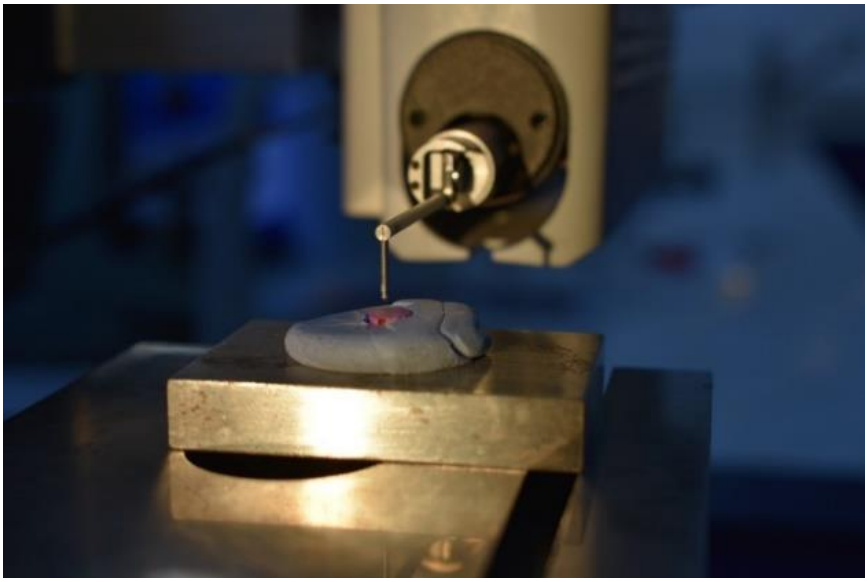

Fig. 1. The MarSurf XR1 computer-based tactile roughness measuring unit

The descriptive statistics, Kolmogorov-Smirnov normality test and data analysis were carried out using IBM SPSS Statistics a (IBM, USA). Intra-class correlation coefficients (ICC) and data distribution were calculated on 95% confidence interval for intra- and inter-operator measurements. The interpretation of results was based on the guideline given by Koo and Li (2016). According to them the values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.90 are indicative of poor, moderate, good, and excellent reliability (10).

RESULTS

A total number of 480 R_a values were measured by the three operators on both occasions. This number is the sum of the four diagonals measured twice on each specimen at both measurement periods by the three operators. The mean surface roughness values and standard deviations (SD) of specimens are shown in tab. I for every operator and measurement period. Data presented normal distribution.

The intraclass correlation coefficients from tab. II show high degrees of inter- and intra-operator reliability of the measurements, all values being higher than 0.75. The highest ICC values were found at the second inter-operator test containing overall measured values of the three operators (0.93) and at the intra-operator test of the first operator (0.90). Surface roughness measurements with the lowest ICC values were found at the first inter-operator test containing overall measurements of all operators (0.79) and at the second operator (0.78).

TABLE I

Mean R_a values of specimens' surface roughness at the two measurement periods

Measurement periods	Specimen	Operator 1 (Average ± SD)	Operator 2 (Average ± SD)	Operator 3 (Average ± SD)
First	S1	0.22 (± 0.07)	0.29 (± 0.10)	0.25 (± 0.06)
	S2	0.24 (± 0.06)	0.21 (± 0.01)	0.28 (± 0.11)
	S3	0.16 (± 0.02)	0.21 (± 0.10)	0.11 (± 0.05)
	S4	0.19 (± 0.07)	0.21 (± 0.03)	0.14 (± 0.01)
	S5	0.37 (± 0.07)	0.43 (± 0.05)	0.71 (± 0.24)
	S6	0.37 (± 0.02)	0.46 (± 0.06)	0.54 (± 0.33)
	S7	0.21 (± 0.03)	0.20 (± 0.06)	0.25 (± 0.09)
	S8	0.22 (± 0.05)	0.31(± 0.09)	0.65 (± 0.41)
	S9	0.16 (± 0.02)	0.32 (± 0.12)	0.22 (± 0.01)
	S10	0.25 (± 0.01)	0.29 (± 0.13)	0.41 (± 0.05)
Second	S1	0.22 (± 0.04)	0.22 (± 0.01)	0.34 (± 0.11)
	S2	0.19 (± 0.05)	0.26 (± 0.05)	0.26 (± 0.09)
	S3	0.14 (± 0.02)	0.18 (± 0.00)	0.22 (± 0.08)
	S4	0.16 (± 0.03)	0.15 (± 0.01)	0.15 (± 0.02)
	S5	0.54 (± 0.10)	0.71 (± 0.23)	0.90 (± 0.26)
	S6	0.39 (± 0.03)	0.71 (± 0.33)	0.40 (± 0.06)
	S7	0.16 (± 0.01)	0.15 (± 0.01)	0.23 (± 0.07)
	S8	0.24 (± 0.04)	0.29 (± 0.06)	0.35 (± 0.09)
	S9	0.16 (± 0.01)	0.15 (± 0.03)	0.28 (± 0.13)
	S10	0.25 (± 0.05)	0.25 (± 0.04)	0.31 (± 0.13)

TABLE II

Intraclass correlation coefficients according to inter- and intra-operator tests

	1st inter-operator (overall)	2nd inter-operator (overall)	1st vs. 2nd inter-operator (overall)	Intra-operator (Operator 1)	Intra-operator (Operator 2)	Intra-operator (Operator 3)
ICC	0.79	0.93	0.86	0.90	0.78	0.87

DISCUSSION

Reliability studies offer information about the reproducibility and repeatability of measurements made by a single operator or multiple operators (4). The necessity of this article is given by the lack of similar reliability studies in field of surface roughness of dental materials. Although, the obtained intra- and inter-operator results had a good and excellent reliability, it is visible that the repeatability of results are not perfect. The performance of operators may vary mildly (tab. II).

It does not exist a standard of minimal

procedures, measurements, samples, subjects, or operators to use for quality ICC estimates. Mehta *et al*. (2018) demonstrated that the increase of subjects in clinical studies beyond 80, does not have an impact on ICC (11). Contrary to clinical interventions, the in vitro research does not require patients' consent and does not raise ethical issues (13). In laboratory reliability studies the number of used samples is variable, frequently the sample size includes 10 specimens (14-16).

Studies found reliable the use of profilometer compared with other devices. Canabarro *et al*. (2009) found similar results of two- and three-dimensional profilometers in analysis of titanium surfaces (7). Other authors also highlighted the good performance of contact devices comparative with alternative methods, but they also recognize the disadvantages of this technology or suggest further examinations (6, 8, 17). The dimension of stylus defines the possible measurement errors. Authors found that the most accurate results are obtained using a 2 µm radius tip (18).

The tactile profilometers have several limitations. The speed of the tip across the surface was proved to be decisive in the success of results (19). If traversing speed of the stylus is too fast, an inaccurate tracking of surface profile may occur, inducing measurement errors (21). The constant contact of the specimen with the tip of the profilometer may cause surface damages, the resulting scratches being detrimental to the results or making the measurements non-repeatable (21). In these cases the incident is caused by the hardness of the analyzed materials and the diameter of the stylus (20). Heurich *et al*. (2009) demonstrated that the tip of the tactile profilometer penetrate the surface of preliminary eroded enamel causing scratches on the surface. Nevertheless, they found the unit suitable for analyzing eroded enamel if the appropriate testing force is being applied (22).

In contact profilometry the R_a value is a commonly used parameter. Although this parameter is easy to measure and it has a good reproducibility in repeated measurements, it gives limited information about the textural characteristics of a profile (12). This method provides quantitative information about heights at regular intervals. In some cases when the original profile of specimen has frequent amplitudes, the stylus sampled profile become distorted with broadened peaks and sharped valleys (23).

The optical devices can be an alternative to tactile measurement. In spite of all their advantages (non-contact working principle, high sensitivity, accuracy, and measurement speed), the physical properties of surfaces (reflectivity, elements which disturb the light path) sometimes influence the results. The results obtained with contact and non-contact methods cannot be always compared (2, 24).

CONCLUSSIONS

As all intra-class correlation coefficients calculated in this pilot study produced high values (>0.75), our null-hypotheses were confirmed. Linear surface roughness measurements of glass ionomer dental materials using a tactile roughness measuring device can be performed with high reliability by single or multiple users. Nevertheless, this device has its own limitations, which derive from its working principle and general characteristics. Further, larger scale studies including other materials are warranted in this topic.

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DIGITAL TECHNOLOGY FOR REMOVABLE PARTIAL DENTURE REALIZATION. CASE REPORT

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DIGITAL TECHNOLOGY FOR REMOVABLE PARTIAL DENTURE REALIZATION. CASE REPORT (Abstract): The conventional fabrication of removable partial dentures is a complex process, which involves a lot of clinical and technological steps. The design of the elements must be performed in order to maintain the homeostasis and to rehabilitate, in optimal conditions, the functions of the stomatognathic system. Digital technology-Computer-aided design/computer-aided manufacturing (CAD/CAM) method for partial denture fabrication brings a lot of advantages: high accuracy of the denture, increases the strength and durability, avoid changes of the volume and, time working economy. This clinical report aims to illustrate the stages of getting a removable prosthesis, using the computerized method and to emphasize that digital prosthesis can be effective and accurate, eliminating or replacing steps that can lead to complications. **Keywords**: REMOVABLE PARTIAL DENTURE, CAD-CAM TECHNOLOGIES, METALLIC FRAMEWORK.

Removable partial denture is a device of great complexity, both in terms of choosing a correct design, adapted to the clinical situation, and in terms of effective technological execution. The elements of this removable prosthesis come in contact with the remaining teeth, but also with the oral mucosa and the bone support. In this case, the choice for the connector design and for the direct and indirect retainers will be made in order to restore homeostasis and the functions of the dento-maxillary system (1). An incorrectly made prosthesis will generate a series of pathological dental, periodontal, occlusal or physiognomic manifestations. The design of the removable prostheses must be realized according to the prosthetic area, not to overload the remaining teeth and the periodontium; thus, all functions altered by edentation will be rehabilitate in optimal conditions. The elaboration of a removable partial denture supposes a succession of clinical and technological steps; the rigorous observance of the steps and the knowledge of their particularities, as well as of the characteristics of the materials used will allow the realization of some prosthetic constructions with optimal resistance and with great clinical longevity. (2). The development of dental materials has diversified the technologies for prosthetic constructions, and knowing the particularities of each method, with their advantages, disadvantages, and limitations, allows choosing an appropriate therapeutic solution and solving the most diffi-

cult clinical situations, in optimal conditions. Modern digital technologies for prostheses realization are currently preferred, because they offer multiple advantages compared to classical methods: decreased number of appointments, high accuracy of the appliance, decreased duration of realization, superior quality control by clinicians and technician, possibility to change the design and to remanufacture without the need to restart the entire process; also, digital solutions allow the application of advanced materials, reduce material costs and casting equipment, so it will allow less wasted alloy, no wasted wax and no wasted investment material (3, 4). The development of the precise design procedures, including computer-aided design and manufacturing CAD/CAM, for fabrication partial dentures is justified because there is still a large group of patients who would benefit on improving the accuracy of these solutions (5, 6).

CASE REPORT

Patient S.O., 59-year-old came to the dentist's office with functional disorders caused by the loss of several masticatory units, both on the maxillary and on the mandible (fig. 1).

Fig. 1. Clinical examination

After performing the clinical and the paraclinical examinations (orthopantomography and model study) the diagnostic was of partial maxillary and mandibular edentation; as a therapeutic solution was chosen, for the maxilla a removable partial denture, with two saddles, reduced palatal plate as main connector and encircling clasps, as elements of retention, support, and stabilization. For the mandible, a removable partial denture was indicated: with two saddles, a lingual bar as connector and also clasps, for the retention

The process for fabricating removable partial dentures has three major steps: the impression procedures, the denture design, and the denture fabrication. The first step consisted into an optical impression of the maxillary and mandibular prosthetic area and occlusal contacts in maximum intercuspal registration (fig. 2).

To realize the prosthetic device, we used the DIGISTELL system, 3D CAD software for removable partial frameworks. The data were sent to the CAD- CAM work unit and, based on the recorded images, the CAD component will analyze the prosthetic area and provide indications which teeth will be applied the clasps, the dental areas to be prepared for the application of the occlusal rests and the teeth for possible coronoplasty; it will also propose a design for the future

prosthesis, indicating the types of connector, the most appropriate clasps for the clinical situation, as well as the relationship with the abutment teeth (fig. 3).

Fig. 2. Optical impression

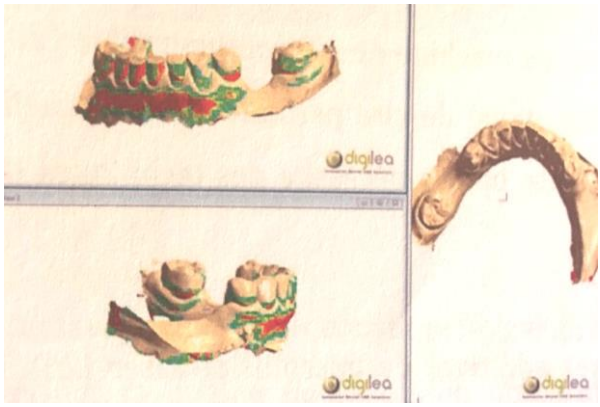

Fig. 3. The design of the future framework

The doctor will make the preparations on the support teeth, will prepare the organic substructure, in order to apply a crown on 2.5 premolar. Then, a new optical impression is registered, with the prosthetic area prepared for the device.

Based on the second impression, virtual models will be developed, corresponding to the new clinical situation, on which virtual frameworks of the future prosthesis will be created (fig. 4).

After establishing the design of the partial removable dentures, the information was transmitted to the CAM unit where the

actual milling of the metal infrastructures is done, using chromium-cobalt-tungsten-based alloy blocks. The framework was checked in the oral cavity, then the dental technician, in the laboratory, made the acrylic component of the saddles and the mounting of artificial teeth. After processing and finishing the prostheses, they are sent to the dental office for verification and intraoral adaptation (fig. 5).

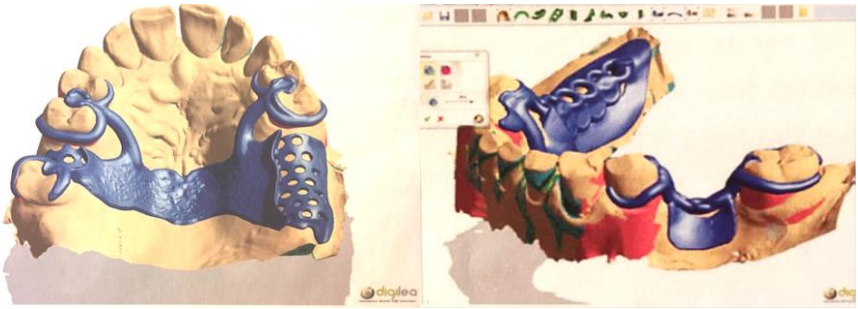

Fig. 4. The models and the wax-up of the frameworks

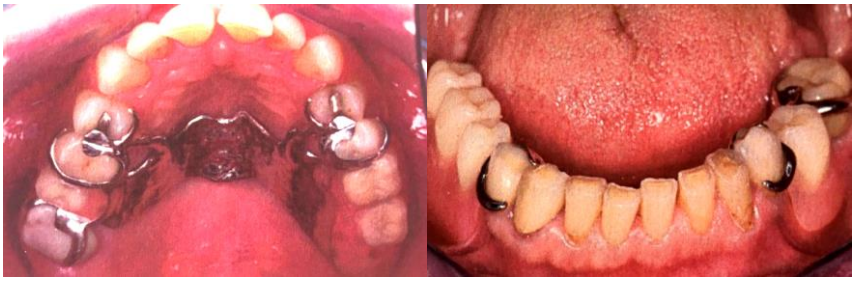

Fig. 5. Intraoral adaptation

DISCUSSION

Making removable partial dentures using CAD-CAM methods has many advantages recording with high precision all the details of the prosthetic area due to the optical impression, designing the framework on a virtual model and analyzing the therapeutic possibility depending on clinical situations, material economy, as a result of eliminating laboratory steps, high accuracy in realizing each element of the prosthesis (7, 8, 9).

For a correct adaptation of the prosthesis, the relationship between elements of retention, support and stabilization and abutment teeth is extremely important. In general, bone resorption at the level of edentulous ridges is asymmetric, which determines an uneven distribution of pressures; therefore, any deviation, even a minor one, of the metal framework geometry can lead to a destabilization of the prosthesis (10, 11).

Computerized technologies eliminate the risk of errors in the design and execution of removable partial dentures, allowing the realization of high-precision prosthetic constructions, correctly adapted and with great longevity over time.

CONCLUSIONS

The individualized treatment of the par-

tial edentation requires an in-depth knowledge of the clinical situation and of the elements of the edentulous prosthetic area, in order to adapt the therapeutic possibilities to the clinical particularities, so as to ensure an optimal morpho-functional rehabilitation. The multitude of prosthetic technologies and the diversification of materials over time, on the one hand make it difficult for the doctor and dental technician to provide the choice of the best solution and, on the other hand, offer a wide range of technological possibilities, allowing individualization of the treatment plan.

Nowadays, there are a large number of devices, technologies and materials that are available to the doctor, but especially to the technician for the realization of removable partial prostheses that correspond to the mechanical, biological, and prophylactic desideratum.

The development of the precise design procedures, including computer-aided design and manufacturing CAD/CAM, for fabrication of partial dentures is justified by many advantages compared to the classical methods.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest and they received no specific funding regarding this scientific research.

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SEA BUCKTHORN FRUIT OIL SUPPLEMENTATION IN ACTIVE RHEUMATOID ARTHRITIS: A FOCUS ON COMPOSITE DISEASE ACTIVITY INDICES AND EULAR RESPONSE

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SEA BUCKTHORN FRUIT OIL SUPPLEMENTATION IN ACTIVE RHEUMATOID ARTHRITIS: A FOCUS ON COMPOSITE DISEASE ACTIVITY INDICES AND EULAR RESPONSE (Abstract): Rheumatoid arthritis is a disabling disease whose treatment options have limitations with regard to efficacy and safety. In this respect, combination of conventional treatment with plant-derived products has attracted considerable interest in recent years. The aim of this study was to assess the impact of sea buckthorn fruit oil supplementation in patients with active rheumatoid arthritis. **Materials and methods:** Eighty patients, diagnosed with active rheumatoid arthritis and treated with a combination of methotrexate (15 mg/week) and diclofenac sodium (50 mg/day), were randomly (1:1) divided into control and experimental groups, the latter being supplemented with sea buckthorn fruit oil (1800 mg/day). The study lasted 12 weeks. The benefits of sea buckthorn fruit oil supplementation were evaluated by various composite disease activity indices (disease activity score in 28 joints, DAS28; simplified disease activity index, SDAI; clinical disease activity index, CDAI) and European League Against Rheumatism (EULAR) response. **Results:** Twelve-week supplementation with sea buckthorn fruit oil significantly ($p< 0.001$) reduced composite disease activity indices in comparison with baseline and control. At week 12, patients in the experimental group showed higher rates of good and moderate EULAR responses than those in the control group. **Conclusions:** According to our results, sea buckthorn fruit oil is a promising option for the adjunctive treatment in rheumatoid arthritis. **Keywords:** SEA BUCKTHORN FRUIT OIL, ACTIVE RHEUMATOID ARTHRITIS, COMPOSITE DISEASE ACTIVITY INDEX, EULAR RESPONSE.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic systemic autoimmune-inflammatory disease characterized by articular and extraarticular manifestations (synovial membrane inflammation, articular cartilage destruc-

tion, periarticular bone erosion and subcutaneous nodules, lung disease, pericarditis, respectively). If inadequately treated, it results in permanent joint deformities, functional disability and poor life quality (1, 2). The drugs used to treat RA, specifically corticosteroids, nonsteroidal anti-inflammatory drugs (NSAIDs), conventional and biological disease-modifying antirheumatic drugs (DMARDs), produce severe side effects. In addition, none of these drugs, alone or in combination, induce a medication-free remission. For this reason, supplementation with plant-derived products to support the efficacy and safety of conventional treatment has attracted considerable interest in recent years (1).

The benefits of coadministration of herbal extracts/phytochemicals with antiarthritic drugs were demonstrated in clinical studies. In RA patients, *Tripterygium wilfordii* Hook.f. extracts improved the therapeutic efficacy of methotrexate (MTX) as evaluated by clinical outcomes and reduction in inflammatory and diagnostic markers (erythrocyte sedimentation rate (ESR), C reactive protein (CRP) and rheumatoid factor (RF), respectively). Supplementation with total glucosides of peony (*Paeonia lactiflora* Pall.) in RA patients, treated with MTX or MTX in combination with leflunomide, reduced liver toxicity caused by DMARDs, ameliorated clinical symptoms, ESR, CRP, RF and lipid profile (3). The combination of dry olive (*Olea europaea* L.) leaf extract with MTX was more efficient than MTX alone in restoring antioxidant defense and reducing oxidative stress, cell damage and interleukin-6 (IL-6) production in early phase RA patients (4). Supplementation with garlic (*Allium sativum* L.) powder in patients with moderate to severe RA, under treatment with DMARDs, resulted in improved clinical symptoms and significant reductions in CRP and tumor necrosis factor- α (TNF-α) compared to patients receiving only DMARDs (5). Also, combinations of pure phytochemicals with antiarthritic drugs were reported to exert better activity and less toxicity than antiarthritic drugs alone. Combined administration of resveratrol with conventional DMARDs induced greater improvement in clinical, biochemical (RF, ESR, CRP, TNF-α, IL-6) and hip fracture (undercarboxylated osteocalcin) markers (6). Sesamin supplementation attenuated pain intensity and significantly decreased CRP, TNF-α, cyclooxygenase-2 (COX-2), hyaluronidase and matrix metalloproteinase-3 (MMP-3)(7). In RA patients, the therapeutic efficacy of sinomenine in combination with MTX was comparable with that of MTX-leflunomide combination but the safety and tolerability (with respect to gastrointestinal and hepatic side effects) were better (8). The combination of curcumin with diclofenac sodium (DicNa) induced better clinical outcomes in comparison with DicNa in patients with active RA (9).

Due to a unique mixture of bioactive compounds, sea buckthorn (*Elaeagnus rhamnoides* (L.) A. Nelson syn. *Hippophae rhamnoides* L., Elaeagnaceae) fruit oil is one of the most valuable plant-derived products. The oil obtained from whole berries is rich in palmitoleic acid (11-37%), an ω-7 fatty acid, other unsaturated fatty acids such as ω-3, ω-6 and ω-9 fatty acids (3-8% α-linolenic acid, 12-18% linoleic acid and 20-53% oleic acid, respectively) and saturated fatty acids (23-40% palmitic acid, < 2% stearic acid), sterols (1200-2300 mg/100 g), carotenoids (350-520 mg/100 g) and tocopherols (up to 160 mg%, α-tocopherol being predominant) (10, 11). Sea buckthorn fruit oil has applications in skin and mucosal disorders (wounds, burns, lesions caused by radiation exposure, hyper pigmentation, infections, itchiness, vaginal

inflammatory atrophy). As nutraceutical, sea buckthorn fruit oil has benefits in the prevention and adjunctive treatment of cardiovascular, liver and metabolic diseases. In cancer patients undergoing chemotherapy and radiotherapy, sea buckthorn fruit oil counteracts some of treatment side effects such as alterations in liver and kidney function and appetite loss (10-12).

The current study was designed to evaluate the efficacy and safety of sea buckthorn fruit oil (SBFO) supplementation in patients with active RA. Disease activity score in 28 joints (DAS28), simplified disease activity index (SDAI), clinical disease activity index (CDAI)and European League Against Rheumatism (EULAR)response were used as efficacy indicators.

MATERIALS AND METHODS

A pilot randomized controlled study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Institutional Ethics Committee of *Grigore T. Popa* University of Medicine and Pharmacy Iasi, Romania (approval no. 68/13.04.2021) and Ethics Committee of the Clinical Rehabilitation Hospital Iasi, Romania (approval no. 13/20.04.2021).All enrolled patients filled in and signed the written informed consent. In brief, 80 patients diagnosed with active RA were randomly classified (1:1) into two groups: control group, treated with MTX (15 mg/week) and DicNa (50 mg/day) and experimental group, receiving the aforementioned medication and SBFO (1800 mg/day, Sea Buckthorn Oil 900 mg, S.C. Hofigal Export Import S.A., Bucharest, Romania). The study lasted 12 weeks. The inclusion and exclusion criteria and baseline characteristics of patients were described in detail elsewhere (Zugravu *et al.*, submitted).

The inflammatory markers (ESR, CRP),lipid profile, glycemia, liver and renal function markers were assessed at baseline and week 12. Venous blood (10 mL) was collected after fasting (10-12 h). A volume of 2 mL was used for ESR measurement (Westergren method, THERMA analyzer, Linear Chemicals, Montgat-Barcelona, Spain). The remaining volume was centrifuged (3000 g, 10 min.) to separate serum and plasma which were further stored at -80°C. Serum CRP was determined by immunoturbidimetry (biochemical analyzer XL 1000, Erba, Lachema S.R.O., Czech Republic). Liver and kidney function markers, lipid and carbohydrate metabolic markers were determined spectrophotometrically (biochemical analyzer XL 1000, Erba, Lachema S.R.O., Czech Republic).

The clinical variables, assessed at baseline and week 12, were the tender joint count (in 28 and 68 joints, TJC28 and TJC68), swollen joint count (in 28 and 66 joints, SJC28 and SJC66) and morning stiffness (in min.) (13, 14). Composite disease activity indices such as DAS28 (15), SDAI andCDAI (13) were also evaluated at the beginning and end of the study. EULAR responses were assessed at week 12 according to improve mentin DAS28 score (15). DAS28, SDAI, CDAIand EULAR responses were calculated as previously reported (13, 15).

Data were analyzed using the SPSS software (version 25). The Kolmogorov-Smirnov test was initially used to assess the normality of data distribution. The differences between the two groups (experimental and control groups) at each evaluation (baseline and week 12) and the differences between the evaluations for each group were examined using either parametric or nonparametric tests. The independent samples *t*-test and Mann-Whitney test were used to compare the differences between experimental and control groups. Within

each group, the comparisons were done using the paired samples *t*-test (normally distributed data) and Wilcoxon signed-rank test (non-normally distributed data). The Chi-square test was applied to assess the relationship between two qualitative variables and two patient groups. Values of *p* less than or equal to 0.05 were considered statistically significant.

RESULTS

The inflammatory markers (ESR, CRP) were not significantly ($p > 0.05$)different between the two patient groups at baseline and week 12. At week 12, CRP decreased in both experimental and control groups; ESR had slightly reduced values in the control group and unexpectedly higher values in the experimental group (tab. I).

TABLE I.
Inflammatory markers at baseline and week 12

Inflammatory marker	Control group	Experimental group	*p* value
ESR (mm/h)			
Baseline	29.57 ± 22.74	27.12 ± 20.59	0.615[a]
(95% CI)	(22.30 – 36.84)	(20.53 – 33.71)	
Week 12	28.38 ± 23.61	32.10 ± 26.46	0.519[a]
(95% CI)	(20.73 – 36.03)	(23.28 – 40.93)	
Change	1.02 ± 12.43	-4.21 ± 20.96	-
p value	0.609[b]	0.229[b]	-
CRP (mg/dL)			
Baseline	17.50 ± 29.95	9.44 ± 19.37	0.158[a]
(95% CI)	(7.92 – 27.08)	(3.25 – 15.64)	
Week 12	14.08 ± 34.44	7.51 ± 9.51	0.258[a]
(95% CI)	(2.92 – 25.25)	(4.34 – 10.68)	
Change	3.81 ± 17.32	2.43 ± 14.18	-
p value	0.177[b]	0.303[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables and 95% confidence interval; ESR, erythrocyte sedimentation rate; CRP, C reactive protein; [a] Independent t test; [b] Paired t test.

At baseline, there were no significant ($p > 0.05$) differences between the two groups with respect to the clinical variables (TJC28, TJC68, SJC28, SJC66, morning stiffness) and composite indices of disease activity such as DAS28 calculated with ESR or CRP (DAS28 (ESR) and DAS28 (CRP), respectively), SDAI and CDAI. In the experimental group, supplementation with SBFO for 12 weeks markedly ($p< 0.001$) improved all clinical variables and indices with significant differences between the values at baseline and week 12. At week 12, all clinical variables were significantly ($p< 0.001$) different between the experimental and control groups. On the other hand, at the end of the study, in the control group, the scores of clinical variables (except TJC28 and morning stiffness) significantly ($p \leq 0.001$) increased indicating higher disease activity and deterioration of patient condition (tab. II).

TABLE II.
Clinical variables at baseline and week 12

Outcome	Control group	Experimental group	*p* value
TJC28			
Baseline	19.72 ± 3.88	18.65 ± 4.58	0.261[a]
(95% CI)	(18.48 – 20.96)	(17.18 – 20.11)	
Week 12	20.43 ± 3.44	12.21 ± 2.83	< 0.001[a]
(95% CI)	(19.31 – 21.55)	(11.26 – 13.16)	
Change	-0.82 ± 3.03	6.94 ± 3.09	-
p value	0.099[b]	< 0.001[b]	-
TJC68			
Baseline	35.65 ± 10.05	32.90 ± 9.68	0.217[a]
(95% CI)	(32.43 – 38.86)	(29.80 – 35.99)	
Week 12	38.48 ± 9.01	19.54 ± 4.54	< 0.001[a]
(95% CI)	(35.56 – 41.40)	(18.02 – 21.05)	
Change	-3.10 ± 5.53	14.37 ± 8.69	-
p value	0.001[b]	< 0.001[b]	-
SJC28			
Baseline	10.62 ± 2.68	9.45 ± 3.10	0.074[a]
(95% CI)	(9.76 – 11.48)	(8.45 – 10.44)	
Week 12	13.05 ± 2.41	5.81 ± 2.39	< 0.001[a]
(95% CI)	(12.26 – 13.83)	(5.01 – 6.61)	
Change	-2.35 ± 2.13	3.89 ± 3.30	-
p value	< 0.001[b]	< 0.001[b]	-
SJC66			
Baseline	15.25 ± 4.35	14.42 ± 4.51	0.408[a]
(95% CI)	(13.85 – 16.64)	(12.98 – 15.86)	
Week 12	20.46 ± 5.08	8.91 ± 3.92	< 0.001[a]
(95% CI)	(18.81 – 22.11)	(7.61 – 10.22)	
Change	-2.35± 3.78	3.89 ± 5.50	-
p value	< 0.001[b]	< 0.001[b]	-
Morning stiffness (min.)			
Baseline	40.12 ± 22.68	40.12 ± 18.30	1.000[a]
(95% CI)	(32.86 – 47.38)	(34.26 – 45.98)	
Week 12	45.12 ± 16.28	21.62 ± 7.64	< 0.001[a]
(95% CI)	(39.85 – 50.41)	(19.07 – 24.16)	
Change	-4.74 ± 16.73	19.45 ± 18.17	-
p value	0.084[b]	< 0.001[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables and 95% confidence interval (CI); TJC28, tender joint count in 28 joints; TJC68, tender joint count in 68 joints; SJC28, swollen joint count in 28 joints; SJC66, swollen joint count in 66 joints; a Independent t test; b Paired t test.

The composite indicesDAS28 (ESR), DAS28 (CRP), SDAI and CDAI were used to assess disease activity. DAS28 > 5.1, 3.2 < DAS28 ≤ 5.1 and DAS28 ≤ 3.2 indicate high, moderate and low disease activity, respectively; DAS28 less than 2.6 is an indicative of remission. SDAI > 26, 11 < SDAI ≤ 26 and 3.3 < SDAI ≤ 11 define high, moderate and low activity, respectively whereas SDAI ≤ 3.3 indicates remission. CDAI > 22, 10 < CDAI ≤ 22, 2.8 < CDAI ≤ 10 and CDAI ≤ 2.8 correspond to high, moderate, low activity and remission, respectively (13). According to the mean values of these composite indices, patients in both groups had high disease activity at baseline (DAS28 (ESR)/(CRP) > 5.1, SDAI > 26, CDAI > 22). At week 12, in the control group, patients still had high disease activity whereas in the experimental group, patients achieved moderate disease activity according to DAS28 (CRP) and SDAI (4.66 ± 0.58 and 25.13 ± 6.70, respectively). However, it is worthy to note that supplementation with SBFO induced significant (p< 0.001) reductions in DAS28 (ESR)/(CRP), SDAI and CDAI compared to baseline and control (tab.III). As shown in fig. 1-3, at baseline, 87.2-97.4% patients in the control group and 89.2-100% patients in the experimental group had high disease activity according to DAS28 (ESR)/DAS28 (CRP)/SDAI/CDAI scores. At week 12, the percentage of patients with high disease activity increased in the control group (from 94.9 to 100% according to DAS28 (ESR), from 87.2 to 97.4% according to DAS28 (CRP), from 97.4 to 100% according to SDAI and CDAI). In the experimental group, 12-week supplementation with SBFO resulted in a marked decline in patients presenting high disease activity (from 97.3 to 64.9% according to DAS28 (ESR), from 89.2 to 13.5% according to DAS28 (CRP), from 94.6 to 48.6% according to SDAI, from 100 to 64.9% according to CDAI) with a concomitant elevation in patients with moderate disease activity (from 2.7 to 35.1% according to DAS28 (ESR), from 10.8 to 86.5% according to DAS28 (CRP), from 5.4 to 51.4% according to SDAI, from 0 to 35.1% according to CDAI).

After 12-week supplementation, SBFO significantly (p< 0.001) improved EULAR-responses calculated with ESR or CRP (EULAR (ESR) and EULAR (CRP), respectively).Thus, in the experimental group, patients attained good and moderate EULAR responses in high rates (EULAR (ESR) good response: 35.1% *vs.* 0% in the control group, EULAR (CRP) good response: 59.5% *vs.* 0% in the control group, EULAR (ESR) moderate response: 56.8% *vs.* 2.6% in the control group, EULAR (CRP) moderate response: 40.5% *vs.* 0% in the control group) (fig.4). Most of the clinical laboratory parameters were not significantly different between the experimental and control groups at the beginning and end of the study. After 12-week supplementation, SBFO reduced total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) and increased triglycerides (TG) but the differences compared to baseline and control were not significant (p > 0.05). Surprisingly, SBFO caused a significant (p< 0.001) reduction in high-density lipoprotein cholesterol (HDL-C). SBFO improved liver function as evaluated by aspartate aminotransferase (AST), alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT) levels. At week 12, in the experimental group, all three enzymes showed lower levels compared to baseline (p > 0.05) whereas higher levels compared to baseline were detected in the control group (p > 0.05). SBFO supplemen-

tation significantly ($p< 0.05$) decreased ALT compared to control. SBFO supplementation also reduced serum levels of urea and uric acid and had little impact on creatinine ($p > 0.05$ compared to baseline). At week 12, urea and creatinine levels were significantly ($p = 0.001$) lower in the experimental group compared to control. A nonsignificant ($p > 0.05$) increase in glycemia and serum alkaline phosphatase (ALP), marker of liver damage, was detected in the experimental group at week 12 (tab. IV). A single adverse event was reported during the study period: one patient in the control group suffered from nausea after MTX administration.

TABLE III.
Composite indices at baseline and week 12

Outcome	Control group	Experimental group	*p* value
DAS28 (ESR)			
Baseline	6.50 ± 0.71	6.34 ± 0.69	0.292[a]
(95% CI)	(6.27 – 6.73)	(6.11 – 6.56)	
Week 12	6.68 ± 0.71	5.31 ± 0.64	< 0.001[a]
(95% CI)	(6.45 – 6.91)	(5.10 – 5.53)	
Change	-0.18 ± 0.46	1.09 ± 0.40	-
p value	0.017[b]	< 0.001[b]	-
DAS28 (CRP)			
Baseline	6.06 ± 0.59	5.81 ± 0.66	0.076[a]
(95% CI)	(5.87 – 6.25)	(5.60 – 6.02)	
Week 12	6.21 ± 0.58	4.66 ± 0.58	< 0.001[a]
(95% CI)	(6.02 – 6.40)	(4.46 – 4.85)	
Change	-0.13 ± 0.37	1.23 ± 0.40	-
p value	0.030[b]	< 0.001[b]	-
SDAI			
Baseline	43.94 ± 7.98	42.30 ± 9.39	0.401[a]
(95% CI)	(41.39 – 46.49)	(39.29 – 45.30)	
Week 12	49.56 ± 8.58	25.13 ± 6.70	< 0.001[a]
(95% CI)	(46.78 – 52.35)	(22.89 – 27.36)	
Change	-5.58 ± 9.72	18.19 ± 4.88	-
p value	0.001[b]	< 0.001[b]	-
CDAI			
Baseline	42.35 ± 9.06	41.35 ± 8.88	0.620[a]
(95% CI)	(39.45 – 45.24)	(38.50 – 44.19)	
Week 12	48.17 ± 7.23	24.35 ± 6.25	< 0.001[a]
(95% CI)	(45.83 – 50.52)	(22.26 – 26.43)	
Change	-5.82 ± 8.73	17.97 ± 5.35	-
p value	< 0.001[b]	< 0.001[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables and 95% confidence interval (CI); DAS28 (ESR), disease activity score in 28 joints calculated with erythrocyte sedimentation rate; DAS28 (CRP), disease activity score in 28 joints calculated with C reactive protein; SDAI, simplified disease activity index; CDAI, clinical disease activity index; a Independent t test; b Paired t test.

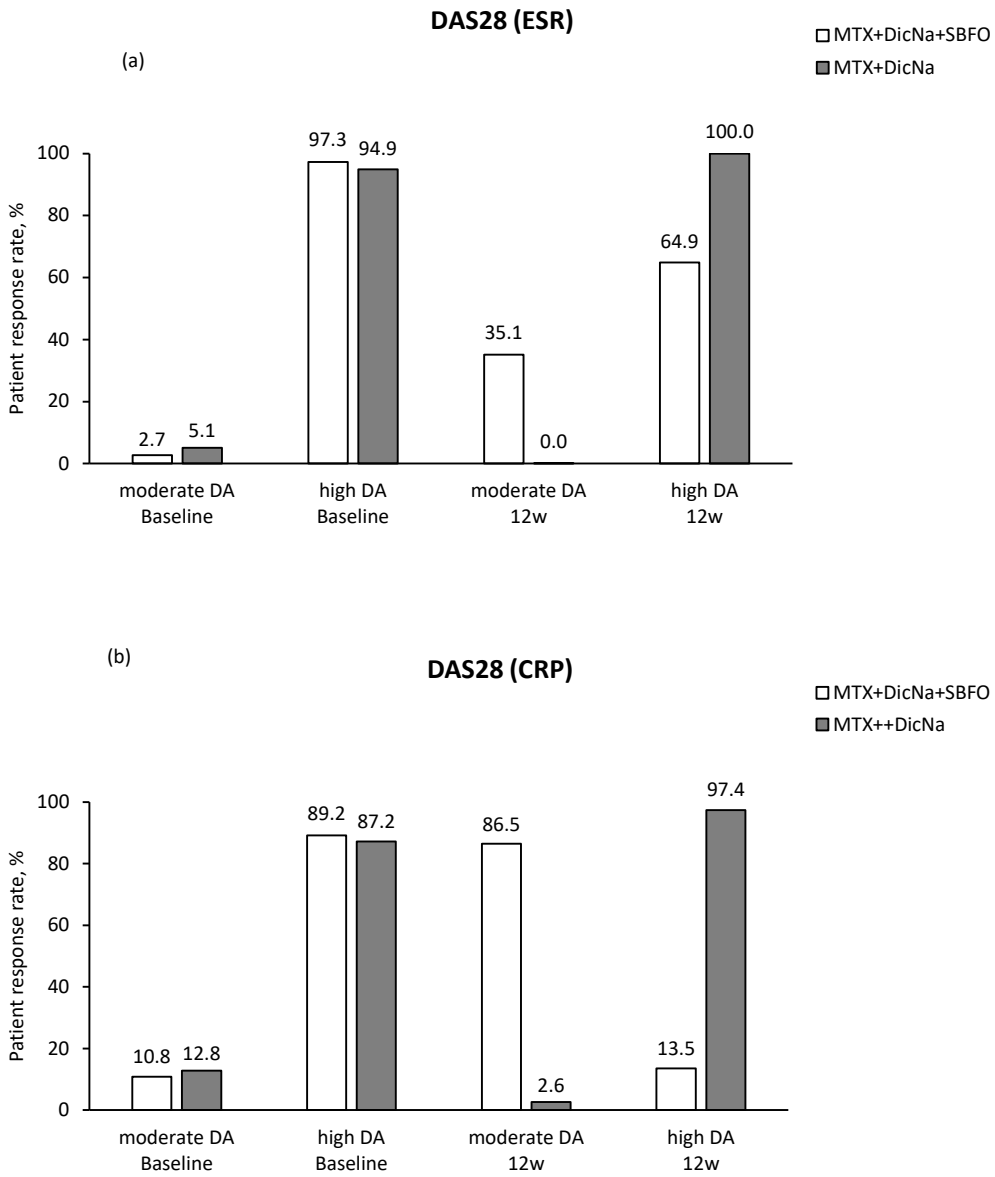

Fig. 1. Differences in DAS28 (ESR) (a) and DAS28 (CRP) (b) between the experimental and control groups (DAS28 (ESR), disease activity score in 28 joints calculated with erythrocyte sedimentation rate; DAS28 (CRP), disease activity score in 28 joints calculated with C reactive protein; DA, disease activity; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

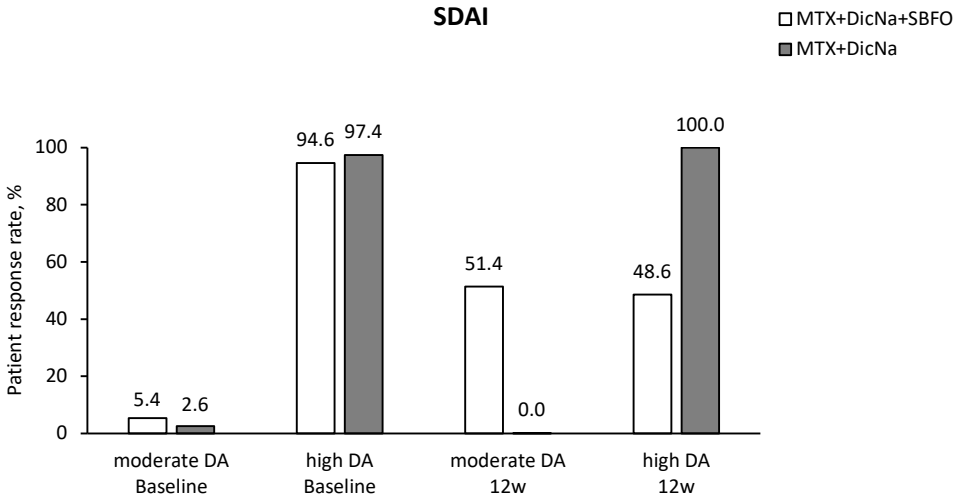

Fig. 2. Differences in SDAI between the experimental and control groups (SDAI, simplified disease activity index; DA, disease activity; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

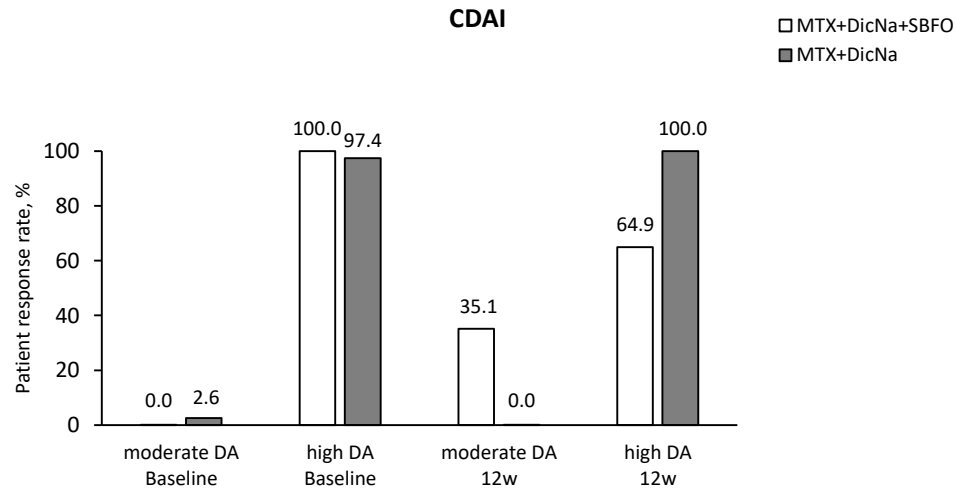

Fig. 3. Differences inCDAI between the experimental and control groups (CDAI, clinical disease activity index; DA, disease activity; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

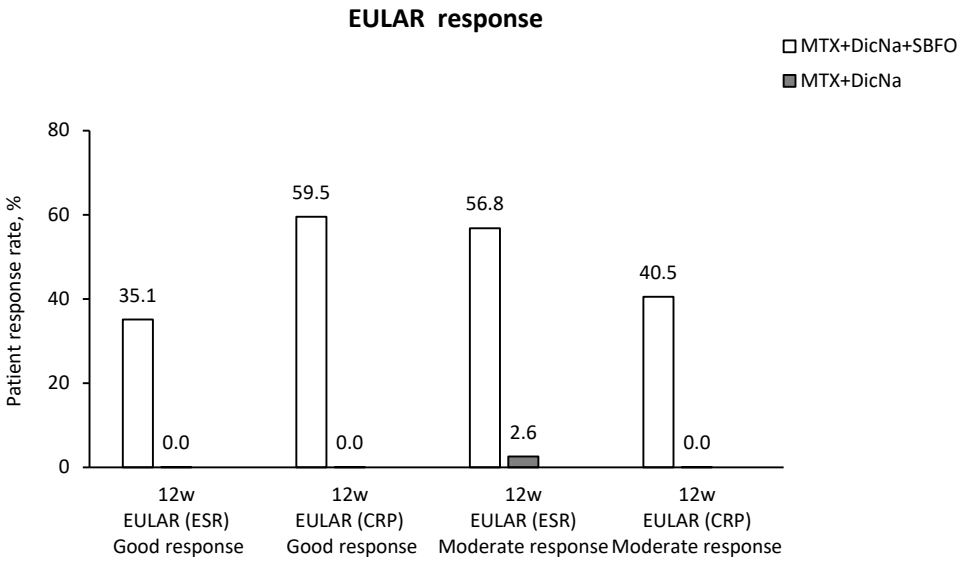

Fig. 4. Differences in EULAR (ESR)/(CRP) responses between the experimental and control groups (EULAR (ESR)/(CRP), European League Against Rheumatism response based on erythrocyte sedimentation rate/C reactive protein; MTX, methotrexate; DicNa, diclofenac sodium; SBFO, sea buckthorn fruit oil)

TABLE IV.

Clinical laboratory parameters at baseline and week 12

Variable	Control group	Experimental group	*p* value
TC (mg/dL)			
Baseline	213.7 ± 44.4	216.30 ± 47.82	0.646[a]
(95% CI)	(199.5 – 227.9)	(201.0 – 231.5)	
Week 12	215.7 ± 44.5	211.30 ± 50.27	0.688[a]
(95% CI)	(201.2 – 230.1)	(194.5 – 228.0)	
Change	-2.92 ± 42.18	6.10 ± 38.23	-
p value	0.668[b]	0.338 [b]	-
HDL-C (mg/dL)			
Baseline	67.16 ± 18.07	72.95 ± 14.84	0.122[a]
(95% CI)	(61.38 – 72.94)	(68.20 – 77.70)	
Week 12	69.66 ± 17.57	65.04 ± 13.40	0.203[a]
(95% CI)	(63.97 – 75.36)	(60.57 – 69.51)	
Change	-2.53 ± 16.29	7.16 ± 12.02	-
p value	0.337[b]	< 0.001[b]	-

Variable	Control group	Experimental group	*p* value
LDL-C (mg/dL)			
Baseline	140.10 ± 35.35	140.20 ± 44.57	0.991[a]
(95% CI)	(128.80 – 151.4)	(126.0 – 154.5)	
Week 12	134.20 ± 35.12	131.80 ± 40.03	0.781[a]
(95% CI)	(122.8 – 145.5)	(118.4 – 145.5)	
Change	5.11 ± 30.78	9.89 ± 34.75	-
p value	0.306[b]	0.092[b]	-
TG (mg/dL)			
Baseline	102.30 ± 46.91	96.36 ± 51.97	0.592[a]
(95% CI)	(87.32 – 117.30)	(79.74 – 113.00)	
Week 12	114.90 ± 52.14	108.30 ± 48.64	0.574[a]
(95% CI)	(97.99 – 131.8)	(92.12 – 124.6)	
Change	-12.92 ± 40.35	-9.35 ± 32.73	-
p value	0.053[b]	0.091[b]	-
GL (mg/dL)			
Baseline	112.50 ± 32.21	103.70 ± 29.81	0.209[a]
(95% CI)	(102.2 – 122.8)	(94.13 – 113.20)	
Week 12	108.50 ± 23.32	106.60 ± 23.13	0.712[a]
(95% CI)	(101.0 – 116.1)	(98.83 – 114.30)	
Change	4.1 ± 27.7	-7.09 ± 26.39	-
p value	0.362[b]	0.111 [b]	-
Urea (mg/dL)			
Baseline	39.70 ± 7.97	33.92 ± 10.74	0.009[a]
(95% CI)	(37.11 – 42.28)	(30.33 – 37.49)	
Week 12	37.04 ± 10.31	31.70 ± 6.71	0.001[a]
(95% CI)	(33.70 – 40.38)	(29.46 – 33.94)	
Change	2.66 ± 6.94	2.21 ± 9.11	-
p value	0.022[b]	0.148[b]	-
Creatinine (mg/dL)			
Baseline	1.03 ± 0.14	0.94 ± 0.11	0.002[a]
(95% CI)	(0.98 – 1.08)	(0.90 – 0.97)	
Week 12	1.04 ± 0.14	0.94 ± 0.09	0.001[a]
(95% CI)	(0.99 – 1.09)	(0.91 – 0.97)	
Change	-0.009 ± 0.076	-0.006 ± 0.090	-
p value	0.443[b]	0.691[b]	-
Uric acid (mg/dL)			
Baseline	3.81 ± 1.46	3.52 ± 1.34	0.382[a]
(95% CI)	(3.33 – 4.28)	(3.07 – 3.97)	
Week 12	3.75 ± 1.21	3.29 ± 0.91	0.065[a]
(95% CI)	(3.36 – 4.14)	(2.98 – 3.59)	
Change	0.05 ± 0.74	0.23 ± 0.91	-
p value	0.643[b]	0.125[b]	-

Variable	Control group	Experimental group	*p* value
AST (U/L)			
Baseline	20.59 ± 5.39	33.23 ± 58.14	0.196[a]
(95% CI)	(18.85 – 22.34)	(13.84 – 52.61)	
Week 12	23.50 ± 12.09	21.16 ± 4.71	0.276[a]
(95% CI)	(19.58 – 27.42)	(19.59 – 22.73)	
Change	-2.90 ± 12.16	12.06 ± 55.88	-
p value	0.145[b]	0.197[b]	-
ALT (U/L)			
Baseline	23.87 ± 10.02	28.28 ± 28.74	0.382[a]
(95% CI)	(20.62 – 27.13)	(18.69 – 37.86)	
Week 12	26.48 ± 12.20	21.00 ± 7.38	0.021[a]
(95% CI)	(22.53 – 30.44)	(18.54 – 23.46)	
Change	-2.61 ± 11.25	7.28 ± 25.39	-
p value	0.156[b]	0.090[b]	-
GGT (U/L)			
Baseline	27.02 ± 17.07	31.70 ± 27.35	0.371[a]
(95% CI)	(21.48 – 32.55)	(22.58 – 40.82)	
Week 12	30.92 ± 22.43	25.98 ± 12.66	0.245[a]
(95% CI)	(23.65 – 38.19)	(21.77 – 30.21)	
Change	-3.90 ± 16.95	5.71 ± 22.95	-
p value	0.159[b]	0.139[b]	-
ALP (U/L)			
Baseline	90.10 ± 22.16	88.04 ± 18.06	0.659[a]
(95% CI)	(83.92 – 97.28)	(82.02 – 94.06)	
Week 12	86.44 ± 20.84	89.97 ± 24.52	0.499[a]
(95% CI)	(79.68 – 93.19)	(81.79 – 98.14)	
Change	3.66 ± 12.89	-1.93 ± 18.60	-
p value	0.084[b]	0.531[b]	-

Values are expressed as mean ± standard deviation for normally distributed variables and median (25th and 75th percentiles) for non-normally distributed variables and 95% confidence interval (CI); TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; GL, glycemia; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; an Independent t test; b Paired t test.

DISCUSSION

According to the clinical variables (TJC28, TJC68, SJC28, SJC66, morning stiffness) and efficacy indicators (DAS28 (ESR)/(CRP), SDAI, CDAI, EULAR(ESR) / (CRP)response), supplementation with SBFO significantly alleviated joint pain, swelling, physical disability and stiffness in RA patients.

ESR and CRP are frequently used to detect and monitor systemic inflammation in routine clinical practice. CRP is a better marker of systemic inflammation compared to ESR. CRP is primarily produced in hepatocytes in response to proinflammatory cytokines (IL-6) whereas ESR is an indica-

tor of plasma level of acute phase proteins (16). At week 12, in SBFO group, CRP decreased while ESR increased compared to baseline values. This discrepancy might result from the fact that, in contrast to CRP, ESR level is also affected by factors that are not associated with chronic inflammation such as age, gender, body mass index, physical activity, common metabolic abnormalities, smoking and alcohol consumption (17).Our results are in agreement with previous clinical investigations reporting little impact of sea buckthorn fruit oil on CRP level. In a double-blinded, randomized, placebo-controlled crossover study (2 × 8 weeks, 4-week washout), sea buckthorn fruit oil supplementation (2000 mg/day) did not induce significant changes in plasma levels of CRP and other markers of inflammation (antitrypsin, orosomucoid, leukocytes). Sea buckthorn fruit oil was a commercially available product obtained from fruit flesh and seeds by supercritical carbon dioxide extraction (18). In obese children, sea buckthorn pulp oil (800 mg/day for 60 days) had weak effect on CRP (19).

In our study, SBFO supplementation (1800 mg daily, 12 weeks) did not significantly influence the lipid profile except HDL-C; the latter significantly decreased in SBFO group at week 12. The decrease in HDL-C in SBFO group was unexpected and might be attributed to the antihypertensive medications used by patients. Thus, 32.4% of patients in the experimental group were under treatment with nonselective β blockers for hypertension in comparison to only 5.1% of patients in the control group. The aforementioned medication has been reported to negatively impact the lipid profile by decreasing HDL-C and increasing TG (20-22). Previous studies investigating the impact of sea buckthorn oil on lipid profile reported inconsistent results. In mice fed a high-fat diet, sea buckthorn pulp oil lowered TG (serum, hepatic), TC (serum), LDL-C (serum, hepatic) but also HDL-C (serum, hepatic) (23). In healthy normolipidemic subjects, administration of sea buckthorn fruit oil obtained by supercritical carbon dioxide extraction (5 g/day, 4 weeks, crossover study) caused no change in plasma lipids (24). In obese children, sea buckthorn pulp oil (800 mg/day, 60 days) significantly improved plasma TC and TG but had weak effect on LDL-C and HDL-C levels (19). A significant increase in HDL-C was detected in atopic dermatitis patients supplemented with sea buckthorn pulp oil (5 g/day, 4 months) (25).

In the present study, at week 12, liver markers (AST, ALT, γ-GT) showed lower levels in SBFO group compared to control, with a significant reduction for ALT.Regarding other biochemical parameters, 12-week supplementation with SBFO (1800 mg daily) did not have a significant impact on ALP, GL, urea, creatinine and uric acid levels. Overall, SBFO was well-tolerated in patients with active RA.

CONCLUSIONS

The present study indicates beneficial effects of SBFO supplementation in active RA with significant improvements in composite disease activity indices and EULAR response. Taking into account boththe efficacy and safety of SBFO, the latter seems to be a promising option for the adjunctive treatment in RA. However, the impact of SBFO supplementation on

various inflammatory and oxidative stress markers needs to be further investigated. Different doses of SBFO should also be evaluated to determine the optimal dosage for the best therapeutic outcome.

CONFLICT OF INTEREST AND FUNDING

The authors declare no conflict of interest and this research received no external funding.

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IMPACT OF TREATMENT ON QUALITY OF LIFE IN PATIENTS DIAGNOSED WITH POTENTIALLY MALIGNANT ORAL DISEASES

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IMPACT OF TREATMENT ON QUALITY OF LIFE IN PATIENTS DIAGNOSED WITH POTENTIALLY MALIGNANT ORAL DISEASES (Abstract): This study **aimed** to investigate the impact of treatment on the quality of life (QoL) in patients diagnosed and treated for potentially malignant oral diseases (OPMDs) such as leukoplakia, oral lichen planus, and actinic cheilitis. OPMDs are chronic diseases with a significant risk of cancerous transformation, affecting 1% to 5% of the global population. Symptoms like pain, burning sensation, trismus, and altered taste adversely affect patients' QoL, compounded by delays in diagnosis. **Materials and methods:** This cross-sectional study, conducted from June 2023 to June 2024, involved 30 patients from the "Sf. Spiridon" Emergency Clinical Hospital in Iasi, Romania. Participants were assessed using a disease-specific QoL questionnaire, which included 20 questions across four subscales: difficulty with diagnosis, physical and functional limitations, psychological and social well-being, and the effect of treatment on daily life. **Results:** Participants consisted of 16 males (53.3%) and 14 females (46.7%), aged between 42 and 75 years, with an average age of 64.61±13.69 years. Patients with oral lichen planus and leukoplakia had a statistically significantly lower quality of life compared to patients with actinic cheilitis ($p = 0.038$). **Conclusions:** The three groups indicated a varied impact of the diseases and treatments on different aspects of life. Implementing disease-specific QoL assessment tools can aid in better understanding and addressing the special needs of OPMD patients, ultimately improving treatment outcomes and patient care. **Keywords:** POTENTIALLY MALIGNANT ORAL LESIONS, LEUKOPLAKIA, ORAL LICHEN PLANUS, ACTINIC CHEILITIS, QUALITY OF LIFE.

The World Health Organization (WHO) defines quality of life as the way a person perceives their position in life, considering the culture, the value system in which they live, as well as their goals, expectations, standards, and concerns. The need to correlate the perception of health with the ability to carry out daily activities led to the formulation of the concept of health-related quality of life. It includes self-perception as a crucial aspect of health assessment and helps to understand the impact of disease on individuals' social lives (1).

Potentially malignant oral diseases

(OPMDs) enhance morbidity and mortality due to the risk of neoplastic transformation (2, 3). Globally, OPMDs (leukoplakia, erythroplakia, oral lichen planus, oral submucosal fibrosis, actinic cheilitis) are reported to affect between 1% and 5% of the population (4). Careful monitoring of these lesions by an experienced specialist is crucial to detect malignant changes in the early stages, thus helping to reduce the incidence of cancer. Patients with OPMDs present with severe symptoms that influence quality of life (QoL). The common symptoms of OPMDs, such as pain, burning sensation, trismus, and altered taste, together with delays in diagnosis, lead to a clear deterioration in the quality of life of patients (5-10).

Patients' perceptions of quality of life are usually measured by questionnaires, which may be general or disease-specific. However, existing general questionnaires do not address these specific issues. A quality of life questionnaire designed for a specific disease is valuable for detecting changes over time, including responses to treatment. The Oral Health Impact Profile (OHIP), Oral Health Quality of Life (OHQL), and Chronic Oral Mucosal Disease Questionnaire (COMDQ) have been utilised in OPMD investigations (11-13).

This study aimed to support ongoing efforts to improve patients' experiences by providing a detailed and comparative assessment of quality of life for patients with OPMD after treatment, using the spicific OPMDQoL questionnaire.

MATERIALS AND METHODS

Research design

The present cross-sectional study was conducted between June 2023 and June 2024 among patients diagnosed and treated for potentially malignant oral lesions. Prior to the initiation of the study, approval was obtained from the research ethics committee of the "Grigore T. Popa" University of Medicine and Pharmacy in Iasi (No. 320/5.06.2023) and the "Sf. Spiridon" County Clinical Emergency Hospital in Iasi, Romania (No. 35/24.04.2023).

Study group

The study included 30 patients under observation who were diagnosed and treated for oral leukoplakia (OL), oral lichen planus (OLP), and actinic cheilitis (AC) in the Department of Oral and Maxillofacial Surgery (OMF) at the "Sf. Spiridon" Emergency Clinical Hospital in Iasi, Romania.

The inclusion criteria for participation in the study were voluntary participation, full completion of the questionnaires, and diagnosis of leukoplakia, oral lichen planus, or actinic cheilitis, while the exclusion criteria were diagnosis of other potentially malignant oral diseases or confirmation of malignancy in lesions through histopathological examination.

The objectives and procedures of the study were explained to the patients, and informed consent was obtained for completing the questionnaire, ensuring the anonymity and confidentiality of the answers, as well as the voluntary nature of participation. Patients received detailed instructions before starting the questionnaire, and any misunderstandings were clarified by the investigators, ensuring full completion of the questionnaires.

Study instrument

The OPMDQoL questionnaire (14) is designed to capture patients' views on how these conditions impact their daily lives. It includes 20 questions divided into four subscales that assess their life and activities after treatment: challenges with the diagnosis, physical and functional limitations,

psychological and social well-being, and the impact of treatment on daily life. Each question uses a five-point Likert scale: 0 for not at all, 1 for a little bit, 2 for somewhat, 3 for quite a bit, and 4 for very much. The total possible score ranges from 0 to 80, with higher scores indicating a lower quality of life.

The Romanian version of the QoL questionnaire for OPMD patients used in this study demonstrated strong internal consistency, with Cronbach's alpha coefficients as follows: 0.768 for difficulty with the diagnosis, 0.813 for physical and functional limitations, 0.887 for psychological and social well-being, and 0.811 for the effect of treatment on daily life.

Statistical analysis

The descriptive statistical analysis was performed using IBM *Statistical Package for the Social Sciences (SPSS) software version 26.0*. Differences between groups were determined using the Chi-square and ANOVA tests. A significance level of p-value<0.05 was considered.

RESULTS

Study group

The study group included 30 patients with OPMD, treated surgically or with medication, of which 14 were female (46.7%) and 16 were male (53.3%), aged between 42 and 75 years and an average age of 64.61±13.69 years. Most patients (16, 53.3%) came from the urban environment. Most patients (16, 53.3%) had completed high school, and very few had higher education (3, 10%). Participants' characteristics are presented in first table.

The responses given by the study participants to the first questionnaire subscale are presented in second table.

TABLE I.

Characteristics of study participants (N=30)

	Participants	Frequency N	Percent %
Variables	**Gender**		
	Male	16	53.3
	Female	14	46.7
	Age (mean ± SD [1])	64.61±13.69	
	≤ 50 years	8	26.7
	> 50 years	22	73.3
	Environment		
	Urban	16	53.3
	Rural	14	46.7
	Educational level		
	Secondary school	11	36.7
	High school	16	53.3
	Higher education	3	10

[1] SD standard deviation

TABLE II.
Patients' responses to the first subscale, Difficulties with Diagnosis

Difficulties with diagnosis	Leukoplakia (n=10)	Oral lichen planus (n=10)	Actinic cheilitis (n=10)	Total	*p*-value
	N (%)	N (%)	N (%)		
Q1. How difficult was it for you to get your mouth condition diagnosed?					
Not at all	2 (20%)	0 (0%)	4 (40%)	6 (20%)	
A little bit	3 (30%)	1 (10%)	2 (20%)	6 (20%)	
Somewhat	3 (30%)	2 (20%)	4 (40%)	9 (30%)	*0.047* *
Quite a bit	2 (20%)	4 (40%)	0 (0%)	6 (20%)	
Very much	0 (0%)	3 (30%)	0 (0%)	3 (10%)	
Q2. How much did the need to visit many doctors for getting your mouth condition diagnosed affect daily life activities?					
Not at all	2 (20%)	1 (10%)	1 (10%)	4 (13.33%)	
A little bit	1 (10%)	1 (10%)	3 (30%)	5 (16.66%)	
Somewhat	3 (30%)	3 (30%)	2 (20%)	8 (26.66%)	0.898
Quite a bit	2 (20%)	4 (40%)	3 (30%)	9 (30%)	
Very much	2 (20%)	1 (10%)	1 (10%)	4 (13.33%)	
Q3. How stressful was it for you to take a variety of treatments before being diagnosed with your mouth condition?					
Not at all	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
A little bit	3 (30%)	1 (10%)	2 (20%)	6 (20%)	
Somewhat	2 (20%)	2 (20%)	3 (30%)	7 (23.33%)	0.854
Quite a bit	1 (10%)	4 (40%)	3 (30%)	8 (26.66%)	
Very much	3 (30%)	2 (20%)	1 (10%)	6 (20%)	
*Significance level of 0.05 (Chi-square test)					

The first question (Q1) indicated that 30% (n=9) of OPMD patients had slight difficulty getting their mouth condition diagnosed. Patients with OLP faced notably more challenges in obtaining a clear diagnosis compared to those with other OPMDs (p=0.047). Because the patients consulted multiple doctors for their diagnosis (Q2), 26.66% reported that this somewhat impacted their daily activities, while 13.33% indicated it had a significant impact. Most patients experienced some stress (Q3) while undergoing various treatments before their diagnosis, although 10% reported not being stressed.

Table III presents the responses given by the study participants to the second questionnaire subscale.

The assessment of patients' physical and functional limitations (tab. III) revealed that 9 patients (30%) experienced moderate pain and discomfort due to their oral condition (Q4). Except for 6 patients diagnosed with OLP, all other patients experienced mild burning sensations when consuming spicy food (Q5). About 16.66% of the patients experienced difficulties opening their mouths widely (Q6). More than half of the patients declared their taste sense didn't change much or at all. Eight patients (26.66%) dealt with some limitation in eating desired foods (Q7), 11 patients (36.66%) somewhat enjoyed their meals (Q8), and 12 patients (40%) reported no dryness of the mouth (Q10).

TABLE III.

Patients' responses to the second subscale, *Physical and functional limitations*

Physical and functional limitations	Leukoplakia (n=10)	Oral lichen planus (n=10)	Actinic cheilitis (n=10)	Total	*p*-value
	N (%)	N (%)	N (%)		
	Q4. How much pain and agony does your mouth condition cause you?				
Not at all	1 (10%)	0 (00.0%)	3 (30%)	4 (13.33%)	
A little bit	1 (10%)	0 (00.0%)	3 (30%)	4 (13.33%)	
Somewhat	4 (40%)	2 (20%)	3 (30%)	9 (30%)	*0.045**
Quite a bit	3 (30%)	3 (30%)	1 (10%)	7 (23.33%)	
Very much	1 (10%)	5 (50%)	0 (00.0%)	6 (20%)	
	Q5. How much burning sensation do you experience while having spicy food?				
Not at all	1 (10%)	0 (0%)	4 (40%)	5 (16.66%)	
A little bit	3 (30%)	1 (10%)	4 (40%)	8 (26.66%)	
Somewhat	3 (30%)	1 (10%)	1 (10%)	5 (16.66%)	*0.023**
Quite a bit	2 (20%)	2 (20%)	1 (10%)	5 (16.66%)	
Very much	1 (10%)	6 (60%)	0 (0%)	7 (23.33%)	
	Q6. How difficult is it for you to open your mouth widely?				
Not at all	1 (10%)	1 (10%)	5 (50%)	7 (23.33%)	
A little bit	5 (50%)	2 (20%)	2 (20%)	9 (30%)	
Somewhat	1 (10%)	2 (20%)	1 (10%)	4 (13.33%)	0.336
Quite a bit	2 (20%)	2 (20%)	1 (10%)	5 (16.66%)	
Very much	1 (10%)	3 (30%)	1 (10%)	5 (16.66%)	
	Q7. How much is your oral condition causing you to limit your desired foods?				
Not at all	3 (30%)	0 (0%)	7 (70%)	10 (33.33%)	
A little bit	3 (30%)	1 (10%)	1 (10%)	5 (16.66%)	
Somewhat	3 (30%)	3 (30%)	2 (20%)	8 (26.66%)	*0.019**
Quite a bit	1 (10%)	5 (50%)	0 (0%)	6 (20%)	
Very much	0 (0%)	1 (10%)	0 (0%)	1 (3.33%)	
	Q8. How much is your mouth condition limiting you from enjoying your meals?				
Not at all	1 (10%)	1 (10%)	6 (60%)	8 (26.66%)	
A little bit	3 (30%)	3 (30%)	1 (10%)	7 (23.33%)	
Somewhat	5 (50%)	4 (40%)	2 (20%)	11 (36.66%)	0.221
Quite a bit	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
Very much	0 (0%)	1 (10%)	0 (0%)	1 (3.33%)	
	Q9. How much does your mouth condition affect your taste sensation?				
Not at all	1 (10%)	7 (70%)	8 (80%)	16 (53.33%)	
A little bit	1 (10%)	2 (20%)	1 (10%)	4 (13.33%)	
Somewhat	4 (40%)	1 (10%)	1 (10%)	6 (20%)	*0.031**
Quite a bit	3 (30%)	0 (0%)	0 (0%)	3 (10%)	
Very much	1 (10%)	0 (0%)	0 (0%)	1 (3.33%)	
	Q10. How much dryness do you feel in your mouth?				
Not at all	0 (00.0%)	4 (40%)	8 (80%)	12 (40%)	
A little bit	3 (30%)	2 (20%)	1 (10%)	6 (20%)	
Somewhat	3 (30%)	2 (20%)	1 (10%)	6 (20%)	*0.042**
Quite a bit	2 (20%)	2 (20%)	0 (0%)	4 (13.33%)	
Very much	2 (20%)	0 (00.0%)	0 (0%)	2 (6.66%)	
*Significance level of 0.05 (Chi-square test)					

Furthermore, the assessment of psychological and social well-being indicated that 17 patients (56.66%) were scared about the possibility of their oral condition progressing to cancer (Q15). Almost half of the patients believed their oral condition had minimal or no effect on their relationships with family and friends (Q13). Furthermore, 66.66% of the patients (n=20, Q17) indicated they felt little to no embarrassment when eating at social events, like parties and functions (tab. IV).

TABLE IV.

Patients' responses to the third subscale, *Psychological and social well-being*

Psychological and social well-being	Leukoplakia (n=10)	Oral lichen planus (n=10)	Actinic cheilitis (n=10)	Total	*p*-value
	N (%)	N (%)	N (%)		
	Q11. How frustrated are you because of your oral condition?				
Not at all	0 (0%)	0 (0%)	0 (0%)	0 (0%)	
A little bit	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
Somewhat	2 (20%)	5 (50%)	2 (20%)	9 (30%)	0.736
Quite a bit	4 (40%)	2 (20%)	5 (50%)	11 (36.66%)	
Very much	3 (30%)	2 (20%)	2 (20%)	7 (23.33%)	
	Q12 . How depressed or low do you feel because of your mouth condition?				
Not at all	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
A little bit	1 (10%)	1 (10%)	2 (20%)	4 (13.33%)	
Somewhat	3 (30%)	5 (50%)	5 (50%)	13 (43.33%)	0.956
Quite a bit	3 (30%)	2 (20%)	1 (10%)	6 (20%)	
Very much	2 (20%)	1 (10%)	1 (10%)	4 (13.33%)	
	Q13. In general, how much is your mouth condition affecting your relationship with family and friends?				
Not at all	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
A little bit	3 (30%)	4 (40%)	5 (50%)	12 (40%)	
Somewhat	4 (40%)	2 (20%)	2 (20%)	8 (26.66%)	0.899
Quite a bit	2 (20%)	2 (20%)	2 (20%)	6 (20%)	
Very much	0 (0%)	1 (10%)	0 (0%)	1 (3.33%)	
	Q14. How much is your mouth condition affecting your satisfaction with life?				
Not at all	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
A little bit	2 (20%)	1 (10%)	4 (40%)	7 (23.33%)	
Somewhat	3 (30%)	6 (60%)	4 (40%)	13 (43.33%)	0.583
Quite a bit	3 (30%)	1 (10%)	0 (0%)	4 (13.33%)	
Very much	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
	Q15. How scared are you about the possibility of your oral condition turning into cancer?				
Not at all	0 (0%)	1 (10%)	1 (10%)	2 (6.66%)	
A little bit	1 (10%)	1 (10%)	2 (20%)	4 (13.33%)	
Somewhat	2 (20%)	2 (20%)	3 (30%)	7 (23.33%)	0.858
Quite a bit	3 (30%)	4 (40%)	3 (30%)	10 (33.33%)	
Very much	4 (40%)	2 (20%)	1 (10%)	7 (23.33%)	

Psychological and social well-being	Leukoplakia (n=10)	Oral lichen planus (n=10)	Actinic cheilitis (n=10)	Total	*p*-value
	N (%)	N (%)	N (%)		
	Q16. How scared are you about the outcome of this condition affecting your life?				
Not at all	0 (0%)	1 (10%)	1 (10%)	2 (6.66%)	0.484
A little bit	2 (20%)	1 (10%)	4 (40%)	7 (23.33%)	
Somewhat	3 (30%)	6 (60%)	3 (30%)	12 (40%)	
Quite a bit	4 (40%)	2 (20%)	1 (10%)	7 (23.33%)	
Very much	1 (10%)	0 (00.0%)	1 (10%)	2 (6.66%)	
	Q17. How embarrassing is it for you to eat foods at parties, functions, or other social gatherings?				
Not at all	0 (0%)	1 (10%)	6 (60%)	7 (23.33%)	0.086
A little bit	5 (50%)	6 (60%)	2 (20%)	13 (43.33%)	
Somewhat	3 (30%)	2 (20%)	1 (10%)	6 (20%)	
Quite a bit	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
Very much	1 (10%)	0 (0%)	0 (0%)	1 (3.33%)	
*Significance level of 0.05 (Chi-square test)					

The impact of treatment on daily life (tab. V) was investigated, revealing that a significant number of patients expressed satisfaction with their treatment (Q19). With the exception of 5 patients (16.66%), the majority reported experiencing only mild or no pain following their treatment (Q18). However, 19 patients (63.33%) noted that their daily routines were disrupted by their treatment appointments (Q20).

TABLE V.

Patients' responses to the fourth subscale, *Effect of treatment on daily life*

Effect of treatment on daily life	Leukoplakia (n=10)	Oral lichen planus (n=10)	Actinic cheilitis (n=10)	Total	*p*-value
	N (%)	N (%)	N (%)		
Q18. How much pain do you experience with treatment of your oral condition?					
Not at all	1 (10%)	1 (10%)	3 (30%)	5 (16.66%)	0.959
A little bit	3 (30%)	3 (30%)	2 (20%)	8 (26.66%)	
Somewhat	3 (30%)	4 (40%)	3 (30%)	10 (33.33%)	
Quite a bit	2 (20%)	1 (10%)	1 (10%)	4 (13.33%)	
Very much	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
Q19. How satisfied are you with the effectiveness of treatment for your mouth condition?					
Not at all	1 (10%)	1 (10%)	1 (10%)	3 (10%)	0.905
A little bit	1 (10%)	2 (20%)	1 (10%)	4 (13.33%)	
Somewhat	3 (30%)	3 (30%)	1 (10%)	7 (23.33%)	
Quite a bit	4 (40%)	3 (30%)	4 (40%)	11 (36.66%)	
Very much	1 (10%)	1 (10%)	3 (30%)	5 (16.66%)	
Q20. How much are your treatment appointments affecting your daily schedule?					
Not at all	1 (10%)	1 (10%)	1 (10%)	3 (10%)	0.808
A little bit	1 (10%)	4 (40%)	3 (30%)	8 (26.66%)	
Somewhat	6 (60%)	2 (20%)	3 (30%)	11 (36.66%)	
Quite a bit	1 (10%)	2 (20%)	2 (20%)	5 (16.66%)	
Very much	1 (10%)	1 (10%)	1 (10%)	3 (10%)	
*Significance level of 0.05 (Chi-square test					

In the present study, it was found that patients with oral lichen planus and leukoplakia had a statistically significantly lower quality of life compared to patients with actinic cheilitis (p=0.038) (tab. VI). Similarly, gender differences were observed, with females reporting lower quality of life scores than males (fig. 1). Additionally, the analysis revealed that patients with a high school education had an adequate quality of life (fig. 2).

TABLE VI.

Patients' scores on the OPMDQoL questionnaire

Score	Leukoplakia (n=10)	Oral lichen planus (n=10)	Actinic Cheilitis (n=10)	*p*-value
	(mean ± SD[1])	(mean ± SD[1])	(mean ± SD[1])	
Difficulties with diagnosis	6.8 ± 3.736	8.70 ± 3.234	6.10 ± 3.315	0.234
Physical and functional limitations	13.1 ± 5.877	15.00 ± 4.830	5.10 ± 5.216	*0.001**
Psychological and social well-being	16.3 ± 6.29	14.10 ± 6.674	12.30 ± 6.056	0.382
Effect of treatment on daily life	6.2 ±1.398	5.70 ± 2.003	6.10 ± 0.738	0.727
Total OPMDQoL	42.40 ± 13.705	43.50 ± 14.002	29.60 ± 10.069	*0.038**

[1] SD standard deviation; *Significance level of 0.05 (ANOVA test)

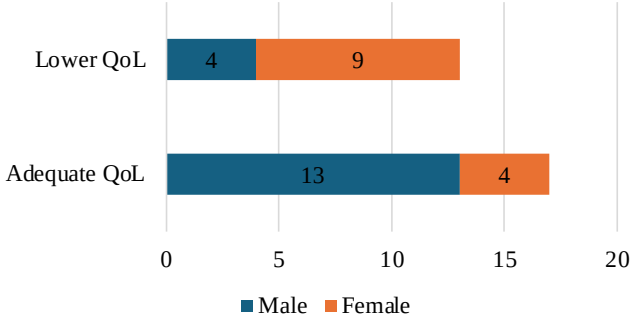

Fig. 1. OPMDQoL scores according to gender

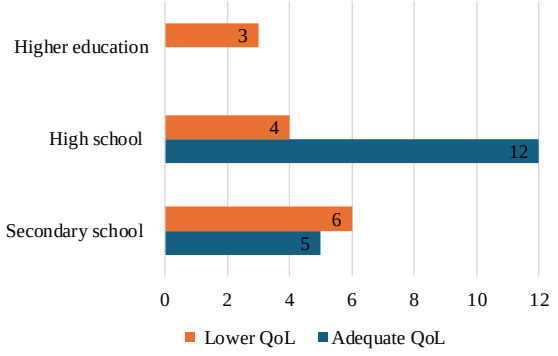

Fig. 2. OPMDQoL scores according to educational level

DISCUSSION

In this study, we analyzed the impact of three distinct OPMDs on the quality of life. According to available literature, this is the first study using a specific tool to compare QoL between the three most common OPMDs (leukoplakia, oral lichen planus, and actinic cheilitis) in Romania. The response rate was remarkable, with all patients agreeing to participate.

According to the OPMDQoL assessment, the first subscale showed that patients with OLP faced statistically significant difficulties in obtaining a clear diagnosis compared to those with OL or AC. This difference can be attributed to the fact that actinic cheilitis is clearly related to prolonged UV radiation exposure, while the etiopathogenesis of oral lichen planus is unclear and multifactorial, involving anxiety, stress, genetic factors, and associations with various systemic diseases. The multifactorial complexity of OLP complicates the diagnosis for dentists.

Participants diagnosed with oral lichen planus or oral leukoplakia exhibited elevated levels of physical and functional limitations in comparison to those with actinic cheilitis. This disparity may be due to the multiple signs and symptoms associated with these conditions, such as pain and burning sensations in the case of OLP and impaired taste perception for patients with OL, symptoms that are not a major concern for patients diagnosed with AC.

In a study published by Tadakamadla *et al.* (8), patients diagnosed with OL reported low scores in this subscale because some were diagnosed incidentally at an early stage during regular dental check-ups. In our case, most of these lesions are unfortunately ignored by patients in the early stages due to the lack of pain, leading to their presentation to the doctor only in advanced stages.

One of the remarkable aspects of this study was that all three OPMDs had similar scores for psychological and social well-being, indicating that the potentially malignant nature of the disease impacted this aspect of their lives. The majority of patients reported feeling frustrated (with 59.99% responding “quite a bit” and “very much”), experiencing some level of depression, and noting that their condition had an impact on their overall life satisfaction. Their primary worry was on the potential for the oral condition to progress into malignancy. Nevertheless, the patients did not notice any negative impact on their social interactions with family and friends, and they did not feel any discomfort when at work, parties, or other social events.

Prolonged treatments without a definitive cure are stressful for OPMD patients. However, no significant differences were observed between the three studied groups regarding the subscale effect of treatment on daily life. Similar results were reported by Kapur *et al.* (15), who compared scores from 45 patients treated surgically for OPMD. Ashshi *et al.* (16), following a 5-year study (2021-2018) at the University of Pennsylvania on a group of 100 patients (53 diagnosed with OLP), presented scores higher than those with associated epithelial dysplasia.

Female patients exhibited lower overall OPMDQ scores, indicating a reduced quality of life, a finding consistent with other studies (8,11,17,18). This result is expected, as women are more prone to anxie-

ty and depression (19).

The limitations of the present study must be acknowledged, particularly regarding the generalizability of the findings. The study sample was composed of subjects from a single teaching hospital in Romania, which may limit the applicability of the results to all OPMD patients in the country. However, the sample size was sufficient to draw valid conclusions and inferences representative of the target population under study.

CONCLUSIONS

Patients with oral lichen planus had higher values in the difficulties with diagnosis subscale than patients with oral leukoplakia and actinic cheilitis. OLP and OL greatly affect the quality of life of those affected, whereas the impact of AC was relatively minor. Patients with AC also had better scores on physical and functional limitations than those with OL and OLP. There were no differences between the three oral conditions with the potential for malignant transformation in the subscales effect of treatment on daily life and psychological and social well-being and a large number of patients declared themselves satisfied with the treatments they received. Therefore, the OPMDQoL instrument can be considered useful in monitoring treatment response. In particular, this tool helps clinicians understand the psychological and social impact of OPMD on their patients' lives.

This information has the potential to motivate patients to quit their bad habits and follow through with treatment. They also contribute to improving treatment outcomes, patient care, oral health education and research.

CONFLICT OF INTEREST AND FUNDING

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CLINICAL AND RADIOLOGICAL STUDY ON PERI-IMPLANT STATUS IN POSTERIOR IMPLANT-SUPPORTED PARTIAL FIXED DENTURES

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CLINICAL AND RADIOLOGICAL STUDY ON PERI-IMPLANT STATUS IN POSTERIOR IMPLANT-SUPPORTED PARTIAL FIXED DENTURES (Abstract). **Aim** of this study was to evaluate the clinical and radiological status of the peri-implant tissues in patients treated with posterior implant-supported partial fixed dentures. **Materials and methods:** The retrospective research was conducted on a cohort of 48 patients (mean age 63.04 ± 10.723 years). The study group included patients with posterior edentulism treated by implant-supported metal-ceramic fixed prosthetic restorations. Socio-demographic data (gender, age group) and anamnestic data were collected on: smoking status; periodontal grade; compliance to the supportive periodontal/peri-implant therapy; OHI index. Clinical and radiological parameters were assessed for 166 implants to establish the status of the peri-implant tissues and prevalence of peri-implantitis. **Results:** 45.8% of patients and 19,9% of implants were diagnosed with peri-implantitis. Significant statistical differences were found between implants without peri-implantitis and implants with peri-implantitis for gender ($p= 0.022^{*}$), age group ($p= 0.018^{*}$), smoking status ($p= 0.007^{**}$), OHI index ($p < 0.001^{**}$), periodontitis stage ($p < 0.001^{**}$), and compliance to periodontal/peri-implant support therapy ($p<0.001^{*}$). **Conclusions:** Although peri-implantitis affects almost half of the patients (45.8%), the proportion of individual implants affected is lower (19.9%). Incidence of peri-implantitis was significantly lower to patients with good oral hygiene, non-smokers, low grade periodontal disease, or patients compliant with periodontal/peri-implant support therapy. **Keywords:** POSTERIOR EDENTULISM, IMPLANTS, PERI-IMPLANTITIS.

INTRODUCTION

Implant-supported posterior partial fixed dentures have provided significant improvements in function, aesthetics, and patient satisfaction when compared with classical fixed prosthetic therapy, while prosthetic success rates were not significantly different between the tooth- and implant-supported posterior fixed partial dentures (1-3). However, various factors that lead to peri-implantitis can compromise the long-term stability of dental implants, which can emerge as primary complications impacting implant therapy success (4, 5). The osseointegration of implants can be impacted by biological, mechanical, or technical complications (6-8). Evaluating peri-implant soft tissues status is essential for the early detec-

tion of inflammation signs, such as edema or bleeding, which may indicate the initial stages of peri-implantitis. Monitoring peri-implant bone through periodic radiographs helps identify bone loss and assess implant stability, preventing advanced complications (9). Preventively, ensuring rigorous oral hygiene, scheduling regular professional cleaning sessions for implant surfaces, and reeducating the patient on proper brushing techniques are crucial. In early peri-implantitis, non-surgical treatments, such as mechanical debridement and the use of antimicrobial agents, can halt the progression of inflammation and prevent bone destruction. Thus, understanding the peri-implant condition in patients with implant-supported partial fixed dentures is crucial for developing effective preventive measures and non-surgical therapeutic protocols to enhance implant survival rates and overall oral health outcomes (10).

The **aim** of this study was to evaluate the clinical and radiological status of the peri-implant tissues in patients treated with posterior implant-supported partial fixed dentures.

MATERIALS AND METHODS

The retrospective research was conducted on a cohort of 48 patients (age: mean age 63.04 ± 10.723 years, range 40-84 years; gender: 22 men, 26 women). The study included patients treated with posterior implant-supported metal-ceramic fixed prosthetic restorations (FPR-IP) 166 implants). The mean follow-up was 6.19 yrs. (range 5-8 yrs.). All subjects were selected from a pool of patients treated by the same implantologist and oral surgery specialist, performing bone addition procedures, implant surgery and prosthetic restorations. Prosthetic loading was performed 3 months after implant stage for patients who did not require alveolar bone addition, and 4-9 months for patients who required bone addition. Inclusion criteria: patients with age over 18 yrs.; posterior edentulism (Kennedy Class I and II); metal-ceramic fixed partial dentures with implant-support (FPR-IP); follow-up 5-8 yrs. Exclusion criteria: cantilever-type FPR-IP; aggressive periodontitis in periodontal history; systemic diseases impacting peri-implant tissues (uncontrolled diabetes mellitus, osteoporosis, metabolic disorders); antibiotic therapy in the last 6 months. The study was conducted in accordance with the requirements of the Declaration of Helsinki from 1975, revised in 2008. Written informed consent was obtained from the enrolled subjects. Socio-demographic data (gender, age groups: 40-60 yrs.; > 60 yrs.) and anamnestic data were collected on: smoking status (smokers; non-smokers); periodontal status (periodontal grade according to the Classification of Periodontal and Peri-Implant Diseases and Conditions, World Workshop 2017); compliance to the supportive periodontal/peri-implant therapy; index OHI (Oral Hygiene Index). Patients were classified into four stages of periodontal disease (I, II, III, and IV) according to the classification to the Classification of Periodontal and Peri-Implant Diseases and Conditions (World Workshop 2017) (11). Patient compliance with periodontal/peri-implant supportive therapy (PST) was evaluated according to the recall intervals proposed by Monje *et al*. (2014) (12): compliant patient (attends PST sessions at intervals of ≤ 5 months); occasional compliant (attends PST sessions at intervals of > 6 months); non-compliant patient. Clinical data on peri-implant soft tissues were collected by clinical examination (13).

Peri-implantitis was diagnosed according to the following criteria (14-15): clinical signs of peri-implant inflammation (erythema, edema, bleeding on probing, and/or suppuration); bone loss of at least 3 mm visible radiographically. Radiological examination was performed by CBCT (Sirona Orthophos XG). Measurements were made by an independent radiologist who was not involved in the study. The peri-implant marginal bone loss (MBL) was calculated at the mesial and distal aspects of each implant using the Sidexis XG/DVT application (Dentsply/Sirona): the distance between the implant-abutment interface and the level of peri-implant marginal bone loss. The highest value was used as a reference for determining the degree of peri-implant bone loss.

Statistical Analysis

Descriptive statistics including frequencies, means and standard deviations were calculated for demographics, clinical parameters, and marginal bone loss. The comparison of the qualitative variables between test group and control was performed by using Chi-squared test. Chi-square test was used to examine the statistical differences between implants with peri-implantitis and healthy implants. All tests of significance were evaluated at the 0.05 error level with *SPSS version 27.0* (IBM, Armonk, NY, USA).

RESULTS

At the patient level, 54.2% of patients are healthy or exhibit a state of peri-mucositis (without peri-implantitis), while 45.8% of patients have peri-implantitis, indicating a significant proportion of affected cases at the individual level. At the implant level, 80.1% of implants are classified as healthy or with peri-mucositis, representing the majority of the analyzed implants, while 19.9% of implants show peri-implantitis. This result reflects a lower incidence of this condition at the implant level compared to the patient level (tab. I).

Significant statistical differences were found between patients without peri-implantitis and patients with peri-implantitis for OHI index (0-1 *vs.* 2-3) ($p= 0.011^{*}$), periodontitis grade ($<0.001^{**}$), and PST compliance (compliant vs. non-compliant) ($p=0.003^{**}$) (tab. II).

Significant statistical differences were found between implants without peri-implantitis and implants with peri-implantitis for gender ($p= 0.022^{*}$), age group ($p= 0.018^{*}$), smoking status ($p= 0.007^{**}$), OHI index ($p < 0.001^{**}$), periodontitis stage ($p < 0.001^{**}$), and PST compliance ($p< .001^{*}$) (tab. III).

TABLE I.

Peri-implant status (per patient; per implant)

Status	Frequency(N)	Percent (%)
Per patient		
Healthy/peri-implantitis	26	54.2
Peri-implantitis	22	45.8
Per implant		
Healthy/peri-mucositis	133	80.1
Peri-implantitis	33	19.9

TABLE II.

Distribution of patients with implant-supported fixed prosthetic restorations based on peri-implant status and socio-demographic factors

	Per patient			
Parameter	Absent peri-implantitis	Peri-implantitis	Total	*p*-value+
Gender				
Male	8 (30.8%)	14 (63.6%)	22 (45.8%)	0.023*
Female	18 (69.2%)	8 (36.4%)	26 (54.2%)	
Age group (yrs.)				
40-60	8 (30.8%)	10 (45.5%)	18 (37.5%)	0.295
>60	18 (69.2%)	12 (54.5%)	30 (62.5%)	
Smoking status				
Non-smoker	16 (61.5%)	12 (54.5%)	28 (58.3%)	0.624
Smoker	10 (38.5%)	10 (45.5%)	20 (41.7%)	
OHI				
0-1	21 (80.8%)	10 (45.5%)	31 (64.6%)	0.011*
2-3	5 (19.2%)	12 (54.5%)	17 (35.4%)	
Periodontitis grade				
I	3 (11.5%)	-	3 (6.3%)	
II	9 (34.6%)	7 (31.8%)	16 (33.3%)	< 0.001**
III	14 (53.8%)	5 (22.7%)	19 (39.6%)	
IV	-	10 (45.5%)	10 (20.8%)	
PST compliance				
Compliant	23 (88.5%)	10 (45.5%)	33 (68.8%)	0.003**
Casual	3 (11.5%)	6 (27.3%)	9 (18.8%)	
Non-Compliant	-	6 (27.3%)	6 (12.5%)	
Total	26 (100.0%)	22 (100.0%)	48 (100.0%)	

+Chi-squared test; *p < 0.05 statistically significant;
**p < 0.01 statistically highly significant

TABLE III.

Distribution of dental implants based on peri-implant status and socio-demographic factors

	Per implant			
Factor	Absent peri-implantitis	Implants with peri-implantitis	Total	*p*-value+
Gender				
Male	59 (44.4%)	22 (66.7%)	81 (48.8%)	0.022*
Female	74 (55.6%)	11 (33.3%)	85 (51.2%)	
Age group (yrs.)				
40-60	43 (32.3%)	18 (54.5%)	61 (36.7%)	0.018*
>60	90 (67.7%)	15 (45.5%)	105 (63.3%)	
Smoking status				
Non-smoker	90 (67.7%)	14 (42.4%)	104 (62.7%)	0.007**
Smoker	43 (32.3%)	19 (57.6%)	62 (37.3%)	
OHI				
0-1	111 (83.5%)	10 (30.3%)	121 (72.9%)	<0.001**
2-3	22 (16.5%)	23 (69.7%)	45 (27.1%)	

	Per implant			
Factor	Absent peri-implantitis	Implants with peri-implantitis	Total	*p*-value+
Periodontitis stage				
I	6 (4.5%)	-	6 (3.6%)	
II	59 (44.4%)	7 (21.2%)	66 (39.8%)	<0.001**
III	64 (48.1%)	4 (12.1%)	68 (41.0%)	
IV	4 (3.0%)	22 (66.7%)	26 (15.7%)	
PST compliance				
Compliant	118 (88.7%)	10 (30.3%)	128 (77.1%)	<.001*
Casual	11 (8.3%)	9 (27.3%)	20 (12.0%)	
Non-Compliant	4 (3.0%)	14 (42.4%)	18 (10.8%)	
Total	133 (100.0%)	33 (100.0%)	166 (100.0%)	
+Chi-squared test; *p < 0.05 statistically significant; **p < 0.01 statistically highly significant				

DISCUSSION

While any implant present in the oral cavity, requiring additional treatment, is considered a "surviving" implant (16), "success" of a dental implant is associated with implants that are functional and satisfactory for patients. Criteria for a successful implant are as follows: immobility, absence of peri-implant bone loss on radiographic images, attached gingiva width ≥ 2mm, absence of peri-implant infection (17). We aimed to evaluate of the clinical and radiological parameters directly associated with peri-implantitis definition as provided by Heitz-Mayfield *et al*. (2018) (14) and Renvert *et al*. (2018) (15). In the investigated group, we observed a peri-implantitis prevalence rate at the implant level of 19.9%, higher than prevalence reported by research groups as follows: 7.3% (18), 9.1% (19), 9.8% (20), 9.83% (5), 9.6% (21), 13.5% (26), and 16% (24). Other research groups reported higher values compared to our results such as 23% (24) and 24,9% (25). At the patient level, we found 45.8% of patients with at least one implant affected by peri-implantitis. Other research groups reported mean values of 13.3% (26), 15.1% (18), 16.3% (19), 18.8% (21), 19.83% (5), 26% (23), 37% (22), and 45% (23). A review found that the prevalence of peri-implantitis ranges from 4.7% to 43% at the implant level and from 8.9% to 56% at the patient level (27). The wide variability in peri-implantitis prevalence rates can be attributed to differences in case definitions and evaluation criteria used by various research groups. However, in our study, the prevalence of peri-implantitis can be explained by the lower compliance of patients with periodontal/peri-implant supportive therapy. Regarding the distribution of peri-implantitis cases based on socio-demographic and population factors, statistically significant differences were observed between the percentages of implants without peri-implantitis and those diagnosed with peri-implantitis among smokers ($p = 0.007^{**}$), oral hygiene levels (OHI index) ($p < 0.001^{**}$), the stage of periodontal disease ($p < 0.001$**), and patient compliance with PST ($p < 0.001$**). The correlation between smoking and increased likelihood of peri-implantitis remains unclear (28). However, some authors categorize smoking as a risk factor as smoking changes the composition of the periodontal microbiota, impacting also the immune system, and increases the incidence and progression of periodontal diseases

associated with higher risk of tooth loss and implant failures (29). Patients with a history of periodontitis who do not fully adhere to supportive periodontal therapy have a significantly higher frequency of severe peri-implantitis cases requiring surgical procedures and/or antibiotic treatments (30).

CONCLUSIONS

Although peri-implantitis affects almost half of the patients (45.8%), the proportion of individual implants affected is lower (19.9%). Incidence of peri-implantitis was significantly lower to patients with good oral hygiene, non-smokers, grade I-II periodontitis, and patients compliant with periodontal/peri-implant support therapy.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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SPINAL METASTASES IN THE MOST COMMON CANCERS

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SPINAL METASTASES IN THE MOST COMMON CANCERS (Abstract): The **aims** of this study are to identify the underlying causes of spinal injuries and the molecular subtypes which are involved in the production of these pathologies. **Materials and methods**: This study is a retrospective one, which covered a period of four months (from January 1st to April 30th, 2015), and involved a total of 50 patients who were diagnosed with non-traumatic spinal cord injuries. These patients received treatment at the "Prof. Dr. N. Oblu" Emergency Clinical Hospital in Iași. **Results**: Despite having a group of just 50 patients we also managed to identify several key factors implicated in bone dissemination of the primary tumor: skeletal homotropism, vertebral bone composition, genetic and epigenetic alterations, bone remodeling imbalance. **Conclusions:** The study highlights the need for a multidisciplinary approach to manage spinal metastases effectively. The interplay between cancer progression, bone health, and patient's quality of life, can help healthcare providers to develop more comprehensive management strategies for individuals affected by metastatic disease. **Keywords**: BREAST CANCER, SPINAL METASTASES, PRIMARY TUMORS, BRONCHOPULMONARY CANCER.

INTRODUCTION

According to alarming data published by the World Health Organization (WHO), the rate of spinal bone metastases in the structure of cancer morbidity has increased significantly over the last 10 years, exceeding 70%, and in the last 2 years has even reached 85%.

This worrying trend places bone metastases in third place in terms of frequency among metastases, second only to lung and liver metastases. It is also worth noting that bone metastatic tumors occur 35-40 times more frequently than primary bone tumors. Metastatic cancer of the spine is an increasingly common problem due to significant advances in the treatment of primary cancers (1-3).

In cases of spinal metastases, the thoracic region is most frequently affected,

accounting for nearly 70% of cases, with a predilection for the T10 vertebral level. Approximately 25% of spinal metastases are found in the lumbosacral region, particularly around the L3 level. The cervical spine is less commonly involved, representing only about 15% of cases. At the vertebral level, the areas most commonly impacted by metastases are the vertebral body and pedicles, which are involved in 85% of cases. The paravertebral space (10-15%) and the epidural and intramedullary/intraspinal spaces (less than 5%) are less frequently affected.

Notably, the posterior part of the vertebra tends to be affected first, followed by the anterior part (2). Metastatic lesions in the spine can present as osteolytic, osteoblastic, or mixed. Spinal tumors are clinically manifested by significant pain, which is the primary reason for referral, along with neurological signs of spinal cord compression by the tumor mass and local or regional spinal deformity. The location of the pain corresponds to the site of the tumor and varies in intensity, progressively worsening over time, influenced by a number of intrinsic and extrinsic factors (2-5). Severe bone destruction can cause pathological fractures, spinal instability, and deformities, which may lead to the most serious complication-spinal cord compression. The onset of neurological symptoms is influenced by factors such as the location of the metastatic tumor on the spine, its position relative to the spinal cord, the speed at which compression occurs, and the vascular anatomy of the spinal cord in the affected area (3). These symptoms usually appear in the later stages and are marked by motor or sensory deficits, along with issues related to sphincter control. Spinal metastases, which affect approximately 70% of cancer patients, are a leading cause of mortality. Autopsy studies reveal alarming statistics, with spinal metastases present in 90% of prostate cancer cases, 75% of breast cancer cases, 55% of melanoma cases, 45% of lung cancer cases, and 30% of kidney cancer cases. Clinically, spinal metastases are most commonly associated with lung (21%), prostate (19%), and breast (12%) cancers, necessitating targeted medical interventions (4). Spinal metastases from breast cancer, like those from other cancers, can cause local pain, spinal instability, pathological vertebral fractures, spinal cord compression, neurological damage, and paralysis, all of which significantly impact patients' quality of life and reduce their lifespan. Accurate histopathological diagnosis is critical to identify the cancer subtype and the genetic mutations involved, guiding the selection of targeted therapies and specific immunotherapy. Percutaneous biopsy is a safe and effective method for obtaining tissue samples needed for diagnosis. The success of this procedure depends on several factors, including the suspected diagnosis, the size and location of the lesion, the imaging guidance used, the operator's experience, the available equipment, and the chosen approach (2-8).

This study aims to identify the underlying causes of spinal cord injury and the molecular subtypes involved in causing these pathologies.

Spinal injuries affect a significant number of people and significantly limit their quality of life.

MATERIALS AND METHODS

This retrospective study, conducted over a four-month period (January 1st - April 30th, 2015), included 50 patients with spinal cord or spine non-traumatic injuries who were

treated at the "Prof. Dr. N. Oblu" Emergency Clinical Hospital in Iași. Initially, the study sample consisted of 58 patients diagnosed with spinal tumors. However, 8 patients were excluded based on the following criteria: lack of surgical treatment due to clotting disorders; severely compromised general health status; life expectancy of less than 3 months; severe associated comorbidities. Data collection and statistical evaluation were based on electronic medical records. Collected data included patient demographics (such as age at breast metastasis (BM) diagnosis), and morphological features of the primary cancer (location, cytopathological features, histopathological subtype (HP), and immunohistochemical (IHC) characteristics). Cytopathological examination was conducted on intraoperative smears, using small specimens obtained during surgery. A small sample (1-2 mm^3) of the biopsy specimen was placed on one edge of a clean, dry, labeled slide, then crushed with the edge of another clean, dry slide, applying sufficient pressure to spread the tissue into a thin film on both slides. The slides were immediately stained with 1% toluidine blue. The remaining biopsy sample was processed for fixation. Surgical specimens were fixed in a 10% neutral buffered formalin solution for 24 hours, dehydrated using acetone and xylene, and embedded in paraffin. Paraffin blocks were sectioned into 3-µm-thick histological slices, which were stained with Hematoxylin-Eosin (HE) for microscopic examination. Additional 3-µm-thick sections were prepared for IHC staining, following a standard immunohistochemical protocol.

RESULTS

The study showed a gender ratio of approximately 1.17: 1 (female to male), with an average age of 57.04 years and a range from 12 to 79 years. Regarding background classification, 52% of patients were from urban areas, while 48% were from rural areas. Ethnic distribution included 82% Romanians and 18% Roma individuals. In terms of education, 20% of patients had postgraduate degrees, 62% had university degrees, 14% had secondary education, and 4% had primary education. The study also examined the duration from the onset of pain to surgery. Surgery was performed within 1 month of pain onset for 56% of patients, between 1 and 3 months for 36%, between 3 and 6 months for 4%, and after more than 6 months for the remaining 4%. Additionally, the study analyzed the type of tumor and its location at various spinal levels. Classification by tumor type shows that 9 patients (18%) had primary tumors and 41 patients (82%) presented secondary tumors. According to tumor site in the spine, 8 patients had cervical (16%), 31 patients (62%) had thoracic, and 11 patients (22%) had lumbar localizations. Our study revealed specific preoperative clinical features of spinal tumor pathology such as spinal pain, paravertebral muscle contracture, radicular pain, paresis or paralysis of a limb, paraplegia and sphincter disorders. Results were categorized by gender.

In male patients, the most common source of metastasis was bronchopulmonary cancer (fig. 1), representing 70% of the analyzed subjects.

One spinal metastasis of small cell bronchopulmonary carcinoma was identified in the selected group of patients (fig. 2), a common cancer in smokers with a 5-year survival prognosis of less than 1% (9).

The incidence of prostate cancer ranks it second among the primary cancers we

encountered as the starting point for spinal metastases diagnosed in the analyzed males (figs. 3, 4). The biopsy revealed metastatic adenocarcinoma. The neoplastic cells were organized in cords and nests, with some areas exhibiting a cribriform growth pattern. These cells had hyperchromatic small nuclei, a few of which contained prominent nucleoli. Immunohistochemical staining showed positive results for prostate-specific antigen and prostate acid phosphatase, while the tumor was negative for CK 7, CK 20, TTF-1, and chromogranin.

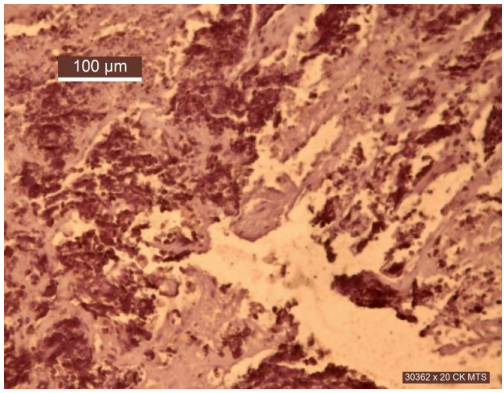

Fig. 1. Metastasis of undifferentiated bronchopulmonary carcinoma. Cytokeratin immunostaining, x200.

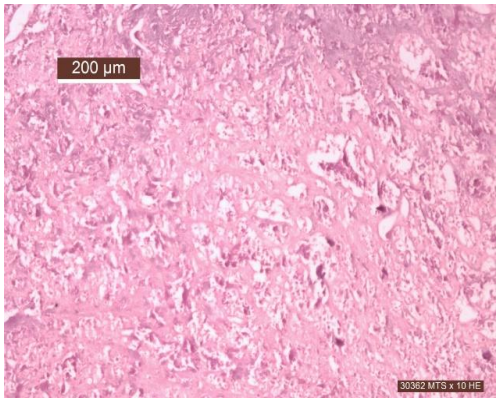

Fig. 2. Osteolytic metastasis of small cell carcinoma. HE staining x100.

After the surgery, the patient was referred to radiation oncology, where he received a total radiation dose of 20 Gy in five fractions, targeting the region from L4 to S3. During the three-month follow-up visit, the patients reported increased strength in his legs and noted improvements in his ability to walk.

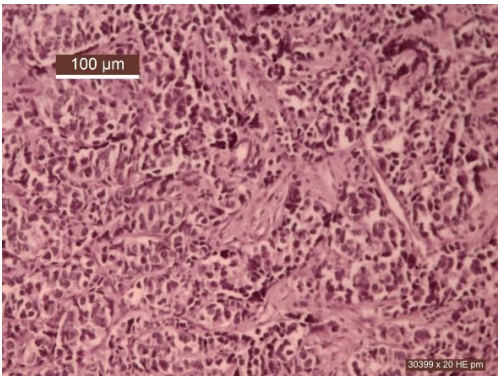

Fig. 3. Metastasis of moderately differentiated prostatic adenocarcinoma in a 68-year-old man. The preserved tubular structure is noted. HE staining, x200.

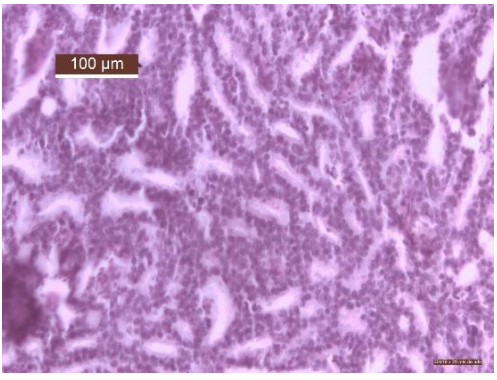

Fig. 4. Metastasis of prostatic adenocarcinoma. HE staining, x200.

As for the female group, breast cancer was the most frequent cancer and 60% of them were over 60 years old (figs. 5, 6, 7). No breast cancer was identified in the male group.

We present in figure 5 a metastasis of ductal breast carcinoma in a 60-year-old woman.

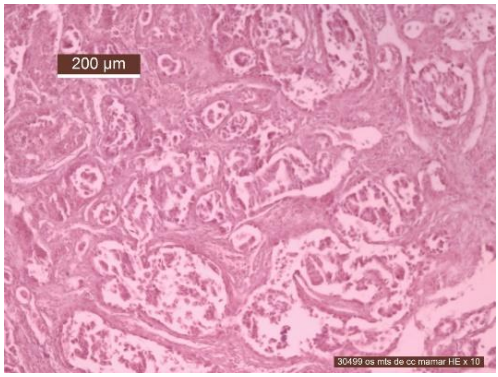

Fig. 5. Osteolytic metastasis of ductal carcinoma. HE staining, x100.

In figure 6 we can see the same metastasis at a higher magnification to note the partially preserved ductal architecture.

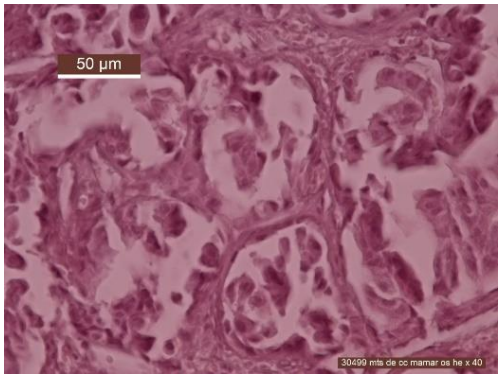

Fig. 6. Spinal metastasis of ductal breast carcinoma in a 60-year-old woman. HE staining, x400.

We considered it appropriate to analyze the parameters recorded in relation to the molecular subtype. For this purpose, we divided the total number of patients into 4 groups according to molecular subtype.

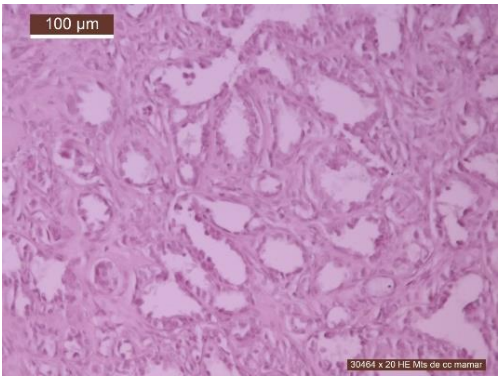

Fig. 7. Spinal metastasis of tubular breast carcinoma in a 68-year-old woman. HE staining, x200.

Based on molecular markers, breast cancer can be classified into molecular subtypes as follows (4, 11):

- Luminal type A is the most common subtype and is characterized by strong ER+ and/or PR+ but HER2- immunostaining. In terms of HP, it has a lower grade (low Ki67 LI) and is hormone responsive with a good prognosis. Type A luminal disease generally only requires endocrine therapy.
- Luminal type B is characterized by ER+ and/or PR+ and HER2+ immunolabels. It correlates with higher grade tumors (high Ki67 LI) and has a worse outcome compared to luminal type A, but also like luminal type A, this type B could be treated with endocrine therapy and chemotherapy.
- HER2+, but ER- is less common, being diagnosed at a younger age. Such cases have a high-grade histopathology and an aggressive outcome, but this outcome could be improved with Herceptin.
- Basal-like type breast cancer (BC) or triple negative breast cancer (TNBC) is an aggressive subtype characterized by high-grade histopathology, propensity for metas-

tasis and a poorer prognosis. Young women are at risk of developing this subtype of BC. Targeted therapies are currently under investigation, but chemotherapy appears to be indicated for most patients.

• Triple-positive BC with ER+/PR+ and HER2+ is a new subtype that can be treated with anti-HER2 agents and chemotherapy.

In the studied group of 27 patients, a number of 16, representing 59.25% were classified in the Luminal A molecular type, while a number of 7 patients, representing 25.92% were classified in the Luminal B type. The fewest patients were classified as HER2 type, 4 patients representing 14.81%.

The distribution of patients according to histopathological type shows a majority number representing 73% classified as ductal, 18.1% as lobular, and 8.9% as mixed.

The second most common cancer in patients is bronchopulmonary cancer as the origin of cancer for the spinal metastases. Figure 8 shows a spinal metastasis with bronchopulmonary origin at the T8 vertebral body in a 61-year-old woman.

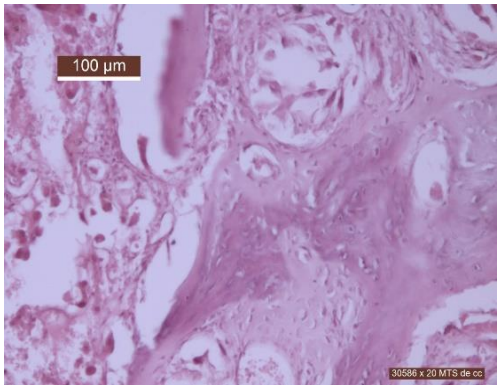

Fig. 8. Osteolytic and osteolytic metastasis of bronchopulmonary carcinoma. HE staining, x200.

DISCUSSION

Human bones are constantly in a state of self-renewal, undergoing a process known as remodeling. This intricate process involves a harmonious interplay between two types of cells: osteoblasts and osteoclasts. Under normal circumstances, these cells work in concert to maintain the bone strength and integrity of our bones (11-13).

However, in the case of metastatic lesions, this delicate balance is disrupted. The presence of cancer disrupts the bone's microenvironment, in favor of the osteoclasts. This disruption triggers a vicious cycle, where the weakened bones provide a favorable environment for tumor growth, which further accelerates bone destruction (12).

This disruption of the bone remodeling process can have serious consequences, leading to bone pain, fractures, and even loss of mobility. Other frequent dissemination sites are represented by lungs or liver (11-14).

Spinal metastasis is a common complication in advanced stages of malignant disease (9). Cadaveric studies reveal that 30% to 90% of cancer patients have spinal metastases at the time of death (10-15). In literature, the average patient age was 63.6 years. This aligns with data showing that the highest incidence of spinal metastases occurs between the ages of 40 and 65, a period when cancer risk is at its peak (15-16).

Spinal metastases typically affect the epidural space and/or vertebral column, while intradural extramedullary and intramedullary metastases are much rarer, accounting for only 5% to 6% and 0.5% to 1% of cases, respectively. The most common histological type involved is adeno-

carcinoma, most frequently originating from the breast and lung. The incidence of bone metastases is notably high in specific cancers.

Prostate adenocarcinoma, which affects up to 70% of men over 80, is the second leading cause of cancer-related death in men. Skull and spinal metastases occur in approximately 10% of prostate cancer patients, though intradural spinal cord tumors from prostate cancer are exceedingly rare. Spinal metastases represent a critical complication of advanced cancers, often leading to severe outcomes such as spinal cord compression, fractures, and neurological deficits. In our study, we analyzed the patterns and characteristics of spinal metastases in a cohort of patients, focusing on primary tumor types, clinical presentation, and outcomes following surgical interventions. The results were further examined in relation to broader literature on spinal metastases, specifically focusing on prostate and breast cancers, which are among the most frequent sources of bone metastasis (14-16).

Approximately 10% of patients with prostate cancer may experience spinal cord compression due to vertebral metastasis. Focal intradural metastasis of the prostate to the spinal cord, however, is extremely rare. An extensive search of English literature on *PubMed* revealed only nine documented cases of patients with intradural spinal cord metastasis (ISCM) from prostate cancer who underwent surgery.

In our study, prostate cancer was identified as the second most common primary tumor responsible for spinal metastasis in male patients. This aligns with established research, which indicates that approximately 10% of patients with prostate cancer develop spinal cord compression due to vertebral metastasis. The clinical presentation in our cohort often included back pain, paraparesis, or sphincter dysfunction, typical signs of vertebral involvement. Biopsy results revealed metastatic adenocarcinoma, with the neoplastic cells exhibiting hyperchromatic small nuclei and positive immunohistochemical staining for prostate-specific antigen (PSA) and prostate acid phosphatase. Following surgical intervention, many patients demonstrated notable improvements in strength and mobility, underscoring the importance of early detection and timely treatment (14).

Interestingly, while our study focused on extradural metastases, it is important to note that prostate cancer can also, though rarely, lead to intradural spinal cord metastasis (ISCM). Extensive reviews of English literature have found only nine documented cases of ISCM from prostate cancer, indicating the extreme rarity of this phenomenon. In contrast, extradural metastases are more common and are a significant cause of spinal cord compression in prostate cancer patients. This finding from the broader literature correlates with our study's emphasis on extradural vertebral metastases as a key cause of spinal pathology in men with prostate cancer. Thus, while ISCM remains a rare presentation, vertebral metastasis leading to spinal cord compression is a frequent and serious concern in prostate cancer patients (10-19).

In our female cohort, breast cancer was the most frequent primary tumor causing spinal metastases, particularly among women over 60 years old. This finding is consistent with data showing that 60-75% of women with advanced breast cancer develop bone metastases. This cancer type is the most frequent cause of bone metastases in women globally. Studies show that

60-75% of women with advanced breast cancer develop bone metastases, with a higher risk in certain subtypes (19). Our study revealed that breast cancer metastases were commonly localized in the thoracic spine, leading to clinical presentations such as spinal pain, radicular symptoms, and in severe cases, paraplegia. The molecular subtype classification in our study provides further insight, with the majority of breast cancer metastases (59.25%) falling under the Luminal A category, followed by Luminal B and HER2-positive subtypes.

A large study analyzing electronic records from various cancer centers in the US found that breast cancer was the leading cause of bone metastases (36%), followed by lung cancer (16%) and colorectal cancer (12%) (20-22).

The identification of molecular subtypes in breast cancer plays a crucial role in understanding metastatic behavior. For example, Luminal A breast cancer is known for a slower progression and a better prognosis, which may explain its prevalence in older women in our study, while HER2-positive breast cancer, though less common, tends to exhibit more aggressive metastatic patterns.

In our study, the majority of women with spinal metastases from breast cancer had ductal carcinoma, which is known for its tendency to metastasize to bones, further reinforcing the connection between this cancer type and bone involvement.

Breast cancer's invasion to bones disrupts the osseous tissue natural rebuilding process, leading to osteolytic lesions. Cancer cells trigger this process by releasing molecules that activate osteoclasts and, interestingly, osteoblasts are also involved, as they also produce factors that stimulate bone breakdown (19). Breast cancer cells initiate the destructive process in the bone by secreting factors such as parathyroid hormone-related protein (PTHrP), which directly affect bone cells. PTHrP, a powerful activator of osteoclasts, is found at elevated levels in an overwhelming 90% of bone metastasis samples. (20). It is the impact of the cancer cells that seems to shift the balance toward tissue breakdown.

Key factors, including parathyroid hormone-related protein (PTHrP) and transforming growth factor-β (TGF-β), play crucial roles in the process of bone destruction associated with metastatic cancer. PTHrP acts as a potent stimulator of osteoclast activity, contributing to bone resorption and further tumor progression. Additionally, TGF-β, released during osteoclast activation, exacerbates the situation by promoting increased production of PTHrP, creating a vicious cycle that accelerates both bone breakdown and cancer advancement.

PTHrP's influence extends beyond bone breakdown. It can directly promote tumor cell growth and angiogenesis through autocrine signaling (21, 22). However, PTHrP's role in prognosis remains a controversy. While some studies suggest its presence in early breast cancer indicates a poorer outcome (22), others show a potential link to improved survival and fewer bone metastases (19-21).

For adding another layer of complexity, the transforming growth factor-β (TGF-β) is responsible. Released by activated osteoclasts, TGF-β further increases PTHrP production in cancer cells (19). This creates a vicious cycle: PTHrP stimulates osteoclasts, leading to bone breakdown and the release of more TGF-β, which in turn increases PTHrP production.

CONCLUSIONS

Cancer metastasis to bones represents a significant challenge in oncology, fundamentally disrupting the natural balance of bone remodeling. This disruption is primarily driven by the interplay between osteoblasts, which build bone tissue, and osteoclasts, which reabsorb it. In cases of metastatic disease, this balance is tipped toward bone breakdown, leading to weakened skeletal structures that further facilitate tumor growth.

In our study, we observed that prostate cancer is the second most common primary tumor responsible for spinal metastases in male patients. This finding aligns with the understanding that among the various sites affected by metastatic disease, spinal metastases are particularly prevalent, especially in advanced stages of cancer. The thoracic and lumbar regions are frequently involved, corroborating existing literature that highlights these areas as common sites for metastatic lesions.

The complications associated with spinal metastases, including spinal cord compression (SCC), fractures, and debilitating pain, pose significant challenges to patient care. The presence of SCC is particularly concerning, as it not only leads to severe neurological deficits but also substantially impacts the functional prognosis and overall quality of life. Our findings indicate that early detection and timely intervention are crucial for mitigating these risks and improving patient outcomes.

Given the serious complications associated with bone metastases, especially the risk of SCC, there is an urgent need for enhanced diagnostic strategies and effective treatment modalities. Interventions that focus on preserving bone integrity and preventing complications can lead to substantial improvements in patient well-being. For instance, surgical interventions aimed at alleviating SCC, followed by radiation therapy, have shown promise in restoring neurological function and enhancing mobility in patients with metastatic disease.

Furthermore, our study emphasizes the importance of understanding the underlying mechanisms of bone metastasis. As we unravel the complex interactions between cancer cells, bone remodeling processes, and the tumor microenvironment, we can better identify therapeutic targets. This deeper understanding is essential for developing innovative treatment approaches that not only address the metastatic lesions themselves but also mitigate the bone-related complications that arise.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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LONGITUDINAL CLINICAL STUDY ON THE PREVALENCE AND RISK FACTORS OF ROOT CARIES IN ELDERLY PATIENTS

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LONGITUDINAL CLINICAL STUDY ON THE PREVALENCE AND RISK FACTORS OF ROOT CARIES IN ELDERLY PATIENTS (Abstract): Dental caries remains one of the most common oral diseases, with a multifactorial etiology involving biological, behavioral, socio-economic, and age-related factors. In elderly patients, physiological changes, systemic diseases, polypharmacy, reduced salivary flow, and gingival recession increase susceptibility to root caries. The **aim** of this study was to evaluate the prevalence of coronal and root caries across different age groups and to identify the etiological factors involved in root caries by comparing caries experience and individual cariogenic risk in adult and geriatric patients. **Materials and methods:** The study included 293 patients examined at the Clinical Teaching Base of the Faculty of Dental Medicine Iași between 2022 and 2026. Clinical assessment included the evaluation of coronal and root caries, gingival recession, and DMFT index. **Results:** Socio-economic factors, systemic diseases, and medication were recorded using a standardized questionnaire. Cariogenic risk was assessed by determining salivary levels of *Streptococcus mutans* and Lactobacilli, resting and stimulated salivary flow, salivary buffer capacity, and caries susceptibility using the modified Snyder test. Statistical analysis was performed using Chi-square and Kruskal-Wallis tests, with the significance level set at $p < 0.05$. Coronal caries showed the highest prevalence in the 71-80-year age group, followed by the 51-60-years group. Root caries was also most frequent in the 71-80-years group, followed by patients over 80 years. DMFT values increased with age, mainly due to the "M" component, reflecting cumulative tooth loss. Systemic diseases, xerostomia-inducing medication, gingival recession, reduced salivary parameters, and increased microbial load were associated with higher cariogenic risk. **Conclusions:** Root caries in elderly patients has a multifactorial etiology, influenced by age-related oral changes, systemic conditions, medication, salivary dysfunction, gingival recession, and cariogenic microorganisms. These findings support the need for individualized preventive and therapeutic strategies in geriatric dental care. **Keywords:** ROOT CARIES, ELDERLY PATIENTS, GERIATRIC DENTISTRY, DMFT INDEX, CARIOGENIC RISK.

INTRODUCTION

Currently, across all medical fields, the emphasis is placed on prevention, aiming to limit disease onset or progression to advanced stages. In dentistry, dental caries remains the most common condition, characterized by high prevalence and a significant impact on patients' quality of life (1, 2). Although often perceived as a minor condition, dental caries is now recognized

as a complex, multifactorial process resulting from the interaction of biological, behavioral, and socio-economic factors (3, 4). Recent studies have shown that the development of dental caries is influenced by factors such as high sugar consumption, poor oral hygiene, reduced salivary flow, insufficient fluoride exposure, and low socio-economic status. In the geriatric population, these factors are amplified by physiological changes associated with aging, including decreased salivary secretion, alterations in salivary composition, reduced self-care ability, and the frequent use of multiple medications (5, 6). Furthermore, age-related systemic conditions, such as diabetes mellitus and cardiovascular diseases, may indirectly affect oral health, increasing susceptibility to caries, particularly at the root surface level. From a microbiological perspective, *Streptococcus mutans* and Lactobacilli play a key role in the etiology of dental caries, with their presence and levels being directly correlated with caries incidence and the intake of fermentable carbohydrates (7,8). Quantitative assessment of these microorganisms is therefore useful not only for evaluating cariogenic risk but also for estimating dietary carbohydrate intake. In addition to these species, other bacteria have been implicated in the carious process, including *Veillonella*, non-mutans oral streptococci (*S. mitis, S. oralis, S. gordonii*), and Actinomyces species, which are capable of acid production at low pH levels (9, 10). The association between these microorganisms and dental caries is closely related to carbohydrate consumption, which is essential for the initiation and progression of the carious process. Levels of *Lactobacillus* and *Streptococcus mutans* may vary significantly even following short-term dietary changes (11, 12). The metabolism of dietary carbohydrates by dental plaque leads to the production of organic acids, which cause demineralization of dental structures (13, 14). This process is particularly relevant at the root level, where cementum and dentin are less resistant to acid attack compared to enamel. Repeated exposure to an acidic environment alters the oral ecosystem, favoring the selection and proliferation of acidogenic and aciduric bacteria (15, 16). While the effects of acid stress on the oral microbiota have been extensively studied, the role of nutrient deprivation has received less attention (17, 18). Studies suggest that under conditions of reduced dietary intake, certain species such as *Streptococcus oralis* and *S. mitis* may proliferate by utilizing salivary glycoproteins as an energy source (19). In this context, the management of geriatric patients requires a comprehensive approach that integrates local, systemic, and behavioral factors. Monitoring these variables is essential for understanding the prevalence and risk factors associated with root caries, contributing to the development of effective preventive and therapeutic strategies tailored to this patient population (20).

The aim of this study was to evaluate the prevalence of coronal and root caries across different age groups and to identify the etiological factors involved in root caries by comparing the degree of caries experience and the individual cariogenic risk between adult and geriatric patients.

MATERIALS AND METHODS

The study was conducted on a sample of 293 patients who attended the Clinical Teaching Base of the Faculty of Dental Medicine in Iași between 2022 and 2026. All participants were included in the study

after providing written informed consent. Clinical examination was performed under standardized conditions, in the dental chair, ensuring adequate illumination and isolation of the operating field. Prior to evaluation, professional oral hygiene procedures were carried out, including manual or ultrasonic scaling followed by professional tooth brushing. The clinical assessment focused on the identification of coronal and root caries lesions, as well as the evaluation of gingival recession. Caries experience was quantified using the DMFT index. For the assessment of cariogenic risk, patients completed a standardized questionnaire that included data regarding socio-economic status, educational level, presence of systemic diseases, and associated medication, with potential impact on the carious process, particularly through alterations in salivary flow or carbohydrate content.

The analysis of local etiological factors included the determination of the Silness and Löe plaque index, evaluation of salivary microbiota, and assessment of salivary parameters. Bacterial load was assessed by determining the levels of *Streptococcus mutans* (Dentocult SM method) and Lactobacilli (Dentocult LB), using standardized microbiological tests.

Salivary parameters were evaluated by measuring resting and stimulated salivary flow rates, as well as buffer capacity, using the Dentobuff method. Salivary flow values were expressed in ml/min, while buffer capacity was assessed colorimetric ally according to the manufacturer's scale. Individual susceptibility to dental caries was determined using the modified Snyder colorimetric test (Arthur Alban), which evaluates the acidogenic activity of oral bacterial flora. The interpretation of results was based on color changes of the culture medium, reflecting acid production levels and, implicitly, cariogenic risk. By correlating clinical, microbiological, and salivary data, the study aimed to evaluate the prevalence of caries lesions and to identify the risk factors involved in the development and progression of dental caries, with a particular focus on root caries in elderly patients.

Statistical analysis was performed in *SPSS version 29.0.* The qualitative data were reported as absolute frequencies and percentages, and the quantitative data were reported as averages and standard deviations. The associations between qualitative data were investigated using the Chi-squared test. The differences between quantitative data in multiple samples were investigated using the Kruskal-Wallis test, after checking the values distribution condition of normality (using the Shapiro-Wilk test). The values of $p < 0.05$ were evaluated as statistically significant, and the values of $p < 0.01$ were evaluated as statistically highly significant.

RESULTS

In order to achieve the objectives of the study, the collected data were statistically analyzed and presented in tables and graphical representations to highlight the distribution of the study group and the prevalence of carious lesions according to the investigated parameters. The study group was characterized from a demographic perspective (gender and age), followed by the analysis of the distribution of coronal and root caries lesions across different age groups. Additionally, the relationship between demographic variables and the studied parameters was evaluated to identify potential statistically significant correlations.

The gender distribution of the study group (tab. I) showed a relatively balanced proportion between male (50.5%) and female patients (49.5%). The age distribution (tab. I) revealed a predominance of the 61-70-year age group (51.9%), followed by the 51-60-year (17.7%), 71-80-year (17.1%), and over 80-year (13.3%) groups.

TABLE I.
The demographical features of the sample

		n	%
Gender	male	148	50.5
	female	145	49.5
Age group	51 - 60 ys	52	17.7
	61 - 70 ys	152	51.9
	71 - 80 ys	50	17.1
	over 80 ys	39	13.3
Total		293	100.0

The comparative distribution on genders by age groups did not reveal statistically significant differences (Chi-square test, p = 0.165) (fig. 1). However, a predominance of male patients was observed in the 51-60 and 61-70 age groups, a relatively balanced distribution in the 71-80 group, and a predominance of female patients in the over 80 group.

Regarding the prevalence of coronal caries (tab. 2), the highest values were recorded in the 71-80-year age group (20%), followed by the 51-60-years group (19.23%), the over 80 years group (12.8%), and the 61-70-year group (8.5%). The prevalence of root caries was highest in the 71-80-years age group (10%), followed by patients over 80 years (5.1%), the 51-60-years group (3.8%), and the 61-70-years group (3.2%).

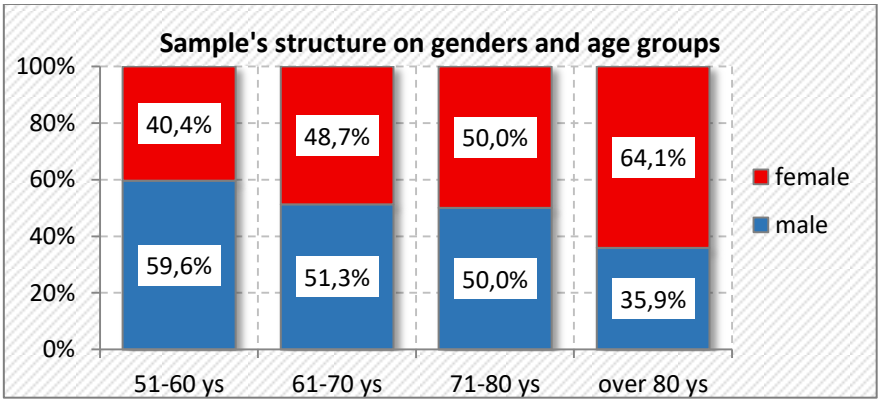

Fig. 1. Patients' distribution by genders and age groups

TABLE II.
The caries distribution by age groups

	Total		Age group								p-value†
			51-60 yrs.		61-70 yrs.		71-80 yrs.		over 80 yrs.		
	n	%	n	%	n	%	n	%	n	%	
Caries	38	12.9	10	19.2	13	8.5	10	20.0	5	12.8	0.085
Root caries	14	4.7	2	3.8	5	3.2	5	10.0	2	5.1	0.279

†Chi-squared test

Following the analysis of caries prevalence, the study further evaluated the degree of caries involvement and identified the factors contributing to the development of root caries. In this context, clinical indicators such as the DMFT index, the presence of gingival recession, socio-economic factors, systemic diseases, associated medication, plaque index, and salivary microbiological parameters were comparatively analyzed.

Comparison of the level of caries involvement

The mean number of carious teeth varied according to age group, with the highest values recorded in patients aged 51-60 years (3.75 teeth), followed by those aged 61-70 years (2.91), 71-80 years (2.3), and over 80 years (1.72) (tab. III). The differences between age groups were statistically highly significant.

Enamel caries lesions showed the highest mean values in the 71-80-year age group (0.44), followed by the 51-60 (0.19), 61-70 (0.14), and over 80 (0.08) age groups. However, the differences between groups were not statistically significant.

For dentin caries lesions, the highest values were observed in the over 80 years age group (1.41), followed by the 51-60-year age group (1.35), 61-70 (0.97), and 71-80 (0.48), the differences between groups being again statistically highly significant.

TABLE III.
The level of caries by age groups

	Age group				Total M ± SD	p-value†
	51-60 yrs. M ± SD	61-70 yrs. M ± SD	71-80 yrs. M ± SD	over 80 yrs. M ± SD		
No. of carious teeth	3.75 ± 2.970	2.91 ± 2.969	2.30 ± 2.735	1.72 ± 1.820	2.80 ± 2.855	0.004**
No. of enamel caries	0.19 ± 0.445	0.14 ± 0.431	0.44 ± 1.514	0.08 ± 0.270	0.19 ± 0.734	0.513
No. of dentin caries lesions	1.35 ± 1.426	0.97 ± 1.617	0.48 ± 1.446	1.41 ± 1.712	1 .01 ± 1.591	<0.001**
No. of root caries lesions - located at cementum level	0.12 ± 0.379	0.64 ± 1.088	1.06 ± 1.476	0.56 ± 0.754	0.61 ± 1.075	<0.001**

†Kruskal-Wallis test, **p < 0.01 Statistically highly significant

Root caries lesions, located at the cementum level, were more frequent in patients aged 71-80 years (1.06), followed by those aged 61-70 years (0.64), over 80 years (0.56), and 51-60 years (0.12). These differences were also statistically highly significant.

The mean number of filled teeth was highest in the 51-60-year age group (2.98), followed by the 61-70 (1.18), over 80 (1.08), and 71-80 (0.32) groups (fig. 2). This distribution was also statistically significant ($p < 0.001$).

The DMFT index showed higher values in patients over 80 years and in those aged 71-80 years compared to the 51-60 and 61-70 age groups (fig. 3). These findings can be explained by an increase in the "M" component of the index, reflecting a higher number of missing teeth due to complications of dental caries. Additionally, patients from rural areas presented higher DMFT values compared to those from urban areas, regardless of age group.

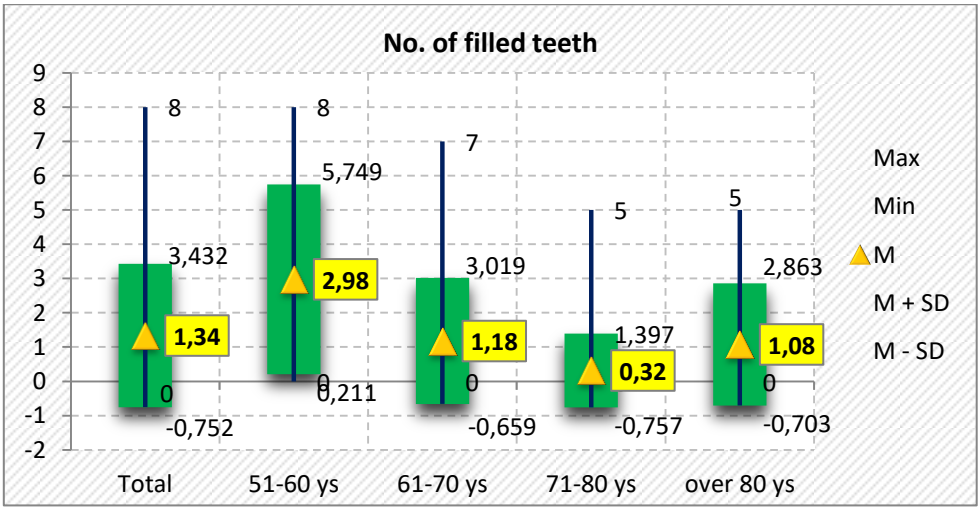

Fig. 2. Distribution of filled teeth by age group

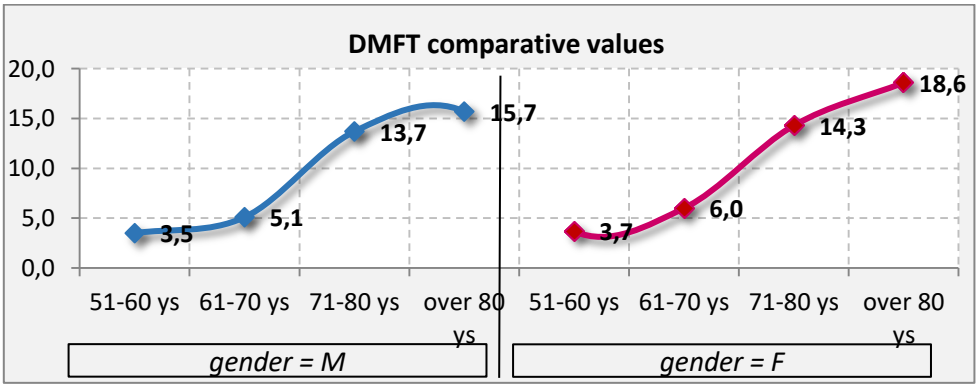

Fig. 3. Distribution of filled teeth by age group and place of residence

Identification of etiological factors of root caries assessment of individual cariogenic risk

Educational level. The distribution of patients according to educational level varied across age groups. In most groups, patients with secondary education predominated, with the highest proportions observed in the over 80-year group (64.1%) and the 51-60-year group (51.9%). These differences were statistically significant (Chi-square test, p = 0.011) (fig. 4).

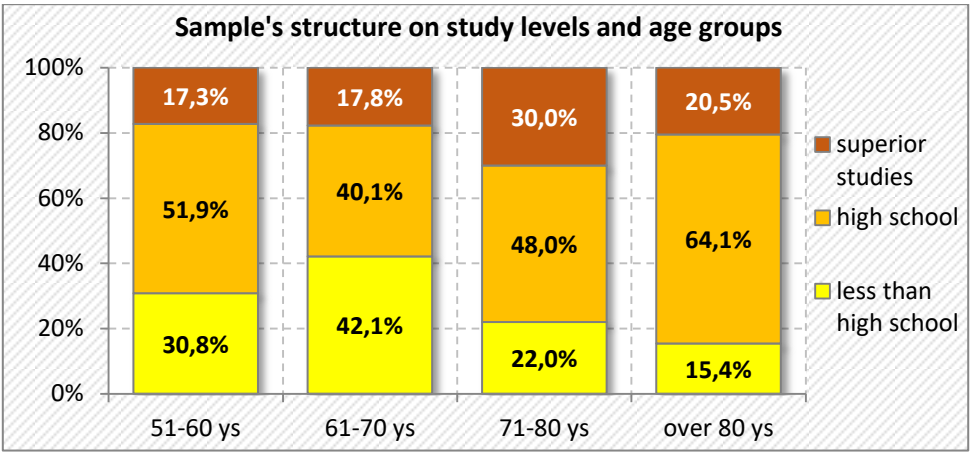

Fig. 4. Distribution of patients by age group and level of education

Place of residence. The majority of patients originated from urban areas, regardless of age group, with the highest percentages recorded in the 71-80-year group (76%) and the 61-70-year group (69.1%). However, the distribution according to place of residence was not statistically significant (Chi-square test, p = 0.075) (fig. 5).

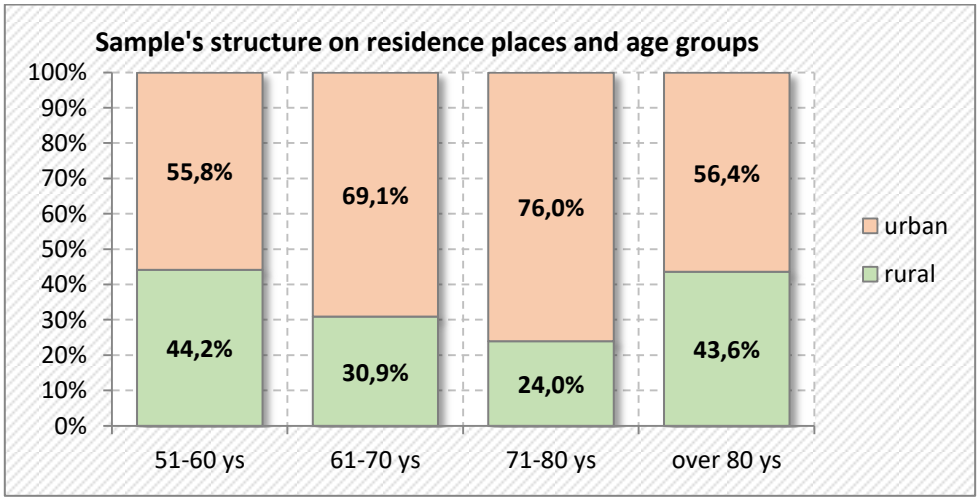

Fig. 5. Distribution of patients by age group and place of residence

TABLE IV.

The presence of associated systemic diseases by age groups

	Total		Age group								p-value†
			51-60 yrs.		61-70 yrs.		71-80 yrs.		over 80 yrs.		
	n	%	n	%	n	%	n	%	n	%	
Bronchial Asthma	28	9.5	3	5.8	16	10.5	6	12.0	3	7.7	0.675
Pulmonary emphysema	12	4.1			4	2.6	2	4.0	6	15.4	0.001**
High blood pressure	162	55.3	12	23.1	83	54.6	33	66.0	34	87.2	<0.001**
Heart rhythm disorders	50	17.1			14	9.2	18	36.0	18	46.2	<0.001**
Angina pectoris	48	16.4			16	10.5	15	30.0	17	43.6	<0.001**
Myocardial infarction	17	5.8			4	2.6	8	16.0	5	12.8	<0.001**
Congenital heart defects	2	0.7			2	1.3					0.600
Valve prostheses	4	1.3			4	2.6					0.288
Chronic hepatitis	104	35.5	16	30.8	48	31.6	18	36.0	22	56.4	0.030*
Liver cirrhosis	12	4.1			10	6.6	2	4.0			0.099
Diabetes Mellitus	30	10.2	1	1.9	21	13.8	7	14.0	1	2.6	0.026*
Epilepsy	9	3.0	1	1.9	6	3.9	2	4.0			0.572
Parkinson's disease	16	5.5			5	3.3			11	28.2	<0.001**
Previous stroke	1	0.4			1	0.7					0.818
Endocrine diseases	6	2.1	3	5.8	3	2.0					0.141
Osteo-articular diseases	53	18.1	7	13.5	28	18.4	12	24.0	6	15.4	0.545
Neuro-motor diseases	27	9.2	2	3.8	14	9.2	3	6.0	8	20.5	0.039*
Renal diseases	35	12.0	5	9.6	15	9.9	5	10.0	10	25.6	0.046*

†Chi-squared test; *p<0.05 statistically significant; **p<0.01 statistically highly significant

Associated Systemic Diseases. Systemic conditions with a potential indirect impact on the carious process were more frequently observed in patients over 80 years of age, particularly renal, hepatic, pulmonary, neuromotor, osteoarticular, and cardiovascular diseases (tab. IV).

Associated Medication. Medication with the potential to reduce salivary flow was more frequently observed in older age groups (tab. V). In almost all cases (with only one exception, the antidepressants) the differences recorded between age groups were statistically highly significant, as it follows: the use of diuretics increased progressively, from 11.5% in the 51-60-year group to 79.5% in patients over 80 years; a similar trend was observed for vasodilators, used by 48.7% of patients over 80 years; beta-blockers were also more frequently used in patients over 80 years (79.5%), followed by the 71-80-year group (60%); ni-

troglycerin use was highest among patients over 80 years (53.8%), compared to younger groups; similarly, Digoxin therapy was most frequent in the over 80-year group (41%); sedatives use was also more prevalent among patients over 80 years (28.2%).

TABLE V.

The distribution of medications with potential to reduce salivary flow rate by age groups

	Total		Age group								p-value†
			51-60 yrs.		61-70 yrs.		71-80 yrs.		over 80 yrs.		
	n	%	n	%	n	%	n	%	n	%	
Diuretics	133	45.4	6	11.5	65	42.8	31	62.0	31	79.5	<0.001**
Vasodilators	63	21.5			23	15.1	21	42.0	19	48.7	<0.001**
Beta-blockers	146	49.8	12	23.1	73	48.0	30	60.0	31	79.5	<0.001**
Nitroglycerin	64	21.8			23	15.1	20	40.0	21	53.8	<0.001**
Digoxin	39	13.3			12	7.9	11	22.0	16	41.0	<0.001**
Anticoagulants	19	6.5			9	5.9	3	6.0	7	17.9	0.007**
NSAIDs	146	49.8	12	23.1	69	45.4	34	68.0	31	79.5	<0.001**
Sedatives	23	7.9	3	5.8	8	5.3	1	2.0	11	28.2	<0.001**
Antidepressants	15	5.1	2	3.8	4	2.6	5	10.0	4	10.3	0.083

†Chi-squared test; *p<0.05 statistically significant; **p<0.01 statistically highly significant

Presence and Severity of Gingival Recession

The average number of teeth with gingival recession was higher in patients aged 51-60 years, followed by those aged 61-70, 71-80, and over 80 years. This decreasing trend with age may be explained by the reduction in the number of remaining teeth. The differences were not statistically significant (p = 0.063) (tab. VI). Regarding the severity of gingival recession, the highest average values were observed in the 71-80-year age group (2.81 mm), followed by the 61-70 (2.08 mm), over 80 (2.07 mm), and 51-60 (1.68 mm) groups; these differences were statistically significant (p = 0.006).

TABLE VI.

The level of caries by age groups

	Age group				Total M ± SD	p-value†
	51-60 yrs. M ± SD	61-70 yrs. M ± SD	71-80 yrs. M ± SD	over 80 yrs. M ± SD		
No. of teeth with gingival recession	5.83 ± 5.223	3.86 ± 4.149	3.34 ± 3.456	3.15 ± 3.305	4.02 ± 4.227	**0.063**
Severity of gingival recession (mm)	1.68 ± 1.274	2.08 ± 1.831	2.81 ± 1.904	2.07 ± 1.904	2.13 ± 1.793	0.006**

†Kruskal-Wallis test, **p < 0.01 Statistically highly significant

DISCUSSION

The results of the present study highlight that caries involvement exhibits an age-dependent profile, with a higher prevalence of coronal lesions in younger age groups and a progressive increase in root caries among elderly patients. This pattern is consistent with current epidemiological trends, which indicate a decline in dental caries in younger populations and an increased burden in older age groups (1).

In this study, the 51-60-years age group presented the highest number of carious teeth, whereas root caries lesions were more frequent in patients aged 71-80 years. This distribution supports the concept that, with advancing age, local factors such as root surface exposure and periodontal changes become major determinants of the carious process (2).

The increased DMFT values observed in older age groups, particularly in patients over 80 years, can be explained by the higher contribution of the "M" component, reflecting tooth loss as a consequence of caries complications. This finding suggests that, in elderly patients, cumulative caries experience is more relevant than the incidence of active lesions and is influenced by previous treatment history and access to dental care.

Regarding lesion localization, the predominance of dentin caries in the 51-60-year group and root caries in the 71-80-years group indicates a shift in the pattern of caries involvement with aging. Recent meta-analyses have reported a global prevalence of root caries of approximately 41%, emphasizing its importance in geriatric oral pathology (4).

Socio-economic factors showed a significant influence of educational level on patient distribution, while place of residence was not significantly associated with caries risk. These findings are partially consistent with the literature, suggesting that education influences oral health behaviors, whereas urban-rural differences are becoming less pronounced due to improved access to healthcare services.

Systemic diseases and associated medication play a crucial role in the development of root caries. In the present study, patients over 80 years exhibited the highest prevalence of systemic conditions and xerostomia-inducing medications (diuretics, beta-blockers, vasodilators, nitroglycerin, and digoxin). These results support previous findings that identify hyposalivation as a major risk factor for root caries, due to reduced buffering capacity and impaired antimicrobial function of saliva (5).

The higher prevalence of xerostomia in older age groups further supports the direct relationship between reduced salivary flow and increased cariogenic risk. Under these conditions, alterations in the oral microbiota favor the proliferation of acidogenic microorganisms, particularly *Streptococcus mutans* and Lactobacilli, which are implicated in the etiology of root caries.

Gingival recession represents another important determinant, through the exposure of root surfaces. Although the number of teeth affected by recession was higher in younger groups, the severity was significantly greater in patients aged 71-80 years. This finding suggests that the severity of gingival recession, rather than its mere presence, plays a key role in the development of root caries.

The plaque index showed higher values in the 61-80-years age groups, without statistically significant differences, indicating that poor oral hygiene remains a consistent factor across all age groups. Epidemiological studies have identified inadequate oral hygiene as one of the strongest predictors of root caries (6).

Microbiological analysis revealed a substantial proportion of patients classified as having moderate to high cariogenic risk, based on the levels of *Streptococcus mutans* and Lactobacilli. Recent studies confirm the role of these microorganisms in the initiation and progression of root caries, although the etiology is considered polymicrobial (2).

Overall, the findings of this study confirm the multifactorial nature of root caries, resulting from the interaction between local factors (dental plaque, gingival recession), systemic conditions (general diseases, medication), behavioral aspects (oral hygiene, diet), and biological factors (saliva, oral microbiota). Modern models of cariogenic risk assessment integrate these variables into predictive approaches aimed at prevention and personalized treatment strategies.

CONCLUSIONS

Caries involvement varies with age, with a predominance of coronal lesions in younger age groups and an increased prevalence of root caries in elderly patients, particularly in the 71-80-years age group.

The DMFT index showed an increasing trend with advancing age, mainly due to the "M" component (missing teeth), reflecting cumulative tooth loss associated with complications of dental caries.

Both systemic and local factors, including general diseases, xerostomia-inducing polypharmacy, and gingival recession, contribute significantly to the development and progression of root caries lesions.

Reduced salivary flow rate and buffering capacity in elderly patients represent key determinants in increasing caries susceptibility, especially at the level of exposed root surfaces.

Increased salivary microbial load, characterized by elevated levels of *Streptococcus mutans* and Lactobacilli, is associated with moderate to high cariogenic risk in the majority of patients.

The overall cariogenic risk is higher in older age groups, particularly in patients aged 71-80 years, confirming the multifactorial nature of dental caries and the need for individualized preventive and therapeutic approaches.

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SELF-MEDICATION-A PUBLIC HEALTH PROBLEM IN ROMANIA NOWADAYS. THE FIRST QUESTS

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SELF-MEDICATION-A PUBLIC HEALTH PROBLEM IN ROMANIA NOWADAYS. THE FIRST QUESTS (Abstract): Self-medication is an important part of the health care system, designed to educate the population in the spirit of responsibility for their own health, to inform and to provide medical and pharmaceutical services. Self-medication has a high prevalence in Romania where people choose to treat themselves by fever, headaches, cough, cold, and pain. **The aim of the study** was to evaluate the situation regarding self-medication in general population, estimated with a questionnaire. **Material and methods**: The study was conducted on 100 respondents aged 18-60 years, patients of an individual family medicine cabinet in Iasi County, North-East Romania. The instrument was a questionnaire consisting of 28 items and structured in two parts: a first part evaluating the respondent's demographic and socioeconomic data, and a second part exploring patient's perception of self-medication, mainly the frequency of use and cause. **Results:** The demographic structure of the study group showed an increased frequency of persons aged 35-44 years (22%), female gender (64%), from urban area (85%); 65% of whom are graduates of upper secondary education, most of them employed (52%) and married (63%). Romanian respondents claim that self-medication is a self-administered treatment (50%), take medicines after consulting websites (32%), for sore throat and fever (24%), because of the lack of money (35%), most of them being anti-inflammatories (30%), unaware of the risks of self-medication (50%), but thinking that one of the main risks could be inappropriate administration (41%), choosing self-medication as first option (12%), but also ask for a physician (69%). **Conclusions:** Self-medication is influenced by the sociodemographic characteristics of individuals such as age, morbidity, health and attitude, stress, and social rules. Getting a look at the elephant meaning this big issue named self-medication, using a questionnaire applied to specific groups of population could an important aspect that can help healthcare professionals to provide personalized counseling based on the specific needs to prevent self-medication risks and adverse effects of inappropriately use of medicines. **Keywords**: SELF-MEDICATION, PUBLIC HEALTH, ANTIBIOTICS, QUESTIONNAIRE.

Self-medication is defined as the administration of medicines by consumers to treat symptoms and mild health disorders recognized by themselves. This definition was included in *Declaration of Alma-Ata*, adopted by UNICEF/WHO experts at Conference of Primary Care in 1978 ("*The people have the right and duty to participate individually and collectively in the planning and implementation of their health care.*") (1). One of the basic characteristics of self-medication is the patient's responsibility for own health, so that Association of the European Self-Medication Industry (AESGP) has replaced the term of "self-medication" with "responsible self-medication" in 1994. It is noteworthy that the self-administration of unqualified medicines (on the recommendation of friends, from the remnants of drugs purchased previously, etc.) cannot be qualified as self-medication and is contra-indicated, although such cases occur very often daily. The notion of self-medication implies improving health in the case of mild illnesses, chronic illnesses ingravescence, or first aid until the physician arrives (2-4).

Applying preventive measures to reduce the risk of illness, detecting symptoms at early stages to prevent illness or disease progression is defined as self-prophylaxis. Self-medication and self-prophylaxis are two parts of the *responsible self-medication* design concept and a component of the primary healthcare. It also included healthy lifestyle, smoking cessation, moderate alcohol consumption, correct medication management, etc. Self-medication is currently a constituent part of the healthcare system designed to educate the population in the spirit of responsibility for their own health, to inform and to provide medical and pharmaceutical services. Self-medication has a high prevalence in Romania where people choose to treat themselves from fever, headache, cough, and cold. The most common medicines used without prescription (OTC) are vitamins and supplements, cough and fever pills, analgesics and antibiotics. Many parents give medicines to their children without any medical advice (5, 6).

Sources of self-medication could be friends, relatives, neighbors, or pharmacists, previous prescriptions or suggestions from commercials. Self-medication could be described as the ability of the population to play an independent and informed role in terms of preventive, diagnosis and treatment activities management that concern themselves. There are many healthcare systems that encourage population for self-prophylaxis and self-care of minor diseases using responsible self-medication to reduce treatment costs, patient and physician time. One of the most important issues linked to self-medication could be the antimicrobial resistance, reported in developing countries where antibiotics are available without prescription, as well as serious incidents due to side effects. To decrease the frequency of them, healthcare professionals should take measures to claim responsible self-medication, achieved by a safe access to medications, correct indications for use and the accessibility of medical consult if needed (7).

The aim of this study was to evaluate the situation regarding the self-medication among a Romanian population group, based on the questioning of the patients. The secondary aims were to raise awareness of the importance of drug use safety issues and to assist professionals in detecting and reporting side effects or other drug-induced problems.

MATERIAL AND METHODS

The study was conducted on 100 respondents, as patients of an individual family medicine cabinet in Iasi County, North-East Romania, aged between 18 and 60 years. The method was the survey of opinion using as instrument a questionnaire consisting of 28 items and structured in two parts: a first part evaluating respondent's demographic and socioeconomic data, and a second part exploring patient's perception of self-medication, mainly the frequency of use and cause. The questionnaires were anonymous to give the participants the opportunity to express their opinions freely.

The questionnaire was handed to each patient separately, explaining the study purpose and taking the oral informed consent before the interview. This consent claimed that participants had no risk if they refuse to participate in the study or cease collaboration during the application of the questionnaire.

Data were processed using *SPSS 20.0.*

RESULTS

1. Demographic and socioeconomic characteristics of participants

Iasi County is in the North-East Romania, Europe, with a population of over 700,000 inhabitants, and a demographic and social structure like the whole geographic area. There is no specific regional pathology, but common colds, diarrheal diseases in young people, and cardiovascular diseases, diabetes mellitus, rheumatic disorders, dental and oncological pathology in elderly adults are predominant (8).

The demographic structure of the study group showed an increased frequency of persons 35-44 years (22%), female gender (64%), from urban area (85%); 65% of them are graduates of upper secondary education, most of them employed (52%) and married (63%) (tab. I, fig. 1).

TABLE I.

Demographic and socioeconomic characteristics of participants, Iasi County,North-East Romania, 2016 (n=100)

Characteristics	Absolute frequency
Age (years)	
18-24	15
25-34	16
35-44	22
45-54	18
> 54	18
Gender	
Male	36
Female	64
Residence area	
Urban	85
Rural	15
Education	
Primary	4
Secondary	31
University	65
Occupation	
Employee	52
Retiring	15
Student	8
House-person	8
Unemployed	7
Maternity leave	3
Other	7
Social status	
Married	63
Unmarried	27
Divorced	5
Widow	5

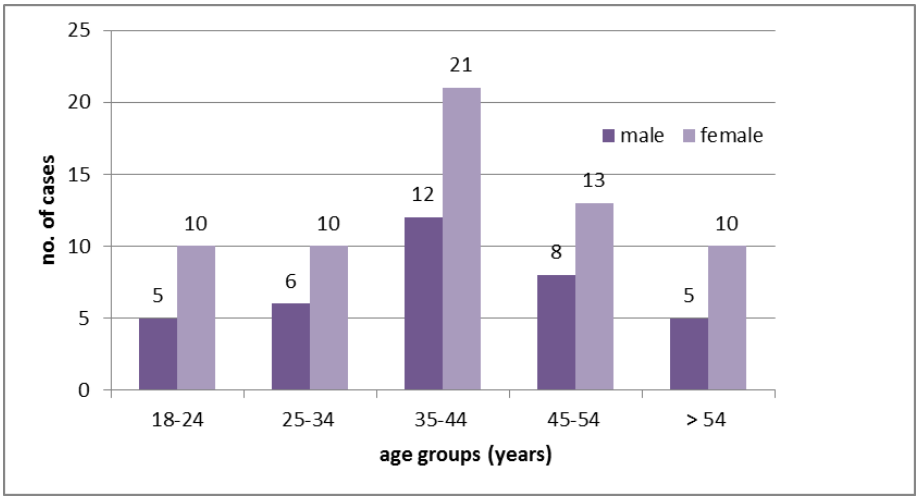

Fig. 1. Distribution by age and gender

2. First quests - items 7-15

Item 7: "Definitions of self-medication by patients". More than half of the respondents said they knew what self-medication means „self-medication treatment" (50%) or "independence in maintaining their own health" (24%). **Item 8:** "The initial source of information that urges the patient to self-medication"**.** 32% of respondents said they were taking medication after consulting websites. 30% of those considered the opinion of their family members about the selection of drugs. Only 11% were given self-medication because of viewing the advertisements (tab. II).

Item 9: "Pathological conditions for which self-medication was applied" were considered cold states (sore throat, cough, fever) - 24%, pains - 19%; headaches, insomnia, asthenia - 15%; dermatitis, acne, mycosis -14%; gastrointestinal tract disorders (vomiting, diarrhea) - 10%; avitaminosis - 6%.

Item 10: "Causes of self-medication". Among the reasons for the self-medication they mentioned: lack of money - 35%; advertising - 20%; permissive legislation - 11%; difficult access to medical services - 7% (tab. IV).

TABLE II

Definitions of self-medication by patients. Sources of information

Items	%
Definitions of self-medication	
Treatment with medication out one's own head	50
Independence in maintaining their own health	24
Purchasing drugs directly from the pharmacy and using them on their own	17
Obtaining medical advice and purchasing medicines in the pharmacy	9
Initial source of information for self-medication	
Internet	32
Relatives, friends	30
Radio advertising	15
Brochures, magazines	12
TV advertising	11

TABLE IV

The most common conditions for which self-medication was used. The main reasons

Items	%
Most common pathological conditions	
Cold states (sore throat, cough, fever)	24
Pains	19
Central Nervous System disorders (head-	15
Dermatological conditions (herpes, acne,	14
Gastrointestinal tract-disorders (vomiting,	10
Avitaminosis	6
Contraception	5
Obesity	4
Quitting smoking	2
Main reasons	
Lack of money	35
Advertising	20
Permissive legislation	11
High costs	20
Accessibility	7
Difficult access to medical services	7

Item 11: "Frequently administered drugs". Regarding the drug most frequently administered without a physician or pharmacist opinion: anti-inflammatory drugs - 30%; antibiotics - 17%; antiallergic drugs - 13%; local topics - 8%; gastric antisecretory drugs - 8%; contraceptives - 7% (tab. V).

Item 12: "To what extent do you know the risks of administering drugs without supervising your physician?". It is noticed that only 15% of respondents knew the risks of self-medication. **Item 13:** "The main risks related to self-medication". Patients answered that inappropriate administration (41%); the appearance of new symptoms or diseases (18%); overdose (15%); dangerous drug interactions (10%) could be some of self-medication risks (tab. VI).

TABLE V

Frequently administered drugs as self-medication

Drugs as self-medication	%
Antibiotics	17
Anti-inflammatory drugs	30
Antiallergic drugs	13
Local topics	8
Gastric anti secretory drugs	8
Contraceptives	7
Antidiarrheal drugs	6
Antitussives	5
Vitamins	6

TABLE VI

Risks of self-medication

Items	%
Risks of self-medication without supervising of a physician	
Not at all	50
To a less extent	35
To a higher extent	15
Main risks related to self-medication	
Inappropriate administration	41
New symptoms or diseases	18
Overdose	15
Drug interactions	10
Aggravation	9
Expiration	7

Item 14: "If you get sick, do you choose self-medication at first?". 88% of respondents said they did not take medication without consulting a physician, while 12% of them considered themselves sufficiently informed to administer their own

medication when suffering of minor affections, thinking they have as many rights and responsibilities as their own health and medical professionals. **Item 15:** "If you get sick, do you consult a physician at first?". 89% of respondents believed that it is necessary to consider the physician's opinion in case of illness, while 11% said they administered medication and the most cited reason for self-medication was previous experience (tab. VII).

TABLE VII.

Self-medication or physician at first?

Items	%
Self-medication at first	
Total disagreement	88
Agreement	11
Total agreement	1
Physician at first	
Total disagreement	11
Agreement	20
Total agreement	69

3. Cronbach alfa calculation

After calculating the Cronbach alpha coefficient, the results showed a value of **0.718** for 12 items taken into consideration (tab. VIII).

TABLE VIII

Cronbach alpha coefficient.

Case Processing Summary

		N	%
Cases	Valid	100	100
	Excluded[a]	0	.0
	Total	100	100.0

a. List wise deletion based on all variables in the procedure

Reliability Statistics

Cronbach's Alpha	No. of items
.718	12

DISCUSSION

Self-medication is an important public health issue, especially in developing countries such as Romania, India, China, and countries where access to healthcare is costly, where it is one of the preferred ways of patients to treat their symptoms. Several studies have shown that self-medication can provoke delays in medical care, leading to paradoxical economic losses due to delayed diagnosis and delayed appropriate treatment. Also, self-medication can lead to drugs interactions that could be prevented if the patient consulted a physician at first. Self-medication for drugs such as antibiotics can lead to antimicrobial resistance (9, 10).

Self-medication is a widespread phenomenon, with an increasing incidence, difficult to quantify, affecting all age groups, but especially people over 65. As for adolescents and young adults, 35% of them use self-medication. Of those over the age of 54, 19% use self-medication. The risks of this practice in elder persons are the same as in younger individuals, but they occur much more frequently and have a higher severity due to the multipathology and multimedication often present in this category of patients as well as the fragility of elders. The study states that self-medication is more frequent among women in all age groups. According to our research, women with higher education and aged 35-49 are more likely to provide medicines to children without a medical consultation. The increasing prevalence of lifestyle and chronic conditions requires education regarding the treatment and effective communication strategies to allow patients to play an active role in chronic disease management or for motivational counseling (11, 12).

Medicines play a key role in ensuring or maintaining people's health, but they are not always used rationally. The rational use of medicines implies their appropriate use. This means that patients administer medications at appropriate, correct doses, when necessary, during the relevant period and at the lowest cost for them, avoiding unnecessary medications or whose use is unlikely to lead to health benefits (13).

More than 50% of countries do not carry a substantive policy to promote the rational use of medicines. In developing countries, less than 40% of patients from state sector and 30% of those from private sector are treated according to clinical guidelines. Excessive and irrational use of antibiotics leads to increased antimicrobial resistance, consequently a growing number of antimicrobial drugs become ineffective in the treatment of infectious diseases. Adverse drug reactions caused by abuse in their use, allergic reactions can lead to aggravation of the disease, undermining patient confidence in the healthcare system. Excessive use of a limited number of medications may result in their lack or inaccessible costs, undermined patient confidence. Unsatisfactory or negative results in treatment, caused by inappropriate use of drugs, may also undermine confidence (14-16).

The alpha coefficient, commonly referred to as *Cronbach alpha* (α), was proposed by the American psychiatric Lee J. Cronbach, in 1951. Although not the only indicator of fidelity, Cronbach alpha is by far the most used and most commonly reported in the literature. There is no absolute standard of the size that a Cronbach alpha coefficient should have to indicate an appropriate fidelity. Values around 0.90 are considered *excellent*, and around 0.80, *very good*, and those around 0.70, *appropriate*. The importance of the Cronbach alpha coefficient can be better understood if we place it on the terms of the relationship between validity and fidelity. A measurement can be reliable, but not necessarily valid. Validity refers to the accuracy of measurement, that is, to its ability to properly reflect what we want to measure and not something else (17).

CONCLUSIONS

Self-medication is influenced by the sociodemographic characteristics of individuals such as age, morbidity, health and attitude, stress, and social rules. Getting a look at the elephant meaning this big issue named self-medication, using a questionnaire applied to specific groups of population could an important aspect that can help healthcare professionals to provide personalized counseling based on the specific needs to prevent self-medication risks and adverse effects of inappropriately use of medicines.

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NEWS

MORPHOLOGIC, MOLECULAR, AND TAXONOMIC EVOLUTION OF RENAL CELL CARCINOMA: A CONCEPTUAL PERSPECTIVE WITH EMPHASIS ON UPDATES TO THE 2016 WORLD HEALTH ORGANIZATION CLASSIFICATION

The recently released 2016 World Health Organization classification now recognizes 12 distinct RCC subtypes, as well as several other emerging/provisional RCC entities. In this paper, the authors provide a conceptual framework for approaching RCC diagnosis and classification. The expanding number of recognized RCC entities in the 2016 WHO classification requires surgical pathologists to integrate clinical, radiologic, gross, and microscopic findings to successful differential diagnoses in RCC classification. In routine clinical practice, accurate RCC classification may have important implications for patients and their families, including prognostic risk stratification, targeted therapeutics selection, and identification for genetic testing (Udager AM and Mehra R. Morphologic, Molecular, and Taxonomic Evolution of Renal Cell Carcinoma: A Conceptual Perspective with Emphasis on Updates to the 2016 World Health Organization Classification. *Arch Pathol Lab Med* 2016; 140(10): 1026-1037).

Doina Butcovan

AN UNUSUAL CASE OF CHOLECYSTODUODENAL FISTULA IN A NONAGENARIAN PATIENT

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AN UNUSUAL CASE OF CHOLECYSTODUODENAL FISTULA IN A NONAGENARIAN PATIENT (Abstract): Acute acalculous cholecystitis is a severe pathology of the gallbladder that associates high morbidity and mortality due to the age of onset and numerous comorbidities frequently seen in a nonagenarian patient. Complications such as cholecystoduodenal fistula is rarely seen in these patients and may be a burden in diagnosis due to atypical symptomatology. We reported a case of 91-year-old woman which was referred to our medical unit with epigastric pain, ferriprive anemia and weight loss and no prior history of gastrointestinal disorders. In the presence of anemia and epigastric pain an upper gastrointestinal endoscopy was performed showing a duodenal ulcer with benign histology. Repeated ultrasonography and computer tomography examinations were performed showing a hypoechoic mass with mixed structure on the topography of the gallbladder and a fistulous tract in direct contact with the duodenum. The diagnosis was difficult to obtain due to poor clinical symptomatology and the possibility of malignancies at this age, still after correlating the laboratory assessments with imaging data we concluded that it was a case of acalculous cholecystitis complicated with cholecystoduodenal fistula and pericholecystic abscess. Our patient was referred to the surgical unit and percutaneous cholecystostomy was performed as a low risk procedure with good outcome in case of our patient. **Keywords:** CHOLECYSTODUODENAL FISTULA, NONAGENARIAN, ACALCULOUS CHOLECYSTITIS.

Cholecystitis is one of the most common diseases of the gallbladder. It is defined as an inflammatory process of the gallbladder which occurs in 90 % of cases because of obstruction of the cystic duct from cholelithiasis defining the calculous form, and in 10 % of cases represented by the acalculous form (1).

Cholelithiasis has a similar incidence in Europe as in U.S., afflicting more than 20 million people annually (2). Due to its tight association with the presence of gallstones the prevalence increases with age, mostly seen in patients over 60-years-old. Recent researches relate to an increased prevalence of gallstones, up to 80 % in patients that are institutionalized, aged over 90 years (3-5).

A good prognosis is seen in patients with uncomplicated forms of cholecystitis without associating severe comorbidities, the development of complications such as perforation or gangrene negatively influencing the prognosis. Cholecystitis affects a wide spectrum of population and may

present different symptoms in extreme categories of age. The symptomatology may vary judging the age of onset, nonagenarians may experience poor clinical signs, without many key physical findings, the complication may occur silently in contrast with young population in which cholecystitis is associated with congenital comorbidities and presents a different clinical panel.

One of the most severe complication of acute cholecystitis is the perforation of the gallbladder usually seen in acalculous form due to underlying diseases which have a higher incidence in elderly patients (age >65 years) (6). Recent data reveal that in elderly patients, bilio-enteric fistulas complicated with liver abscess are rarely seen and the symptomatology may be atypical due to an increased number of comorbidities and reduced biological reserves. Also, the surgical treatment is controversial in these patients due to high mortality rates, percutaneous cholecystostomy (PC) being in some cases the most convenient and minimal risk procedure option in favor of laparoscopic cholecystectomy(LC) (7).

Taking in consideration the data presented in the literature, we intend to report a case of acute acalculous cholecystitis complicated with duodenal fistula and pericholecystic abscess in elderly patient.

CASE REPORT

A 91-year-old Caucasian woman, with prior history of diabetes type 2 and hypertension, was referred to our Institution of Gastroenterology and Hepatology in February 2017 for recent history of epigastric pain, lack of appetite, vomiting, nausea, and weight loss (3 kg in the last month), slow transit constipation without signs of gastrointestinal bleeding. The patient denied both alcohol and drug administration at home. Physical examination found pallor teguments, arrhythmic cardiac sounds and right upper quadrant localized tenderness. Electrocardiogram showed atrial fibrillation with no signs of ischemia, chest X-ray ruled out pleural and lung lesions.

Patient's first laboratory assessment showed severe ferriprive anemia (7 g/dL), leukocytosis (12×10^9/L), hyperglycemia (124 mg/dL), abnormal liver tests (TGP = 495 U/L, TGO= 459 U/L, GGT= 634U/L, total bilirubin =1.61 mg/dL, alkaline phosphates = 781 U/L), inflammatory syndrome (CRP= 6.43 mg/dL), amylase 73 U/L, lipase 70 U/L, and negative blood culture. Renal function and uric acid levels were within normal range. Tests for hepatitis C and B turned out negative. Abdominal ultrasound was performed showing a poorly defined hypoechoic mass with echogenic gas bubbles inside surrounding an indistinct gallbladder outline, no typical gallstones images were detected (fig. 1).

Intrahepatic biliary ducts were dilated at a maximum of 12 mm, the common bile duct with a diameter of 9 mm and no ascites was seen. To investigate the cause of severe anemia in the presence of epigastric pain, we performed upper gastrointestinal endoscopy that revealed an ulcer situated in the descending part of the duodenum with shallow fibrinous necrotic base and blood oozing in the nearby area (fig. 2). The ultrasound results and the endoscopic findings raised the suspicion of cholecystic abscess with duodenal fistula. To sustain our presumptive diagnosis, the patient undergoes abdominal computed tomography (CT) that showed a liquid hypodense mass, poorly demarcated, without identifying the gallbladder, gas bubble inside and in direct contact with the second part of the duodenum consisting with a cholecystoduodenal fistula (fig. 3).

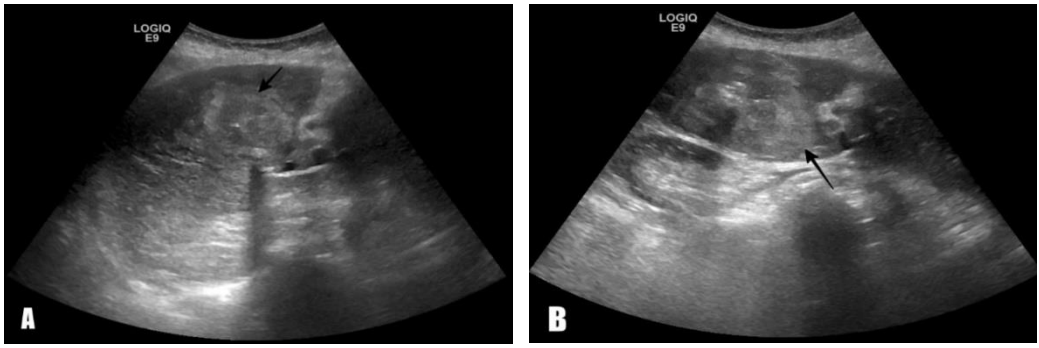

Fig. 1. A. Abdominal ultrasound showing in the examined window a hypoechoic liver mass with echogenic gas bubbles inside poorly demarcated on the topography of the gallbladder, dilated intrahepatic ducts. **B.** Abdominal ultrasound showing in the examined window the absence of gallbladder visualization.

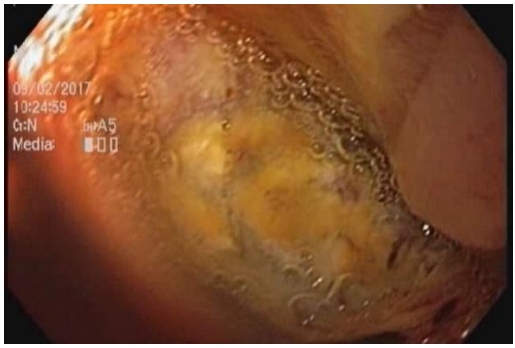

Fig. 2. Upper gastrointestinal endoscopy showing a giant duodenal ulcer in the descending part with shallow fibrinous necrotic base and blood oozing.

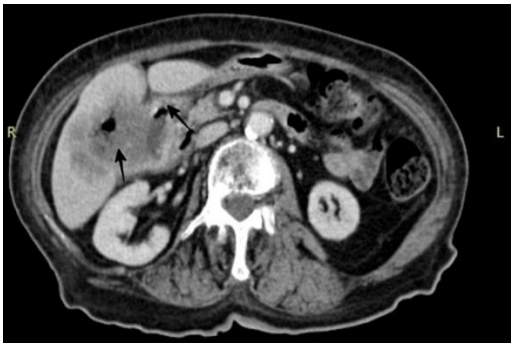

Fig. 3. Abdominal CT showing a pericholecystic abscess with duodenal fistula

At this point our suspicion of *acute acalculous cholecystitis* (AAC) complicated with pericholecystic abscess and duodenal fistula was confirmed and the patient was referred to surgical unit to assess the best treatment. In our unit, the treatment began with proton pump inhibitor (PPI) prescribed for the gastric ulcer, empirical antibiotherapy, iron replacement, volemic repletion and antalgic drugs.

Based on the age of the patient, the traumatic stress, atypical symptoms and the elevated risk of complications, the surgeons decided the PC is optimal as first line treatment instead of LC.

DISCUSSION

AAC is a severe condition with high mortality and morbidity with different clinic manifestation in comparison with the calculous form due to its multiple pathogenic mechanism, rarely seen in young and healthy patients (8-11). It is associated with an increased number of comorbidities such as ischemia, heart failure, diabetes mellitus, bile stasis, surgery and it has an atypical

onset in elderly patients with severe complications (gangrene, perforation) if it is misdiagnosed (12-14).

Ultrasound examination is needed in first stages of cholecystitis for a thoroughly diagnosis, but in some cases when complication occur such as gas formation in the wall or inside the gallbladder visualization can be impaired requiring computed tomography and for particular situations when direct observation of the fistulization tract in the adjacent organs, endoscopic retrograde cholangiopancreatography (ERCP) is to be considered a possible method of choice. One of the most severe complications of AAC is perforation of the gallbladder being first described by Duncan et al in 1884 and classified in three types, in the late nineties being updated by Anderson et al, adding the fourth stage consisting with cholecystobiliary fistula (15). The clinical symptomatology may differ judging the degree of perforation of the gallbladder, in type I peritonitis occurs, type II form pericholecystic abscess, type III and IV evolve with duodenal perforation and fistulization (16). In our case the perforation was a type III with atypical symptoms frequently seen in elderly patients.

Pain in the right hypochondrium, fever and leukocytosis may occur as classical symptoms, but in the case of our patient the fever was absent with negative blood cultures while the abdominal pain had epigastric localization. The clinical symptoms and the severe anemia required further investigations. The upper endoscopy findings showed a giant ulcer in the second part of the duodenum. Biopsies samples were taken from the margins of the duodenal ulcer to exclude the possibility of a malignant ulcerative tumor with perforation and fistula in the gallbladder.

In the elderly peptic ulcers are a frequent and a severe pathology with a risk of bleeding that increases constantly above age of 75 years. Comorbidities and the use of non-steroidal-anti-inflammatory (NSAI) drugs are the major risk factor in peptic ulcer, in 10 % of the cases bilio- duodenal fistulas being a severe complication. The medical history of our patient did not show NSAI drug administration and the comorbidities did not consist in a substantial risk of duodenal ulcer, the ulcerative process being the outcome of the bilio-duodenal fistula. Although there is a substantial risk of ulcers in elderly, in 90 % of cases fistulation occurs after the erosion of the wall of the gallbladder in the presence of a gallstone (15).

The lack of visualization of the gallbladder and a hypoechoic liquid mass with bubble gas described by the ultrasound firstly raised the suspicion of emphysematous cholecystitis with gas gangrene because of anaerobic bacterial infection. There were predisposing factors such as diabetes type 2 and ischemia judging the age of the patient and the cardiovascular risk factor. At the time of diagnosis, the clinical symptomatology was atypical but couldn't rule out the diagnosis of emphysematous cholecystitis. In this stage of diagnosis an improvement was brought by the CT scan. However, in our case the gas in the gallbladder could have been derived either from the fistula or could be formed in site of the gallbladder as a result of anaerobic bacterial infection, following that the result of analyzed drainage liquid collected in the surgical unit to conclude the nature of the gas formation.

As possible explanations for lack of visualization of the gallbladder we hypothesized some common conditions such as

previous cholecystectomy, unusual shape or positioning, obscure image due to gas in the gut or contraction of the gallbladder due to lack of food intake frequently seen in elderly patients (17). The medical history and abdominal ultrasounds previously performed before the admission in our hospital showed a normally shaped gallbladder with thin walls and no signs of gallstones present at the moment of examination. Before the CT scan result ruled out a possible malignant tumor with duodenal invasion, our hypothesis was related to a possible gallbladder neoplasm or cholangiocarcinoma taking in consideration the gender and the age of the patient. A common characteristic in patients with gallbladder cancer is the strong association with gallstones and cholelithiasis, multiparty or increased body mass index. Chronic inflammation of the gallbladder induced by large gallstone (2-3 cm) may be considered either the emerging point of the carcinogenic process or an associative risk, studies suggesting that prophylactic cholecystectomy is not to be recommended in asymptomatic patients (18). Although there is an elevated risk associated with large gallstones, gallbladder cancer may mimic the same symptomatology in elderly patients making the differential diagnosis to be complex even in the presence of advanced radiological imagining. The best treatment was decided by the surgical and radiological unit judging upon the age, comorbidities, and high-risk procedure in case of our patient.

According to the recent data from the literature in AAC, LC is a safe procedure and it is proven to decrees the hospitalization stay. LC performed in the acute onset still associate's high morbidity (up to 41 %) and mortality (up to 4.5 %) because of several comorbidities in elderly patients and anesthetic risk. It is preferred to delay the intervention for 6- 8 weeks and perform minimal risk procedure such as PC (19). PC may be considered a safe procedure in elderly patients with associated comorbidities and increased surgical risk. Is a radiological procedure that does not need a surgical unit, a percutaneous catheter is inserted in the gallbladder under radiological guidance in the calculous form of acute calculous cholecystitis it also gives the possibility of stone dissolution and extraction. A study made by Kierkegard et al included 56 patients who were undergoing this procedure revealed that in 80.4 % of them PC was a definitive treatment option (20). Other studies considered that in critic elderly patients with severe sepsis, PC is a time delay procedure until the LC can be performed (21). Judging upon the prognosis of other comorbidities, limited survival and low recurrence rate of AAC, PC with percutaneous drainage of the pericholecystic abscess is to be considered the optimal surgical treatment in this case.

CONCLUSIONS

Our case emphasizes the fact that AAC may present, especially in elderly patients, as a severe condition, with redoubtable complications, such as perforation, pericholecystic abscess and cholecystoenteric fistula. Clinical picture may not be typical, the diagnosis being either established after several investigations or only intraoperative. Complications might imply high mortality if left untreated, especially in elderly patients; in the same time, advanced age and comorbidities are reasons for which caution in using surgical therapeutic arsenal is required.

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PROGNOSTIC VALUE OF PREOPERATIVE LYMPHOCYTE RATIO IN HOSPITALIZED GERIATRIC PATIENTS WITH EXTRACAPSULAR HIP FRACTURES: PRELIMINARY RESULTS

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PROGNOSTIC VALUE OF PREOPERATIVE LYMPHOCYTE RATIO IN HOSPITALIZED GERIATRIC PATIENTS WITH EXTRACAPSULAR HIP FRACTURES: PRELIMINARY RESULTS (Abstract) Despite the development of modern implants and multidisciplinary approach to extracapsular proximal femoral fractures in the geriatric population, mortality remains high. Our aim is to identify the factors that influence the mortality of these patients to optimize their treatment. **Material and methods:** We analyzed for 12 months' geriatric patients with extracapsular fractures of the proximal femur in terms of age, sex, place of origin, type of fracture, treatment type, duration of preoperative interval variation of hematological values and in-hospital mortality. **Results:** We identified 262 geriatric patients with a mean age of 76.29 years ± 12.14. Sex ratio was 2/1 for the female patients. 7/224 (3.1%) of operated patients and 9/38 of functional treated patients (15.8%) died during hospitalization (p=0.005). The duration of preoperative interval ranged between 1 and 19 days, with no significant differences in the average recorded types of fractures (p=0.355). In the case of deceased patients, we noticed a slight decrease in lymphocytes count, while operated patients, recorded a slight increase (p=0.091). ROC curve showed the best predictability of the percent lymphocyte ratio for mortality (AUC=65%; IC95: 0,523-0,777). **Conclusions:** Preoperative lymphocyte ratio may be a prognostic factor for mortality during hospitalization of geriatric patients with extracapsular fractures of the proximal femur. **Keywords:** LYMPHOCYTE, HIP FRACTURE, MORTALITY, GERIATRIC, PROGNOSIS.

Trochanteric fractures represent a significant source of morbidity and mortality for geriatric population despite development of modern implants and multidisciplinary approach (1).

Despite numerous attempts to outline the factors influencing mortality during hospitalization, the results were inconclusive. Several factors were considered: patient age (2, 3), associated comorbidities (4) delay of surgery (5-7), hemoglobin level at admission (8), nutritional status (9-10). The purpose of the study was to determine the prognostic value of preoperative lymphocyte ratio for mortality during hospitalization for geriatric patients with extracapsular fractures of the proximal femur.

MATERIAL AND METHODS

We reviewed all consecutive geriatric patients with extracapsular fractures of the proximal femur, admitted to Orthopedic

Clinic of "Sf. Spiridon" County Clinical Emergency Hospital from Iasi between January and December 2015. We analyzed demographic characteristics of patients, type of fracture, type of treatment, preoperative interval, hematological profile at admittance and preoperative day and mortality during hospitalization.

Data were collected from patients' clinical observation sheets and by using Unique Integrated Information System (UIIS). A two-sided p - value of 0.05 was considered significant for all tests. All analyses were conducted using *SPSS 21.0* (SPSS, Inc., Chicago, IL, USA).

RESULTS

We identified 262 geriatric patients with a mean age of 76.29 ± 12.14 years. The gender distribution highlights the increased number of female patients (66.4%), sex ratio is 2/1; 88.5% of basicervical fractures, 78.6% of complex trochanteric fractures, 65.6% of fractures pertrochanteric and 63.6% of trohantero-diaphyseal fractures were women, while in males prevails avulsion fractures of the trochanter (54.5%) and intertrochanteric fractures (53.8%) (p=0.05). 57.1% of patients were from rural areas.

In this study group, 85.5% of patients were operated and 14.5% received functional treatment; 7/224 (3.1%) patients operated and 9/38 patients treated functionally (15.8%) died during hospitalization (p=0.005) (fig. 1, 2).

Among deceased patients during hospitalization (n=13), 69,5% were pertrochanteric fractures and 30,8% basicervical fractures (p=0,035).

Distribution of cases by age showed no significant differences depending on the type of treatment used and the evolution during hospitalization (p=0.551).

Operated patients were more frequently engaged in age groups 70-79 years (29.5%) and 80-89 years (41.1%).

Among patients with functional treatment, 39.5% were aged 80-89 years. Most deaths were noted in patients aged 80-89 years (61.5%) (fig. 3, 4).

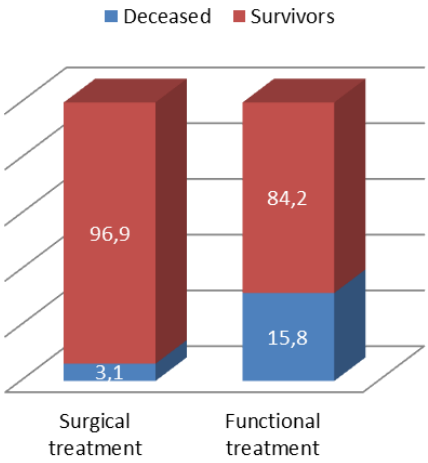

Fig. 1. The percentage of survival according to type of treatment

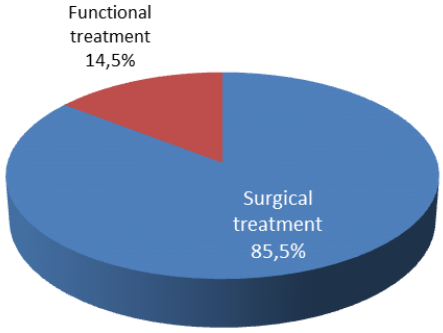

Fig. 2. Type of treatment in the cohort

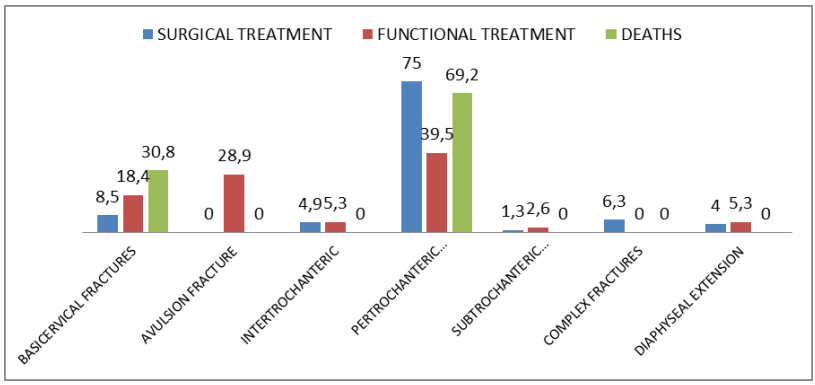

Fig. 3. The correlation between the type of fracture, the method of treatment and death

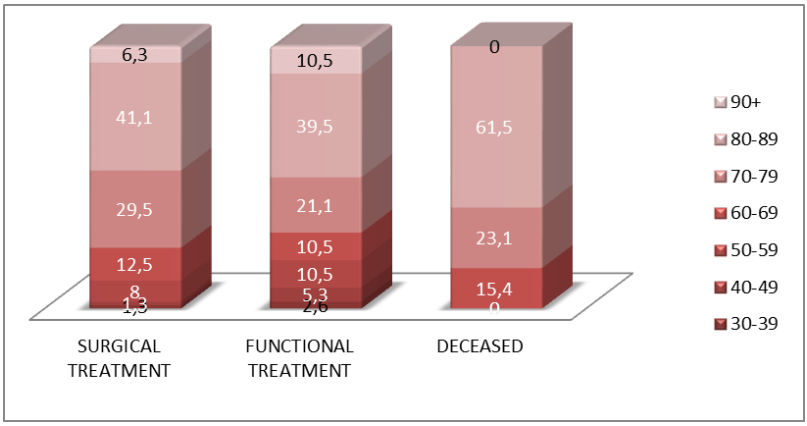

Fig. 4. Distribution of cases by age, type of treatment and survival

Pre-op interval ranged from 1 to 19 days, with no significant differences between types of fractures (p=0.355): the highest average value was found in 9 patients with fractures diaphyseal extension (6, 56 ± 4.19 days). The lowest preoperative interval was found in patients with intertrochanteric fractures (3.73 ± 1.56 days).

Variation of hematological values was not statistically significant for the deceased patients except lymphocytes ratio.

Although the number of lymphocytes at the time of operation recorded an average grown compared to admission, the differences were not significant (1.27 *vs.*1.81; p=0.143) (fig. 5).

During hospitalization, patients with functional treatment recorded decrease of the percentage of lymphocytes by an average of about 11 units, while patients operated recorded decrease about 10 units. Deceased patients have registered a decrease by 7 units (p=0.463).

In patients with functional treatment and deceased we observed a slight decrease in lymphocytes count and a slight increase in operated patients (p=0.091) (fig. 6)

ROC curve highlights the best predictability of lymphocite ratio for mortality (AUC=65%; CI95: .523-.777). The remaining parameters do not provide statistically acceptable predictability (AUC<0.600) (fig.7).

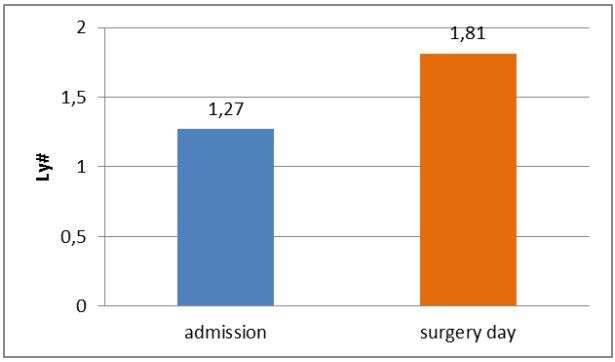

Fig. 5. Evolution of the average level of lymphocytes

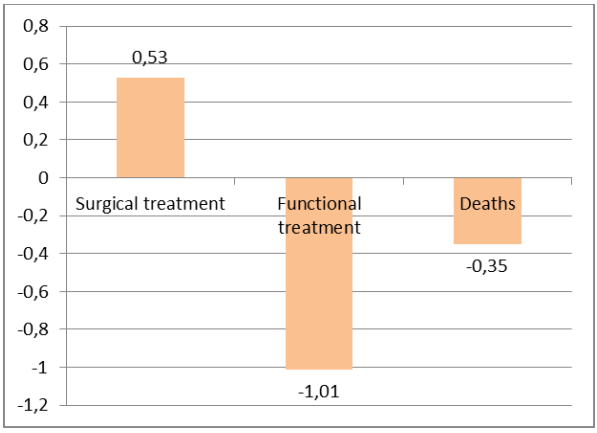

Fig. 6. The average level of lymphocyte per the method of treatment and survival

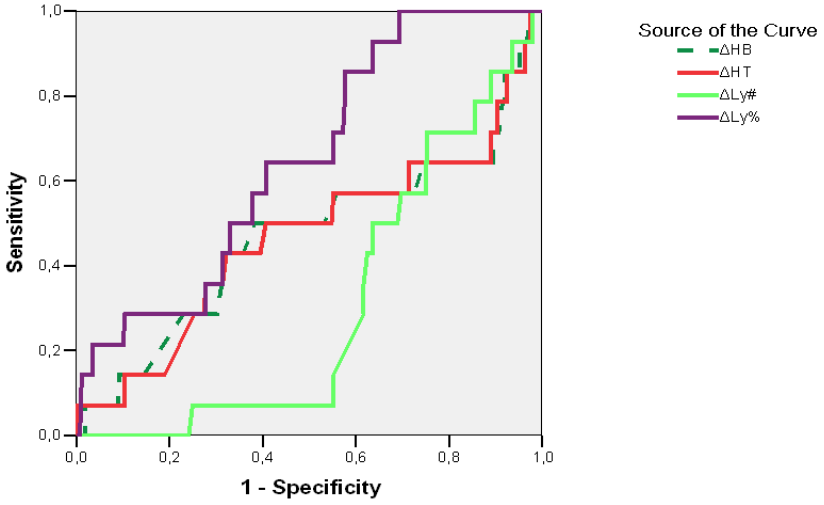

Fig. 7. The ROC Curve

DISCUSSION

This study analyzes the factors that influence hospital mortality of geriatric patients with extracapsular fractures of the proximal femur.

Our results regarding the influence of gender on mortality were in discrepancy with the literature (1-4, 11-12): we have noticed an increased mortality among female patients.

Influence of preoperative interval on mortality is a controversial topic, results of studies being discrepancy (5-7). In our study, we did not find a link between mortality and duration of preoperative interval and we recommend appropriate preoperative detrimental to surgery in the first 24 hours.

Our results regarding the influence of lymphocytes count on mortality, although inconclusive, confirmed results described by Kumar (9), among deceased patients were recorded lower values of lymphocytes with about 7 units (p=0.463). Lymphocyte ration was a significant predictor for mortality within hospital stay.

CONCLUSIONS

Present study demonstrates the prognostic value of the variation of lymphocytes percentage in assessing the risk of death during hospitalization geriatric patients with extracapsular fractures of the hip.

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DETECTION OF AMINOGLYCOSIDE AND MACROLIDE RESISTANCE MECHANISMS IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS*

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DETECTION OF AMINOGLYCOSIDE AND MACROLIDE RESISTANCE MECHANISMS IN METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (Abstract): Methicillin-resistant *Staphylococcus aureus* can become resistant to many different classes of antibiotics. **Objective:** To characterize aminoglycoside and macrolide resistance mechanisms in MRSA strains in relation to antibiotic susceptibility patterns. **Materials and methods:** We tested 86 MRSA strains using multiplex PCR for detection of genes *mecA, aac(6')-Ie/aph(2"), ant(4')-Ia, aph(3')-IIIa, ermA, ermC and msrA.* **Results:** There was a prevalence of *msrA* (32.5%), *ermC* (30.2%) and *aph(3')-IIIa* (61.6%) genes, which are less frequently reported in MRSA. Most *msrA* genes was detected in PVL positive strains (92.8%) and was associated only with non-MDR strains, while *ermC* genes were associated with MDR strains. PVL producing strains were characterized by the presence of *aph(3')-IIIa* (93.1%) and *msrA* genes (93.1%), being phenotypically susceptible to clindamycin. **Conclusions:** Detection of aminoglycoside and macrolide resistance genes allowed us to establish the concordance between genotypic and phenotypic methods and to correlate the presence of certain resistance genes with the type of circulating strain and the production of virulence factors. **Keywords**: MRSA, MULTIPLEX PCR, AMINOGLYCOSIDE, MACROLIDE.

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common nosocomial pathogens, responsible for a broad spectrum of infections, which can become resistant to different classes of antibiotics. Aminoglycosides play an important role in the treatment of staphylococcal infections (1). There are several mechanisms for aminoglycoside resistance, the most frequent being inactivation by enzymatic modification that involves over 50 enzymes and multiple phenotypes (2). The genes encoding for those enzymes are usually found on plasmids and transposons. The majority of aminoglycoside modifying enzymes seen in MRSA strains are encoded by genes *aac(6')-Ie/aph (2"), ant(4')-Ia* and *aph(3')-IIIa* (2, 3). Resistance to gentamicin, kanamycin, and tobramycin is mediated by a bi-functional enzyme displaying acetyltransferase *aac(6')* and phosphotransferase *aph(2")* activity. The adenyltransferase *ant(4')-Ia* inactivates neomycin, kanamycin, tobramycin and amikacin, while the

phosphotransferase *aph(3')-III* inactivates neomycin (1, 2). Susceptibility testing against a range of aminoglycosides can help establish the resistance patterns that are unique for each enzyme (4).

Macrolide resistance in *S. aureus* can be caused by altered ribosomal target, active efflux or antibiotic inactivation. The most common mechanism is target modification mediated by one or more erythromycin ribosome methylase (*erm*) genes that confer resistance to macrolide, lincosamide, and streptogramin B (MLS_B phenotype) (5). The macrolide streptogramin resistance (*msr*) drug efflux mechanism causes the MS_B phenotype (5, 6).

Given the frequent association of methicillin resistance with multiple resistance to other non-beta-lactam antibiotics, in this study we aimed to implement, for the first time in the Microbiology Laboratory of UMPh "Grigore T. Popa" Iasi, a multiplex PCR assay for identification of MRSA strains resistant to aminoglycosides and macrolides, in order to describe the resistance mechanisms in relation to the antibiotic susceptibility patterns of MRSA strains.

MATERIAL AND METHODS.

We have tested 86 community-acquired (CA-MRSA) and hospital-associated (HA-MRSA) isolates from "St Parascheva" Clinical Infectious Diseases Hospital, Iaşi, collected from localized and systemic infections during a 3 year' period (2008-2010) and previously tested for *lukF/S-PV* genes encoding Panton Valentine leukocidin (PVL) (7). In this study, we performed three multiplex PCR reactions, each at a total volume of 50 µL, using 5 µL of template DNA, *ImmoMix*^{TM}*Red Master Mix* (*Bioline, UK*), primers at 50 pmol/reaction (*Metabion international*, Germany) selected from the DNA sequences previously published (8-12**).** Amplification was performed using *Corbett Palm-Cycler* (*Qiagen*, USA) under thermal cycling conditions described in tab.I and analysis was done by electrophoresis in 2% agarose gel, at 130V, for 35 minutes. In addition to PCR tests, phenotypic methods were also used as previously described (7) and antimicrobial susceptibility patterns were evaluated.

TABLE I

Protocol used for detection of macrolide and aminoglycoside resistance genes (adapted from (8-12))

Amplified gene	Sequence (5'-3')	Thermal profile
mecA	F-CCTAGTAAAGCTCCGGAA	1 cycle: 5 min. at 95°C 30 cycles: 2 min. at 95°C 30 sec. at 58°C 30 sec. at 72ºC 1 cycle: 7 min. at 72ºC
	R-CTAGTCCATTCGGTCCA	
aac(6')-Ie/aph(2")	F-GAAGTACGCAGAAGAGA	
	R-ACATGGCAAGCTCTAGGA	
ant(4')-Ia	F-CAAACTGCTAAATCGGTAGAAGCC	1 cycle: 5 min. at 95°C 30 cycles: 30 sec. at 95°C 10 sec. at 58°C 30 sec. at 72ºC 1 cycle: 3 min. at 72ºC
	R-GGAAAGTTGACCAGACATTACGAACT	
aph(3')-III	F-GGCTAAAATGAGAATATCACCGG	
	R-CTTTAAAAAATCATACAGCTCGCG	

Amplified gene	Sequence (5'-3')	Thermal profile
ermA	F-TATCTTATCGTTGAGAAGGGATT	1 cycle: 5 min. at 94°C 30 cycles: 1 min. at 95°C 30 sec. at 55°C
	R-CTACACTTGGCTTAGGATGAAA	
ermC	F-CTTGTTGATCACGATAATTTCC	
	R-ATCTTTTAGCAAACCCGTATTC	
msrA	F-TCCAATCATAGCACAAAATC	
	R-AATTCCCTCTATTTGGTGGT	

RESULTS

The results of aminoglycoside and macrolide resistance genes are presented in tab. II. The most common macrolide resistance gene was *msrA* (32.55%), associated only with non-multidrug resistant (MDR) strains. Phenotypically, 26 of 28 of those strains were resistant to erythromycin. Particular for those strains was the association with PVL toxin production (26/28). All erythromycin–resistant PVL positive strains carried only *msrA* gene, being phenotypically susceptible to clindamycin. During the study we have observed a significant increase in the proportion of the strains carrying *msrA* gene from one year to another (p = 0.02) and a significant decrease in the proportion of the strains carrying *ermA* gene (p = 0.01). *ErmA* and *ermC* genes were detected in equal proportions (30.23%) and were associated with MDR strains. All *ermC* strains were phenotypically resistant to erythromycin and the majority (25/26) were resistant to clindamycin. Twenty of the 26 *ermA* positive strains were phenotypically resistant to erythromycin and only half were resistant to clindamycin. Macrolide resistance genes were also detected in strains phenotypically susceptible to erythromycin (3 strains showed *ermA* gene and 2 strains were carrying *msrA* gene). We have observed a significant association of *ermA* gene with HA-MRSA strains (the gene was present in 60% of HA-MRSA strains and in only 22.58% of CA-MRSA strains, p=0.001), while in contrast, *msrA* genes were associated with CA-MRSA isolates (23/28 *msrA* positive strains, p=0.008). Detection of macrolide resistance genes showed a concordance between phenotypic and genotypic methods of 94.18% for erythromycin and 82.55% in case of clindamycin. The sensitivity of phenotypic detection of resistance to erythromycin was 91.25% and lower for clindamycin (73.07%).

Of the total MRSA isolates, 6 (6.97%) were negative for aminoglycoside resistance genes and carried PVL genes. The most frequent aminoglycoside resistance gene was *aph(3')-IIIa* (61.62%). This gene was the only one associated with PVL producing strains (93.1%, p<0.001). Genes *aac(6')-Ie/aph(2")* were present in 27.9% of strains, while gene *ant (4')-Ia* in only 3.4% of strains. Genes *aac(6')-Ie/aph(2")* and *ant(4')-Ia* were associated with MDR strains, while gene *aph(3')–IIIa* was found both in MDR and in non-MDR strains. In one case, the strain was resistant to gentamicin phenotypically, but none of the 3 genes was present, suggesting the involvement of another mechanism of resistance. Also, in one case, the gene *aac(6')-Ie/aph(2")* was detected in a strain with phenotypic susceptibility to gentamicin.

TABLE II

Results for macrolide and aminoglycoside resistance genes detection

	Macrolide resistance				Aminoglycoside resistance			
Detected gene	*ermA*	*ermC*	*msrA*	Negative	*aph(3')*	*aac(6')/aph(2")*	*ant(4')*	Negative
No. of strains	26	26	28	6	53	24	3	6
PVL +	0	0	26	3	27	0	0	2

DISCUSSION

The presence of resistance genes can be correlated with data on antibiotic resistance profiles of CA-MRSA, HA-MRSA and PVL producing strains and can be used to adjust the application of therapeutic regimens in our region. This study reveals that *ermA* gene was more commonly found in HA-MRSA (60%) strains, data consistent with those described by Pruneau *et al.* and Sun *et al*. (13, 14). Contrary to reports of *ermA* gene being the dominant *erm* gene in *S. aureus*, our results showed the same proportion for *ermA* and *ermC* genes. No combination of *ermA* and *ermC* was detected. Per Sun *et al*., *ermA* gene was frequently detected in HA-MRSA isolates (86.9%) while the detection rates of *ermB* and *ermC* genes were lower, and the mentioned study also revealed the triple combination of *ermA, ermB* and *ermC* in 17.8% of HA-MRSA isolates (14). In a study by Schmitz *et al*. the *ermA* gene was more common in MRSA isolates (88%) than MSSA (38%), and occurred mainly in strains with constitutive MLSB expression, while *ermC* was more common in MSSA (47%) than in MRSA (5%), occurring mainly in strains with inducible expression (5). Another particularity of tested strains was the high percentage of MRSA strains harboring *msrA* gene, mainly in CA-MRSA isolates. Contrary to our findings, Sun *et al* reported that CA-MRSA isolates only possessed the *ermA* gene (14) and Schmitz *et al.* detected the *msr* genes only in MSSA isolates (13%) (5). Similarly, Shittu *et al.* identified the *ermA* gene in all erythromycin-resistant MRSA isolates, while *msrA* gene was detected in MSSA isolates (15). Since isolates with MS_B phenotype are susceptible to lincosamides and 16-membered ring macrolides, detecting resistance genes is useful for timely and appropriate choice of antibiotics (16). Three MRSA isolates were susceptible to erythromycin even though they possessed the *ermA* gene. Similar discordance was found by Sun *et al* who stated that this could be caused by mutation in the coding or promoter region of the genes (14). Wong *et al* also reports the case of an erythromycin- and clindamycin-susceptible phenotype in an *ermA* positive strain and discovered, through DNA sequence analysis, a 48-bp deletion in Tn554 harboring *ermA* (6). In our study, there was no strain resistant to erythromycin that did not possess resistance genes. However, such cases were reported by Sun *et al*, when resistance genes were located on small plasmids, occasionally lost (14).

Interestingly, the most frequently identified gene implicated in aminoglycoside resistance in our study was *aph(3')-IIIa* (61.62%). This gene is infrequently found in *S. aureus*. Sun *et al* reported the presence of

this gene in 5.6% of HA-MRSA and no CA-MRSA isolates (14). Gene *ant (4')-Ia* is rarely detected in MRSA strains and this is in accordance with our findings (3.48%). Thus, Sun *et al*, detected *ant(4')-I* gene in 9.1% of CA-MRSA isolates (14). In our study, gene *aac(6')-Ie/aph(2'')*, frequently reported as predominant, was present in only 27.9% of isolates. Contrary to our findings, Sun *et al* reported that the most common aminoglycosides resistance gene was *aac(6')-aph(2'')*, which was found in 95.3% of HA-MRSA and 45.5% of CA-MRSA (14). Rahimi *et al.* also found a predominance of *aac(6')-aph(2'')* gene (65.6%) (1).

CONCLUSIONS

Characterization of macrolide and aminoglycoside resistance mechanisms revealed the particularities of MRSA strains in our region. The isolates showed a high prevalence of *msrA*, *ermC* and *aph(3')-IIIa* genes, which are less frequently reported in MRSA. The majority of *msrA* genes was detected in PVL positive strains (92.8%) and was associated only with non-MDR strains, while *ermC* genes were associated with MDR strains and phenotypic resistance to clindamycin (92.3%). PVL producing strains were characterized by the presence of only *msrA* genes (being phenotypically susceptible to clindamycin) (93.1%) and *aph(3')-IIIa* (93.1%).

Multiplex PCR techniques for detection of genes encoding resistance factors in MRSA isolates permit rapid identification of strains with associated resistance to non-beta lactam antibiotics and may improve diagnosis and help adjust therapeutic regimens and preventive measures.

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NEWS

GENETIC SUSCEPTIBILITY TO INSULIN RESISTANCE ASSOCIATED WITH LOWER ADIPOSITY

The researchers tried to demonstrate the relationship between adiposity, genetic susceptibility to insulin resistance or insulin secretion and the response to GH treatment in short children born small for gestational age(SGA).The study was realised on 89 short prepubertal SGA children treated with GH for one year in a multicentre study. Combined multiallele gene scores based on single nucleotide polymorphisms with known associations with lower insulin sensitivity and insulin secretion were analysed. The results showed that during GH treatment, GS-In Res was related to a lesser decline in trunk fat and a higher trunk- limb fat ratio at 1 –year. GS-In Sec was positively associated with truncal fat (Thankamony A[1,2], Jensen RB[1], O'Connell SM[3] et al. Adiposity in children born small for gestational age is associated with β-cell function, genetic variants for insulin resistance and response to growth hormone treatment. *J Clin Endocrinol Metab.* 2015 Nov 20: jc20153019).

Diana Tatarciuc

SIMULTANEOUS UNILATERAL RADIAL SHAFT FRACTURE WITH DISTAL AND PROXIMAL RADIOULNAR JOINT DISLOCATION, AN EXTREMELY RARE CASE REPORT

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SIMULTANEOUS UNILATERAL RADIAL SHAFT FRACTURE WITH DISTAL AND PROXIMAL RADIOULNAR JOINT DISLOCATION, AN EXTREMELY RARE CASE REPORT (Abstract): Till now we know two fracture pattern in forearm fracture dislocation, Galeazzi fracture dislocation which is defined as fracture of radial shaft with distal radioulnar joint (DRUJ) dislocation and Monteggia fracture dislocation which is ulnar fracture with radial head dislocation. During this case report we present a case with simultaneous distal and proximal radioulnar dislocation and radial shaft fracture which is extremely rare fracture pattern and could be a new entity in forearm bones fracture dislocation. **Keywords**: RADIAL SHAFT FRACTURE, RADIAL HEAD, DISTAL RADIO ULNAR JOINT (DRUJ)

The fracture of radial shaft simultaneously with proximal and distal radioulnar joint dislocation in an extremely rare event in children and adults. This pattern doesn't conform to Galeazzi, Monteggia and Essex-Lopresti fractures (1-3). We did not found any other similar cases during our search. During this case report we present a case with simultaneous distal and proximal radioulnar dislocation and radial shaft fracture

CASE REPORT

A 52 years old man admitted in our emergency ward after a motor vehicle accident. The patient was motorcycle rider and had a crash with a car. There was sever pain and restriction in range of motion in both right wrist and elbow joint. Also patient had a severe pain and deformity in right forearm. Supination and pronation in elbow and wrist were absent. Also flexion and extension in both joint were painful.

In plain radiography a radial shaft fracture, anterolateral dislocation of radial head and dorsal dislocation of ulnar head with mild ulnar plus was apparent. No other fracture was determined (fig. 1).

Patient was transferred to operation room and open reduction with Dynamic compression plate was done, the elbow joint was checked with fluoroscopy which was reduced after radial shaft fixation. Then a long arm cast in neutral flexion of elbow and forearm supination was applied (fig. 2). A long arm cast was applied in supination of forearm and neutral position of elbow. The post-operative radiographies were acceptable. After four weeks the cast was removed and physiotherapy started. Radiographs were taken at 3rd, 6th and 12th weeks and then at 3 monthly intervals until 10 months.

The fracture was united and patient was able to 80° extensions and 75° flexion at wrist, 80° of supination and pronation at the fore-arm during 12th week visit.

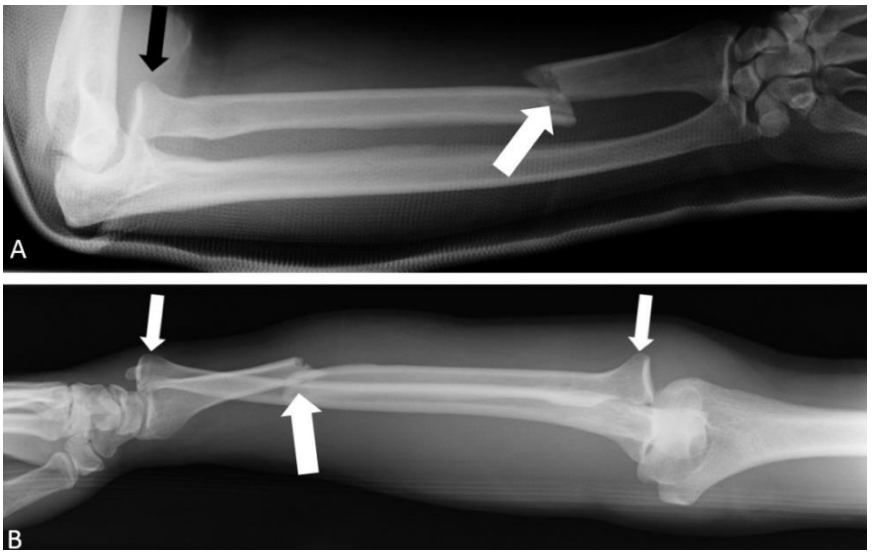

Fig. 1. Pre-operative lateral (A) and Anteroposterior (B) x-rays. The dislocation of distal and proximal radioulnar joints (arrows) and the radial shaft fracture are apparent.

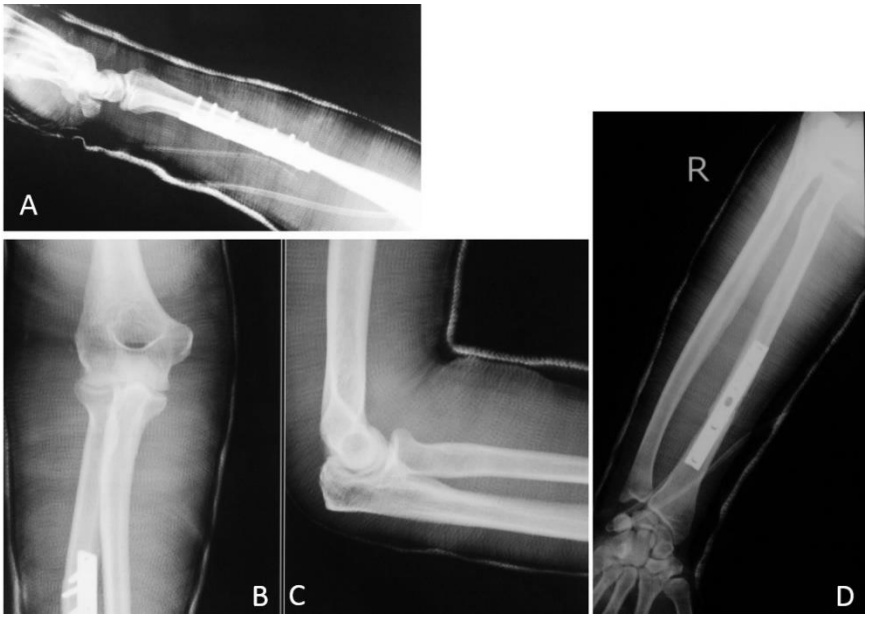

Fig. 2. Post-operative x-rays. Lateral (A) and anteroposterior (D) X-rays of distal radioulnar joint (DRUJ) and lateral (C) and anteroposterior (D) X-rays of elbow joint. The radial shaft is fixed with 3.5 DCP plate, distal and radioulnar joint are reduced.

DISCUSSION

During this case report we presented a patient with dislocated distal and proximal radioulnar joint simultaneously with radial shaft fracture. This pattern was not reported in any previous case reports. Distal radioulnar joint dislocation often is associated with unstable radial fracture which was firstly described by cooper in 1822 and named as Galeazzi fracture-dislocation after Ricardo Galeazzi's presentation case series in 1934 (1). Also isolated radial head dislocation is associated with proximal ulnar fracture which was described by Giovanni Battista Monteggia, in 1814 (2). The Essex-Lopresti injury is characterized by a fracture of the radial head with concomitant disruption of the distal radioulnar joint (DRUJ) and rupture of the interosseous membrane (IOM) (3).

There are few case reports on simultaneous dislocation of proximal and distal radioulnar joint (4-9). In these cases, there are either pure dislocation of distal and proximal radioulnar joints (4, 6-9) or simultaneously with elbow dislocation or radial head fracture (5). But there is no report on proximal and distal radioulnar joint dislocation with radial shaft fracture (10-13). This case is the first report of such a fracture pattern.

It seems that hyper pronation is the causative force for the simultaneous dislocation of proximal and distal radioulnar joint. When the patient falls onto dorsiflexed wrist, pronated forearm and extended elbow, he might rotate the body over the affected limb which is fixed to ground. This rotation can cause much pronation that can disrupt triangular fibrocartilage complex and also varus force that cause disruption of annular ligament. But fracture of radial shaft during each stage of this process can interfere the described mechanism and prevent to dislocate the proximal or distal radioulnar joints. In our case the fracture of radial shaft was fixed with Dynamic Compression Plate. The radial and ulnar head were reduced after plating of radial shaft. A long arm cast was applied in supination of forearm and neutral position of elbow. The post-operative radiographies were acceptable and final outcomes was favorable during follow-ups.

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NEWS

BIOLOGICS IN RHEUMATOLOGY: BRITISH GUIDE UPDATE

On August 24th, in *Rheumatology*, the British Society for Rheumatology (BSR) and British Health Professionals in Rheumatology (BHPR) Standards, Guidelines and Audit Working Group published an updated guideline for prescribing biologics to the patients with axial spondyloarthritis (including Ankylosing Spondylitis). The group recommended the inclusion in biological therapy not only patients with Ankylosing Spondylitis meeting the modified New York criteria, but also those with non-radiographic disease, but only in cases with real evidence of inflammation on MRI or a raised C-reactive protein. Also, they advised the clinicians to switch from one TNF- inhibitor to another anytime during the treatment, reducing the time between BASDAI measurments to 4 weeks. Regarding the problem of choosing one TNF-inhibitor or another for axial spondyloarthritis, the studies the group reviewed did not demonstrate an important difference in terms of efficacy between infliximab, golimumab, etanercept, certolizumab and adalimumab. Response evaluation of the treatment in patients with TNF-inhibitors should be realised every 3 to 6 months, and the responsive ones must be reassessed every 6 months after. It is important to acknowledge that the non- responsive patients (those with no clinical response by 6 months or if the response is not maintained at two consecutive assessments) should be discontinued. The working group did not answered though to an important issue, if the anti-TNF therapy could prevent radiographic progression or not. (Hamilton L, Barkham N, Bhalla A et al. BSR and BHPR guideline for the treatment of axial spondyloarthritis (including ankylosing spondylitis) with biologics. *Rheumatology.* 2016 . doi:10.1093/rheumatology/kew223.233-6)

Luana - Andreea Macovei

IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL ADENOCARCINOMA

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IMMUNOHISTOCHEMISTRY IN ENDOMETRIAL HYPERPLASIA AND ENDOMETRIAL ADENOCARCINOMA (Abstract) **Aim**: Endometrial hyperplasia is a proliferation of endometrial glands due to the prolonged stimulation with estrogens of the endometrium that occurs in women receiving exogenous estrogens, with anovulatory cycles, or in patients with ovarian tumours with estrogen secretion. **Material and methods**: The study performed by the authors included 575 patients with endometrial hyperplasia and 163 patients with endometrial adenocarcinoma admitted to the "Cuza-Vodă" Obstetrics and Gynaecology Clinical Hospital of Iasi, between 2005-2007. **Results**: There were selected, for these immunohistochemistry reactions, 22 cases of simple hyperplasia without atypia, 26 cases of complex hyperplasia without atypia, 23 cases of endometrial adenocarcinoma of endometrioid type, well differentiated, 22 cases of endometrial adenocarcinoma of endometrioid type, moderately differentiated, and 19 cases of non-endometrioid adenocarcinomas represented by nine clear cells and 10 serous endometrial adenocarcinomas. Estrogen receptors have been positive in about 85-90% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI). In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), the estrogen receptors were positive in approximately 70-85% of the tumour cells. **Conclusions**: Endometrial hyperplasia, especially complex endometrial hyperplasia with atypia, increase the risk for endometrial adenocarcinoma, and their early detection becomes mandatory under cancer prevention. Well-differentiated endometrioid endometrial adenocarcinomas were ER and PR-positive, so that the ER expression correlated with the PR expression. Well-differentiated endometrioid endometrial adenocarcinomas (GI) in the studied group also showed a higher content of ER and PR compared to the endometrial moderately-differentiated endometrioid endometrial adenocarcinomas (GII). In non-endometrioid adenocarcinomas, represented by clear-cell endometrial adenocarcinomas, the ER content was reduced and the PR expression was negative. Serous adenocarcinomas failed to show an immunohistochemically expression for ER and PR. **Keywords**: IMUNOHISTOCHEMITRY, ADENOCARCINOMA, ENDOMETRIUM.

The link between endometrial hyperplasia and endometrial carcinoma was mentioned in several studies. Some of them have shown a development of the endometrial carcinoma in 2% of the complex endometrial hyperplasia without atypia and

20-30% of the complex atypical hyperplasia, therefore the complex atypical hyperplasia is a precursor of endometrial carcinoma (1).

Pesce and al. (1) describe two subtypes of endometrioid adenocarcinoma, represented by adenocarcinoma with trophoblastic differentiation and adenocarcinoma with Sertoli form differentiation. The endometrioid adenocarcinoma with trophoblastic differentiation has giant multinucleated cells, of the syncytiotrophoblastic type, that are immunoreactive to HGG, while the cells of the endometrioid adenocarcinoma with Sertoli form differentiation are similar to the ovarian tumours with the same differentiation type.

The expression of PTEN (Phosphatase and tensin homologue deleted on chromosome ten) is an oncosupressor gene of the same class of p53, rbp and APC recently identified in the Cz 10q23 (LI) region, which encodes a protein with 403 amino acids, of the multifunctional phosphatase type, with a very wide tissue nuclear expression, which is involved in the regulation of the cell cycle and apoptosis. PTEN's tumour suppressor gene mutation has been reported in approximately 50-83% of endometrial adenocarcinoma.

PTEN is expressed in most human tissues. PTEN deletions and mutations occur not only in endometrial carcinomas but also in glioblastoma, prostate carcinoma, lung carcinoma and mammary carcinoma, and it is mostly associated with invasiveness.

PTEN inactivation in endometrioid adenocarcinomas and in many other types of tumors cannot be explained only on the basis of the observed mutations. This observation suggests that the expression of PTEN is suppressed at transcriptional and translational level through other mechanisms (12).

With the help of the immunohistochemically evaluation of the estrogens (ER) and progesterone (PR) it is better appreciated their distribution in tumour tissues and normal peritumoral tissues. In endometrial carcinoma, the ER/PR status, in particular PR, correlate with histological differentiation and survival. In well-differentiated carcinoma, the ER/PR positivity is more common and it is associated with a better prognosis. It is believed that an ER/PR positive endometrial carcinoma responds better to hormonal therapy (9).

Cyclooxygenases COX-1 and COX-2 are enzymes which play an important role in the synthesis of prostaglandins from the arachidonic acid. COX-2 expression has been related to carcinogenesis and the COX-2 specific inhibitors that have antitumour effects. The immunohistochemically expression of COX-2 has been reported in a variety of normal and neoplastic tissues.

Her-2/neu/cerbB2 oncogene is part of the EGFR genes family. The detection of Her-2/neu in gynaecological tumours is important, both as a prognostic marker and also to qualify the Herceptin therapy. The normal endometrial glands are weakly to moderately positive for Her-2/neu. Compared with normal endometrium, 9% of the endometrial adenocarcinoma demonstrate a stronger positivity for Her-2/neu.

Ki-67 is a nuclear factor of the cellular proliferation, with protein structure with two isoforms.

P53 is a tumour suppressor gene, located on the short arm of chromosome 17. P53 is most frequently positive in the papillary serous adenocarcinoma and also in other carcinomas with poor prognosis, with a high degree of malignancy and high prolif-

eration index (2).

K-ras oncogene mutations have been detected in 10-25% of endometrial carcinomas and 17% of the atypical hyperplasia (3). K-ras activation is an early event in the carcinogenesis of endometrial carcinoma. Studies have shown that K-ras oncogene is an independent negative prognostic factor (4).

MATERIAL AND METHODS

The study included 575 patients with endometrial hyperplasia and 163 patients with endometrial adenocarcinoma admitted to the "Cuza-Vodă" Obstetrics and Gynaecology Clinical Hospital of Iasi, between 2005-2007.

The collection, processing and interpretation of the information required for the clinical-statistical study on knowing the peculiarities of the cases were performed using data recorded in the case report forms and in the computer system.

The immunohistochemistry reactions performed on the cases in the hyperplasia and endometrial adenocarcinoma group in our study had in view the assessment of the hormone receptors for estrogen (ER) and progesterone (PR), the PTEN gene protein, the Her2/neu (cerb-2)/ gene protein, the nuclear factor of Ki-67 cell proliferation, cyclooxygenase 2 (COX-2), metalloproteinases (MMP-2 and MMP-9).

The data used as study material from the patient charts refer to the division of cases into age groups, their distribution according to their residence environments, according to their profession, to their admission reasons, to personal physiological history, to personal pathological history, and to family history relevant for endometrial carcinoma.

The age of patients with endometrial carcinoma is an important prognosis factor independent from other parameters. In our study, the patients with endometrial carcinoma were aged 30 to 70, with an average age of 58.94 years. The highest percent – 29.3% - are patients over 65 years old. The percent of patients younger than 50 was 6.84% of the cases. The socioeconomic status can be perceived as a risk in developing endometrial adenocarcinoma, as well as a condition for the increase in the rate of women who submit to specialty consultation, due to a high education level enabled precisely by their high standard of living. Moreover, the low socioeconomic level is a factor affecting negatively the subsequent monitoring of patients who can develop endometrial adenocarcinomas.

RESULTS

There were selected, for these immunohistochemistry reactions, 22 (3,82%) of simple hyperplasia without atypia, 26 (4,52%) of complex hyperplasia without atypia, 23 (4%) of endometrial adenocarcinoma of endometrioid type, well differentiated, 22 (3,82) of endometrial adenocarcinoma of endometrioid type, moderately differentiated, and 19 (3,3%) of non-endometrioid adenocarcinomas represented by nine clear cells and 10 (1,74%) serous endometrial adenocarcinomas.

Estrogen receptors were present in different percentages in the studied hyperplasia and endometrial adenocarcinomas (table I). The marking recognized amount is represented by the nuclear marking.

In simple endometrial hyperplasia without atypia, estrogen receptors were positive in 85-95% of the glandular epithelial cells. In complex endometrial hyperplasia without atypia, the estrogen receptors were positive in 70-90% of the glandular epithe-

lial cells.

Estrogen receptors have been positive in about 85-90% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI). In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), the estrogen receptors were positive in approximately 70-85% of the tumour cells. The estrogen receptors in clear-cell endometrial adenocarcinomas were positive in 40-45% of the tumour cells. In serous endometrial adenocarcinomas, the estrogenic receptors were negative in the tumour cells and they showed positivity only in the remaining glandular epithelium (table I).

TABLE I.

Results of the study of ER and PR hormone receptors

Histological type		No. Cases	ER %	PR %
Endometrial hyperplasia	Simple hyperplasia without atypia	22	85-95	85-95 %
	Complex hyperplasia without atypia	26	70-90	80-90
Endometrioid ADK	Well-differentiated endometrioid ADK (GI)	23	85-90	90-95
	Moderately-differentiated endometrioid ADK (GII)	22	70-85	80-85
Non-Endometrioid ADK	Clear-cell endometrial ADK	9	40-45	0
	Serous cells endometrial ADK	10	0	0

The cases of simple endometrial hyperplasia without atypia expressed progesterone receptors in 85-95% of the glandular epithelial cells. In complex endometrial hyperplasia without atypia, the progesterone receptors were positive in 80-90 % of the glandular epithelial cells.

Progesterone receptors were positive in 90-95 % of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI).

In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), the progesterone receptors were positive in 80-85 % of the tumor cells.

In serous endometrial adenocarcinomas, the progesterone receptors were negative in the tumour cells and they showed positivity only in the remaining glandular epithelium.

The progesterone receptors were negative in clear-cell endometrial adenocarcinomas and they were positive only in the remaining glandular epithelium (table I).

In simple hyperplasia and complex hyperplasia without atypia, PTEN showed regional positivity in the glandular epithelial cells. The reaction was negative in a single case of complex hyperplasia without atypia. PTEN showed regional positivity in the tumour cells of the endometrial adenocarcinoma tumor of endometrioid type, well differentiated (GI) and moderately differentiated (GII).

In serous endometrial adenocarcinomas, PTEN gene protein was positive in rare tumor cells and diffusely positive in the remaining glandular epithelium.

The PTEN gene protein was regionally positive in the tumour cells of the clear-cell endometrial adenocarcinomas.

In terms of the immunohistochemical expression of C-erbB2 (Her2/neu/cerb-2 gene protein), in endometrial adenocarcinomas of endometrioid type and in clear-cell endometrial adenocarcinomas of our lot, the reaction to cerb-2 was negative. In serous endometrial adenocarcinomas, cerb-2 showed regional positivity in tumor cells, scored with 2+.

The positivity of Ki-67 reaction was approximately 2-15% of the glandular epithelial cells in the simple endometrial hyperplasia without atypia. In complex endometrial hyperplasia without atypia, Ki-67 was positive in about 3-10 % of the glandular cells. Ki-67 was positive in about 25-30% of the tumour cells of the well-differentiated endometrial adenocarcinomas of endometrioid type (GI). In endometrioid-type endometrial adenocarcinomas moderately differentiated (GII), Ki-67 was positive in approximately 20-40 % of the tumor cells. Ki-67 was positive in about 60-65% of the tumor cells in the clear-cell adenocarcinomas. In serous endometrial adenocarcinomas, Ki-67 was positive in about 50-60 % of the tumour cells.

In simple endometrial hyperplasia without atypia, COX-2 is variably expressed in glandular epithelial cells from weak regional positivity to diffuse positivity. COX-2 shows a weakly positive regional reaction in the glandular epithelial cells of the complex endometrial hyperplasia without atypia. In the well-differentiated endometrial adenocarcinoma of the endometrioid type (GI) and moderately differentiated ones (GII), COX-2 is expressed diffusely in all the tumor cells. COX-2 is diffusely present in the cytoplasma of the tumour cells of the serous endometrial adenocarcinomas.

In simple endometrial hyperplasia without atypia, MMP-2 showed only in one case a weak positive reaction in the epithelial cells and a positive reaction in the stromal cells. In complex endometrial hyperplasia without atypia, MMP-2 showed only in one case a regional positive reaction in the epithelial cells and a regional positive reaction in the stromal cells. In well-differentiated endometrioid endometrial adenocarcinomas (GI) MMP-2 showed positive reaction in rare tumor cells and negative reaction in stromal cells. In moderately-differentiated endometrioid endometrial adenocarcinomas (G II), MMP-2 showed weak regional positive reaction in tumor cells only in one case and negative reaction in the stroma in all the cases. In serous endometrial adenocarcinomas, MMP-2 showed weak positive reaction in tumor cells and stromal cells. In clear-cell endometrial adenocarcinomas, MMP-2 has shown a negative reaction in both tumor cells and stroma.

MMP-9 showed a regional positive reaction in the epithelial cells and a regional positive reaction in stromal cells in simple endometrial hyperplasia without atypia. In complex endometrial hyperplasia without atypia, MMP-9 showed regional positive reaction in the epithelial cells and regional positive reaction in the stromal cells. In well-differentiated endometrioid endometrial adenocarcinomas (GI), MMP-9 showed regional positive reaction in the tumor cells and negative reaction in stromal cells. In moderately-differentiated endometrioid endometrial adenocarcinomas (GII), MMP-9 showed, in 2 cases, regional positive reaction in tumor cells and negative reaction in stromal cells in all cases. In one case it showed diffusely positive reaction in tumor cells and regional positive in stromal cells. MMP-9 showed a diffuse

positive reaction in tumour cells and stromal cells of serous endometrial adenocarcinomas. In clear-cell endometrial adenocarcinomas, MMP-9 showed weak positive reaction in tumor cells and stromal cells.

DISCUSSION

The completion of the histopathologic diagnosis in endometrial hyperplasias and adenocarcinomas with the immunohistochemical study has unquestionable value in assessing the prognosis of these lesions (13).

The results of the immunohistochemically study of ER and PR, PTEN, MMP-2, MMP-9, C-erbB-2, COX-2 and Ki 67 showed the value of these markers in differentiating the complex endometrial hyperplasia from the endometrioid adenocarcinomas.

The benign squamous metaplasia adenocarcinoma presents immunohistochemically positivity for ER and PR and it is rarely positive at Ki-67 and p53.

The malignant squamous metaplasia adenocarcinoma presents rare positivity for ER and PR and it is positive at Ki-67 and p53. No aspects of stromal invasion are identified at the serous intraepithelial carcinoma. In terms of immunohistochemistry, tumour cells are positive for Ki-67 and p53 and with focal positivity for ER and PR.

In our study, in simple and complex endometrial hyperplasia with no atypia, PTEN showed regional positivity in glandular epithelial cells, and in only one case of complex hyperplasia with no atypia the reaction was negative.

Both in the well-differentiated endometrioid endometrial adenocarcinomas (GI) and in the moderately differentiated adenocarcinomas (GII) in our cases, the expression of PTEN was regionally positive in tumour epithelial cells.

In weakly differentiated adenocarcinomas, represented by serous adenocarcinomas and clear-cell adenocarcinoma, the PTEN expression was weak.

In a study on the development of endometrial carcinomas, show that after various molecular alterations, represented by microsatellite instability and mutations of PTEN, K-ras and beta-catenin, the endometrial cells are transformed in endometrioid carcinoma, and the modification of the expression of p53 protein and the loss of LOH (loss of heterozygosity) on different chromosomes, leads to the development of endometrial non-endometrioid tumours (5).

PTEN is an early change that occurs in the pathogenesis of the endometrioid endometrial adenocarcinoma (6). These studies demonstrate that the immunohistochemically identification of PTEN protein is an important marker for endometrial premalignant lesions.

The PTEN mutations are important in the pathogenesis of the endometrioid endometrial adenocarcinoma. These mutations are shown in the normal mucosa, in postmenopausal women and in complex endometrial hyperplasia, aspects which suggest that PTEN mutations show an early change that occurs in the pathogenesis of the endometrioid endometrial adenocarcinoma (6). These studies demonstrate that the immunohistochemically identification of PTEN protein is an important marker for endometrial premalignant lesions (10).

Some authors (7) have demonstrated that the number of the progesterone receptors is dependent on the concentrations of estradiol and the progesterone in the serum. It was found that PR is more often positive in endometrial hyperplasia without atypia compared to the hyperplasia with atypia.

PR is expressed at a lower level in the endometrial carcinoma compared to the endometrial hyperplasia.

In endometrial carcinomas, the Her-2/neu positivity is correlated with ER negativity and it is associated more commonly with the existence of metastasis. This suggests that Her-2/neu positivity is associated with a much more aggressive evolution (8).

In our study, regional positivity scoring 2+ was found only in the serous endometrial adenocarcinoma, which is considered a weakly-differentiated carcinoma. In the clear-cell carcinomas and the endometrioid adenocarcinomas under study, the C-erb-2 reaction was negative.

In our cases, in endometrial hyperplasia, the MMP-2 and MMP-9 expressions were different, with a better expression of the MMP-9 than that of the MMP-2. MMP-9 showed regional expression in glandular epithelial cells and in stromal endometrial cells, both in simple hyperplasia with no atypia and in complex hyperplasia with no atypia. MMP-2 showed regional positivity in glandular epithelial cells only in 50% of simple and complex hyperplasia with no atypia, which was accompanied by a positive reaction in the endometrium stroma.

The antigenic profile of endometrial adenocarcinomas is different. The endometrioid adenocarcinoma is positive to ER and PR, negative to C-erb B-2 and it shows low positivity for Ki67. The non-endometrioid adenocarcinoma is negative to ER and PR, positive to C-erb B-2 and it shows a high Ki67 proliferation risk. The endometrioid adenocarcinoma is associated more frequently with PTEN mutations, compared to the non-endometrioid adenocarcinoma. In endometrial adenocarcinomas, MMP-9 is expressed more intensely than MMP-2 and it correlates with a low degree of differentiation of adenocarcinomas. In our study, COX-2 shows a positive diffuse reaction in most endometrial adenocarcinomas and in non-endometrioid adenocarcinomas (11).

In the endometrial hyperplasia of the cases under study, the expression of COX-2 showed a weak positive reaction regionally in glandular epithelial cells. An exception was a case of simple glandular hyperplasia with no atypia, in which the COX-2 was positive diffusively in glandular epithelial cells; this reaction can be explained by the patient's young age, correlated to her hormonal status (active genital period).

In all endometrial adenocarcinomas in our study, the COX-2 showed a positive reaction diffused in the tumoral cells, except for a case of endometrioid endometrial carcinoma GII, which showed a weak positive regional reaction.

In our cases, we noticed a positive correlation between the level of the Her-2/neu signal and the COX-2 expression in the serous endometrial carcinoma.

CONCLUSIONS

In our case studies, well-differentiated endometrioid endometrial adenocarcinomas (GI) and moderately differentiated (GII) ones had a relatively low content in the ER and PR of the tumor cells compared to the simple and complex hyperplasia without atypia. Well-differentiated endometrioid endometrial adenocarcinomas were ER and PR-positive, so that the ER expression correlated with the PR expression. Well-differentiated endometrioid endometrial adenocarcinomas (GI) in the studied group also showed a higher content of ER and PR compared to the endometrial moderately-differentiated endometrioid endometrial adenocarcinomas (GII).

In non-endometrioid adenocarcinomas,

represented by clear-cell endometrial adenocarcinomas, the ER content was reduced and the PR expression was negative. Serous adenocarcinomas failed to show an immunohistochemically expression for ER and PR.

The loss of alleles and mutations in p53 have been detected not only in endometrial carcinoma but also in atypical hyperplasia. These findings suggest that p53 inactivity is a late event in the endometrial carcinogenicity.

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OUTCOME OF INTERNAL HEMIPELVECTOMY IN A PATIENT WITH MULTIPLE MYELOMA - CASE REPORT

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OUTCOME OF INTERNAL HEMIPELVECTOMY IN A PATIENT WITH MULTIPLE MYELOMA: CASE REPORT (Abstract). A patient treated for thyroid cancer was diagnosed with multiple myeloma in the context of trauma. After the pelvic tumor devascularization she underwent internal hemipelvectomy Type I + IIA Enneking Classification using polymerizing bioneutral gel embolization, "Glubran 2". The purpose of this case report is to demonstrate the importance of careful selection of treatment methods, together with adequate physical therapy in order to obtain favorable long-term results. **Keywords**: HEMIPELVECTOMY, MYELOMA, RECOVERY.

Internal hemipelvectomy is a surgical limb-sparing procedure used to treat malignant tumors of the pelvic ring. The most commonly used classification for hemipelvectomy is that by Enneking classification (1, 2), which distinguishes 4 types depending on the anatomical components removed, combinations of different types being also possible. We report the case of a female patient with multiple myeloma in association with multiple malignancies (3),who underwent Enneking Type I+IIA internal hemipelvectomy on January 21, 2008. The importance of adjuvant treatments and rehabilitation methods on patient outcome is discussed.

CASE REPORT

Patient E.G., 47-year-old female, was diagnosed on October 25, 2004 with nodular goiter and underwent surgical removal of the thyroid tumor. On April 13, 2007, the patient suffered an injury by falling from a height of about 1m with impact on the right hemipelvis. At physical and radiological examination a massive tumor involving the entire right iliac fossa was detected. The tumor was highly vascularized and its extra-compartmental component was displacing the iliac muscle together with the adjacent arteriovenous vascular groups.

On June 15, 2007 an angiography was performed. It detected a tumor occupying the right iliac fossa, highly vascularized, receiving blood supply from emerging vessels from right hypogastric artery and right lumbar artery IV. Tumor embolization was performed using Glubran 2, a bioneutral polymerizing gel,a total tumor devascularization being obtained (figs. 1, 2). On January 21, 2008, after a surgical biopsy was performed, the patient was diagnosed with myeloma. On the same day, the patient un-

derwent internal hemipelvectomy type I (iliac radical resection with gluteal muscles and sciatic nerve preservation) and IIA (excision of the whole acetabulum), according to Enneking classification (fig. 3).

The resection of the right hemipelvis was performed with the following margins 17x16x10 cm, together with the collection of a tumor mass measuring 11x10x9cm, which was consisting of translucent yellow-brown bulky tissue.

Macroscopically, the tumor occupied a part of the iliac fossa, expanding beyond the cortical component to the surrounding soft tissues and reaching the sacroiliac resection edge.

Microscopically, the osteomedullary tissue was infiltrated with a population of plasma cells. Rapid tests, together with immunohistochemistry studies confirmed the diagnosis of myeloma.

After surgery a rehabilitation program was initiated. After the removal of suture threads, 20 days after surgery, the patient was started on active and passive range-of-motion hip mobilization pool exercises. With the aid of two crutches, the patient resumed walking with left leg weight bearing and right leg in flexion up to 60 degrees, considering the possible risks of early right hip mobilization. A strengthening exercise program for gluteal and right thigh muscles was also started. Forty days after surgery, the patient began the progressive weight bearing on the affected limb. One year after surgery the patient started to walk without using any equipment. The patient presents a leg shortening of 8.7 cm together with a limitation of rotation movements. Up to now, the patient did not present any complication, did not require administration of analgesics, and has already exceeded the average survival rate, seven years after surgery.

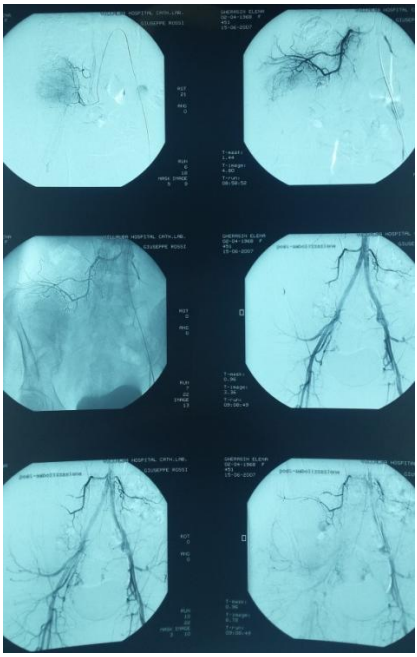

Fig 1- Angiography showing tumor blood supply

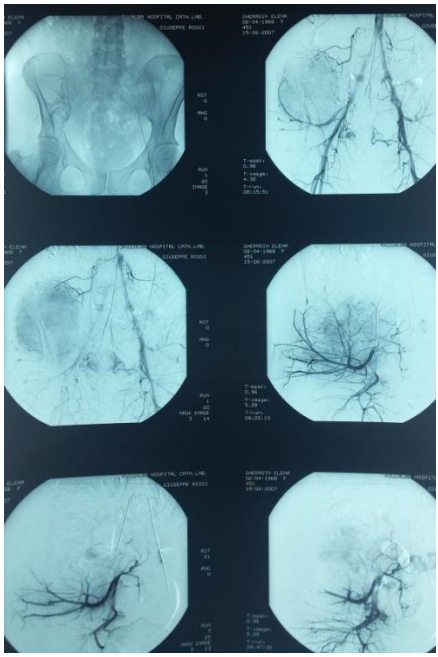

Fig 2 – Embolization using Glubran 2 gel

Fig. 3. Internal Hemipelvectomy type I+IIA Enneking

DISCUSSION

Oliveira et al. (1) state that with adequate physical therapy patients who undergo internal hemipelvectomy without further reconstruction may walk again after the pelvic fibrous plaque forms in about 1-3 months after surgery. Our patient did so 40 days after surgery. She showed no recurrences or complications, a satisfactory functional outcome and a favorable long-term prognosis, which demonstrated a pattern of myeloma evolution after internal hemipelvectomy similar to that of other types of malignancies reported in the literature (1, 2, 4, 5). The patient does not require any pain medication unlike the patients who undergo amputation, is in good psychological state and socially integrated, results in agreement with previous studies (2, 4, 5).

CONCLUSIONS

In our patient, internal hemipelvectomy was the treatment option that spared the affected limb and avoided amputation, which is a traumatic radical alternative. Our particular case demonstrates a good functional recovery, regaining the ability to walk independently. The lack of complications and recurrences confirms the appropriate therapeutic selection. Indications for reconstruction should be reconsidered and individualized in order to achieve a long-term survival at the cost of weaker functionality.

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ADDITIONAL DEMOGRAPHIC AND CLINICAL EVIDENCES ON THE RELEVANCE OF THE SYSTEMIC THERAPY IN ALCOHOL DEPENDENCE

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ADDITIONAL DEMOGRAPHIC AND CLINICAL EVIDENCES ON THE RELEVANCE OF THE SYSTEMIC THERAPY IN ALCOHOL DEPENDENCE (Abstract). The modern treatment for alcohol dependence is still problematic, in many cases with the costs exceeding benefits. In these conditions a new management approach was developed lately, known as the systemic therapy. In this way, the crystallization and practical transposition of this new treatment approach is represented by the Clubs of Alcoholics in Treatment. These clubs are in fact a form of psycho-social intervention consisting of multi-family communities in order to maintain long-term abstinence from alcohol and to change their lifestyle and behavior. Thus, in the present paper we were interested in understanding the demographics of this systemic theory and how these aspects are influencing the final results of the therapy, as well as studying/confirming how relevant is this systemic approach on the management of alcohol dependence. Our results presented in this report bring additional evidences for the superiority of the systemic, multi-family approach of alcohol-related problems, as complemented to the standard medicinal therapy. Moreover, the data collected from patients in this study might suggest that patients with a higher educational level and therefore better capacity of understanding the information, with family support, and also with a better occupational insertion, have accepted to follow The Clubs of Alcoholics in Treatment program, with a subsequently better evolution. **Key words:** ALCOHOL DEPENDENCE, SYSTEMIC THERAPY, DEMOGRAPHICS.

Alcohol consumption is a universal widespread activity. In this way, some numbers regarding alcoholism provided by the National Institute of alcohol abuse and Alcoholism are showing that in 2013, 24.6 % of people ages 18 or older reported that they engaged in binge drinking in the past month, while 6.8 % reported that they engaged in heavy drinking in the past month (1). In addition, nearly 88,000 people (approximately 62,000 men and 26,000 women) die from alcohol-related causes annually, making it the third leading preventable cause of death in the United States (2,3).

However, the alcohol consumption patterns are variable. As a matter of fact, excessive consumption of alcohol can take many forms, from daily consumption of large quantities to repeated episodes of consumption with intoxication or from alcohol addic-

tion behavior to consumption resulting in impairments of health status. However, there is not a very clear distinction between social/occasional drinking of alcohol and abusive consumption. From the medical point of view, there are two notions that are associated with consumption in excess of alcohol: addiction and abuse (4).

There are various approaches in the literature regarding the management of the alcoholism (5,6). One of these approaches is regarding the problem of alcohol AS based on the ecological and social systemic theory, specifying that consumption of alcohol is actually a type of behavior, a way of life. The crystallization and practical transposition of this ideology is represented by the Clubs of Alcoholics in Treatment. These clubs are in fact a form of psycho-social intervention consisting of multi-family communities in order to maintain long-term abstinence from alcohol and to change their lifestyle and behavior (7). Moreover, alcoholism has a huge impact at the individual level, through medical, socio-familial and financial problems involved, but at the same time it is a major public health problem. Thus, alcoholism and the problems arising from it require a complex therapeutic approach, not only medical but also social and psychotherapeutic, centered both on the individual and his family.

Moreover, there are studies bringing evidence in support of a community-based management in post-hospital phase. For example, Mann and his collaborators have published a study that evaluated a model of community-based treatment combining a six-week period of hospitalization with one year of ambulatory therapy. The results of the research have shown that a subgroup of alcoholics appear to benefit from the community-based programs. This subgroup is characterized by a good social adjustment and their relatives' desire for active participation in the treatment (8).

In this way, in the present paper we were interested in understanding the demographics of this systemic theory and how these aspects are influencing the results of the therapy, as well as studying/confirming how relevant is this systemic approach on the managements of alcohol dependence.

MATERIAL AND METHODS

The present study involved clinical therapeutic evaluation of 85 patients with alcohol dependence within a period of 3 months. The patients evaluated in the study were in the evidence of the Clinical Psychiatric Hospital Socola in Iaşi. The patients have been diagnosed with alcohol dependency in accordance with the criteria DSM-IV tr. These patients presented or not psychiatric or somatic comorbidity (with the exception of those specified in the exclusion criteria). All patients received standard medication for alcoholism (mood stabilizers, benzodiazepines and vitamin therapy).

Patients included in the study were patients aged between 18 and 63 years, with alcohol dependency (according to DSM-IV tr), who have expressed their desire for the cessation of alcohol. The following exclusion criteria have been used: patients who have completed treatment or psychotherapy for alcohol dependence during the past 90 days, patients without discernment, other associated somatic decompensated conditions or which could worsen the patient clinical status during the participation in the study.

After screening, patients were distribut-

ed into two approximately equal groups (based on their consent to participate in the therapy carried out in the Clubs of Alcoholics in Treatment). Thus, 42 patients with alcohol dependence have received standard medical treatment and entered the program of Hudolin systemic psycho-social intervention and 43 patients with alcohol dependence have received only standard medical treatment.

The evaluation of patients with alcohol dependence was performed with the AUDIT scale (The Alcohol Use Disorders Identification Test) and the quality of life scales (QOL 16 The Quality of Life Scale).

The AUDIT scale (Alcohol Use Disorders Identification Test) is a questionnaire which can be self managed, containing 10 items and assessing the behavior and problems associated with alcoholism, as well as harmful consumption of alcohol (items 1 to 3), the behavior associated to consumption (items 4-6), adverse reactions to alcohol (items 7-8) and the problems associated with alcohol (items 9-10). Items 1-8 scores vary from 0 to 4, while items 9-10 may be scored 0, 2 or 4. The scores range between 0 and 40, higher scores being associated with a greater risk. Scores between 8 and 12 indicate a harmful consumption, while the scores 13-40 indicate alcohol dependency. Studies performed on this scale show a high sensitivity and specificity (9). The level of alcohol dependence and consumption prior to inclusion in the study was assessed through this scale at the time of the inclusion in the study.

The quality of life scale QOL 16 (The Quality of Life Scale) originally developed by Flanagan in the 1970s (10), is a scale used to evaluate the quality of life in people suffering from chronic conditions. Is a Likert Scale whose items have 7 response possibilities ranging from: delighted (=7) satisfied (=6), rather satisfied (=5), mix (=4), rather dissatisfied (=3), miserable (=2), awful (=1). It is composed of 16 items, with an additional item compared to the original version, namely the 16th item, which refers to the independence degree and self-care ability. The other five evaluated fields include: physical and material well-being, relations with others, social, civic and community activities, and personal development and fulfillment, recreational activities. It is a self-managed scale whose total scores range between 16 and 112. Higher scores indicate an increased level of quality of life.

DATA ANALYSIS

The sampling included as stages the determination of the sampling frame (screening of Socola Hospital patients) and the statistical analysis with appropriate use of statistical tests. There were used descriptive methods (for demographic data analysis), comparative methods (single Factor Anova test), as well as prospective methods for tracking the evolution in time of the patients. The data were loaded and processed using the statistical functions in Microsoft Excel (Microsoft Corp.) and SPSS16 statistical analysis package (SPSS Inc.). The results are expressed as mean ± standard deviation (SD). Statistical interpretation took as reference point the value of 0.05, p (Sig.) <0.05 with statistical significance.

RESULTS

In this way, several preliminary results of the current study will be presented, with particular reference to the demographics obtained, as well as changes in quality of life (up to 3 months).

Analysis of demographic data

In terms of gender, all patients included in the study were male. Regarding the age of the patients included in the group who benefited from the combined therapy (systemic plus pharmacological), the average age was 48.6 ± 10.2, with the standard error of 1.53, while the patients who received standard therapy only, had a relatively close average age, 42.7 ± 10.5, with an standard error of 1.55.

Also the statistical analysis for the background of origin shows a slightly higher percentage of people coming from rural areas (52.17%), when compared to patients who come from urban areas (47.82%) within the group treated with standard medication, while if referring to the patients who opted for systemic therapy, a higher percentage of people with urban background (63.63%) was registered, as compared to rural areas (36.36%).

In terms of marital status, in the patients included in the associated therapy, the percentage of married patients is the highest (70.45%), followed by divorced patients (13.63%), unmarried (9%) and widowers (6.81%). In patients who were included in the standard therapy, most patients were unmarried (44%), followed by married (28%), divorced (24%) and widower patients (4%).

Also, most patients from both groups have an average education level and none of them is unstudied. Thus, for patients who have opted for the combined therapy, a percentage of 38.63% have not graduated from high school, 27.27% of them have graduated from 12 classes, a percentage of 20.45% have post-secondary and faculty studies and only 2.2% have only 8 classes or less. Also, in the case of the second group, most patients have not graduated from high school (44%), followed by patients with 8 classes or less (41%), 11% have graduated from high school and only 4% of them have post-secondary studies.

In both groups of patients, most of them were without occupation (47.72% in the group with combined therapy and 69% in the lot with standard therapy). Close percentages between the two batches were recorded for the retired patients (22.72 % in the group with combined therapy vs. 24% in the group with standard therapy).

Only the percentage of employee patients clearly differ for the two groups, a much higher percentage of employees being recorded in the group subjected to combined therapy (29.54%), as compared to the group that received the standard treatment (7%).

In both groups most patients were smokers, and the proportion between smoker and non-smoker is preserved in the two batches, as 61.36% patients were smokers in the combined therapy group, when compared to 69.56% in the standard therapy group.

The AUDIT scale was applied to all patients at the inclusion in the study. However, there were no significant differences in terms of statistics between the 2 groups of treatment, which is important as it proves the homogenity of the two batches ($F(1,89) = 3.94$, $p = 0.11$) (fig. 1).

Also, **the scale for the quality of life** QOL 16 was applied to each visit. On the baseline, the means for the scale between the two groups of patients were very close, with the mean for the combined therapy group being 74.8 ± 18.64, while for the standard therapy group it was 73.6 ± 9.6. As seen in fig. 2, there were no significant differences between the quality of life scale scores of the two groups of patients

(F=0.11, p=0.7).

Still, the application of the QOL16 scale at one month, highlighted significant differences between the two groups, with a higher mean for the combined therapy patients (88.1 ± 15.4), when compared to 80.25±14.4, the mean of the other group of study (F=4.29, p=0.04) (fig. 3).

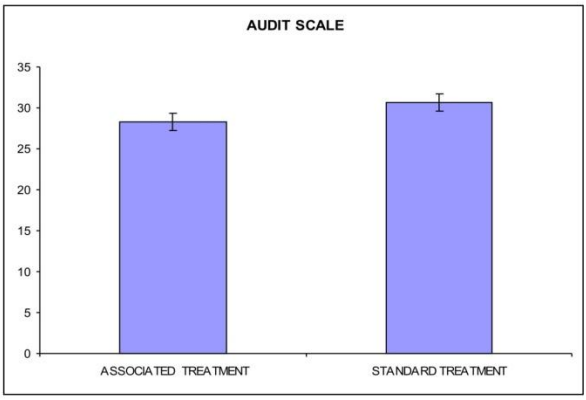

Fig. 1. Comparison among AUDIT scale scores for the two gropus of patients.

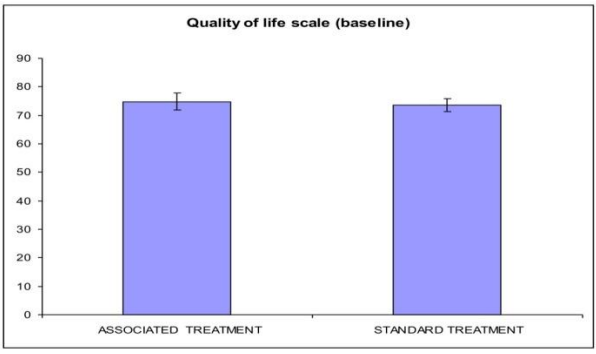

Fig. 2. Comparison between QOL16 scores at baseline for the two groups of patients.

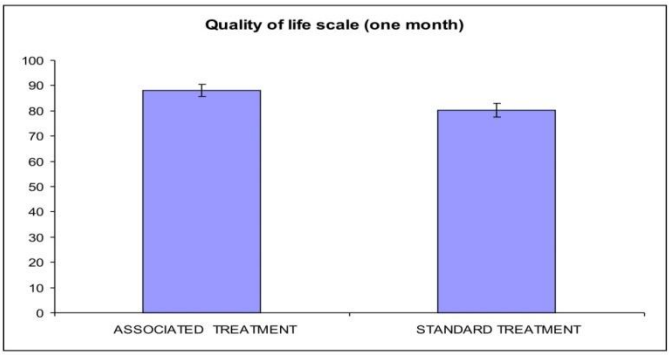

Fig. 3. Comparison between QOL 16 scale scores – one month between the two gropus of patients.

Consequently, the QOL 16 scale was applied at periods of 3 months and increased statistical significant differences between the 2 groups of patients were also obtained (F=10.71, p= 0.01). Moreover, as it can be seen in the chart below, the difference between average values was approximately 10 points (fig. 4).

Fig. 4. Comparison between QOL16-3 months between the two groups of patients.

DISCUSSION

In this way, the data presented in this article is providing additional evidences for the superiority of the systemic, multi-family approach of alcohol-related problems, as complemented to the standard medicinal therapy.

Also, regarding the demographical results we obtained in the current report, our study was applied only on male population, as a result of the division profile where the sampling was made. There is also a predominance of the urban environment in the case of the group that benefited from systemic therapy, and this could be explained by the particularities for the location of the Clubs of Alcoholics in Treatment, as they are organized mainly in the city, while the patients from rural areas have somehow a reduced access possibility.

Also, in terms of marital status, the majority of patients who have opted for combined therapy were married (70.45%), while the majority of patients who followed standard therapy were unmarried (44%). In fact, one of the features of the systemic approach to alcoholism is the active participation of the family in the recovery process of the addicted persons. The same issue could be interpreted also by the fact that marital status can confer an additional motivation (either through the encouragement and support of the family, either by responsibility and commitment assumed by the patient in front of his family) in committing to this type of therapy. At the same time, it may be that the spouse is more available and more interested in participating at these meetings than a partner without marital commitment.

With regard to the level of education, a relative symmetry for the considered groups has been noticed. No patient in the study was unschooled. The group of less schooled patients is the one that received just the standard therapy, while most patients with intermediate and higher level of education were in the category of patients which benefited from the combined therapy. We can speculate, as a result of these aspects, that the level of education could be a positive factor in the patient's desire to

become abstinent, as well as in the capacity of understanding the potential benefits brought by a systemic approach, thus giving the dependent patient an extra motivation towards the acceptance of new solutions to the problem of alcoholism.

From the point of view of their occupation, the majority of patients in both groups were without occupation. This aspect could be interpreted differently. Either alcohol dependence has led or contributed to the loss of the service, or adherence to a service is a protective factor for alcoholism. Considering that most patients who were employed were in the combined therapy group, the explanation that the service could further motivate the individual wishing to treat himself by maintaining a certain social status and financial support, as well as fear of dismissal, may be justified.

Further analysis of tobacco consumption, has revealed that the majority of patients in both treatment groups were smokers. Incidentally, this was to be expected considering that there is a well-known correlation between alcohol consumption and tobacco. In this way, it is estimated that 80% of alcohol addicted persons smoke. In our study, we found smaller percentages of smokers (a general percentage of 65%) than in the general population, with a slightly higher percentage (almost 70%) in the standard treatment group. The results of studies that examined the connection between smoking and alcohol showed that nicotine not only correlates with alcohol consumption, but may also be a prediction factor for the severity of alcoholism (11, 12).

Additionally, our statistical data showed very clearly the homogenity of the studied groups, which constitutes a necessary prerequisite for comparing the results obtained in the course of research (13). In this way, no significant differences between groups were found in terms of demographic data.

The severity of alcohol dependence was assessed using the AUDIT scale. This was done for all the patients from the study, even from the beginning (before patients were divided into two groups of treatment). The AUDIT scale assesses the behavior and problems associated with alcoholism and harmful alcohol consumption. The application of this scale revealed high overall scores, indicating an increased severity of the addiction.

Also, very importantly, calculating the total scores means after the distribution of patients in different groups revealed similar mean values (around 30 for both groups), without significant statistical differences. This indicates the correct prerequisites for our research performed here, having homogeneous groups of study and a high level of alcoholism severity for the both groups of patients. In fact, this is supported by the scientific data showing that increased scale scores predict the severity of alcoholism (14, 15).

Thus, the data collected from patients in this study might suggest that patients with a higher educational level and therefore better capacity of understanding the information, with family support, and also with better occupational insertion, have accepted to follow The Clubs of Alcoholics in Treatment program with a subsequently better evolution. Also, it seems that the systemic therapy associated with standard therapy provides additional value in terms of improving the quality of life.

CONCLUSIONS

The data presented in this study shows the additional benefits that systemic therapy provides for patients with alcohol related problems and their families. In this way,

our results can be resumed into the following conclusions:

1. The systemic method additionally improves the quality of life in patients with alcohol dependence, when it is associated with standard therapy.

2. The improvement in the quality of life is initiated on the very beginning of therapy, starting with the first month of systemic therapy associated with standard treatment.

3. The systemic method brings also long-lasting benefits, as demonstrated by the increase in time for the quality of life scale scores.

4. Patients with a higher educational level, with family support, and also with better occupational insertion have a better evolution.

5. The systemic method has exhibited a beneficial role in the post-hospital therapeutic phase management for patients with alcohol dependence, when it is associated with standard therapy.

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ZINC IMBALANCE AND THE OPPORTUNITY OF ZINC SUPPLEMENTATION IN ALCOHOLICS AND DRUG USERS

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ZINC IMBALANCE AND THE OPPORTUNITY OF ZINC SUPPLEMENTATION IN ALCOHOLICS AND DRUG USERS (Abstract): Zinc deficiency is well-documented to be associated with alcoholism, being considered a consequence of unbalanced eating habits in such patients. The present review focuses on zinc imbalance in drug addicts and on the possible beneficial role of zinc administration as dietary supplement. All relevant literature published up to May 2015 was searched. The search was performed using the keywords "zinc" plus "alcoholism", "addiction", "dependence", and the name of different drugs of abuse (classes or representatives). Human and animal studies were included. In alcoholism, zinc deficiency is not only due to malnutrition and malabsorption which are characteristic to this condition, as there is evidence of increased elimination and altered distribution of zinc. In opioid addicts, zinc deficiency is also commonly encountered and opioid administration in animals determines changes in zinc distribution. Studies regarding the zinc status in other drug abusers are inconsistent. Zinc deficiency in drug addicts also seems to affect some neural mechanisms involved in the downward spiral of addiction, as decreased zinc is associated with depression-like symptoms, known to induce drug-taking behavior. Moreover, zinc deficient animals are resistant to dynorphin-induced feeding. **Conclusion**: Based on evidence in the medical literature, the present review pleads for a beneficial role of zinc supplementation in alcoholics and opioid-users, not only in order to correct or prevent a metabolic imbalance, but also to influence some cerebral mechanisms involved in drug-taking behavioral patterns.
Keywords: ZINC, DEPENDENCE, ADDICTION, ALCOHOLISM.

Zinc, the second most important trace element in humans (after iron) (1), plays mainly structural and catalytic roles (2). It is important for the antioxidant defence, as a co-factor of Cu-Zn superoxide dismutase (3). Zinc finger proteins acting as transcription factors regulate gene expression (4). The brain is one of the organs with highest Zn^{2+} levels, containing approximately 1.5% of the estimated 2-3 g of zinc in the human body. Within brain zinc is nonuniformly distributed: the olfactory bulb has the highest level, followed by the frontal and parietal cortices (5). Within the central nervous system, zinc influences synaptic plasticity, hormone release, and nerve impulse transmission (6).

Zinc deficiency affects over 15% of world population and the prevalence of inadequate intake varies from 7.5% in high-income regions to 30% in South Asia (7). More than 80% of pregnant women

have a zinc intake lower than recommended (8). Severe deficiency is rare, but mild and moderate zinc deficiency is widespread. Deficiency symptoms are rather nonspecific, including growth retardation, male hypogonadism, delayed wound healing and cell-mediated immune dysfunction (9), but response to repletion occurs very fast (10). Disorders mainly related with brain dysfunction (behavioral changes, depression, emotional instability, anxiety, socializing deficits, impaired memory and learning, anorexia, autism spectrum disorders, attention deficit hyperactivity etc.) are frequently associated with zinc deficiency (11, 12). In some of these conditions, it is debatable if decreased zinc is a consequence or a cause. Under moderate zinc deficient diet, brain zinc is maintained within normal limits, due to increased uptake (13).

Addiction is a chronic, relapsing disorder, characterized by loss of control over drug-taking. The association between zinc deficiency and alcoholism is well-known, being considered a consequence of unbalanced eating habits in such patients. There is also evidence of decreased zinc levels in opioid-users. On the other hand, zinc deficiency reduces food intake and causes depressive-like symptoms, known to induce drug-taking behavior. In this context, the present review focuses on zinc imbalance in drug-addicts and alcoholics and aims at discussing the possible beneficial role of zinc administration as dietary supplement in such persons.

All relevant literature up to May 2015 was searched. The search was performed using the keywords “zinc” plus “alcoholism”, “addiction”, “dependence”, “smoking” and the name of different drugs of abuse (classes or representatives). Human studies on the status of zinc and other metallic trace elements possibly interacting with zinc in addicts (alcoholics including) were included. Animal studies on the interaction between addictive substances and zinc metabolism were also considered.

In order to discuss the opportunity of zinc supplementation, searching strategies were consequently adapted.

RELEVANT FINDINGS

There are many studies reporting low zinc levels in drug addicts and drug users, most of them referring to alcoholics and opiate users. To our knowledge, studies on zinc levels in nicotine addiction or cigarette smoking are scarce, whereas those referring to zinc status in users of psycho stimulants or cannabis are absent. Low zinc status in case of different types of drug addiction is documented to represent rather a consequence of the toxic capacity to influence zinc distribution and elimination than a factor predisposing to drug-seeking behavior; however, malnutrition is common among drug addicts (14), especially in women (15), and this might represent an explanation for the low zinc levels in these subjects.

A. ***Alcohol***

a. *Human studies*. Decreased serum zinc in alcoholics has been widely reported and known for a long-time. About 90% of alcoholics, with or without liver disease, were reported to have inadequate dietary zinc intake (16). It is believed that long-term ethanol consumption is responsible for zinc malabsorption, since it was shown that decreased zinc absorption only occurs in patients with alcohol-induced cirrhosis, where $ZnCl_2$ labeled with 65Zn was absorbed in a reduced proportion compared to controls (37±17% vs. 55±16%, $p<0.01$),

whereas in non-alcoholic cirrhotic patients zinc absorption was similar to that of healthy controls (47±11%) (17). Similar results on reduced zinc absorption were also reported by Dinsmore et al. (18). In delirium tremens patients, zinc is decreased in both serum and erythrocytes (19). Alcohol users' exhibit increased urinary zinc elimination (20); the reduced zinc-binding capacity of albumin in patients with decompensated alcoholic cirrhosis is probably responsible for this (21). Controversial data regarding zinc metabolism in alcoholic cirrhosis have also been published: Mills et al. (22), reported increased intestinal absorption, whole-body content and total daily elimination of zinc in alcoholic cirrhosis, but with no changes in the biological half-life of zinc.

b. *Animal studies*. Interestingly, one study investigating the effect of 20% ethanol solution administration (intragastric, 2 g/kg daily) for five days, revealed that, on the day 5 animals presented higher zinc levels in serum and heart, but decreased zinc concentration in brain, lungs, kidney, liver, spleen and muscles (23).

B. ***Opioids***

a. *Human studies*. Low serum zinc levels are reported in heroin users, with a slight tendency to increase during detoxification, yet not reaching control level (0.815±0.144 mg/L on admission for treatment and 0.859±0.205 mg/L at the end of hospitalization in opiate users vs. 1.118±0.114 mg/L in controls) (24). Moreover, zinc deficiency is correlated with the period of abuse in heroin addicts (25). Low serum zinc level is accompanied by increased serum copper level (26), the same being reported for the intraerythrocytic levels of zinc and copper. The antagonism between copper and zinc in both intestinal absorption levels and biological actions is well documented (27); the competition between zinc and copper at the level of DMTa transporter seems to be responsible for this. Copper facilitates zinc elimination though urine, but copper excess facilitates zinc storage (28). The antagonism between zinc and cupric bivalent ions occurs in the conditions of certain chemical similarities between them. So, as decreased zinc is accompanied by increased copper, it is reasonable to consider that zinc deficiency has better conditions to more clearly manifest in opioid users. Zinc level in the cerebrospinal fluid of former heroin addicts is lower than in controls, but generally within normal ranges (29). During methadone detoxification in opioid addicts, zinc elimination is strongly increased (600±50μg/g creatinine in patients vs. 300±50μg/g creatinine in controls, $p<0.001$), while copper elimination is slightly, but significantly decreased (23±3μg/g creatinine in patients, $p<0.05$ vs. controls) (30). On the other hand, heroin samples on the illegal market were shown to contain variable amounts of trace elements, including zinc, but these have little contribution to zinc intake and product toxicity (31) and they are believed to appear from the metallic container used in the processing/cutting stage (32).

b. *Animal studies*. Morphine administration in rats decreases the serum zinc level, but it increases liver cytosolic zinc and metallothionein (a family of cysteine-rich proteins involved in both zinc and copper metabolism) levels. If the effect of morphine on liver metallothionein levels is mediated mainly by the morphine effect on specific receptors (being antagonized by naloxone), zinc accumulation in the does not recognize this type of mediation, as

naloxone does not alter it; however, glucocorticosteroid and β-adrenergic mediation seem to be at least in part responsible for the increase in zinc and metallothionein liver levels, as their antagonists (RU 486 and labetalol, respectively) diminish that increase (33). Increased liver metallothionein levels (3-folds vs. control) were also reported by other authors (34), these being accompanied by increased brain and kidneys metallothionein levels (5 and 2-fold, respectively vs. controls). Interestingly, one study on the effect of morphine escalating doses in mice (intraperitoneal; 15 mg/kg on day 1; 30 mg/kg on day 2; 45 mg/kg on day 3; 60 mg/kg day 4; 50 mg/kg on day 5) showed on day 5 animals presented increased serum zinc concentration and increased zinc levels in the heart, spleen and muscle, but decreased in the brain, lungs, kidney, and liver (23). Intracerebroventricular administration of the δ-opioid agonist (D-Pen2, D-Pen5) enkephalin results in decreased Zn^{2+} content in parietal cortex, hippocampus and striatum; the effect is time- and dose-dependent and it is antagonized by naloxone pre-treatment (5).

C. ***Nicotine addiction and smoking***

a. *Human studies*. A positive association between smoking and serum zinc concentration ($p=0.0003$) was evidenced, but only in women (35), whereas other studies report no influence of smoking habit on zinc levels in urine (36), placenta, cord blood and maternal blood of women at delivery (37). However, the hair of tobacco smokers contains less zinc, calcium, magnesium, iron, and copper compared to controls (38).

b. *Animal studies*. Infusion of 50 mg nicotine for 21 days in rats did not modify serum zinc levels, but increased Cu-Zn superoxide dismutase activity (39).

DISCUSSION

In alcohol addiction the associated malnutrition and malabsorption are considered the cause of lower magnesium and zinc levels. However, increased urinary zinc elimination, probably related to decreased albumin capacity to bind zinc and zinc altered distribution, is also involved. Eating-related characteristics, as well as kinetic particularities, may also explain the tendency to zinc deficiency in opioid addicts. On the other hand, when discussing the eating habits, it should not be disregarded that zinc deficiency is associated with anorexia and zinc ions are important regulators of eating and liquid-intake patterns. For instance, acute third ventricle injection of minute zinc amounts (3 to 300 pmol) induces a dose-dependent antidipsogenic action in dehydrated rats, almost complete at the highest dose. The effect is reversed by naloxone, indicating that it may be, at least in part, due to stimulation of central opioid peptides (40). Zinc-deficient animals have low levels of hypothalamic dynorphin (a leucine-enkephalin opiate peptide and a potent inducer of spontaneous feeding) compared to *ad libidum*-fed controls and are also relatively resistant to dynorphin-induced feeding (41). Zinc ions inhibit anorexigenic proopiomelanocortin neurons in the hypothalamic arcuate nucleus (42). All these suggests that in zinc deficiency-induced anorexia appetite dysregulation is mediated via the endogenous opioid system, including alterations in receptor affinity, a post-receptor defect and alterations in the dynorphin synthesis and/or release.

Decreased zinc influences certain mechanisms responsible for the downward

spiral of addiction, as it is associated with depression-like symptoms (11, 12), which are known to induce drug-taking behaviour (43). Normalizing zinc level in zinc-deficient opioid-users and alcoholics may reduce the use of drug by attenuating depressive symptoms.

As to opioid dependence, there are studies revealing increased naloxone-precipitated withdrawal by zinc chelators (in mice) (44) and decreased withdrawal due to zinc administration during the induction phase of morphine-dependence (in rats) (45). This is an argument for a possible beneficial role of zinc supplementation in opioid users or addicts and it can be explained by zinc ions capacity to decrease opioid binding to μ-opioid receptors, as there is evidence that Zn^{2+} lowers the affinity of different ligands to receptors (46). The idea is reinforced by the low levels of zinc in opioid users.

Assessment of trace element status is often disregarded in drug users. Zinc deficiency is considered a consequence of reduced intake or impaired absorption in alcoholics, but it seems to be also related to increased elimination. In drugs addicts zinc deficiency is not just a dietary imbalance with metabolic consequences, but it also may contribute to the persistence of drug-taking behavior by inducing depressive symptoms. Also, zinc deficiency favors anorexia, which may contribute to further imbalances.

CONCLUSIONS

Our present review pleads for a beneficial role of zinc supplementation in opioid users and alcoholics. This is based not only on the low zinc levels documented in ethanol and opioid users, but also on the fact that correcting zinc status may improve the eating habits and lower the craving by attenuating depressive-like symptoms. The low toxicity of orally-administered zinc salts is an additional argument. The status of trace elements in drug users should be more carefully assessed.

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THE EFFECT OF COBALT CHLORIDE PRECONDITIONING ON PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY

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THE EFFECT OF COBALT CHLORIDE PRECONDITIONNING ON PACLITAXEL-INDUCED PERIPHERAL NEUROPATHY (Abstract) **Introduction**: Recent studies indicate that Cobalt Chloride (CoCl2) modulates mitochondrial activity. There is emerging data suggesting that Paclitaxel-induced peripheral neuropathy (PIPN) is a consequence of the drug's mitochondrial toxicity. AIM: to assess the effect of CoCl2 preconditioning on PIPN in an animal model. **Material and method**: PIPN was induced by 7 daily consecutive i.p. Paclitaxel (PXT) administrations. Male BALB/c mice were divided into three groups as follows: group A - CoCl2 (12.5 mg/kg b.w.) for three weeks (preconditioning) followed by 1 week of PXT, group B - saline for three weeks, followed by 1 week of PXT and group C - saline for four weeks. Thermal and mechanical allodynia were assessed by means of paw withdrawal latency (PWL). **Results**: In group A, CoCl2 preconditioning lead to a decrease in both thermal and mechanical PWLs. 7 days after the last dose of PXT, however, values returned to normal in group A and allodynia for both thermal and mechanical stimuli was noted in group B ($p<0.05$). **Conclusions**: CoCl2 preconditioning seems to protect against PIPN. Although CoCl2 administration decreased thermal and mechanical PWLs, subsequent P administration did not lead to the persistent mechanical and thermal allodynia that was noted in the P-alone group. Further studies are required for determining the exact relationship between CoCl2 and PIPN. **Keywords**: PACLITAXEL, PAINFUL NEUROPATHY, COBALT CHLORIDE

Painful peripheral neuropathy (PPN) is one of the most frequent side effects of Paclitaxel (PXT) – a chemotherapy drug used in the treatment of breast, lung, ovarian and germinal cancers. Its mechanism involves blocking the cell cycle, through centrosomal impairment, induction of abnormal spindles and suppression of spindle microtubule dynamics (1). This antitumor mechanism does not explain, however, the pathogenesis of PXT-induced peripheral neuropathy (PIPN). Recent research suggest that there is a connection between PIPN and its mitochondrial toxicity (2,3).

Cobalt chloride (CoCl2) is a chemical compound widely used in research due to its ability to induce chronic HIF-1 (hypoxia inducible factor-1) activation and, thus, to mimic cellular hypoxia by activating the genes responsible for adapting to low oxygen concentrations. Moreover, CoCl2 stabilizes this factor, which is usually inactivat-

ed once normal oxygen concentrations are again detected. Studies suggest that hypoxic preconditioning has cardio- (4), reno- (5) and neuroprotective (6) effects.

A 2012 study (7) indicates that CoCl2 preconditioning (due to hypoxia adaptation) leads to increased physical performance and enhances mitochondrial metabolism in mice. Other studies, such as the one of Jones et al. (8) demonstrate the protective effect of CoCl2 against perinatal hypoxic-ischemic lesions.

In a recent study, we showed that daily CoCl2 administration for 3 weeks has anti-inflammatory effects in a murine pain model and leads to a decrease in pain behavior, especially during the second phase of the formalin test (9).

The present study aims to investigate the effects of CoCl2 preconditioning on Paclitaxel-induced peripheral neuropathy.

MATERIAL AND METHODS

Animals: 24 male BALB/c mice, weighing 27 to 30 grams were used. Throughout the experiment, the animals had ad libitum access to water and food; the mice were habituated with the testing environment 2 hours prior to testing. The study design and experimental procedures described in this article are in accordance with the European Council Directive 2010/63/EU and follow the guidelines for investigations of experimental pain in conscious animals issued by the University of Medicine and Pharmacy "Gr. T. Popa".

Drugs used in the experiment: CoCl2 (Sigma-Aldrich Chemie BmbH, Germany) and Paclitaxel (Sindan Pharma, Romania); the substances were administered intraperitoneally; doses are in mg per kg body weight (mg/ kg b.w.).

Thermal and mechanical allodynia were assessed by:

Hargreaves, or plantar, test (Hg) was used in order to assess thermal allodynia. Mice were placed in transparent Plexiglas cases. Underneath the heat-conducting floor, a mobile radiant heat source (Plantar Test-37370 UgoBasile) was placed directly under the animal's paw; we measured the time between applying the heat stimulus and the moment when the animal withdraws its paw – response latency to thermal stimuli (paw withdrawal latency – PWL) (10).

The von Frey test, performed by means of the automatic von Frey method, (vF), was used to assess mechanical allodynia. Mice were placed in transparent cases with a wire-mesh floor. Underneath, a mechanical device with a thin filament (Dynamic Plantar Aesthesiometer 37450, UgoBasile) was used to apply increasing pressure onto the plantar side of the animal's paw. We measured the time between applying pressure and the moment when the animal withdraws its paw – response latency to mechanical stimuli (paw withdraw latency – PWL) (11).

The average of three different measurements for each paw (right and left) was used for statistical evaluation, both for thermal and for mechanical allodynia. Hyperalgesia was quantified as the percentage decrease in withdrawal latency (DWL) when compared to baseline:

% DWL = (baseline value - measured value) x 100 / (baseline value).

Study design: PIPN was induced by intraperitoneal administration of 2 mg/kg b.w. PXT for 7 consecutive days. The mice were divided into 3 groups (n = 8 mice/group) as follows: group A – CoCl2 (12.5 mg/kg b.w.) daily, for 3 weeks (preconditioning), then PXT for a week; group

B – 3 weeks of daily saline administration, then PXT for a week; group C – 4 weeks of daily saline administration. The CoCl2 dose was selected in accordance with published data (9). Thermal and mechanical allodynia were evaluated at the beginning of the experiment (baseline), then weekly during CoCl2/saline treatment and twice a week after PXT administration and until the end of the experiment. All tests were performed before the daily administration of CoCl2/PXT/saline. The animals were monitored for 7 weeks.

Statistical analysis: The results are expressed as DWL or as average±standard error. Statistical evaluation was performed by means of the SPSS v.20 software – ANOVA analysis with post-hoc Tukey test. Significance level was set at .05.

RESULTS

Effects on mechanical allodynia

Chronic CoCl2 administrations lead to a progressive decrease of the response latency for mechanical stimuli (vF test). Thus, after two weeks of treatment, PWL average for group A was 6.91±0.12s, while in groups B and C, the average values were 7.84±0.15s and 7.96±0.41s, respectively ($p<0.02$); after three weeks, the average PWL for group A was 5.77±0.26s, while for groups B and C, PWL averages were 7.94±0.08s and 7.31±0.47s, respectively ($p<0.01$). At that time, the CoCl2 treatment group had a response latency 24.8% lower compared to the beginning of the experiment (fig. 1). ANOVA analysis indicated a significant substance effect for days 14 and 21 of the experiment with $F(2, 5.3)=4.73$, $p=0.02$ at day 14 and $F(2, 19.9)=12.56$, $p<=0.0001$ at day 21.

After the last PXT dose (for groups A and B), in the 28th day of the experiment, the response latency of CoCl2 preconditioned mice increased close to baseline values. Thus, in the last day of chemotherapy (CHT), the average was 7.63±0.51s for group A, 7.33±0.43s for group B and 7.41±0.4s for group C ($p>0.05$). Two weeks after CHT, in the Paclitaxel-alone group (group B), a significant decrease of response latency for mechanical stimuli was noted, with average values of 5.69±0.53s, 27.5% lower than baseline values ($p=0.02$); at that time, the average value for group A was 8.01±0.69s and the average value for group C (control group) was 7.52±0.42s. Subsequently, sensitivity to mechanical stimuli increased only for PXT treated mice, with a maximum 41% decrease in response latency compared to baseline ($p=0.002$); for the CoCl2 preconditioned group, maximum decrease in averages after PXT treatment was 2.9% when compared with baseline (7.44±0.5s). At the end of the experiment, the average values of group B remained 13% lower than baseline, while in the other two groups, average values varied by 1-3% when compared with baseline (fig. 1.).

There were no statistically significant differences between baseline values in the three groups.

Effects on thermal allodynia

Chronic CoCl2 administration determined a transient decrease of response latency to thermal stimuli (Hg test). After the first week of CoCl2 treatment, PWL average in group A was 6.34±0.23s, while in groups B and C is was 8.06±0.43s and 7.92±0.23s respectively ($p=0.03$); ANOVA analysis indicated a significant substance effect for day 7, with ($F(2, 14.6)=9.08$, $p=0.001$). Subsequently, response latency values of CoCl2 treated mice increased and, after the third week, reached a higher

than baseline average (fig. 2.).

After the last PXT dose (for groups A and B), in the 28th day of the experiment, the response latency of CoCl2-treated mice remained constant, while for the mice treated with PXT alone, a decrease of 18.65% was noted. Starting with the 6th day after CHT, the difference between group B and the other two groups became statistically significant. Mice treated only with PXT had lower response latencies, with up to 45% decrease when compared to baseline ($p<0.05$ for all measurements) (fig. 2.). At that time point, in group A, average values were similar to baseline. In the last day of the experiment, the average value in group B was 3.92±0.43s, while in groups A and C, it was 10.81±0.92s and 9.01±0.81s, respectively.

There were no statistically significant differences between baseline values in the three groups.

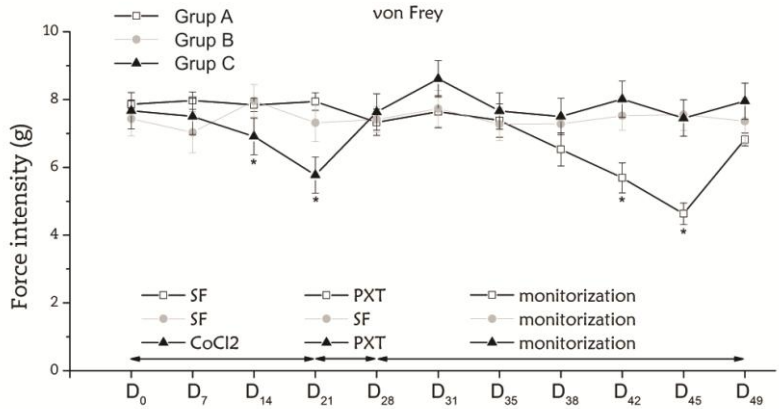

Fig. 1. The effect of CoCl2 on response latency after mechanical stimuli. For each group, treatment and motorization time-frames are mentioned. * - $p<0.05$ - Tukey post-hoc

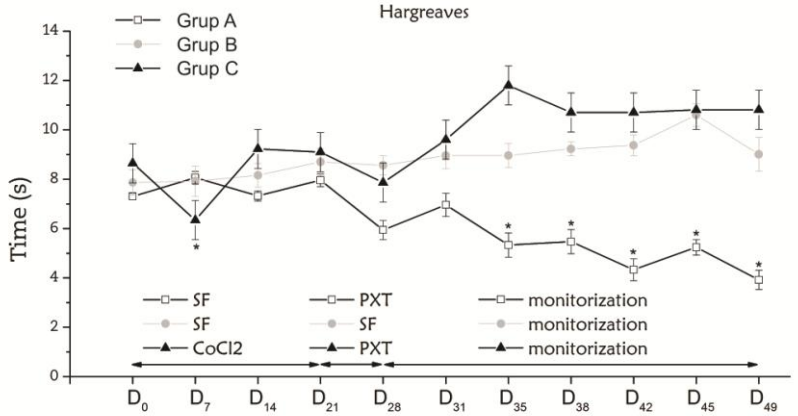

Fig. 2. The effect of CoCl2 on response latency after thermal stimuli. For each group, treatment and motorization time-frames are mentioned. * - $p<0.05$ - Tukey post-hoc

DISCUSSION

CoCl2 induced thermal and mechanical allodynia after one week of administration; thermal allodynia was transient, while mechanical allodynia persisted until the end of CoCl2 treatment (3rd week of the experiment). These results are in accordance with published data (9) and indicate a neurotoxic effect of CoCl2, possibly due to the substance's affinity for the nervous tissue (12). However, after PXT administration, response latency after both thermal and mechanical stimuli returned to baseline values in CoCl2 group and significantly decreased in the PXT-alone group. In group B, a significant and persistent thermal and mechanical allodynia appeared after CHT, with response latencies 41% lower than at baseline. The protective effect of CoCl2 preconditioning can be explained by CoCl2's stimulating effects on mitochondria.

Several studies suggest that PXT induced neuropathy is a result of its mitochondrial toxicity. For example, a paper published in 2014 indicated that, by blocking the sigma-1 receptors (which are involved in mitochondrial calcium homeostasis) in a mouse PXT neuropathy model, PIPN is can be prevented (13). Another study revealed the presence of mitochondrial anomalies in A and C fibers by microscopy analysis of the saphenous nerve; this led the authors to suggest that mitochondrial modifications are the main mechanism responsible of PIPN (3). Janes et al. suggested that the presence of PIPN is strongly related to mitochondrial superoxide dismutase inactivation and to decreased ATP production in the axons of the primary sensitive neurons (14).

There are several in vitro studies highlighting the effect of PXT on mitochondria. For instance, Xiang et al. performed in-vitro administration of PXT to isolated mitochondria and measured the organelle's membrane potential ($\Delta\psi m$) by means of flow citometry. The results showed a dose-dependent decrease in potential value; these changes were not observed when the experiment was carried out with Cisplatin or actinomicine D (15).

Another in-vitro study revealed the direct influence of PXT on mitochondria isolated from human cancer cells (neuroblastoma), in which PXT administration lead to the release of cytochrome C. Thus, the authors suggested that even the apoptotic effect of PXT may result from its direct interaction with the mitochondria (16).

Even though the exact mitochondrial component responsible for PIPN is unknown, all the above-mentioned studies point toward a common mechanism: mitochondrial toxicity in the axons of the primary sensitive nerves following a loss of mitochondrial bioenergetics (14). Furthermore, a recent study (17) assessed the effect of two mitochondrial modulators - ascorbic acid and curcumin – on pain; results showed that these substances decreased pain behavior in the second phase of the formalin-induced pain test, considered to be an equivalent of neuropathic pain (18).

Our results can also be explained by the beneficial effects of hypoxic preconditioning in itself. In humans, training in a hypoxic environment was associated with improvements in physical performance (7). Oxidative stress generated during CoCl2 administration is involved in nociceptive processing (19), and may also lead to adaptive processes that subsequently protect against PIPN. Furthermore, a recent study suggests that HIF activity in itself protects against thermal pain (21). Taking into account the fact that ischemia is known to play a part in other types of neuropathy, such as diabetic (22) or optic (23), adapting

to hypoxia may be involved in PIPN pathogenesis.

CONCLUSIONS

CoCl2 preconditioning protects against PIPN. Although CoCl2 administration had an initial hyperalgesic affect, subsequent PXT treatment did not lead to persistent thermal and mechanical allodynia. By contrast, mice that were not preconditioned and were treated with PXT alone experienced intense and persistent allodynia for both thermal and mechanical stimuli. Further studies are required for establishing the exact relationship between CoCl2 and PIPN.

ACKNOWLEDGEMENTS

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NEWS

POSSIBLE IMPLICATIONS OF NI ON ORAL IL-1B-INDUCED INFLAMMATORY PROCESSES

Nickel (Ni) is one of the main metal elements in orthodontic and prosthetic devices. Different effects of Ni are described ranging from an induction of local inflammation to allergy and cancerous/mutagenic properties. Inflammatory reactions are frequently observed in the oral cavity, but the interrelationship of Ni with those events is still unknown. The authors carried out an in vitro study to annlyze the impact of Ni on inflammation processes. Human gingival fibroblasts (HGFs) ($n = 6$) were exposed to a pro-inflammatory environment using interleukin-1 beta (IL-1β) and additionally stimulated with different Ni(II) concentrations (400 and 4000 ng/ml). At varying time points the expression of pro- and anti-inflammatory as well as matrix degeneration proteins, i.e. MMPs, were analyzed. Proliferation assays, wound healing tests and the detection of NF-κB activation were conducted. Unstimulated HGFs served as control. The experiments showed that low clinical average Ni(II) levels did not alter pro-inflammatory cytokines significantly compared to control ($p > 0.05$). Instead, a 10-fold higher dose up-regulated these mediators significantly in a time-dependent manner ($p < 0.01$). This was even more pronounced combining both Ni(II) concentrations with an inflammatory condition ($p < 0.001$). The mRNA data were supported by proliferation and wound closure assays ($p < 0.001$). However, the combination of both stimuli induced contradictory results. Analyzing NF-κB activation revealed that the results may be in part attributed to NF-κB. The authors of this study suggested that Ni(II) has various modifying effects on IL-1β-induced inflammatory processes depending on the concentration (Lina Gölz, Stefan Bayer, Ludger Keilig, Andreas Jäger, Helmut Stark, Christoph Bourauel, Werner Götz, Stilla Frede, Jochen Winter, Dominik Kraus. Possible implications of Ni(II) on oral IL-1β-induced inflammatory processes. *Dental Materials* 2014; 30(12):1325-1335).

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CLINICO-EPIDEMIOLOGICAL STUDY OF ENDOMETRIAL HYPERPLASIA – A RISK FACTOR FOR THE DEVELOPMENT OF ENDOMETRIAL CARCINOMA?

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CLINICO-EPIDEMIOLOGICAL STUDY OF ENDOMETRIAL HYPERPLASIA – A RISK FACTOR FOR THE DEVELOPMENT OF ENDOMETRIAL CARCINOMA? (Abstract): **Aim:** Metaplasia is defined as a transformation of an adult epithelial or conjunctive cellular type into another adult cellular type. Endometrial hyperplasia and particularly complex atypical hyperplasia exposes to a high risk of development of the endometrial carcinoma, being considered a lesion precursory to the same. Endometrial hyperplasias are risk factors for the development of endometrial carcinoma and their prophylaxis implies their accurate diagnosis, taking into account that the adenocarcinomas diagnosed in an advanced stage, whose therapeutic context differs from the early stages, have a much lower chance of survival. **Material and methods:** Our study aimed at highlighting objective criteria in establishing the morphological diagnosis and in evaluating the prognostic elements. The studied batch included 875 patients with endometrial hyperplasia and 263 patients with endometrial adenocarcinoma, who were admitted between 2003 and 2007, and the histopathologic diagnosis was obtained by processing the hysterectomy pieces. The presence of this tumour was at its highest level half-way through the study, which was in 2005. **Results:** According to the study, there was a higher proportion of patients with endometrial carcinoma from the urban environment (58.2%) than the ones from the rural environment (only 41.8%). Depending on their age, most cases of endometrial adenocarcinoma were diagnosed in 53-year old patients, with an average age of 58.94 years. Our study, made of the two batches of endometrial adenocarcinomas, shows that between the endometrial and non-endometrial adenocarcinoma there are significant differences related to the patients' age, the morphological aspect of the carcinoma, the architectural degree, the nuclear degree of tumours and the invasion in the myometrium. **Conclusions:** Our study proves that endometrial hyperplasia is a frequent diagnosis in peri- and postmenopausal patients and is frequently identified following investigations for an abnormal uterine bleeding. The age of patients with endometrial carcinoma is an important prognostic factor independent of other parameters. The difference between complex hyperplasia with no atypias and complex hyperplasia with atypias is important, because atypical complex hyperplasia is considered the precursor of endometrial adenocarcinoma. **Keywords:** ENDOMETRIAL HYPERPLASIA, ENDOMETRIAL ADENOCARCINOMA, ENDOMETRIAL CANCER, MYOMETRIUM INVASION.

We witness a world-wide increase in the incidence of corpus uteri tumours. Endometrial carcinoma is one of the most frequent invasive neoplasms, developed in the genital tract.

Endometrial hyperplasia and particular-

ly atypical complex hyperplasia runs a high risk of malignifying and is considered a lesion precursory to endometrial adenocarcinoma.

The current possibilities of diagnosing the endometrial carcinoma in early stage do not allow the identification of all cases.

According to international statistics, the cases of endometrial carcinoma identified early can benefit from a less aggressive treatment with encouraging results for the patients.

In the current gynecological practice, numerous uterine lesions appear that can be associated with abnormal uterine bleeding.

As regards uterine haemorrhages, the morphologic sublayer most frequently found is endometrial hyperplasia and endometrial carcinoma.

Endometrial hyperplasias are risk factors for the development of endometrial carcinoma and their prophylaxis implies their accurate diagnosis.

The *treatment* of patients with endometrial hyperplasia must consider the patient's age and the type of hyperplasia.

In patients under 40, a conservative medicamental treatment is recommended, as well as periodical biopsic check-up, as these patients have a low risk of developing atypical hyperplasia or endometrial carcinoma.

As regards atypical hyperplasia that is diagnosed through biopsy, a uterine curettage is indicated to exclude an endometrial carcinoma. Patients who want to maintain their fertility, in case of atypical hyperplasia, are treated with hormones and are monitored periodically through endometrial biopsy. The treatment is surgical if the diagnosis is carcinoma.

In perimenopausal patients without risk factors for endometrial carcinoma, monitoring is recommended, and in perimenopausal patients with risk factors, endometrial biopsy is recommended. In patients with atypical hyperplasia, hormonal treatment or hysterectomy is recommended. The risk of becoming a carcinoma is lower in perimenopausal patients than in postmenopausal patients. During hormonal treatment, endometrial biopsy is recommended every three months.

In postmenopausal patients over 55 years old, in case of abnormal uterine bleeding, endometrial biopsy and fractionated curettage are recommended. This evaluation of the endometrium is recommended because postmenopausal patients run a high risk of atypical hyperplasia and endometrial carcinoma.

In metaplastic papillary hyperplasia, the treatment consists of polypectomy or curettage. Hysterectomy is advised if the patient is postmenopausal and the lesion is extended. Metaplastic papillary hyperplasia runs a high risk of turning into endometrial carcinoma.

We are facing a large number of endometrial carcinomas, diagnosed in an advanced stage, whose therapeutic context differs from the early stages and whose chance of survival is much reduced.

In this context, we intended a clinical-morphological study of endometrial hyperplasias and endometrial carcinoma, with the purpose of establishing a clinical-pathological correlation between the two lesions and of elaborating a correct diagnosis, with a view to evaluating the prognostics of these cases.

This study was performed on a representative batch of patients, which included 875 endometrial hyperplasias and 263 endometrial carcinomas, manifested clinically through abnormal uterine haemorrhage.

MATERIAL AND METHODS

The analysed batch included 875 patients with endometrial hyperplasia and 263 patients with endometrial adenocarcinoma. They were admitted between 2003 and 2007 in "Cuza Vodă" Obstetrics and Gynecology Clinical Hospital of Iaşi.

The histopathologic diagnosis was obtained by processing the hysterectomy pieces.

The classification divides hyperplasias into two categories – simple and complex – each having two sub-categories, represented by hyperplasias with atypias and without atypias.

The 875 patients with endometrial hyperplasia were admitted for dysfunctional haemorrhages, uterine fibroid, premenopause and postmenopause haemorrhages.

Of the 875 patients, 739 had simple hyperplasia, 14 complex hyperplasia without atypias, 8 complex hyperplasia with atypias, 93 simple hyperplasia associated with complex hyperplasia without atypias, 21 simple hyperplasia associated with complex hyperplasia with atypias.

Even if the number of admitted cases operated for different gynaecological conditions increased in "Cuza Vodă" Hospital of Iaşi between 2003 and 2007, this was not the case for endometrial carcinoma. The presence of this tumour was at its highest level half-way through the study period, which was in 2005 (77 cases of the total cases of 1,813 surgical interventions, namely 29.3%), and values twice as low being registered in 2003 (36 cases) and 2007 (37 cases).

As a matter of fact, endometrial adenocarcinoma is only 2.87% of the total of 9,100 gynecological conditions, which needed surgery.

RESULTS

In the performed study, we observed a higher proportion of patients with endometrial carcinoma from the urban environment (58.2%) than the ones from the rural environment (only 41.8%).

Our study revealed that the average age of the patients diagnosed with endometrial carcinoma is of 58.94 years (58.94+/-8.194 years).

Of the total number of patients included in the study, 25% are between 33 and 53 years old. And half of them (percentile 50) are up to 58 years old.

Most cases of endometrial adenocarcinoma in our study were 53-year old patients (modal or dominating value).

In our study, 117 patients were at least 50 years old, which means 44.48% of the cases diagnosed with endometrial adenocarcinoma. Of the 263 patients, 242 (namely 92% of the total number) were menopausal.

The results of the study showed that abnormal uterine bleeding was present in all the 263 cases, as a singular symptom or associated with pelvic pain.

Abnormal uterine bleeding was reported in 67.7% of the cases (representing 178 patients) and the association of this main symptom with pelvic pain was found in 32.3% of the cases (representing 85 patients).

The pathological history was divided into two main categories. The first category refers to the non-gynecological pathological history and the second category to the gynaecological history. Patients who mentioned non-gynecological conditions in their history are 14.45% (38 patients) and the ones who suffered from gynecological conditions represent 42.20% of the total cases (111 patients).

From the point of view of the morphological aspects in endometrial adenocarcinomas in our study batch, of the 263 cases of endometrial adenocarcinomas, in 177 cases the coexistence of hyperplastic or atrophic changes in the endometrium adjacent to adenocarcinomas was reported, in relation with whom these tumours developed. Adenocarcinomas were divided into two batches.

Batch X included 39 patients (14.82%) with endometrial adenocarcinomas associated with hyperplasia, and batch Y of 138 cases (52.47%) with adenocarcinomas developed on an atrophic endometrium. In 86 cases (32.7 %), no hyperplastic or atrophic aspects of the endometrium were identified.

The histopathological diagnosis was established on histological sections of the surgically resected pieces. In all cases, the histological grading and the myometrium invasion were established.

Batch X is represented by patients between 40 and 60 years old, with an average of 49.9 years. There were two patients under 40, 14 patients were between 40 and 49 years old, 10 patients between 50 and 49 and 6 patients with ages of at least 60 years.

In batch X, the following cases were identified microscopically: 22 endometrioid adenocarcinomas without squamous metaplasia and 10 endometrioid adenocarcinomas with squamous metaplasia, of which 7 endomerioid adenocarcinomas with benign squamous metaplasia (adenoacanthoma) and 3 endometrioid adenocarcinomas with malignant squamous metaplasia (adenosquamous endometrioid carcinoma) and 7 secretive endometrioid adenocarcinomas. All the 39 cases of endometrioid adenocarcinomas developed in patients with endometrial hyperplasia.

In grading the endometrioid adenocarcinomas, we used the FIGO grading system, based on which we evaluated the architectural aspect of the tumour correlated with the microscopic nuclear grading system. According to the grading system based on the architectural criterion, endometrioid adenocarcinomas in batch X had 3 malignity degrees. 24 cases belonged to the 1st degree of architectural malignity, 5 cases to the 2nd degree and 3 cases to the 3rd degree.

Endometrioid adenocarcinomas with a low degree of malignity – 1st degree – are characterized by a predominating glandular aspect, with delimited edges and absence of necrosis. They have a solid tumour growth, up to 5% of the tumour volume. Moderately differentiated 2nd degree endometrioid adenocarcinomas have a solid tumour growth, between 6 and 50% of the tumour volume. Adenocarcinomas of high malignity degree – 3rd degree – have a solid architectural aspect, infiltrated tumour edges and tumour necrosis areas in over 50% of the tumour volume.

Microscopic nuclear grading was measured based on cytological criteria represented by nuclear pleomorphism, the size of nuclei, prominent nucleoli and the chromatin aspect.

In the analysed study batch, 24 endometrioid adenocarcinomas were classified as belonging to the 1st degree of nuclear malignity, 5 adenocarcinomas to the 2nd degree and 3 adenocarcinomas to the 3rd degree of nuclear malignity.

The myometrium invasion in 28 cases of endometrioid adenocarcinomas from Batch X was in less than 50% of the myometrium thickness and only in 4 cases it was in more than 50% of its thickness.

Batch Y included patients between 50 and 80 years old, with the age average of 63.34 years. There was one patient under 50, 44 patients were between 50 and 59 years old, 71 patients were between 60 and 69

years old, 28 patients were between 70 and 79 years old, and one 80-year old patient.

In batch Y, the following cases were identified microscopically: 138 cases of endometrial adenocarcinomas developed on an atrophic endometrium. Of these, 12 were endometrioid adenocarcinomas with or without squamous metaplasia, 49 were serous adenocarcinomas, 36 were clear cell endometrial adenocarcinomas, 9 were mucinous endometrial adenocarcinomas, 2 were squamous endometrial adenocarcinomas and 30 were mixed endometrial adenocarcinomas.

As regards the myometrium invasion, in 34 cases of endometrial adenocarcinomas of the 2nd group, it was in less than 50% of the myometrium thickness and in 111 cases in over 50% of its thickness.

86 cases (32.7 %) of endometrial adenocarcinomas in which the hyperplasia or atrophy of the endometrium near the tumours was not identified show microscopically aspects of endometrioid adenocarcinomas. They are represented by 49 well differentiated endometrioid adenocarcinomas, 27 moderately differentiated endometrioid adenocarcinomas and 10 cases of poorly differentiated endometrioid adenocarcinomas.

Our study, made of the two batches of endometrial adenocarcinomas, shows that between the endometrioid adenocarcinoma and the non-endometrioid adenocarcinoma there are significant differences as regards the patients' age, the morphological aspects of the carcinomas, the architectural degree, the nuclear degree of tumours and the invasion in the myometrium.

The value of the endovaginal ultrasound examination is relevant in the diagnosis of carcinomas. In the 263 patients, the local vaginal examination combined with abdominal palpation and correlated with endovaginal ultrasound established the uterus size.

In 205 cases (77.95%), the uterus was normally sized – between 4 and 6 cm – in 27 cases (10.27%) it was enlarged – over 6 cm –, and in 31 cases (11.78%) it had reduced sizes, of less than 4 cm.

In 249 (94.68%) patients with endometrial carcinoma in our study, the thickness of the uterus established by the endovaginal ultrasound was of 15 mm on average, with limits between 5 and 65 mm. In 14 patients (5.32%) with endometrial carcinoma, the thickness of the uterus established by the endovaginal ultrasound was under 5 mm, with values between 2 mm and 4.5 mm.

The observation charts of the 14 patients were examined in detail with regard to their history, clinical signs, menopausal status and if they had uterine curettages done within 3 months immediately before the endovaginal ultrasound. All patients were on menopause, with ages between 60 and 69 years, and the histopathologic examination identified different types of endometrial carcinomas.

In all 14 cases uterine curettage had been performed before the endovaginal ultrasound, less than 3 months before.

DISCUTION

The clinical and morphological study of the 875 endometrial hyperplasia cases and 263 endometrial adenocarcinoma cases under study reveals a few aspects that need to be highlighted and discussed in the light of the recent data in the specialized literature.

Our study proves that endometrial hyperplasia is a frequent diagnosis in peri- and postmenopausal patients, it is frequently identified following investigations for abnormal uterine bleeding, which are as-

pects that need to be taken into account by the gynecologist in routine consults.

In the studied batch, 84.45 % of the cases were normal hyperplasia, 1.60% complex hyperplasia without atypias, 0.94% complex hyperplasia with atypias, 10.62% simple hyperplasia associated with complex hyperplasia with atypias.

The study reveals that in the several types of hyperplasia can be associated in the endometrium, which we found in 114 cases.

The largest number of hyperplasia cases in our study batch was represented by simple hyperplasia (739 cases, 84.45%) and there was no case of simple hyperplasia with atypias.

The age of patients with endometrial carcinoma is an important prognostic factor independent of other parameters. The highest proportion – 29.3% (77 cases) – is seen in patients over 65 years old. In patients younger than 50 years old, the proportion was of 6.84% of the cases. Endometrial adenocarcinoma is a rear malignant tumour in Caucasian women under 40, but its incidence is gradually growing after menopause and reaches a maximum between 70 and 79 years of age. In women of the black race, the maximum incidence of endometrial adenocarcinoma is signalled in perimenopause, with a rate of 85 cases in 100,000 women annually.

Abnormal uterine bleeding associated in some cases with pelvic pain represented the main symptom for which patients came in for medical check-up and specialized investigation, in all the patients in our study batch.

The normal endometrium is under 5 mm thick in post-menopause and its limits must be regular and visible in the entire uterine cavity.

In interpreting the endometrium thickness, in our statistics we considered the literature data, which show that the endometrium that is thicker than 5 mm in postmenopause is associated with high probability of the existence of hyperplasia or endometrial carcinoma, and any focal lesion of the endometrium, identified in endovaginal ultrasound needs endometrial biopsy, regardless of the endometrium thickness.

Most carcinomas developed in patients with abnormal uterine lesions in postmenopause had a focal growth pattern. In focal lesions in the endometrium, the hysteroscopic resection is superior to uterine curettage in obtaining representative endometrium fragments.

Our results confirm the data in the specialized literature, which show that in postmonopausal patients an endometrial carcinoma can be associated with an endometrium thinner than 5 mm.

Nevertheless, the cases published in literature are rare. Only 14 authors that reported cases of endometrial adenocarcinomas in postmenopausal patients, where endovaginal ultrasound showed the endometrium thickness under 5 mm.

In postmenopause any focal thickening noticed or even suspected in endovaginal ultrasound must be considered pathological.

The evaluation of the endometrium thickness by endovaginal ultrasound is a current investigation method in the diagnosis of endometrial lesions and plays an important role in the screening of endometrial adenocarcinoma. Endovaginal ultrasound can reveal in over 90% of the cases the existence of a postmenopausal proliferative endometrium lesion if the endometrium thickness is over 5 mm.

If an endometrium thinner than 5 mm with regular aspect is visualized in endo-

vaginal ultrasound in patients with high risk or abnormal uterine bleedings, the clinician must perform uterine curettage for the histopathological diagnosis.

The pulsed Doppler endovaginal ultrasound identifies the presence of endometrial adenocarcinoma accurately, which determined the reduction of false positive results.

Our study supports the literature data regarding the fact that well differentiated and moderately differentiated endometrial adenocarcinomas generally invade the myometrium under 50% of its thickness. The myometrium invasion in 28 cases of endometrioid adenocarcinomas in batch X was in less than 50% of the myometrium thickness and only in 4 cases it was in over 50% of its thickness. The myometrium invasion in 34 endometrial adenocarcinoma cases in batch Y was in less than 50% of the myometrium thickness, and in 111 cases in over 50% of its thickness.

The studies published in literature mention that the tumour infiltration of the myometrium is associated with an unfavourable prognostic. Adenocarcinomas limited only to the endometrial mucous have a rate of survival of over 95% in 5 years.

CONCLUSIONS

The management of patients with endometrial hyperplasia and endometrial carcinomas is complex. Our study emphasized the importance of multidisciplinary approach, with the involvement of gynaecologists, anatomopathologists, immunologists and oncologists in the diagnosis and therapy of these lesions for the increase in the survival rate of patients.

The study suggests the supervision of cases of endometrial hyperplasia, especially if they are associated with risk factors incriminated in the etiology of corpus uteri carcinoma.

The clinical, histopathological, immuno-histochemical and prognostic aspects of certain representative batches of endometrial hyperplasia and endometrial adenocarcinomas were evaluated.

Endometrial hyperplasia and endometrial carcinomas appear frequently in peri- and postmenopause and are associated with abnormal uterine hemorrhages, which impose a full evaluation by the gynecologist.

Endometrial adenocarcinomas are under two forms represented by type I or endometrioid adenocarcinoma and type II or non-endometrioid adenocarcinoma, with differences regarding epidemiology, morphological aspect and evolution between them.

Endometrioid adenocarcinoma develops in young patients, on a hyperplastic endometrium, in a hyperestrogenic context, it is well differentiated microscopically, with little invasion in the myometrium, without tumour emboli in lymphatic vessels and with favourable prognostic, and non-endometrioid adenocarcinoma develops in postmenopausal patients on an atrophic endometrium, with high malignity degree, invasive in the myometrium and in the lymphatic vessels and with a reserved prognostic.

Complex atypical hyperplasia is a precursor only to endometrioid adenocarcinomas, as all the other microscopic variants of adenocarcinoma develop on an atrophic endometrium.

Endovaginal ultrasound can guide the diagnosis in very many cases to the existence of endometrial hyperplasia and endometrial carcinoma in peri- and postmenopause, if the endometrium is over 5 mm thick.

When an endometrium thinner than 5 mm with regular aspect is visualized in endovaginal ultrasound in peri- and post-

menopausal patients with high risk and abnormal uterine bleedings, who had a biopsy performed less than 3 months before, the clinician must perform uterine curettage for the histopathological diagnosis and to exclude endometrial carcinoma.

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PERSONALITY TRAITS RELATED TO CHIPS CONSUMPTION: A CASE-CONTROL STUDY IN ADOLESCENTS FROM ROMANIA

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PERSONALITY TRAITS RELATED TO CHIPS CONSUMPTION: A CASE-CONTROL STUDY IN ADOLESCENTS FROM ROMANIA (Abstract): In this study it is investigated whether there is a relation between personality traits of adolescents and consumption of chips. **Material:** The study was performed on 2 groups of adolescents: a study group – 248 adolescent (43.1% male and 56.9% female) who consume chips two or more times/day and a control group – 481 adolescent (51% male and 49% female) who do not consume chips at all. Case-control samples were selected from 2908 teenagers. **Method:** The method was case-control observational inquiry and consisted in applying 2 questionnaires: Freiburg Personality Inventory (Cronbach`s alpha=0.802) and CORT (Cronbach`s alpha =0.910) from which it was extracted 1 item Q107 (frequency of chips consumption). **Results:** The personality profile of the study group is different from the personality profile of the control group; significant statistical differences (Significance=0.000) between the group of study and the control group for nervousness (chi square - χ^2=22.516), aggressiveness (χ^2=22.877), excitability (χ^2=27.132), domination (χ^2=21.152) and extraversion (χ^2=9.770) were found. **Conclusion:** The results suggest a relation between personality traits of adolescents and consumption of chips. **Keywords:** CHIPS, PERSONALITY, ADOLESCENTS, OBSERVATIONAL STUDY.

Teenage is a developmental stage in human life when integration is followed by recycling that lasts for the rest of the life (1). Consumption of potato chips tends to increase with the progress in technology (2). Being addictive (3), chips consumption is a diet risk behavior in adolescence. The aim of the performed study was to investigate whether personality traits of adolescents relate to the excessive consumption of chips.

MATERIAL AND METHOD

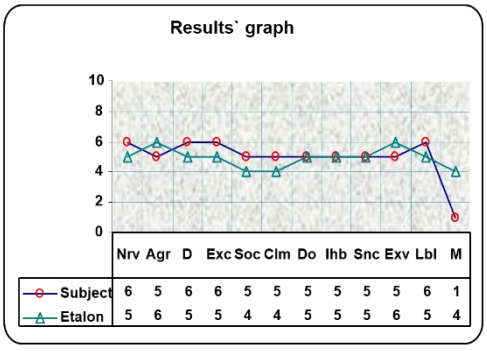
This study was performed on sample of 2908 teenagers (51.5% females, 48.5% males, aged 15-19 years) provided by an urban area in south-western Romania. The ethical approval of the University Ethical Committee was asked for and obtained. The study conducted was a case-control observational inquiry and it consisted in applying 2 questionnaires: CORT questionnaire ("**Co**mportamente cu **r**isc la **t**ineri"–"Risk behaviors in young people" - 126 items of risk behaviors with Cronbach`s alpha index=0.910) (4), and FPI (Freiburg Personality Inventory - 212 items with Cronbach`s alpha index=0.802,

normalized for the Romanian population) (5). From CORT questionnaire it was considered only item Q107, referring to chips consumption as diet risk behavior. Item Q107: "How many times per week do you consume chips?" had 5 possible answers depending on the level of risk. For the study the groups with risk 0 (control – 481 adolescents who do not consume at all chips; 51% females, 49% males) and risk 4 (case – 248 adolescents who consume 2 or more times/day chips; 57% females, 43% males) were chosen. Personality bipolar traits measured by Freiburg Personality Inventory consisted in Nervousness (Nrv), Aggressiveness (Agr), Depression (D), Excitability (Exc), Sociability (Soc), Calm (Clm), Domination (Do), Inhibition (Inh), Sincerity (Snc), Extraversion (Exv), Emotional lability (Lbl). Masculinity (M) was not taken into consideration due to the risk of misinterpreting this trait by Excel 2003 Program. Both questionnaires were applied individually and anonymously, in the same 1 hour and a half session. The informed consent of each student to participate was asked for and obtained. A personality profile was built by an Excel 2003 Program considering the median frequency value of the intensity of all the 9 ranks of each personality dimension. This personality profile could be compared with the etalon (profile established and standardized for the adult Romanian population). The personality traits of the 2 groups of teenagers for chips consumption (Q107=0 and Q107=4) were analyzed and compared. The Body Mass Index (BMI) was considered for the group of study and for the control group.

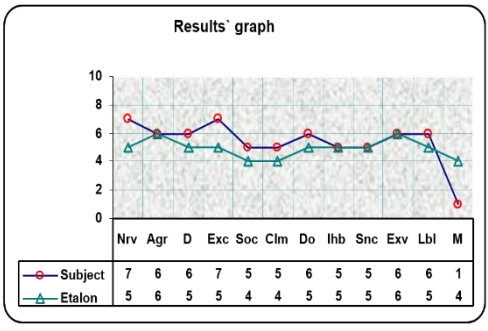
Statistical work (Chi square–χ^2 and gamma correlation- γ) was performed using a *SPSS Statistics 20*.

RESULTS

The established personality profiles of adolescents considering the median value of frequency for the control-group and the study-group were different.



Q107=0



Q107=4

Fig. 1. Personality profiles of control-group (Q107=0) and study-group (Q107=4)

The study-group has 5 personality traits (Nrv, Agr, Exc, Do and Exv) with higher intensity than the control-group. In the profile of the study-group 2 personality traits (Nrv and Exc) were outside the etalon interval (4-6) with the registered ranks=7. All the personality traits for the control-group were included in the etalon interval (4-6) (fig. 1).

The 9 intensity ranks of the personality

traits reduced to 3 (1=1-3 -low intensity; 2=4-6 -the average or etalon interval and 3=7-9 -high intensity of the trait) offered the possibility of applying Chi square. A significant statistical difference between the control-group (adolescents with no consumption of chips) and the study-group (adolescents with high level consumption of chips) was registered for certain personality traits (Nrv, Agr, Exc, Do, Exv) after applying Chi square (tab. I). The calculated gamma correlation coefficients indicated positive correlations: Q107-Nrv, Q107-Agr, Q107-Exc, Q107-Do and Q107-Exv (tab. II).

TABLE I

Chi square test Q107 (0 and 4) - personality traits

Chi-Square – χ^2	Value	*df*	Asymptotic Significance (2-sided)
Q107 * Nrv	22.518[a]	2	0.000
Q107 * Agr	20.877[a]	2	0.000
Q107 * Exc	27.132[a]	2	0.000
Q107 * Do	21.152[a]	2	0.000
Q107 * Exv	9.770[a]	2	0.008

Note. a. 0 cells (0%) have expected count less than 5. The minimum expected counts are 24.97 (q107 * Nrv), 25.38 (q107 * Agr), 24.03 (q107 * Exc), 27.75 (q107 * Do) and 29.78 (q107 * Exv).

TABLE II

Gamma (γ) correlation Q107 (0 and 4) - personality traits

Ordinal by Ordinal Correlations	γ	Asymptotic Standard Error[a]	Approx Sig.
Q107 * Nrv	0.352	0.069	0.000
Q107 * Agr	0.328	0.068	0.000
Q107 * Exc	0.363	0.066	0.000
Q107 * Do	0.332	0.067	0.000
Q107 * Exv	0.233	0.076	0.003

Note. a. Not assuming the null hypothesis;
b. Using the asymptotic standard error assuming the null hypothesis.

Nervousness was the only personality trait which registered a weak statistically significant difference between females and males ($\chi^2 = 7.869$, Significance = 0.02, γ = -0.357, Significance = 0.005). No significant statistical difference and no γ correlation between females and males were registered for other traits of the personality profile. There was also no statistically significant difference of personality profile depending on age or BMI.

DISCUSSION

The results of this study are consistent with recent studies and appear to complement previous results. Recent studies supported the hypothesis that personality can affect dietary intake (6, 7). A result of the study conducted (personality profile and statistically significant difference between

case-control groups) was that adolescents who had high chips consumption also had high spontaneous aggressiveness (Agr) and reactive aggressiveness (Do), reduced tolerance to frustration (increased Exc) and increased somatic-affective resonance (Nrv) and extraversion (Exv). Nervousness and excitability were clearly excessive and were outside the etalon interval (ranks 7) in the study-group profile. Another study demonstrates that personality traits (neuroticism, extraversion, openness, agreeableness, and conscientiousness) are associated with dietary intake. These associations were stronger in female than male subjects (8). In the performed study, only nervousness was higher -with weak statistical significance- in the female than the male subjects. In the conducted study, the high level of excitability (reduced tolerance to frustration) found in the study-group (females and males) involves increased reaction to a stimulus and a feeling of stress. In other research study on this topic, 33% of the subjects answered that they consumed higher amounts of chips when they are under stress (9). A limitation of this study is that it is an observational one and it approaches qualitative aspects only.

CONCLUSIONS

The study conducted demonstrates a specific personality profile of the population of adolescents with excessive chips consumption and it suggests a relation of certain personality traits with chips consumption behavior.

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CLINICAL-EPIDEMIOLOGICAL STUDY ON THE INCIDENCE OF POSTOPERATIVE COMPLICATIONS AFTER PULMONARY RESECTION FOR LUNG CANCER

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CLINICAL-EPIDEMIOLOGICAL STUDY ON THE INCIDENCE OF POSTOPERATIVE COMPLICATIONS AFTER PULMONARY RESECTION FOR LUNG CANCER (Abstract): **Aim:** To determine the relationship between the characteristics of a group of lung cancer patients, lung cancer surgeries, and the incidence of early and/or late postoperative complications. **Material and methods:** The study included a group of 115 lung cancer patients aged 40 to 85 years, 76.5% men, 60.91% living in urban areas, who received surgical treatment in the interval January 2013-August 2014. Data were collected from electronic medical records and survey sheets and processed and interpreted using Pearson correlation index (p-value of 0.05 was considered statistically significant and of <0.005 highly statistically significant), and chi^2, y, 1c = 25% tests. All statistical analyses were performed using the SPSS statistical software package version 13.0. **Results**: Of the 115 patients 7.8% were in the 40 -50 years age group and 87.8% in the 51-75 years age group ($p \leq 0.001$). Most patients were males (76.5%, p <0.001), and 60.9% of the patients were living in urban areas ($p \leq 0.05$). The difference in the prevalence of smokers (76.5%) *vs.* non smokers (23.5%) was highly statistically significant (p <0.005). Active smoking of one pack of cigarettes per day for more than 10 years was recorded in 54.8%. The presence of co morbidities (17 events) and the distribution of neoplastic lesions in the five anatomical areas required the performance of lobectomies, bilobectomies or pneumectomies. The incidence of early (14 entities) and late postoperative complications (7 entities) depended on patient's general condition, nature of co morbidities, location and stage of neoplastic lesions, and surgical complexity. **Conclusions**: Lung cancer, in various locations and stages, most commonly affected males aged 40-85 years, from urban areas, smokers, with various co morbidities. Surgeries performed in these patients were often laborious and with a relatively low incidence of postoperative complications. **Keywords**: LUNG CANCER, CANCER SURGERY, CO MORBIDITIES, POSTOPERATIVE COMPLICATIONS.

It has been long recognized that surgery has an important role in healing when the cancer is located in the organ of origin (1, 2). In the case of late interventions, with risks of metastasis, surgery has to be associated with other therapeutic procedures. Thus, curative surgery, radical or conservative, may be replaced with a tumor size reduction surgery, removal of metastases or palliative or reconstructive and rehabilitation surgery (3, 4, 5).

Because in practice about two thirds of

cancer patients present with advanced disease oncologic surgery has to be part of a multimodal approach associating depending on circumstances various pre- and postoperative adjuvant therapies (6,7,8).

Depending on patient's general condition, malignant disease stage, anatomic location and histological type, surgical procedures are associated with or followed by various complications with minor or alarming implications that require the surgeon and the multidisciplinary team to consider how treatment might affect patient's quality of life (9, 10, 11).

MATERIAL AND METHODS

The study included a group of 115 lung cancer patients, aged 40-85 years, 88 (76.5%) males and 27 (23.5%) females, 70 (60.9%) from urban areas and 45 (39.1%) from rural areas, who underwent surgery (lobectomy + bilobectomy: 77.8% and pneumonectomy: 25.2%) in the interval January 2013 - August 2014 at the IInd Surgical Clinic, Department of Cardiac and Thoracic Surgery of the Iași Regional Cancer Institute.

Data were collected from electronic medical records and survey sheets and processed and interpreted using Pearson correlation index (*p*-value of 0.05 was considered statistically significant and of <0.005 highly statistically significant), and chi^2, y, 1c = 25% tests. All statistical analyses were performed using the SPSS statistical software package version 13.0.

RESULTS AND DISCUSSION

Age distribution of the 115 lung cancer patients who received surgical therapy showed that 7.8% were in the 40-50 year age group, 4.3% in the 76-85 year age group (significant difference: p≥0.05) compared to 87.8% in the 51-75 year age groups (very highly significant difference compared to the previous ones, p <0.001) (tab. I, fig. I).

TABLE I
Age group distribution of the study patients

Age group	No.	%	Cumulative percentage
40 – 45	3	2.6	7.8%
46 – 50	6	5.2	
51 – 55	12	10.4	87.8%
56 - 60	20	17.4	
61 - 65	28	24.3	
66 – 70	23	20.0	
71 – 75	18	15.6	
76 - 80	4	3.5	4.3%
81 - 85	1	0.9	
Total	115	100.0	-

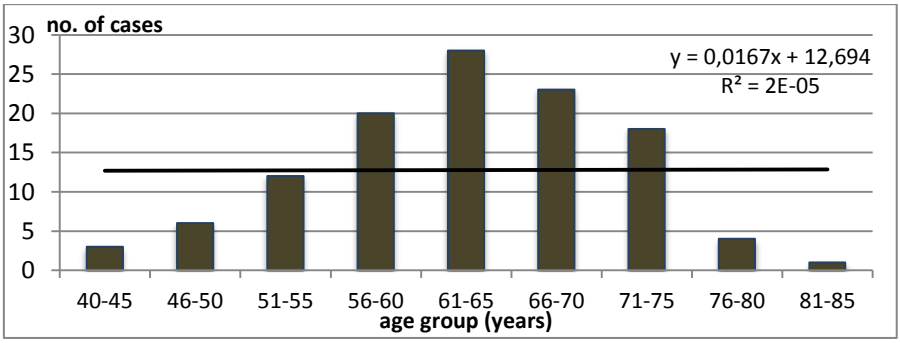

Fig. 1. Age group distribution of the surgically treated lung cancer patients

Sex distribution showed highly significant differences ($p<0.001$) in favor of males (76.5% males and 23.5% females).

Urban-rural distribution showed that 60.9% of patients lived in urban areas compared 39.1% in rural areas ($p \leq 0.05$).

The intensity and duration of smoking proved to be a major risk factor for lung carcinogenesis.

Eighty-eight (76.5%) of the 115 study patients were **active smokers,** of which 54.8% smoked one pack of cigarettes per day, 52.2% for over 10 years, and 20.9% smoked 2 packs per day for more than 10 years. The difference between the prevalence of smokers (76.5%) and nonsmokers (23.5%) was highly statistically significant ($p <0.005$) with the mention that the 27 (23.5%) non smokers reported: intensive long-term exposure to passive smoking (19: 70.3%); construction work, inhalation of dust and particles (6: 22.2%) to which a miner and a carpenter (3.7% each) were added (tab. II).

TABLE II

Assessment of the intensity and duration of smoking in the 115 lung cancer study patients

Intensity (no. packs cigarettes/day)			**Duration (years)**			
			<10		**>10**	
Packs/ day	No. cases	%	No. cases	%	No. cases	%
1	63	54.8	3	2.6	60	52.2
2	24	20.9	5	4.3	19	16.6
≥3	1	0.9	-	-	1	0.9

Note: active smokers: 88 (76.5%); nonsmokers: 27 (23.5%); passive smokers: 19 (70.3%); constructors: 6 (22.2%); miners: 1 (3.7%); carpenters: 1 (3.7%).

Types of surgery and postoperative course depended on the presence of co morbidities, often with associative action in the same patient (12). In the 115 patients included in the study we identified 17 categories of co morbidity with variable frequency from 33.9% for hypertension, to 8.7% and 7.8% for heart failure, tuberculosis, anemia, and type 2 diabetes mellitus.

Two patients (1.7%) presented synchronous lung cancer and 6 (5.2%) metachronous cancer (rectum: 2; skin: 2; breast, ENT, 1 case) (tab. III).

The distribution of neoplastic lesions in the 5 anatomic areas showed that the most common location was in the right upper lobe (37.4%) followed by the left upper lobe (29.5%) and right lower lobe (16.5%). The incidence decreased to 8.7% for the left lower lobe and 7.9% for the right middle lobe (tab. IV, fig. 2).

The types of surgery (lobectomy, bilobectomy, or pneumonectomy) depended on the location and stage of the tumor (13). Right upper lobectomy was performed in 27% of the 115 cases, followed by left upper lobectomy and left pneumon-ectomy in 14.8% and 14.0%, respectively. Right lower lobectomy, right pneumon-ectomy and left lower lobectomy were performed in 12.1%, 11.3% and 10.4% of the cases. Middle lower bilobectomy and right middle

lobectomy were performed in a similar number of cases (6/5.2%). Thus, lobectomy and bilobectomy were performed in 86 (77.8%) and pneumonectomy in 27 (25.2%) of the 115 patients, with a moderately significant difference ($p \leq 0.05$) (tab.V).

TABLE III

Frequency of complications in our study group

No.	Comorbidity	No. cases	%
1.	Hypertension	39	33.9
2.	Heart failure	10	8.7
3.	Tuberculosis	9	7.8
4.	Anemia	9	7.8
5.	Type 2 diabetes mellitus	9	7.8
6.	COPD	8	6.9
7.	Ischemic cardiomyopathy	8	6.9
8.	Thrombophlebitis lower extremities	6	5.2
9.	Obesity	6	5.2
10.	Myocardial infarction	5	4.3
11.	Hepatitis C	5	4.3
12.	Hepatitis B	4	3.5
13.	Bronchiectasis	3	2.6
14.	Stroke	3	2.6
15.	Chronic bronchitis	2	1.7
16.	Pulmonary emphysema	2	1.7
17.	Pulmonary fibrosis	2	1.7
18.	Synchronous cancer (lung)	2	1.7
19.	Metachronous cancer (rectum 2; skin: 2; breast: 1;ENT:1)	6	5.2

TABLE IV

Anatomical distribution of neoplastic broncho pulmonary lesions in the 115 surgically treated patients

No.	Anatomic area	No. of cases	%
1.	Right upper lobe	43	37.4
2.	Left upper lobe	34	29.5
3.	Right lower lobe	19	16.5
4.	Left lower lobe	10	8.7
5.	Right middle lobe	9	7.9
Total		**115**	**100.0**

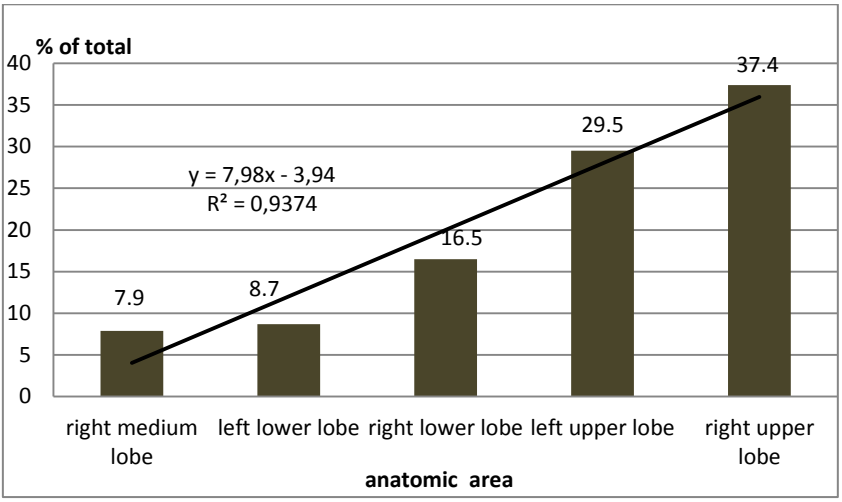

Fig. 2. Anatomical distribution of neoplastic bronchopulmoary lesions in the surgically treated patients

TABLE V

Types of surgery (lobectomy or pneumonectomy) performed in the 115 study patients

No.	Category	No.	%
1.	Right upper lobe	31	27,0
2.	Left upper lobe	17	14,8
3.	Left pneumonectomy	16	14,0
4.	Right lower lobe	14	12,1
5.	Right pneumonectomy	13	11,3
6.	Left lower lobe	12	10,4
7.	Right middle and lower lobe	6	5,2
8.	Right middle lobe	6	5,2
9.	Total lobectomy+bilobectomty	86	77,8
10.	Total pneumonectomy	29	25,2

Postoperative complications (14 entities) showed a variable dispersion and resulted from numerous factors related to patient's general condition, nature of co morbidities, location and stage of cancer lesions and surgical complexity (14, 15).

Of the early complications the following had an incidence of 6 to 11.3%: subcutaneous emphysema, atrial fibrillation, lung expansion defects and lobar or segmental atelectasis. An incidence of 2.5 to 4.3% was recorded for nosocomial pneumonia, multiple organ dysfunctions, including cardio respiratory arrest, recurrent nerve paralysis and anemia. Cardiac arrhythmias, prolonged air leak, hemorrhage, ischemic stroke, acute respiratory failure and myocardial infarction were reported in 0.8 to

1.7% of the cases.

Late postoperative complications showed a relatively low incidence (0.8 to 4.3%) and were represented by: postoperative empyema, bronchial stump fistula, pleurisy, pulmonary embolism, pneumothorax, bronchopneumonia and sepsis (tab. VI).

TABLE VI

Postoperative complications

A. Early

No.	Complication	No. of cases	%
1.	Subcutaneous emphysema	13	11.3
2.	Atrial fibrillation	8	6.9
3.	Lung expansions defects	7	6.0
4.	Lobar or segmental atelectasis	7	6.0
5.	Nosocomial pneumonia	5	4.3
6.	Multiple organ dysfunctions with cardio respiratory arrest	5	4.3
7.	Recurrent nerve paralysis	4	3.5
8.	Anemia	3	2.6
9.	Cardiac arrhythmia	2	1.7
10.	Prolonged air leak	2	1.7
11.	Hemorrhage	2	1.7
12.	Ischemic stroke	2	1.7
13.	Acute respiratory failure requiring prolonged intubation and temporary tracheostomy	2	1.7
14.	Myocardial infarction	1	0.8

B. Late

No.	Complication	No. of cases	%
1.	Empyema in the operated hemithorax	5	4.3
2.	Bronchial stump fistula	4	3.5
3.	Pleurisy	3	2.6
4.	Pulmonary embolism	2	1.7
5.	Pneumothorax	2	1.7
6.	Bronchopneumonia	1	0.8
7.	Sepsis	1	0.8

CONCLUSIONS

Our study showed a significantly higher incidence of lung cancer in various locations in men aged 40-85 years, living in urban areas, long-term active smokers, and presenting varied and multiple co morbidities. All patients received surgery, often laborious, and the incidence of early or late postoperative complications was relatively low.

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VENOUS PULMONARY THROMBOEMBOLISM AND ATHEROTHROMBOSIS: NEED FOR A NEW APPROACH

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VENOUS PULMONARY THROMBOEMBOLISM AND ATHEROTHROMBOSIS: NEED FOR A NEW APPROACH (Abstract): Pulmonary venous thromboembolism and atherothrombosis are traditionally considered as distinct diseases. As the two entities share common risk factors and mechanisms current experimental and clinical studies support their mutual causal relationship. For the clinician, the current concept requires a different clinical, laboratory and therapeutic approach. The patient with a first arterial or venous thrombosis should be fully assessed for the risk of future clinical events, and, simultaneously addressed to aggressive preventive non-pharmacological and pharmacotherapy intervention. In this context, antiplatelet agents and statins have a potentially beneficial role. **Keywords**: VENOUS THROMBOEMBOLISM, ATHEROTHROMBOSIS, INTEGRATED APPROACH, RISK FACTORS, PREVENTION.

CONCEPTUAL EVOLUTION

The relation between the complications of atherosclerosis (ATS) and pulmonary venous thromboembolism (VTE) is a current topic as both entities are of major importance in the prevalence and potentially fatal consequences, and according to studies, are frequently associated in the same patient. The literature of recent years supports the "VTE-atherothrombosis concept", a dynamic point of view with three stages of development (1). The classical approach supports two distinct categories of diseases based on some different types of arguments. The hemodynamic arguments refer to vascular occlusion in areas with different shear stress regimens, and pathological arguments are based on the different structure of thrombi. Venous thrombi are mainly composed of erythrocytes and fibrin, while arterial systemic thrombi are platelet-rich. Epidemiological studies also make a clear distinction between the mechanisms involved and risk factors. The distinction is also therapeutic as anticoagulation is the cornerstone of treatment in VTE while antiplatelet agents play a key role in prevention of atherothrombotic events. The classic paradigm started to be questioned at the beginning of the 21st century when epidemiological studies suggested that the dichotomy is an artificial simplification (2-5). This new stage is based on data related to a significantly increased incidence of VTE compared to the general population in the elderly and patients with a history of

peripheral arterial disease, hypertension, and dyslipidemia. The observations referring to unprovoked VTE in over 30% of the cases also support the new point of view (6). Other studies suggest common risk factors for arterial and venous thrombotic events, such as hiperhomocysteinemia, thrombophilia, or first refer to the benefit of statins in VTE (7,8). These observations recall Macfarlane's assertion in the 70s that "thrombosis is hemostasis in the wrong place" (9). In other words, the prothrombotic status secondary to platelet activation, coagulation, and increased fibrin turnover is a common event in atherothrombosis (ATT) and VTE. Based on this evidence, Prandoni examined whether asymptomatic carotid ATS is predictable for the risk of VTE (5). The study considered to be a reference for the evolution of the concept, included 299 patients with spontaneous and provoked deep vein thrombosis (DVT) and with no athero-thrombotic events, and 150 control subjects, examined by ultrasound for carotid plaques detection. The authors concluded that asymptomatic carotid lesions had significantly higher prevalence in the subgroup with provoked DVT, suggesting the potential of ATS to induce VTE. The hypothesis of an association between arterial and venous thrombotic events develops simultaneously in many prospective studies in order to elucidate the relationship direction: ATT-VTE or VTE-ATT? Data published after ‘2000s are crucial for the evolution towards the modern conceptual stage. The current paradigm argues that VTE is part of "pan-cardiovascular syndrome" that includes all clinical manifestations of ATT as there are common risk factors and mechanisms. It is concluded that between VTE and ATT there is not a simple association but a mutual causal relationship (6).

EVIDENCE FROM TRIALS

1. Atherosclerosis and risk of VTE: is there a relationship?

A first group of observations refers to the impact of subclinical ATS on the risk of VTE. There are two reference prospective population trials with the same objective as the study by Prandoni, but with conflicting results. Atherosclerotic Risk in Communities, which included asymptomatic adults aged 45-64 years, does not support the relationship. The Cardiovascular Health Study, addressed to elderly over 65 years, states low risk of VTE irrespective of etiology only related to carotid ATS but without evidence of subclinical ATS in other arterial territories (10,11). Another group of studies addresses the issue of atherothrombotic cardiovascular events (CVE). There are consistent published data but differently supporting the positive relationship with increased risk of VTE. There are interesting observations published by Eliasson et al. in a retrospective study of 23,976 consecutive autopsies in patients with objectively verified arterial and venous thrombosis. The authors suggested a heterogeneous relationship since the risk of VTE was confirmed in patients with thrombosis of the aorta and its branches, except the coronary arteries. An inadequately motivated explanation of these results is related to the ‘90s when fewer therapeutic options for acute coronary syndromes were available, and thus many patients did not survive long enough to develop VTE (12). The results are contradictory to Tromsø and HUNT-2 studies supporting that family history of acute myocardial infarction (AMI) is significantly and independently associated with the

risk of VTE (13,14). More recent data confirm the high risk of VTE during the first 3 months after AMI or stroke, then it decreases but remains statistically significant (15).

2. VTE and the risk of atherothrombosis: is there a relationship?

One of the first relevant prospective studies belongs to Prandoni showing a doubling in the risk of atherothrombotic symptomatic events following an unprovoked VTE episode compared with a secondary VTE (15.1% *versus* 8.5%), irrespective of age and other cardiovascular risk factors (16). To the contrary, Spencer stated that the relation between unprovoked VTE and AMI is age-dependent. Thus, patients younger than 40 years without atherosclerotic risk factors had a 4-fold higher incidence of AMI over 10-years of follow-up as compared to the control group (17). In addition, the study highlighted the risk of VTE recurrence within 8 years of warfarin discontinuation (30% of cases) and an about 40% increase in the subsequent risk of all-cause death (17,18). Reference data are also available from a prospective Danish trial on a cohort of more than 200,000 patients followed-up for 20 years (19). The same temporal trend was noticed when VTE was related to AMI and stroke, with an excess of relative risk for major CVE during the first year of follow-up (1.6-fold increased risk for AMI, 2.6-fold increased risk for stroke in DVT cohort, and 2.6-2.93-fold increased risk in patients with pulmonary embolism). The risk was still increased by 20-40% over the 19 years of follow-up. In a meta-analysis summarizing the literature results published until 2010, Becattini stated that patients with unprovoked VTE have a 1.5-fold higher risk of arterial CVE over 10 years than patients with provoked VTE and the general population. The meta-analysis highlights common mechanisms such as thrombogenesis, endothelial dysfunction, and inflammation. These mechanisms are supported by the modern paradigm that is focused on the causal relationship between VTE and ATT (20,21). For a clinician, these studies imply a careful monitoring of cardiovascular risk factors in patients with a history of unprovoked VTE when considering an optimal primary prevention of atherothrombotic events concomitant with recurrent venous thrombotic event prophylaxis (22).

VTE- ATHERO-THROMBOSIS CONCEPT: CLINICAL ASSOCIATION OR CAUSAL RELATION?

Abandoning classical mechanistic thinking and the need of an integrated approach of these two entities were recognized by the North American Thrombosis Summit in 2011. At this point, the relationship is reviewed in terms of pathogenesis, clinical and therapeutic management. The new concept supports VTE and ATT clinical events as a continuous spectrum of the same disease in the same patient and the risk is increased in a mutual manner.

Common risk factors and common mechanisms?

The statement that the patients with atherothrombotic events are at increased risk of VTE and *vice versa* is based on several studies that support common risk factors. Age is also a powerful risk factor in VTE, as Worcester study show an exponential increase in incidence in both sexes (23). Ageno's meta-analysis of over 65,000 patients included in 21 studies adds valuable information regarding the relation between

the major risk factors and VTE (24). Obesity expressed by body mass index doubles the risk of VTE. This observation is consistent with other studies on cohorts of women, where obesity significantly increases the risk of DVT and triples the risk of unprovoked VTE (6). Hypertension is associated with a 50% increase in the risk of VTE, a relation admitted inconstantly by other studies because of the different ways of reporting high blood pressure. Diabetes mellitus is associated with a 42% increase of VTE incidence, regardless of the trials included in the meta-analysis and type of diabetes (24). As to dyslipidemia, only decreased HDL doubles the risk of VTE and triglycerides are on average 21 mg% higher. However, smoking has a controversial influence. Ageno does not support its role, while two recent studies confirm previous results on the effect dependent on the daily number of cigarettes. Therefore, more than 25 cigarettes doubles the risk of VTE, and more than 35 cigarettes triples it (25,26). Information is extended to other risk factors for ATT. Although there are data supporting that the metabolic syndrome increases the risk of VTE by up to 84%, it is not clear whether actually it is its components, mainly abdominal obesity and non-alcoholic fatty liver, that have a major role, (6, 22). There are also controversies about the influence of age, prevalence in males, and involved mechanism. The central role of prothrombotic status activated by athero-genic mediators released by adipocytes (leptin, IL-6, TNF-α) has been stated. The VLDL trigger effect on platelet activation and PAI-1 gene expression or hyperglycemia effect on the structure and function of fibrin, generating a clot more resistant to fibrinolysis (27), is discussed. Microalbuminuria is suggested as an independent risk marker for VTE and its recurrence by the Dutch trial PREVEND. The study states a direct relationship between the amount of urinary albumin and the annual incidence of VTE (28). Hormone replacement therapy is classically linked to both a triple risk of atherothombotic events and a double risk of VTE, and Pomp's study confirms the synergistic role of smoking (1.29). Nutritional factors are supported by numerous studies. In a cohort of 14,962 patients followed-up for 12 years, daily consumption of fruits and vegetables was associated with decreased risk, whereas dietary intake of red and processed meat doubled the incidence of VTE. Eating fish at least once a week is also demonstrated to reduce the risk by 30-45% (Steffen LM cited by 6). Other prospective studies independently associate stress with increased risk of VTE in men (30). An interesting idea is suggested by Sorensen demonstrating increased risk of VTE within the first 3 months in patients with cardiovascular diseases that are potential sources of thrombi (AMI, heart failure, atrial fibrillation, especially right heart valvulopaties) in the absence of DVT (31). Finally, risk factors and clinical conditions such as hiperhomocysteinemia, thrombophilia, antiphospholipid syndrome, malignancy, chemotherapy, specific acute infections (AIDS, *Chlamydia pneumoniae*), intestinal inflammatory diseases, etc. have been linked with both arterial and venous thrombotic events (22). If the data on traditional risk factors are largely consistent, the underlying mechanisms responsible for this association are controversial. The classic triad of Virchow is still valid in VTE, but it is now believed that stasis and hypercoagulability are critical as experimentally the vascular endothelial surface is initially intact (27).

Activation and endothelial injury play a crucial role as a minor damage unprovoked by immobilization or surgery increases by 5-fold the risk of DVT (32). Based on experimental models of venous thrombosis and using electron microscopy studies, Mackman shows the role of inflammation in endothelial activation and injury, irrespective of hypoxia. It results in P-selectin, E-selectin and von Willebrand factor expression. Activated endothelial cell captures leukocytes, platelets and microparticules derived from activated monocytes. Both microparticules (membrane fragments) and neutrophils express tissue factor. It is considered that tissue factor activity is crucial for the activation of coagulation cascade and fibrin-rich, erythrocyte-rich thrombi formation (33,34). Venous wall inflammation is an early phenomenon, present in patients with uncomplicated varicose veins being induced by oxidative stress similarly to damage of the arterial wall (35). Clinical studies support the role of inflammation linking the intestinal inflammatory diseases, vasculitis or genetic polymorphisms of interleukins with the increased risk of VTE (6). For example, ARIC study demonstrates an increase by 76% of VTE risk related to C-reactive protein (36), and JUPITER trial states a 43% lower risk in patients with initially increased C-reactive protein level treated with rosuvastatin (37). Recent data also support the role of inflammation in promoting atherogenesis, destabilization of atheroma plaque, and atherothrombotic complications. The focus is on neutrophils, as there is a direct link between neutrophil count, neutrophil-related biomarkers (myeloperoxidase, elastase), and cardiovascular risk factors (38). Activated platelet-derived microparticles also seem to be involved in coronary events by inducing and stimulating massive platelet and fibrin deposition (39). All evidence supports the causal relationship between VTE and ATT, centered on the role of inflammation.

HOW THE CURRENT CONCEPT SHOULD BE APPROACHED IN PRACTICE?

In clinical practice, is fundamental to use an integrated clinical, laboratory and therapeutic approach for assessing each patient with a first arterial or venous thrombotic event. According to this concept, VTE is an early event that expresses a generalized vascular disease, present from the beginning in both types of circulation. As a result, therapeutic interventions should be similar as for a patient with severe ATS, with emphasis on active detection and optimization of risk factors. A routine screening for asymptomatic ATS following a VTE episode remains a goal of future studies (1). To the opposite, patients with atherothrombotic CVE are an underrecongnized population at risk for VTE. The analysis of the Healthcare Cost and Utilization Project Nationwide Inpatient Sample database found that more than 1 million patients with AMI and stroke develop VTE (6). Identification of VTE high risk subgroup in this large category of patients may be an additional target in its primary prevention. According to evidence from trials, major cardiovascular risk factors optimization could also be an additional way of prevention (1,6). When referring to laboratory assessment, an ambitious goal is to determine individual vulnerability inherent in venous thrombosis. Although in practice there are multiple parameters for coagulation and fibrinolysis assessment, most patients with VTE have normal tests (40). The development of sensitive tests specific to

other pathogenic pathways is a currently studied direction. Platelet membrane phospholipid, inhibition of leukocyte-released tissue factor or monocyte recruitment are some of the studied targets. Also, platelet clot volume or the particular fibrin clot phenotype characterized by a highly branched network structure with lower clot permeability and prolonged lysis time are suggested as common mechanisms linking arterial and venous thrombosis (40,41). For therapy, the major objective remains prophylaxis optimization. The multicenter, multinational trial ENDORSE points out that 51.8% of hospitalized patients have increased risk of VTE, and yet, the rate of suitable prophylaxis is reduced – 58.5% in surgical patients and 39.5% in high risk medical patients (42). Therefore, for vulnerable patients the U.S. medical system recommends implementation of computerized algorithm-based strategies, and the introduction of this issue to health policies (43). The anticoagulation benefit is extended to prophylaxis of recurrences, so as in patients with unprovoked VTE an indefinite period is recommended (6). The potential for primary prevention of AMI (reducing the incidence by 39%) is an additional reason according to a randomized trial in healthy men at high cardiovascular risk (5). A largely debated direction is the role of aspirin in primary and secondary prevention of VTE. The potentially beneficial effect is based on inhibition of platelet activation and aggregation together with interfering with acetylation of protein molecules such as fibrinogen (39). Early studies and the related APTc meta-analysis indicate a 25-30% decrease of VTE incidence in hospitalized patients at high risk treated with aspirin. The same data suggest the prevention of VTE recurrence when aspirin is given to patients at risk higher than 2% for coronary heart disease or stroke who had discontinued the oral anticoagulant therapy (44). Two recent trials with similar design support the benefit of aspirin in secondary prevention of unprovoked VTE. The WARFASA prospective study demonstrates that aspirin 100 mg/day reduces the recurrence rate by about 40% without increasing bleeding risk in patients who had completed 6 to 18 months of oral anticoagulation treatment (45). Similarly, the ASPIRE study states a 32% reduction in the rate of recurrent VTE and by 34% of major CVE without clinically relevant bleeding risk during 37.2 months of follow-up (46). However, current guidelines do not recommend antiplatelet prevention given the higher net benefit of anticoagulant therapy, including new anticoagulants. The role of antiplatelet agents for long-term prevention of VTE remains to be defined, but it is a cheap, well-known, widely available, more secure therapy, with extended benefits in the primary prevention of atherothrombotic events when anticoagulation is contraindicated. Finally, a highly debated and controversial topic is the role of statins in VTE. The potential beneficial effect is based on the antithrombotic role mediated by the decrease in platelet activation, up-regulation of thrombomodulin expression by the endothelial cells, increased susceptibility of fibrin to lysis, as well as on the anti-inflammatory role focused on reducing tissue factor, C-reactive protein, IL-6 and IL-8, MCP-1 expression (47). A secondary analysis in the JUPITER trial supports the benefit of rosuvastatin 20 mg/day in decreasing by 43% the risk of symptomatic VTE in healthy subjects. Two recent meta-analyses admit the benefit of statins in primary prevention of VTE, but at a much lower level (about 11%). The need to adjust data related to selected population, objec-

tive, associated therapy, age, statin type, etc. account for the attenuated optimistic results of previous studies (48,49). The only way to clarify the role in primary VTE prevention is to initiate a randomized prospective trial, with venous thrombosis as primary endpoint (50). Recently, information about the beneficial effect of statins has been supplemented with data on secondary prevention. Two recent Danish studies state a reduction by about 50% in the rate of recurrent VTE associated with a 30% reduction in the incidence of CVE and the relationship is dose dependent but not influenced by antivitamin K therapy. As with the primary prevention, the results need to be clarified by prospective randomized placebo-controlled trials (51, 52).

Evidence from clinical studied suggest that a new integrated approach should be applied to patients with venous or arterial thrombosis. An individualized attitude based on comprehensive assessment of total prothrombotic burden is required in order to provide a more efficient antithrombotic prevention.

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SINGULAR CASE OF THIRD VENTRICLE METASTASIS OF COLORECTAL CARCINOMA - CASE REPORT

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SINGULAR CASE OF THIRD VENTRICLE METASTASIS OF COLORECTAL CARCINOMA CASE REPORT(Abstract): Third ventricle tumors are uncommon central nervous system lesions and unusual locations for metastatic colorectal cancer. We present a case of a 68 year old woman with a solitary 3rd ventricle lesion found on a computed tomography scan of the brain and the synchronous mass of the right colon. The aim in this case was local control of cerebral lesion with pathological diagnosis. Surgery was followed by a short term good evolution but with sudden death. **Keywords**: BRAIN METASTASES, THIRD VENTRICLE, COLORECTAL CANCER.

Metastatic brain tumors from colorectal cancer are relatively rare. The incidence ranges from 1.9 to 3.5 percent of all metastatic brain tumors (1) with a median value of 2.3 percent in the latest studies (9). Left-sided primary colon tumors were predominate and the cerebellum was the most common area of brain involvement. Tumors of the ventricular system account for less than 1% of intracranial lesions and 18.5% of them occur in the third ventricle (16). The most frequent lesions are colloid cysts (55%) and gliomas (19%), other tumors are uncommon (7). Those statistics data denote a very rare and extremely rare localization of brain metastases in the 3rd ventricle. Furthermore this location represents a challenge in terms of medical and surgical approach(12).

CASE REPORT

A 68 year old female presented with headache with a short term onset (apparently one week before admission - according to family anamnesis) associated with dizziness and mixed aphasia. She was admitted in neurology department and she had performed a contrast enhancement computed tomography (CT) scan of the brain which showed a lesion located on the 3rd ventricle topography, well defined with polycyclic aspect (fig. 1).

The laboratory studies denoted an iron deficiency anemia: Hb= 9.7g /dl; MCV=72;

MCHC= 30.6 g/dl. She was transferred in neurosurgical department and shortly after that she became comatose. A new CT scan has been performed and it revealed a hemorrhage around the 3rd ventricle lesion, in the 4th ventricle and internal active hydrocephalus predominant on the left lateral ventricle (fig. 2). We performed an external ventricular drainage (EVD) on the left side and after patient stabilization (the same day) we did a contrast enhancement brain MRI (fig. 3).

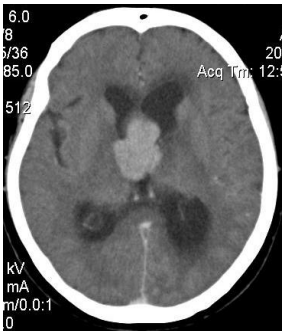


Fig. 1. Contrast enhanced CT scan showing a lesion in the 3rd ventricle

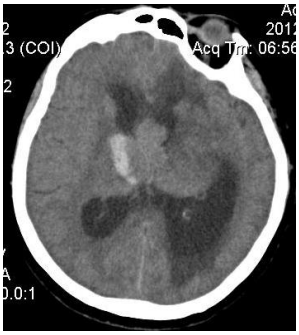
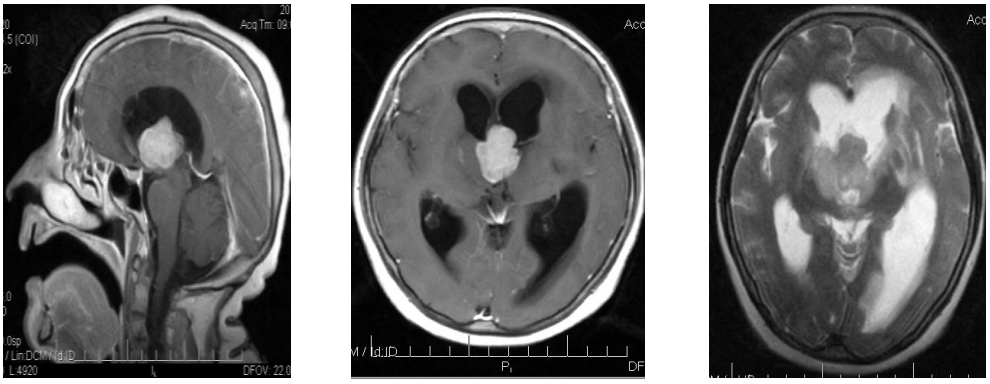


Fig. 2. Hemorrhage around the ventricular mass with active hydrocephalus



a. b. c.

Fig. 3. a, b T1WI C+ , c T2WI lobulated lesion on the topography of the 3rd ventricle with enhancement and active hydrocephalus prevalent on the left lateral ventricle.

In order to assess the origin of the anemia we performed an abdominal ultrasound examination followed by an abdominal CT scan which confirmed the presence of a tumor located on the ascending colon with a diameter of 92/53/90 mm (fig.4). Our next step was to proceed to the second stage intervention aiming to resect as much as possible from the tumor. Previously to the second intervention we removed the external ventricular drainage. We performed a precorronary transcallosal ap-

proach. The intraoperative histological examination revealed moderately differentiated adenocarcinoma.

Her postoperative course was relatively good in the first day with progressive general worsening after that. The CT scan showed the persistence of the hydrocephalus (fig 5). A new EVD was placed and the patient had a slight recovery. Unfortunately the evolution with cardiac arrhythmia and decease of the patient was registered 4 days after the tumor removal.

Fig.4. Abdomino-pelvic CT scan: lesion located on the right colon

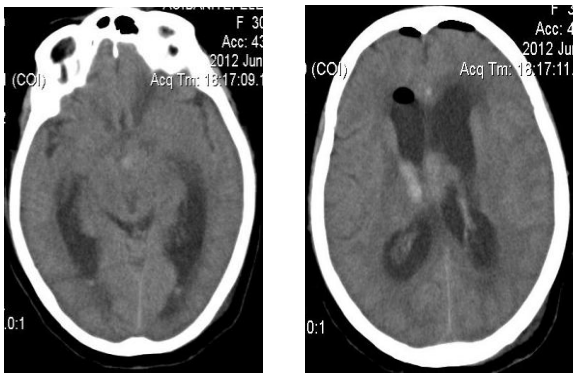


Fig. 5. CT scan : persistence of the hydrocephalus, slight hemorrhage around located on the right colon the 3rd ventricle residual lesion

DISCUSSION

Brain metastases are the most common intracranial tumors in adults; the latest reports revealing that 10 - 40 % of the cancer patients will develop lesions in this specific location (14). The most common primary cancers metastasizing to the brain are lung cancer (50%), breast cancer (15%–20%), unknown primary cancer (10%–15%), melanoma (10%) and colon cancer (5%) (8, 11).

The incidence of 3rd ventricle lesions is very low (4). We haven't found yet cases of 3rd ventricle colorectal metastasis mentioned in literature. As far as we know it was a singular case. Colorectal cancer usually metastasize in the liver or in the lung, very rarely as primary brain metastasis. In our case the histopathological result revealed moderately differentiated adenocarcinoma with epithelial tumoral cells showing cytological features of neoplasia. It presented areas of papillary fronds arranged around a fibro-vascular core (Fig. 6a) and expressing epithelial anaplasic cells arranged as irregular gland-like structures in a back-to-back fashion. (fig. 6b) In the light of the results of the abdominal examination the probability for a metastatic tumor of gastrointestinal origin is very high and according to some studies the median survival after surgery for gastrointestinal brain metatstasis is 9.1 months (1) while after radiosurgery is 6.7 months (2), so the surgery may be more beneficial than other oncologic therapies in this specific case.

The patient was diagnosed with anemia previously to the intervention but the swift neurological deterioration allowed us to find the cause of anemia after EVD.

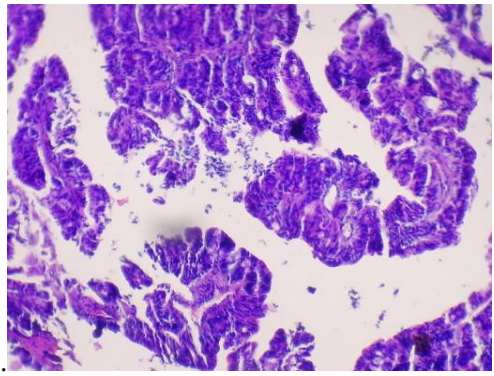
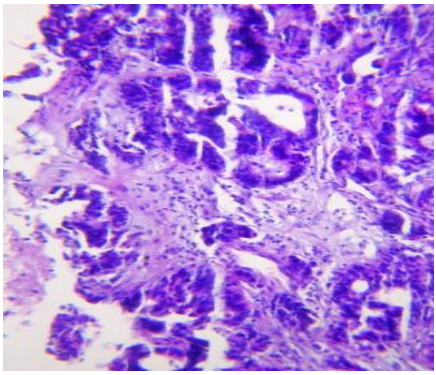
a.  b. 

Fig. 6. a. Tumoral cells form papillary fronds b. expressing epithelial anaplasic cells arranged as irregular gland-like structures

From the beginning the synchronized diagnosis of a brain metastasis and colorectal cancer indicates a late stage gastrointestinal tumor and consecutively a poor prognosis (3). The latest data found that gastrointestinal tract tumors (already metastasized in the brain) were associated with a shorter median survival than even melanoma (6).

The patient presented with acute obstructive hydrocephalus due to both - tumor extension per se and intraventricular bleeding, imposed EVD and surgical intervention with the aim of ventricle repermeabilisation by tumor debulking and histopathological confirmation.

Technical considerations. We have chosen a transcallosal approach on the left side (5, 13). We did an "S" shaped incision starting from the left side, from the incision for the EVD with a parasagittal craniotomy centered on the former burr hole for EVD. We distinguished the superior sagittal sinus which presented a voluminous venous dilatation without any important drainage. Parasagittal dissection of adherences between the 2 hemispheres was followed by a 2 cm long callosotomy with ultrasound aspirator and entrance in the ventricular system and evacuation of the CSF under pressure. The choroid plexus was visualized and the inferior one quarter of the septum seemed infiltrated, the fornix was pushed upward and the cleavage plane was difficult to find. We resected the tumor with the aid of ultrasonic aspirator until we saw the lateral wall of the third ventricle. The tumor was well vascularised, adherent to the choroid plexus and veins while in the center it had a grey, fatty aspect.

We considered it was too dangerous to continue debulking because of the absence of the cleavage plane, the presence of the adherences to the wall of the ventricle and difficulties in visualization of the bleeding sources. We have chosen the classic intervention and not the stereotactic biopsy because of the presence of obstructive hydrocephalus and we didn't chose also the endoscopic approach (10,15) because of the likelihood of bleeding and poor control of hemostasis. Intra operative there were no major complication but the resection was stopped because of the lack of the differention in between the tumor mass and the diencephalic structures.

The initial evolution after surgery was good with no more deficits. The unfortunate evolution with cardiac arrhythmia and death

was registered. The patient did not suffer of another new hemorrhage or recent ischemia, on the control CT scan and she presented no hydroelectrolytic disturbances. She also didn't respond to the replacement of EVD. Sudden death is cited in lesions that are infiltrating the diencephalon.

CONCLUSIONS

The 3rd ventricle metastases are unusual lesions with difficult evolution and with poor prognosis especially when they are diagnosed synchronically with a primary intestinal lesion and they may be complicated with sudden death.

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SMOKING GENERATED PM $_{2.5}$ EXPOSURE AMONG TRANSYLVANIAN STUDENTS

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SMOKING GENERATED $PM_{2.5}$ EXPOSURE AMONG TRANSYLVANIAN STUDENTS (Abstract)**:** A WHO project initiated in 2004, tries to identify and reduce the number of smoking third year university students attending medical training in Medicine, Dentistry, Pharmaceutical sciences as these participants can become role models that patients can look up to. **Aim**: we proposed to make a general view regarding smoking and exposure to smoke within our medical University, in contrast with the existing legislation regarding smoking within public institutions of the Universities. Along with the investigations held at our university we extended these to other tree, non medical universities. **Materials and methods:** using an TSI Pack Aerosol Monitor unit to measure the total PM $_{2.5}$ we determined the air quality in several target locations of our University in holiday season and during full didactical periods. The average values were later compared and assessed in a series of statistical tests. **Results:** analyzing the holiday period our university head the most polluted air showing the P.M. 2.5 of 0.016 mg/m^3. In the following there were analyzed the recordings from the didactic period where the registered values were significantly higher ($p<0.0001$) in comparison with the readings from the holiday season. From several points within the universities there were reading 7 times higher than in the holiday season. **Conclusions:** the results show two evident conclusion, that there is smoking within the buildings of the universities and within our university the non-smoking students are totally exposed to exhaled cigarette smoke. **Keywords:** SMOKING, STUDENT, PM $_{2.5}$, SECOND HAND SMOKING EXPOSURE.

According to WHO estimates, about 6 million people die annually due to smoking, of which about 5.5 million smokers, and the remaining 500.000 people due to passive smoking. WHO initiated a study in 2004 (called Global Health Professions Student Survey), which reached until 2010 approximately 50 countries and focused on identifying smokers and reducing smoking among students of medical universities (medicine, pharmacy, dentistry, nursing, etc.) starting from their 3rd year of studies, considering that as they will become doctors, dentists, pharmacists, they will be considered role models by thousands of patients. Romania joined this study in 2010 (1).

Another recent study performed in Romania identified smoking as an important issue among young adults aged over 19 years. This even called tobacco smoking a

lifestyle component of young people in Romania, its incidence increasing by 15% from the age group of 14-18 years to the age group of over 19 years (2).

Among the toxic emissions of smoking there are several substances, some of which are known carcinogens. Additionally, cigarette combustion products are known to be small, suspended particles, especially those under 2.5 μm (fine particle - PM $_{2.5}$) that penetrate as deep as the alveoli. A significant association between these particles and premature mortality has been reported in several studies. Worsening of chronic cardiovascular and respiratory diseases have been reviewed, as well as the occurrence of impaired lung function and airway irritation syndrome, with symptoms such as cough, shortness of breath, wheezing in otherwise healthy children and adults. This is why exposure to passive smoking - second hand smoking receives a special importance in the etiology of many diseases, not only among smokers, but also among non-smokers (3).

In support of non-smokers from all over Europe come a series of laws that prohibit smoking in somewhat restricted or more extensive areas. According to 2013 WHO reports, smoking prevalence in Romania is 25% and it is proving to be a European country to make some efforts in the adoption of laws to protect its citizens, both non-smokers and smokers (4).

Nevertheless, legislation in force still allows smoking in bars, restaurants, discos, and in public places, even in public institutions, in specially designated areas (5). This means that the Romanian citizen could be protected to some extent by legislation from exposure to cigarette smoke.

Since the strengthening of the smoking habit occurs somewhere around 18-19 years of age, and because students from medical universities may be key figures for future patients, we focused on this population, establishing certain key questions (research question).

The study evaluates compliance with the regulations against smoking in universities in Romania that aim to protect non-smoking students and employees. It will answer the question whether the University of Medicine and Pharmacy complies with the above mentioned legislation more or less than other, non-medical universities?

MATERIAL AND METHODS

Measurements of PM $_{2.5}$ particle concentrations were performed with an AM510 TSI Pack Aerosol Monitor device (6, 7). Recordings of PM $_{2.5}$ values came from central buildings of four universities. These buildings were previously evaluated, and potentially contaminated sites were identified (4-5 for each). Then, for each of the four to five predefined sites, monitoring was performed for 10-14 minutes by recording data every second. This has been done first during the holidays (minimum, constant employee activity), thus obtaining a baseline data for the following: site A - 3521 data from the central building of a state held medical university (medicine, pharmacy, dentistry, nursing) in Transylvania; site B- 3426 data from a state held university with non-medical profile, site C - 3045 data from a private university, site D - 3483 data from another private university with environmental profile. The averages of the PM $_{2.5}$ measurement values of the four universities were compared. Data were processed using Microsoft Office Excel.

The next step was to carry out measurements at site A (medical university) during full teaching activities, between

Tuesday and Thursday, from 10 to 12 and 14 to 16 hours, maintaining the sites of measurement. Recording was performed every 30th second. The evolution of the $PM_{2.5}$ particles from the first to the second measurement was noted and compared using statistical tests for nonparametric, unpaired data (Mann Whitney U) using the Graph Pad Instat software.

In case of the A university, the evolution of values from the teaching period compared to the holidays was also analyzed for each measurement site.

RESULTS

In the first phase, a pre-screening of the central buildings of the four universities has been performed, in order to establish potentially contaminated sites. Following establishment of these sites, the aerosol monitor has been deployed. The results of concentration measurements of fine particles during holidays placed site A (Transylvanian medical university) first. The 3521 measurements performed at five different areas, with a total measurement time of 58 minutes and 41 seconds averaged a value of 0.0168 mg/m^3. Second place, very close to the first, was taken by the non-medical state held university (site B). Its 3426 measurements collected from five different points in 57 minutes and 6 seconds, resulted an average of 0.0153 mg/m^3. In case of the private university – site C – the average value was 0.0140 mg/m^3 from 3045 measurements performed in four areas, and a total measurement time of 50 minutes and 45 seconds.

The lowest concentration was found at site D, (private university with environmental profile), where an average of 0.0059 mg/m^3 was calculated from 3483 measurements collected from five different points in 58 minutes and 7 seconds. All measurements from all areas were recorded every second (tab. I).

TABLE I

Average $PM_{2.5}$ measurements of the four universities in Transylvania

No.	University	Meas. No.	Meas. time	Average mg/m^3
1.	A –state held medical	3521	58:41	0.0168
2.	B –state held non-medical	3426	57:06	0.0153
3.	C – privately held	3045	50:45	0.0140
4.	D –privately held ecological	3483	58:07	0.0059

Next we went on to monitor the teaching period of university A. In order to obtain the most relevant results, we focused on maximum activity intervals during the week (Tuesday, Wednesday and Thursday), between 10 and 12, and 14 and 16 hours. The second set of measurements was not taken in the same place on the same day. Results obtained in the interval between 10 and 12 were compared for each measurement area with those recorded between 14 and 16 hours on another day (tab. II).

E- stair case 1st-2nd level, administrative zones neighborhood, next to a coffee machine; F- men's restroom, 1st floor; G- women's restroom, 1st floor; H- ground

floor corridor, doorman rooms neighbor- hood

TABLE II

Comparison of PM $_{2.5}$ value medians of the measurement areas

No.	Meas. area	Meas. No. 10-12/14-16	Median 10-12 h mg/m^3	Median 14-16 h mg/m^3	Mann W.U. test
1.	E	246/238	0.0258	0.0513	p<0.0001
2.	F	240/240	0.01051	0.01058	p=0.413
3.	G	237/240	0.0105	0.0124	p<0.001
4.	H	237/238	0.0258	0.0513	p<0.0001

Significant increase	No significant result	Significant decrease

So we see that in two of the above areas (E and G) the values increase significantly, there is no change (F) in one area and in one area (the one next to the doorman's room) (area H) there is a decrease during the second interval.

The next step was to compare the values obtained during the holidays with those obtained during full teaching activities for each of the measurement areas. For the teaching period, we used the values of both of recorded time intervals from one measurement area. The results show a significant increase in all recorded parameters at each measurement area; in one area there is even a 7-fold increase compared to the holiday period (tab. III).

DISCUSSION

Although in 2010 Romania joined the project initiated by the WHO - Global Health Professions Student Survey (GHPSS) – its results, the country report cannot be found between the reports from the other participating countries. This would provide information about smoking habits of students of the medical university, and about their desire to stop this habit. In their knowledge effective prevention programs could be developed. This is why the value of this study is important, because it shows objectively the presence of PM $_{2.5}$ particles from tobacco smoke inside the main building of the evaluated university (A) (1).

TABLE III

Comparison of PM $_{2.5}$ value medians of the holiday period and teaching period

No.	Meas. points	Meas. No. Hol./Tea.	Holiday median (mg/ m^3)	Teaching period median (mg/ m^3)	Mann W.U. test
1.	E	801/484	0.0127	0.0383	p<0.0001
2.	F	686/480	0.00934	0.0105	p<0.001
3.	G	686/477		0.0115	p<0.001
4.	H	612/475	0.0087	0.0567	p<0.0001

E- stair case 1st-2nd floor, administrative zones neighborhood, next to a coffee machine; F- men's restroom, 1st floor; G- women's restroom, 1st floor; H- ground floor corridor, doorman rooms neighborhood

very significant-7x

Moreover, comparing the values measured at different universities, it emerged that the medical university (A) has the most polluted air in the holiday period, which incriminates the administrative and ancillary staff. Private universities have complied more rigorously with regulations (5) concerning smoking prohibition in public buildings and institutions (C and D), but it should not be neglected that these are equipped with smoke alarms. But the law is broken not only in Romania: a study using the same methodology and measurements, performed at certain hospitals in Hungary to measure $PM_{2.5}$ with a TSI Aerosol Monitor Pack, showed that employees are smoking in their premises, even if it is strictly forbidden (8). A recent study in Romania, speaks of the smoking habit of young adults as a "lifestyle component" (2). Our study demonstrates as well that besides employees, students also greatly contribute to increased values of $PM_{2.5}$. This is demonstrated both by the significant increase in two of the measurement areas (E and G) of the medians of the 10-12 and 14-16 intervals, and by the comparison of values recorded during the teaching period to the values of the holiday period, which were statistically significantly higher when students were present. Therefore a program encouraging smoking cessation, as well as enforcement of existing legislation would be welcome.

Lastly, the not so very good position of Transylvanian universities regarding clean air is suggested by a study conducted in 32 countries in 2008; the study measured and analyzed the $PM_{2.5}$ values in a total of 1822 bars and restaurants, among others, in also Romania. Unfortunately, our country was in second place regarding air pollution by $PM_{2.5}$. It is to be noted the air quality in New Zealand regarding $PM_{2.5}$ contents. The restaurants in this country have cleaner air than some of our universities (0.008 mg/m^3, as compared to 0.0168 mg/m^3 at university A, 0.0153 mg/m^3 at university B and 0.0140 mg/m^3 at university C) (9).

CONCLUSIONS

This study may add to the WHO project initiated in Romania in 2010, proving objectively the presence of smoking within the Medical University. It turned out that both employees (based on the results of the holiday period), and students (based on the results of the teaching period) smoked at the premises of the Medical University. This also reveals the importance of informing the smoker, as well as the non-smoker students to promote cessation of active smoking, and avoid secondhand smoke, and in order to develop prevention programs and effective interventions.

Legal regulations regarding smoking in public institutions are often violated by university students, and staff both at the medical university, as well as other universities. A smoke alarm system may help law enforcement, thus preventing student-employee exposure to second hand smoke. The method of measuring fine particles can be used to test a future intervention among students.

ACKNOWLEDGEMENTS: The study was supported by the Fogarty Foundation NGO in collaboration with the Davidson Medical College North Carolina and the Medical School of Semmelweis University Budapest, and it is part of an international grant “Building Capacity for Tobacco Research in Romania”. Thank you goes out to the managements of the universities for allowing the recording of the presented data.

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NEWS

MATHEMATICAL MODEL EXPLAINS BACTERIA- HOST INTERACTIONS IN LYME DISEASE

The Lyme disease is a borreliosis characterized in early stage, by a rash on the skin named erythema migrans, caused by the interactions between bacteria and the host immune response. In this early stage the antibiotic treatment is most effective. The cutaneous rash has three separate morphologies: homogenous erythemas, central clearing rash with a peripheral ring erythematosus and characteristic central erythemas with a bull's-eye appearance. When the aspect of rush it's not characteristic, the diagnosis is difficult. Using a mathematical model that explains bacteria- host immune response interactions, researchers Vig and Wolgemuth show that the morphology of rash and the time during spreading are linked with the rates of bacterial replication and macrophage clearing. Also, this model revealed that the antibiotic treatment clears spirochetes from the dermis in the first week for all type of erythemas, but evolution of rush morphology is not an indicator of the efficacy of the therapy.The researchers suggest that the mathematical model could be able to explains bacteria- host interactions in other bacterial infection and could be used to develop guided treatment for Lyme disease (Vig DK, Wolgemuth CW. Spatiotemporal evolution of erythema migrans, the hallmark rash of lyme disease. *Biophys J*. 2014; 106(3):763-8).

Cătălina Luncă

ESTIMATION OF OCHRATOXIN A IN THE HUMAN BLOOD OF ROMANIAN POPULATION

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ESTIMATION OF OCHRATOXIN A IN THE HUMAN BLOOD OF ROMANIAN POPULATION (Abstract): Ochratoxin A (OTA) is a toxic secondary metabolite of fungi belonging to the *Aspergillus* and *Penicillium* genera, its presence in human blood being the primary indicator of exposure. **Aim:** In the present study we determined OTA in 38 blood samples collected from healthy Romanian subjects of both genders. **Material and methods:** The OTA was determined through the direct, competitive, solid-phase immunoenzymatic method; the minimum quantification limit for determining OTA in serum samples was 0.0289 ng/mL. **Results:** The positive sample percentage was 100%. OTA concentrations varied between < 0.04 ng/mL and 1 ng/mL. There were no significant differences between OTA concentrations in men versus women (0.24 ± 0.20 ng/mL versus 0.17 ± 0.15 ng/mL, p=0.3527). **Conclusions:** The analyzed blood samples exhibit a very high degree of exposure to OTA, but in only approximately 10% of the subjects exceeded 0.5 ng/mL, considered the threshold for OTA-induced renal pathology. **Keywords:** OCHRATOXIN A, BLOOD SAMPLES, IMMUNOENZYMATIC METHOD

Ochratoxins (A, B, C) are a group of compounds with related chemical structure, a isocoumarin derivate being linked to L-b-phenylalanine by an amide bond.

OTA is a hepatotoxic, nephrotoxic, carcinogenic, teratogenic, immunotoxic and neurotoxic compound that contaminates foods before harvesting or, more frequently, during storage (1). The mycotoxin is naturally produced by *Penicillium verrucosum* in temperate areas and by *Aspergillus ochraceus* and, more rarely, by *Aspergillus carbonarius* in tropical regions. The toxicity of OTA results from the inhibition of protein synthesis, disruption of calcium homeostasis, increased lipid peroxidation, mitochondrial dysfunction, and also through the effects at the level of transcription and transduction (2).

The purpose of this paper is to determine OTA in blood samples collected from Romanian healthy volunteers through a

solid-phase direct, competitive immunoenzymatic method.

MATERIAL AND METHODS

Reagents and equipment. STAT FAX 303 Plus ELISA microplate readers, (Awareness Technology, USA).

All reagents were multianalyte, supplied in a compact kit:

1. Calibrators with OTA in the concentration range 0.0 – 0.40 ng/mL: each kit contains multianalyte calibrators with values attributed through a reference method that attests that the values of the analytes were established according to reference materials certified by WHO for 6 levels of concentration (0.0, 0.02, 0.05, 0.1, 0.2, 0.4 ng/mL), in liquid conditioning form, that can be used as such.

2. Reagent kit for the quantitative determination of OTA, code DE991OCH01MS (Demeditec Diagnostics GmbH, Germany) containing all components for the ELISA reaction necessary for the determination of OTA.

Analyzed samples. Blood samples were collected from 38 healthy volunteers from Iasi, Romania. This study was approved by the Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy and was carried out in accordance with standard operation procedures. Written informed consent was obtained from all subjects before initiation of the study.

Method. The quantitative determination of OTA in human serum samples was realized through a solid-phase, direct, competitive immunoenzymatic method. The standards (calibrators) or samples are added to the polystyrene microwells coated with high-affinity antibodies and, if present, OTA couples with the antibodies. Next, OTA conjugated to the enzyme horseradish peroxidase (HRP) is added, forming bonds with the antibodies that have not yet been occupied by the OTA from the standard or sample. After a reaction incubation interval, the content is removed, the microwells are washed, and the chromogenic (3,3',5,5'-tetramethylbenzidine) (TMB) substrate is added. TMB soluble substrates yield a blue color when detecting HRP. The intensity of the color is directly proportional with the quantity of conjugate and inversely proportional with the quantity of OTA present in the standard or in the sample. The reaction is stopped by adding an acid solution that changes the color from blue to yellow. The intensity of the color is spectrophotometrically determined at 450 nm with a 630 nm reference filter.

The above described method for OTA analysis was linear for the concentration range 0.0-0.5 ng/mL, with the regression coefficient (R) = 0.968058367. The minimum detection limit was 0.0133ng/mL and the minimum quantification limit for OTA determination in serum samples was 0.0289 ng/mL.

Sample preparation. To determine OTA in human blood, the blood is collected in vacutainer serum tubes; the blood is centrifuged, the serum is separated and exposed to ethanol extraction: 750 µl absolute methanol is added to 250 µl serum. The mixture is homogenized, left at room temperature, centrifuged at 3500 rpm for 5 minutes and only the supernatant is used for OTA determination.

Procedure. The successive work steps to determine OTA in blood samples are described in tab. I.

Statistical analysis. The statistical analysis was performed using one-way analysis of variance (ANOVA) and Pearson's r. Tukey test was used for multiple comparisons (software *Stats Direct* version 2.6). A value of $p < 0.05$ was considered significant.

TABLE I

Procedure for the determination of OTA from serum

	BLANK	S_1 (0.0 ng/ml)	S_2 (0.02 ng/ml)	S_3 (0.05 ng/ml)	S_4 (0.1 ng/ml)	S_5 (0.2ng/ml)	S_6 (0.4 ng/ml)	Sample
S_1 (0.0 ng/ml)	-	100µl	-	-	-	-	-	-
S_2 (0.02 ng/ml)	-	-	100µl	-	-	-	-	-
S_3 (0.05 ng/ml)	-	-	-	100µl	-	-	-	-
S_4 (0.1 ng/ml)	-	-	-	-	100µl	-	-	-
S_5 (0.2 ng/ml)	-	-	-	-	-	100µl	-	
S_6 (0.4 ng/ml)	-	-	-	-	-	-	100µl	
Sample	-	-	-	-	-	-	-	100µl
Assay diluent	-	200µl	200µl	200µl	200µl	200µl	200µl	200µl
	100µl of contents from each mixing well of microtiter plate was transferred to corresponding Anti OTA antibody coated well from reaction microplate							
Incubation	30 minutes at ambient temperature (22ºC).							
Wash	Decant the contents of the wells. Wells were washed 3 times with washing buffer PBS-Tween (pause between washes was 60 seconds). After the last wash was absorbed excess moisture by blotting on filter paper.							
OTA HRP-conjugate	-	100µl	100µl	100µl	100µl	100µl	100µl	100µl
Incubation	30 minutes at ambient temperature (22ºC).							
Wash	Were carried out three successive washes with washing buffer PBS-Tween. After the last wash was absorbed excess moisture by blotting on filter paper.							
Substrat solution TMB	-	100µl	100µl	100µl	100µl	100µl	100µl	100µl
Incubation	10 minutes at ambient temperature (22ºC).							
Stopping the reaction with acidic solution	-	100µl	100µl	100µl	100µl	100µl	100µl	100µl
	Reaction contents were homogenized by 2-3 successive pipetting.							
	Optical density was read at $\lambda = 450$ nm with 630 nm reference filter.							

RESULTS

The study population comprised 9 males (23.68%) and 29 females (76.32%) with an average age of 38.63 ± 13.08 years, 38.24 ± 13.61 for women and 39.88 ± 11.87 for men.

The majority of the study population (28 subjects) was living in urban areas (74.69%) whereas 10 patients (26.31%) were from rural areas. All study participants were white Caucasians and had a average Body Mass Index (BMI) of 22.98 ± 1.83 kg/m^2 for women and 23.36 ± 1.96 kg/m^2 for men, with no statistical difference between sexes.

All 38 plasma samples exhibited the presence of OTA in concentrations ranging between 0.04 ng/mL and 1 ng/mL. The average OTA concentration in the examined plasma samples was 0.22 ± 0.19 ng/mL. Seven subjects (18.42%) exhibited OTA concentrations less than 0.1 ng/mL, 27 subjects (71.05%) between 0.1 - 0.49 ng/mL, 3 subjects (7.89%) between 0.5 - 0.99 ng/mL, and 1 subject (2.63%) of 1 ng/mL (fig. 1). There were no significant differences in OTA concentrations between men and women (0.24 ± 0.20 ng/mL versus 0.17 ± 0.15 ng/mL, p=0.3527). Our results indicated no correlations between OTA concentrations and age or BMI.

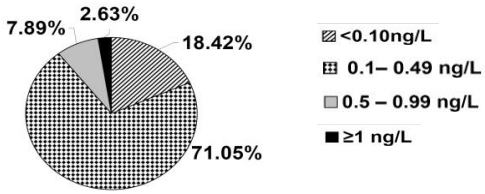

Fig. 1. The distribution of OTA concentration in blood samples from healthy Romanian subjects

DISCUSSION

OTA can be present as a natural contaminant of food and, as a consequence, it can be traced in human blood, breast milk and

animal-based products. In European diet, cereals (barley, wheat, corn and oat) and their derivatives are viewed as the major source of OTA ingestion, representing approximately 50% of the total input (1).

The importance of determining OTA plasma concentrations is related to the fact that OTA levels higher than 0.5 ng/mL are linked to the development of renal diseases (3).

The nephrotoxic action of OTA in humans was demonstrated by the occurrence of Balkan endemic nephropathy (BEN) and is associated with renal and urethral carcinoma, and, less frequently, with carcinoma of the bladder (1, 4).

Still, there are insufficient epidemiological and clinical data to demonstrate the carcinogenic potential of OTA in humans and it is considered that other nephrotoxic agents like aristolohic acid could be involved in BEN (5).

Similar studies using a detection limit of 0.025 ng/mL identified OTA in 324 of the 341 analyzed blood samples. 62% of the samples contained OTA concentrations lower than 0.2 ng/mL, while 17 samples (5.2%) presented concentrations higher than 0.50 ng/mL, indicating a low exposure of Italian population (6).

European studies reported high percentages (97%) of OTA positive blood samples at comparable detection limits, data that are in agreement with our results (7, 8). In similar studies conducted in African or Near East countries, these percentages were lower; thus, in Morocco, using a detection limit of 0.08 ng/mL the percentage of positive samples was 60%, while in Lebanon, using a detection limit of 0.60 ng/mL the percentage dropped to 33% (9, 10).

As to the difference in plasma OTA concentrations between men and women, the results are heterogeneous. Several authors indicated that plasma OTA concentration is higher in men than in women, whereas other studies revealed no significant differences between sexes (11, 12). Giuseppe et al. (2012) signaled a significant age-sensitive difference between these values in men and women, differences that were not found in our study (6).

CONCLUSIONS

In conclusion, our results indicate an increased level of exposure to OTA (100% of the blood samples), but only approximately 10% of the subjects exceeded 0.5 ng/mL considered the threshold for OTA-induced renal pathology.

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NEWS

ANEMIA- RISK OF DEMENTIA IN THE ELDERLY

According WHO criteria's, anemia is defined by a level of hemoglobin concentration less than 13 g/dL for men and 12 g/dL for women. This illness affects a quarter of adults over the age of 65. Another condition common in the elderly pathology is dementia. A study published in online issue of *Neurology*®, the medical journal of the American Academy of Neurology, show that anemia is associated with increased risk of dementia. The study group which includes 2552 elderly adults was investigated for anemia and cognitive disorders and memory for 11 years period. At baseline, 393 participants had anemia. At the end of the study, 23% of the patients with anemia have associated dementia. Only 17% of people without anemia have developed dementia. The increased risk of developing dementia for older adults with anemia has remained significant after adjusting for age, race, sex and education. These results suggest that anemia is a target for preventing cognitive disorders (Hong CH, Falvey C, Harris TB et al. Anemia and risk of dementia in older adults. Findings from the Health ABC study. *Neurology*, 2013 DOI: 10.1212/WNL.0b013e31829e701d).

Cătălina Luncă

EPIDEMIOLOGICAL ASPECTS OF HYDATIDOSIS IN CHILDREN, IN SOME AREAS OF NORTH-EASTERN ROMANIA

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EPIDEMIOLOGICAL ASPECTS OF HYDATIDOSIS IN CHILDREN IN SOME AREAS OF NORTH- EASTERN ROMANIA (Abstract). Hydatid cyst is a major public health problem in the pediatric population, both in terms of its frequency and of its endemic character in certain regions. **The aim** of the study was to present the incidence of hydatidosis in children who were hospitalized in pediatric clinics specialized in surgery of the Emergency Hospital for Children "St. Maria" from Iasi. **Material and methods:** Prospective study conducted in the interval 2008-2012 in a series of 194 children patients from Moldova, Romania. **Results:** The peak incidence of the hydatid disease is located around the age of 11-14 years (33%) in male patients (55%) from rural areas (79%) and families with low socioeconomic level. The hepatic localization of the hydatid cyst (50.51%) among children was more common than pulmonary localization (16.49%). Liver and lung localization was found in 22.16% of cases. **Conclusions:** Hydatidosis remains a major health problem requiring drastic measures to limit the spread and transmission of the parasite in humans, primarily by reducing the number of stray dogs. **Keywords**: *ECHINOCOCCUS GRANULOSUS*, HYDATIDOSIS, HYDATID CYST, CHILDREN.

Echinococcosis/hydatidosis are parasitic diseases in humans caused by *Echinococcus granulosus larvae* (1, 2). In the form of cysts, localized mainly in the liver, lung and not infrequently other organs, through the private development and its complications the parasite becomes the cause of various clinical and sometimes quite severe manifestations, which have turned this parasitosis into an important chapter of human pathology. Dog's role in the spread of this disease is well established (3), and from this point of view, Romania has its future assured, creating a huge reservoir of parasites, due to the large number of stray dogs (1).

The disease in humans and animals is produced by the larval form of cestode *Echinococcus granulosus* which as an adult parasitizes the small intestine of carnivores. The hydatid infection rate in our country among human and animal populations in recent years is downright alarming and is mainly due to lack of health education (4).

Hydatid disease is transmitted gastrointesinally through food infected with *Tae-*

nia echinococcus eggs (5). The main location of the hydatid cyst is the liver in a proportion of 50-60%, the second location being the one represented by the lung in a percentage of 30-40% (6).

MATERIAL AND METHODS

The study is retrospective and included a group of 194 patients, 107 males and 87 females, aged 1-18 years, hospitalized in pediatric clinics specialized in surgery of the Emergency Hospital for Children "St. Maria" from Iasi between the years 2008-2012 and diagnosed with hydatidosis. The aim of the study was to present the incidence of hydatidosis in children. Studying the admissions of the clinic, which generally concentrates the specific pathology from Moldova, we managed to constitute a statistically representative sample.

The study database was performed using the data obtained from clinical observation sheets of selected patients, paraclinical lab results and records of paraclinical and ultrasound investigations results and protocols related to surgery performed. Data from patients' medical observation sheets were entered into a Microsoft Excel spreadsheet.

RESULTS

The current study was conducted on a sample size of 194 patients diagnosed with hydatid cyst. Distribution on backgrounds (area of origin) showed differences in favor of rural areas. There is a higher proportion of patients from rural areas, a total of 154 children (79%) as compared to those in urban areas, 40 children (21%).

Distribution by sex of the value of prevalence recorded in our study shows a predominance of male versus female. Of the 194 patients, 107 were males (55%) and a total of 87 patients (45%) were females.

Fig. 1. Distribution of cases by area of origin

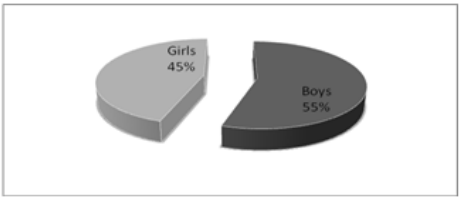

Fig. 2. The distribution of cases by sex

Regarding the distribution by age group, the hydatid cyst is found in children of different ages, showing an increased incidence in children of 11-14 years (33%) and 7-10 years (31%). The incidence is lower in toddlers and preschoolers (4%). The highest rate of hydatid cyst occurred in children with ages between 7-9 years, 11-13 years and 16-17 years.

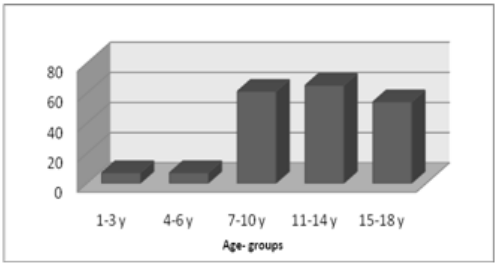

Fig. 3. Distribution of cases by age group

The possible source of infestation was the contact with the animal (dog) and the non-observance of the rules of hygiene.

Domestic animals were present in the household of patients studied, a situation which is significantly associated with an increased risk of parasites. Domestic animals are intermediate or final hosts for a large number of parasites including *Echinococcus granulosus*, the dog transporting parasite eggs on its fur (2).

The diagnosis was set through abdominal ultrasound, pulmonary X-rays and the parasitic etiology of these cystic formations was confirmed by the presence of anti-*Echinococcus granulosus* antibodies in the patient's blood.

The location of hydatid cysts was as follows: hepatic in 98 patients (50.51%), pulmonary in 32 children (16.49%), hepatic and pulmonary in 43 cases (22.16%). The number of cysts was variable: 131 children had one cyst (79.87%), 63 children (38.41%) had more than one cyst, of which 7 (4.26%) had localizations in several organs (liver, lung, spleen). Double and multiple localizations are relatively quite common and can lead to serious complications. Treatment of hydatidosis was extremely difficult, 34 patients needed surgical treatment, mainly cystectomy and for 88 children antiparasitic chemotherapy was the only method of treatment.

After surgery the disease may relapse in many cases. Given that relapses may occur after a few years these patients must be followed through imaging and immunological examinations (7).

DISCUSSION AND CONCLUSIONS

The peak incidence of hydatid disease is located around the age of 11-14 years in male patients, from rural areas coming from families with low socioeconomic level. The rural origin of most patients supports the inclusion of the disease in zoonoses (18), these patients being more exposed to a contact with pets. Hepatic location of hydatid cyst in children was more common than pulmonary location, a situation reported in other studies as well (9, 10).

Hydatid cyst is a major public health problem in the pediatric population, both in terms of frequency and of its endemic character in certain regions (11). In recent years there has been an increased incidence which proves that sanitary hygiene compliance is not rigorous, and an increase in the number of stray dogs (12).

Hydatid cyst, a parasitic disease with ubiquitous geographic distribution, far from being eradicated in our country requires a simple treatment method, with satisfactory results for both doctor and patient, both from a clinical evolutionary point of view and socio-economically.

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NEWS

NOSOCOMIAL INFECTIONS IN A SURGICAL INTENSIVE CARE UNIT

Medical and surgical intensive care unit patients represent two different populations and require different treatment approaches. The aim of this study was to investigate the parameters associated with mortality in medical and surgical intensive care units. A prospective cohort study of adult patients admitted to a medical and surgical intensive care unit teaching hospital over an 11-month period was performed. Factors associated with mortality were explored using logistic regression analysis. In total, 827 admissions were observed, and 525 patients >18 years old and with a length of stay >24 h were analyzed. Of these patients, 227 were in the medical and 298 were in the surgical intensive care unit. The surgical patients were older ($p<0.01$) and had shorter lengths of stay ($p<0.01$). The mortality in the intensive care unit (35.1 vs. 26.2, $p = 0.02$) and hospital (48.8 vs. 35.5, $p<0.01$) was higher for medical patients. For patients in the surgical intensive care unit, death was independently associated with the need for mechanical ventilation, prognostic score (SAPS II), community-acquired infection, nosocomial infection, and intensive care unit-acquired infection. For patients in the medical intensive care unit, death was independently associated with the need for mechanical ventilation and prognostic score. Authors concluded that, although the presence of infection is associated with a high mortality in both the medical and surgical intensive care units, the results of this prospective study suggest that infection has a greater impact in patients admitted to the surgical intensive care unit. Measures and trials to prevent and treat sepsis may be most effective in the surgical intensive care unit population. (Toufen C Junior, Franca SA, Okamoto VN, et al. Infection as an independent risk factor for mortality in the surgical intensive care unit. *Clinics (Sao Paulo)*. 2013;68(8):1103-1108.)

Alina Manole

PRENATAL DIAGNOSIS OF GONOSOMAL ANOMALIES: LIMITATIONS OF THE FISH METHOD AND GENETIC COUNSELING DIFFICULTIES IN 15 CASES

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PRENATAL DIAGNOSIS OF GONOSOMAL ANOMALIES: LIMITATIONS OF THE FISH METHOD AND GENETIC COUNSELING DIFFICULTIES IN 15 CASES (Abstract): Prenatal diagnosis (PD) by FISH or cell culture is today an important tool for the prevention of chromosomal anomalies. A difficult issue is prenatal detection of gonosomal anomalies. Most gonosomal anomalies neither affect life expectancy nor cause psychomotor retardation, but sexualization disorders and the lack of reproductive potential are a constant finding. **Aim:** This study aimed at identifying the medical problems the specialists and the parental couple are faced with at the time of the diagnosis of fetal gonosomal anomalies. **Material and methods:** This retrospective study (2004-2012) was conducted in the Prenatal Genetic Diagnosis Department of "CuzaVoda" Maternity by FISH technique in 1685 pregnancies. The AneuVysion probes were used for identifying and enumerating chromosomes 13, 18, 21, X, and Y via fluorescence in situ hybridization (FISH) in interphase nuclei obtained from amniotic fluid. **Results:** Fifteen fetuses were selected in which we were faced with difficulties interpreting the number of gonosomes: monosomy X (5 cases), pseudomosaicism XX/XY (3), trisomy XXY (3 cases), trisomy XYY (1 case), 45,X/46,XX mosaicism (1 case) and triploidy XXX (2 cases). Later, by repeating the analysis, 2 cases with pseudomosaicism XX/XY were excluded. A case highlighting the limitations of the FISH test was that of a fetus in which the FISH test revealed trisomy XXY, while postnatal karyotyping showed a six cell line mosaicism (marker and ring X chromosomes). **Conclusions:** All parental couples received nondirective genetic counseling, respecting the individuals' dignity and rights of self-determination. Parents received information on the natural course of the disease, treatment options, and psychological support and were involved in their child's recovery.
Keywords: PRENATAL DIAGNOSIS, FISH, MONOSOMY X, TRISOMY XXY, TRISOMY XYY.

Chromosomal diseases, with an incidence of approximately 1% newborns, high severity, chronic and disabling course, can be prevented by prenatal cytogenetic diag-

nosis. It uses minimally invasive, expensive, laborious techniques of embryonic or fetal cells study, requiring a rigorous selection of cases. The indications for the prenatal diagnosis of chromosomal abnormalities are: visceral anomalies at ultrasound examination, advanced maternal age, positive biochemical screening (abnormal double or triple test), family history of chromosomal disease, and a balanced structural chromosomal abnormality in a parent (1). Fetal cells are obtained by chorionic villi biopsy, amniocentesis or cordocentesis. Classical prenatal chromosome analysis requires *in vitro* culture of fetal cells with a waiting period of 2-3 weeks. An alternative method is the fluorescent in situ hybridization (FISH), based on the hydrogen bonds formed between the DNA of the analyzed chromosome (target DNA) and fluorescently labeled DNA probe (2). Sex chromosome abnormalities are common genetic diseases, affecting 1/400 - 1/500 live births. Increased frequency is correlated with minimal phenotypic changes. Gonosomal abnormalities detected by prenatal diagnosis create difficulties in genetic counseling related to the impossibility of estimating the severity of clinical features, with the decision-making process becoming a complex medical act (3). Prenatal medicine has brought great benefits related to the reproductive choice knowing the particular circumstances, but also generated ethical controversies. Prenatal diagnosis of chromosomal disease puts the prospective parents in a position to choose between recognizing the severity of the genetic disease, understanding and accepting the chronic illness without a pathogenic treatment, or terminating the pregnancy by therapeutic abortion. Severe chromosomal disease (trisomy 13, trisomy 18 or triploidy) is not marked by these dilemmas, because keeping the pregnancy is not put into question. Instead, gonosomal anomalies allow in most cases a fairly normal life. Thus, for the gonosomal anomalies detected prenatal, the genetic counseling must be completed by appropriate support, helping the parents to deal with psychological problems.

The aim of our study was to detect the diagnostic difficulties of gonosomal abnormalities by FISH method in prenatal diagnosis and to identify the genetic counseling issues and bioethical dilemmas in parental couples who have a child with a sex chromosome abnormality.

MATERIAL AND METHODS

This 9-year retrospective study (2004–2012) targeted the gonosomal anomalies identified in the Department of Prenatal Diagnosis of the Iasi "CuzaVoda" Maternity Hospital. Prenatal diagnosis by FISH method in interphase nuclei obtained from amniotic fluid was performed in 1685 pregnant women. Amniocentesis was performed between 16 and 27 weeks of amenorrhea (WA). FISH method used fluorescently labeled DNA probes specific for chromosomes 13, 18, 21, X and Y (AneuVysion Multicolor DNA Probe Kit Vysis CEP 18/X/Y - alpha satellite / LSI 13/21). In 15 cases gonosomal abnormalities were detected or there were difficulties in interpreting the number of fluorescent signals corresponding to the two sex chromosomes: X and Y (tab. I).

TABLE I
Particularities of the study group

No.	Patient	Analysis motivation	Gestational age (GA)	Results	Pregnancy outcome
1	FE	child with DS	20	46,XX/46,XY denied by repeat test	healthy newborn 46,XY
2	TI	CPC	24	46,XX/46,XY denied by repeat test	healthy newborn 46,XY
3	BN	CH, FA	17	45,X	TA
4	LM	ATT	17	45,X	newborn ♀ ST
5	GV	HC	16	45,X	TA
6	GI	ATT	18	47,XYY	newborn ♂ clinically normal
7	DT	CPC	26	47,XXY	newborn ♂ abnormal, CCM
8	JI	ADT	17	45,X	SA, fetus with CH
9	BI	ATT	16	47,XXY	newborn ♂ without KS features
10	VG	ATT	19	46,XX/46,XY	SA, fetus with IC
11	CG	ADT	18	69,XXX	TA fetal malformations
12	SE	DH, OH, SUA	27	69,XXX	TA fetal malformations
13	AE	ADT	18	45,X/46,XX	SA
14	RŞ	VSD, ASCA	22	45,X	SA
15	AG	ATT, AMA	18	47,XXY	newborn ♂ without KS features

GA - Gestational age; **DS**–Down syndrome; **CPC**– choroid plexus cyst; **CH**– cystic hygroma; **FA** - fetal anasarca; **ATT**– abnormal triple test; **AMA**– advanced maternal age; **ADT**– abnormal double test; **DH**– diaphragmatic hernia; **OH**–oligohydramnios; **SUA**– single umbilical artery; **VSD**– ventricular septal defect; **ASCA**– aberrant subclavian artery; **TA** – therapeutic abortion; **TS**–Turner syndrome; **CCM**– complex chromosomal mosaicism: 48,XY,+mar,+mar[29]/47,XY,+mar[14]/48, XY,r(X),+mar[10]/49,XY,r(X),+mar,+mar[3]/46,XY[3]/50,XY,r(X),r(X),+mar,+mar[3]; **SA**– spontaneous abortion; **KS**–Klinefelter syndrome; **IC**–intersex condition.

RESULTS

Six of our cases showed X monosomy. Five were homogeneous forms (single fluorescent gonosomic signal corresponding to chromosome X) and in the sixth case we found a 45,X/46,XX mosaicism (presence of only one gonosomic fluorescent signal corresponding to X chromosome in 60% of the cells). The evolution of pregnancies was the following: one ended with the birth of a baby girl with Turner syndrome (monosomy X confirmed by postnatal chromosome analysis), three ended in miscarriage (one with cystic hygroma), and two ended in therapeutic abortion due to cystic hygroma. In three cases we found both cells with two fluorescent signals corresponding to X chromosomes and cells with a signal

for chromosome X and a signal for Y chromosome. Pseudomosaicism 46,XX/46, XY was not confirmed by repeat analysis in two cases, proving that the first amniotic fluid samples were contaminated with maternal blood. Both pregnancies ended in normal male babies. In the last case, repeat analysis confirmed the 46,XX/46,XY mosaicism, indicating a true hermaphroditism. The intersex stated was confirmed by anatomopathological examination, the pregnancy ending in miscarriage. In three cases we found a trisomy XXY, the FISH test showing two green signals corresponding to X chromosome and a red signal for Y chromosome. Two pregnancies ended in two baby boys, postnatal chromosome analysis confirming the trisomy XXY. In another case we were faced with one of the limitations of FISH technique in prenatal diagnosis. Thus, although in most fetal cells we detected three fluorescent signals corresponding to gonosomes, we also identified cells with extra small green fluorescent signals. Due to the small number, these cells were interpreted as artefacts. Although the fetus presented choroid plexus cysts, there were no reasons for therapeutic abortion and pregnancy ended with the birth of a baby boy weighing 2750 g and measuring 47 cm in height.

Clinical examination showed microcephaly with scaphocephaly, hypertelorism, epicantus, bilateral cryptorchidism and hypotonia. Postnatal chromosomal analysis revealed mosaicism with 6 cell lines:

48,XY,+mar,+mar/ 47,XY,+mar/48,XY ,r(X),+mar/

49,XY,r(X),+mar,+mar/ 46,XY/50,XY, r(X),r(X),+mar,+mar

The ratio of the six cell lines was: [29]/[14]/[10]/[3]/[3]/[3]. In one case we found a trisomy XYY (a single green signal for chromosome X and two red signals for chromosome Y), the pregnancy ending with the birth of a clinically normal baby boy. Postnatal chromosome analysis disclosed the 47,XYY formula. In two cases we identified three green signals corresponding to X chromosome, but also three fluorescent signals for chromosomes 13, 18 and 21, proving a triploidy: 69,XXX. Both pregnancies were terminated, and the anatomopathological examination revealed fetuses with multiple congenital anomalies and severe intrauterine growth retardation.

DISCUSSION

Globally, gonosomal abnormalities have a high incidence, being the most common chromosomal pathology, affecting 1/500 live births. The most common abnormalities are monosomy X, trisomy X, trisomy XXY and trisomy XYY (4, 5).

Monosomy X was the most common gonosomal abnormality in our group. It affects 1/2500 women and causes Turner syndrome, characterized by short stature, primary amenorrhea, infertility and normal intellectual development in most cases. Although phenotypic changes are not very severe, 90% of fetuses with cystic hygroma and monosomy X shows fetal anasarca, causing spontaneous abortion (10% of spontaneous abortions in the first trimester) (4, 6). The prenatal detection of monosomy X is usually accidental and, due to good integration into society, it is not an indication for therapeutic abortion, except for the cases with cystic hygroma (7). In one of our cases the pregnancy had a normal evolution; the girl has a favorable outcome and benefits from growth hormone therapy.

Trisomy XYY affects 1/1000 boys and is a disease diagnosed fortuitously, because unless high stature and possibly hypo fertil-

ity are present, no other particular clinical signs are present (4, 8). In our case the family decided to keep the pregnancy, which was ended by the birth of a clinically normal baby boy.

Trisomy XXY causes Klinefelter syndrome, the most common cause of male chromosomal infertility. The disease affects 1/1000 boys and is characterized by hypogonadotropichypogonadism, but close to normal intellectual development. Two of the trisomy XXY cases detected by us were homogeneous, and at the time of birth the children showed no clinical manifestations. In the third case we identified a complex mosaicism with six cell lines and additional X chromosome fragments (marker and ring X chromosomes). To diagnose such a case is impossible by using only the FISH technique, the anomaly being mosaic and the marker chromosomes representing X chromosome fragments that do not contain centromeric sequences (the region where the probe corresponding to the chromosome X is attached). Polysomy XY with more than two X chromosomes generates mental retardation and birth defects, features identified in our cases. Also, additional ring X chromosomes induce microcephaly, growth retardation, genital abnormalities and mental retardation (4, 9).

Triploidy, characterized by the presence of an extra haploid set of chromosomes, generates a severely altered phenotype, which usually induces miscarriage (4). In the cases of triploidy diagnosed by FISH technique are identified three fluorescent signals corresponding to the gonosomes, and three signals for chromosomes 13, 18 and 21. Both pregnancies with triploidy in our study showed three signals for X chromosome, and ended in miscarriages, the fetuses presenting multiple congenital anomalies.

In all cases there were some genetic counseling issues (related to the type of chromosomal abnormality) and difficulties in providing psychological support. Communication of an abnormal result in prenatal chromosome analysis is a difficult process, inducing various reactions from the parents: confusion, guilt, anxiety related to child's development and integration into society, helplessness, rejection of the baby. In the first instance, most couples chose termination of a pregnancy with pathological gonosomal abnormalities, even if only minor development features were modified.

Reaction to diagnosis depends on culture, education, religion and economic status of the couple. Overcoming obstacles imposed discussions with parents so that they understood the significance of the diagnosis and took an informed decision about the pregnancy (10, 11, 12). Depending on the evolutionary potential, the pregnancies could be divided into two groups. One group included the cases with trisomy XXY, trisomy XYY and monosomy X without cystic hygroma, characterized by a positive development and satisfactory integration in society. In these cases, during genetic counseling it is explained to the family that there is no reason for therapeutic abortion (10, 11, 12, 13). This strategy was applied by us in our eight such cases. The other group included the cases of triploidy and monosomy X with cystic hygroma. In both situations, the pregnancy has a negative prognosis, ending in a miscarriage with the fetus having multiple congenital anomalies; pregnant women require exceptional attention, particularly when the pregnancy is "special", being obtained after several attempts (7). Thus, the severe progression of the disease should be explained,

with its negative repercussions on the reproductive potential of women, the best option being the therapeutic abortion. In fact, in our cases parental couples have opted for therapeutic abortion, the anatomopathological findings confirming the malformations. In the cases with 46,XX/46,XY pseudomosaicism there were genetic counseling difficulties in the first instance related to accepting the intersex status associated with true hermaphroditism, but repeat tests confirmed the contamination of the first amniotic fluid sample with maternal blood (14).

Another problem we were faced with is the legitimacy of abortion. In Europe there are countries that do not allow abortion (Malta, Ireland), countries that support therapeutic abortion when the fetus has non-viable birth defects or the pregnancy is dangerous for the mother (this approach is applied in Cyprus to 28 WA, 24 WA in Finland and United Kingdom and 22 WA in Spain) and countries that allow abortion on-demand (mostly to 12 WA, except Romania 14 WA, 18 WA in Sweden and 10 WA in Slovenia). In all therapeutic abortion cases, pre-and post-abortion counseling support is necessary (15).

CONCLUSIONS

Our study, aiming to detected gonosomal abnormalities by FISH technique, enabled us on the one hand, to highlight the limits of this type of diagnosis, and on the other hand, to reveal the genetic counseling difficulties. The main limitations are related to the impossibility of correctly quantifying the gonosomal mosaics and interpretation difficulties for some 46,XX/46,XY mosaicism types. Genetic counseling in prenatally detected gonosomal anomalies faces difficulty in convincing the couple that the fetus has a chromosomal abnormality with minor changes in phenotype that allows a normal integration in society, so that they can get over the psychological trauma induced by prenatal diagnosis and choose to maintain the pregnancy. On the other hand, when non-viable abnormalities or with a minimal development potential are identified, the possible negative consequences to maintaining the pregnancy should be objectively presented.

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NEWS

CORTICOSTEROIDS IN TUBERCULOSIS THERAPY

Tuberculosis (TB) is a major public health problem which determining 1.4 million deaths in each year. The steroids as an associated treatment with anti-TB drugs are routinely used for reduce inflammation, only in certain types of TB. Professor Critchley and collaborators from St George's, University of London have made a systematic review and meta-analysis to estimate the steroids capacity for reduced mortality in all forms of tuberculosis. The researchers examined results from 41 previous trials on the efficacy of corticosteroids therapy in some forms of TB (pericarditis, meningitis, peritonitis, pleurisy and pulmonary TB also). Patient's age, the comorbidity, the types, the doses and the duration of corticosteroids therapy have varied. The results show that steroids therapy has reduced mortality by 17%, regardless of the form of tuberculosis or type of anti-TB therapy. However, researchers say that further studies are necessary for the evaluation if the benefits of steroids therapy for all TB forms would outweigh the risk of side effects, especially for multidrug resistant TB cases (Critchley JA, Young F, Orton L. Corticosteroids for prevention of mortality in people with tuberculosis: a systematic review and meta-analysis. *The Lancet Infectious Diseases*, 2013; 13 (3): 223).

Cătălina Luncă

MULTIPLE MYELOMA WITH BILATERAL HUMERUS LOCATION CASE REPORT

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MULTIPLE MYELOMA WITH BILATERAL HUMERAL LOCATION: CASE REPORT (Abstract): Multiple myeloma, also known as Kahler's disease or myelomatosis, is a type of cancer that begins in plasma cells. It is the second most common hematologic malignancy (13%) and accounts for 1% of all cancers. A mnemonic sometimes used to remember the common tetrad of multiple myeloma is *CRAB*: C = Calcium (elevated), R = Renal failure, A = Anemia, B = Bone lesions. **Case report**: We present the case of an 70- year-old male with bilateral humeral location. Diagnosis was difficult because of atypical X-ray findings in the right shoulder, the classic finding being punched-out lesions. ESR, blood, renal and liver tests were atypical. **Conclusion**: Although the diagnosis of multiple myeloma is easy, being based on blood and kidney tests and X-ray examination, in some cases an accurate diagnosis is obtained only by pathological examination. **Keywords:** MULTIPLE MYELOMA, PLASMA CELLS, HUMERUS.

Multiple myeloma (MM) is a cancer of plasma B cells, also known as Kahler's disease or myelomatosis. It is the second most common hematologic cancer (13%) and accounts for 1% of all cancers (1).

The exact cause of multiple myeloma is not known, and most myeloma patients are elderly.

It is located in the bone marrow, especially in the vertebrae, ribs, skull, pelvis and femur and is accompanied by numerous clinical manifestations including anemia, bone lesions, hypercalcemia, renal dysfunction and compromised immune function. A mnemonic sometimes used to remember the common tetrad of multiple myeloma is *CRAB*: C = Calcium (elevated), R = Renal failure, A = Anemia, B = Bone lesions" (2).

Diagnosis is based on the presence of neoplastic plasma cells in the bone marrow or elsewhere extramedullary together with the presence of multiorgan dysfunctions.

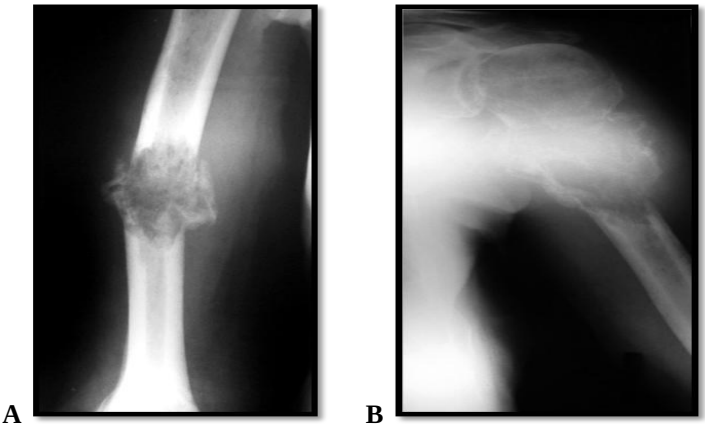
CASE REPORT

Patient, aged 70 years, reported about two months earlier a traction injury of the left upper limb and about 1.5 months ago one of the right upper limb for which he presented at the Câmpulung Moldovenesc Hospital, Suceava County, where he was diagnosed with pathological fracture of the middle third of the right humeral shaft and of the proximal third of the left humeral shaft, both treated by plaster splint. Because after about two months both fractures were not consolidated, the patient was referred to our department.

The patient underwent laryngeal surgery

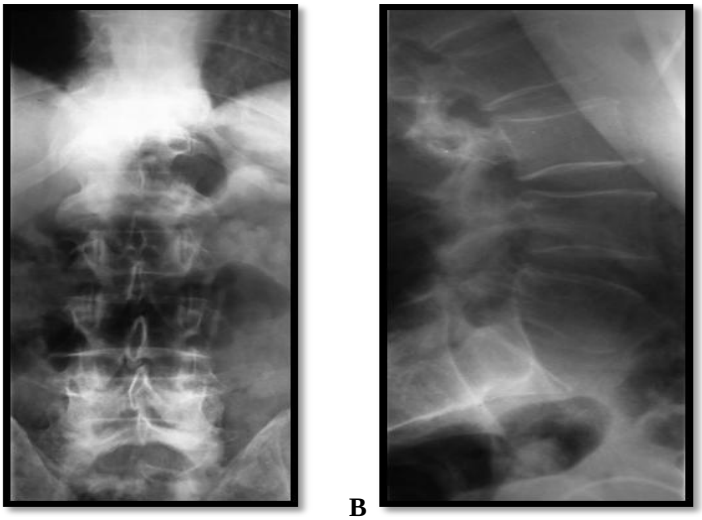
30 years ago and surgical removal of a rib segment about 15 years ago, but was unable to provide detailed information. The patient had a first-degree relative with leukemia.

Physical examination showed an increase in volume of the left shoulder and right arm in the middle third. Radiographs showed in the right humerus a relatively well-defined osteolytic area of approximately 30 mm accompanied by fracture (fig. 1A), and in the left humerus a poorly-defined osteolytic area covering the entire proximal humeral epiphysis, with moderate periosteal reaction and extension to the neighboring soft tissues (fig. 1B). Thoracic radiography showed the absence of the lateral arch of the VIII-th right rib of about 8 cm, and lumbar column radiography revealed no osteolytic lesions (fig. 2). Abdominal and pelvic ultrasound revealed hepatic steatosis, homogeneous spleen and prostatic hypertrophy.



A **B**

Fig. 1. X-ray - anterior-posterior view: A – right arm, B – left shoulder



A **B**

Fig. 2. Lumbar column X-ray,: A – anterior-posterior view, B – lateral view

Laboratory tests detected a slight increase in granulocyte count (70,1/%), moderately increased ESR (16 mm/h); total proteins and the other biological tests were normal.

Scintigraphy revealed hyperfixing sites projected in areas of the middle 1/3 of the right humeral shaft, proximal third of the left humerus, very heterogeneous, which appeared to exceed the bone contour. It also detected linear hyperfixed areas at the level of the xiphoid appendix; moderate hyperfixing sites in the posterior arches of the CIX and CX right ribs; heterogeneous hyperfixing in the lumbar spine with moderate hyperfixing al the level of the right costovertebral joints CVIII, CIX and CX. It was concluded that in the context of recent trauma and in the absence of other pathological hyperfixing sites, a traumatic etiology seemed more likely but a tumoral etiology could not be ruled out. (fig. 3).

Contrast enhanced MRI showed the presence of an expansive, solid, heterogeneous formation in the upper third of the left humerus, which disrupted the bone cortical with invasion of the brachial biceps and triceps muscles; with invasion of the left scapula, mainly the superficial muscle plane (supra and infraspinatus muscles), and subscapular muscle (fig. 4A). In the right humerus multiple well-defined sites of "gadolinophilic" lesions of 2 to 30 mm, disseminated intraosseously in the upper two-thirds were seen (fig. 4B).

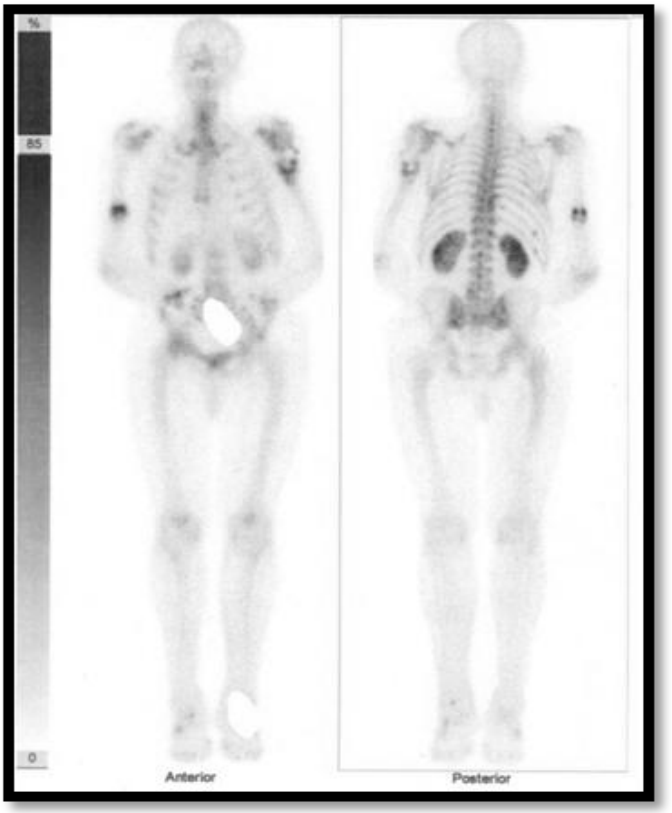


Fig. 3. Bone scan - full body.

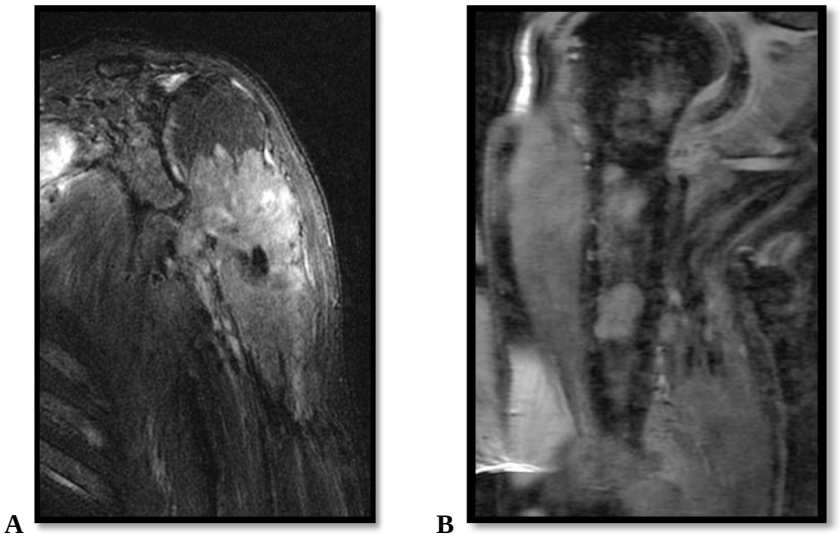


Fig. 4. Contrast enhanced MRI: A – left shoulder; B – right arm.

Pathological examination of hematoxylin-eosin stained slides revealed bone tissue of osteolytic appearance and tumor fragments consisting of a monomorphic plasma cell population, which, in the left humerus, also infiltrated the muscle, these findings being conclusive for the diagnosis of multiple myeloma.

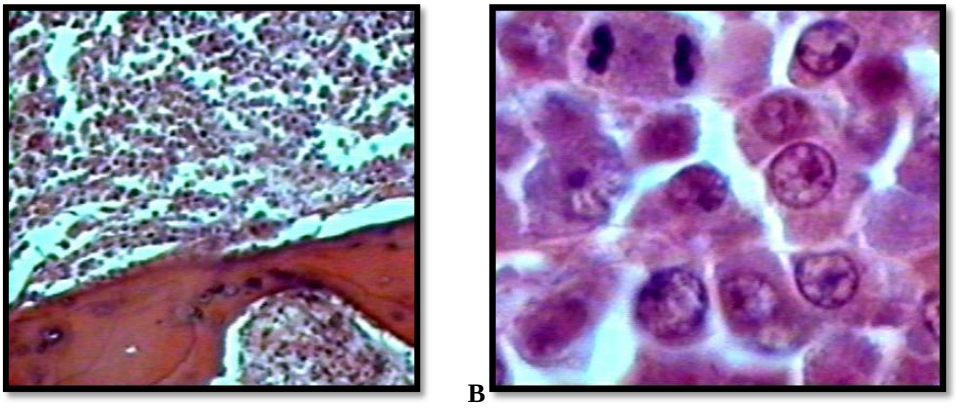


Fig. 5. Multiple myeloma, H & E staining (A - Ob. 20x, B – Ob. 90x)

DISCUSSION

The most common locations of MM include the ribs, vertebrae, pelvis and skull cap (3). In our case it was located in the humerus.

In our patient the diagnosis was difficult due to the development of an atypical radiologic image picture in the left shoulder; radiologically, MM is characterized by the presence of "punched-out" osteolytic lesions prevalently located in the shaft of long bones (4).

MM diagnosis can be made with pretty much certainty on the basis of laboratory tests. In our case these were not conclusive enough. MM is characterized by normochromic anemia, normocytic anemia, elevated ESR, hypercalcemia, elevated serum proteins, Bence-Jones protein in urine + (5), while our patient presented only a slight increase in granulocyte counts (70,1/%) and a moderately increased ESR (16 mm/h), kidney and liver tests being normal.

In our patient, diagnostic certainty came from pathological examination performed on tissue biopsy taken from the left shoulder and proximal third of the right humerus.

MM is a malignant neoplasm of plasma cells that remains incurable by conventional chemotherapy, median survival being 2-3 years (6). It is usually chemosensitive, but frequently enters a plateau phase of variable duration (6).

The presented case had an atypical progression with quite important extramedullary extension. The cases with extraosseous extension of an intramedullary tumor nodule as a result of cortical erosion and subsequent spread beyond the periosteum are not rare, bur soft tissues involvement is exceptional occurring in rare forms of IgD MM (7).

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PATHOLOGY OF THE OPTIC NERVE INJURY

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PATHOLOGY OF THE OPTIC NERVE INJURY (Abstract): The optic nerve injury is produced by damaging the optic nerve within a craniocerebral trauma. The anatomo-pathology and the pathology of posttraumatic visual impairments are fundamental data for the therapeutic indications. The microscopic examination reveals only the particular cases of very serious or severe extensive lesions. Damage to the vessels of the optic nerve, damage and break of the optical fibers are responsible for immediate visual impairments and secondary hematomas and edemas involving. The factors for the late appearance of visual impairments are numerous and often confounding. **Material and methods:** In the present research, we have a female patient D.A., aged 29, diagnosed with closed fracture in the right frontal lobe, irradiated to the optic canal; blindness in the right eye. **Results:** Break of the optical fibers is a result of bone fragments or of traction and torsion. Damage to the vessels of the optic nerve represents the main cause of blindness for most authors. Traction and torsion of the vessels at the moment of injury may cause thrombosis and small hemorrhages which lead to severe ischemia of the optical fibers. **Conclusions**: The optic nerve injury is produced by damaging the optic nerve within a craniocerebral trauma, through an indirect mechanism most of the times. **Keywords:** CRANIOCEREBRAL TRAUMA, OPTIC NERVE, VISUAL IMPAIRMENTS

The optic nerve injury is produced by damaging of the optic nerve within a craniocerebral trauma. The microscopic examination reveals only the particular cases of very serious or extensive lesions; the anatomical and clinical examination is usually missing. Only the neurosurgical intervention undergone in order to explore the nerve can provide information macroscopically (1, 2).

The macroscopic examination finds specific relations by highlighting cause and effect, but sometimes the examination undergone during the surgical intervention is incomplete; moreover, opening the optic nerve sheath is rarely in the literature (3, 4, 5).

There are several causes distinguished of immediate and late visual impairment. Immediate visual impairments are divided, clinically speaking, into complete and partial impairments. Within the optic nerve traumas the main cause that can be associated with different degrees is *optic nerve damage* - can be a bone injury as a conse-

quence of an injury to the optic canal or a direct injury. The direct injury to the optic nerve is visible; it can be a result of a foreign body or it can occur as a result of bone fractures. Optic nerve injury by fracturing the canal has a frequency which varies according to different authors: Rollet, Paufique, Barkan, Lazorthes (1, 3) consider them to be frequent injuries; Wagemann, Berlin, Strieff, Genet and Turner (7, 8) insist on the rare occurrence of this type of injury. This disagreement occurs because of insufficient radiologic examinations and due to the fact that some patients underwent a surgery while others were treated medically.

It is important to establish the cause-effect relationship between the bone fracture and the injury to the optic nerve. The opinions are different. In some cases, the cause effect relationship is obvious: the nerve is compressed by bone fracture in the canal area, the canal and the nerve are displaced, and bone fragments penetrate the nerve while the sheaths are breaking down – a fracture without an evident displacement (6, 7, 8).

According to other authors (9, 10), the optic canal frequently has a very small and reversible deformation due to a flattened diameter. These deformations can also be permanent as some radiographies show it – they can injure the optic nerve. In rare cases, the bone lesions accompany the optic nerve injuries without being the causal factor.

MATERIAL AND METHODS

The female patient D.A., aged 29, has the following diagnosis: - closed fracture in the right frontal lobe, irradiated to the optic canal; blindness in the right eye; right frontal flap, disease of the scalp skin; bone flap with lateral pedicle; lumbar puncture with convenient intracranial hypotension. Dura mater is separated from the roof to the little wing along which a fracture is found parallel to the posterior border unfolding into numerous fragments together with the inferior, superior and external fragment. It follows frontal ablation of the orbital roof and bone fragment compression and 75 % of the circumference of the optic canal is liberated. Inside, an ethmoid cell is opened and many bone fragments are found inside; these fragments are ablated.

RESULTS AND DISCUSSION

The ethmoid cell is obstructed. Haemostasis is assured. The dura is suspended and the anatomical plans are restored. The diagnosis is established intraoperative; interruption of the right optic nerve syndrome; frontal vault fractures irradiated to the optic canal.

Break of the optical fibers are a result of bone fragments or of traction and torsion. Minassian (4) reported a case of injury to the optic nerve without fracturing the canal. Nucci et al. (6) makes the assumption that there may be optic nerve contusions with interrupted nerve fibers caused by the relative motion of the encephalon in the skull, producing tractions and torsions at the level of optic canal and junction areas. Nevertheless, this hypothesis cannot be demonstrated surgically.

Damage to the vessels of the optic nerve represents the main cause of blindness for most authors (7, 8). Traction and torsion of the vessels at the moment of injury may cause thrombosis and small hemorrhages which lead to severe ischemia of the optical fibers.

Functional disturbances of the optic nerve generate general physico-pathological reactions and some spontaneous remissions

of immediate blindness can be explained. These functional disorders are in connection with the blocking of subdural and subarachoid space; this way, the passing of fluid along the nerve is prevented; the flow of the fluid elements can be restored only after the decompression of these inextensible areas – the optic canal and Zinn's ligament, accordingly.

Castros (6) claims that immediate visual impairments can also occur within the traumatic retinal angiopathy through venous reflux (Purtscher's retinitis) and due to retinal posttraumatic ischemic syndrome, through central retinal artery thrombosis.

Secondary appearance of impairments or blindness is a consequence of the initial perturbations, but which lack clinical expression or the examination does not reveal it. Hematomas can lie inside three levels: inside the duramater and extradural, inside the subdural space and in the optic nerve. These hematomas are frequent inside the subdural space; they do not have specific limits and are easily evacuated. Subdural hematoma or sheath hematoma can stretch along the walls of the nerve, and this may cause severe compromised vascularization; hematic compressive lesions may lead to necrosis of nerve substance; it may also lead to thrombosis of small vessels of piamater. The arterioles break in the vaginal space may produce intra-nervous hematomas; they can organize themselves in order to produce fibrotic glial tissue and adjacent necrosis. These intravenous hematomas can explain the unsystematised amputations of the visual field (6, 7).

Edemas apparently seem that are early lesions and not immediate ones. Juge (4) finds cases in which edemas appear 3 months after the initial trauma. Due to the rigidity of the bone canal, the edema causes a veritable nerve strangulation, so that during the decompression it can be seen the trace left on the nerve sheath. In edematous forms, the postoperative treatment with corticosteroids is compulsory.

Late impairments are often unknown due to the fact that they evolve gradually and they are clinically latent. These forms have a certain importance because they get good results after the surgical intervention.

Gliosis scar tissue is consecutive to the attrition of the optical fibers and nerves' vessels and they are represented, at least at the beginning, by histological repairs of the damage. New lesions are produced due to the fact that these scarring processes can extend.

Perioptic arachnoiditis were described by Marinescu and Cazaban (3). They can occur in nervous system disorders, traumas, tumors and infections, and the general conditions of the body (intoxications, infections, especially facial cavities infections). Arachnoid neoformations form a construction of the nervous tissue and they tend to comprise the optic nerve and chiasm. Sometimes incisures can be seen as a result of the strangulation of the optic nerve or chiasm. So, the adhesions press the nervous tissue transacting it. At the same time, the circulation is disturbed: the arteries appear to be atrophic, the flow in the arterial circulation is diminished; the veins are dilated and congested, so the return flow is not normal. The mechanic and vascular elements contribute to the anatomic and functional alteration of the nervous cells.

Chronic swelling of the nerve – it was reported in other observations (8, 10).

Progressive development of a bone callus is an exceptional lesion. Streiff (4) cited a case where the diagnosis was established

radiologically, 5 years after the trauma took place, even if the patient had lost his vision immediately after the injury. Functional disturbances of the optic fibers or of the vascularisation of the nerve – it seems that they play an important role. Also cavernous carotid aneurysms, according to Brihaye (6) or ophthalmic artery aneurysm were detected.

CONCLUSIONS

The optic nerve injury is produced by damaging the optic nerve within a craniocerebral trauma, through an indirect mechanism most of the times. In these cases, the fracture of the skull base, with or without the involvement of the optic canal, determines immediate bilateral or unilateral blindness, stationary or slightly regressive. The fracture trajectory may or may be not visible through imagistic diagnosis at the level of the canal or the optical hole, and it is not compulsory to have dilacerated or compressive bone fragments.

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SEVERE ENTEROVIRAL ENCEPHALITIS COMPLICATED BY ENCEPHALOMALACIA - CASE PRESENTATION

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SEVERE ENTEROVIRAL ENCEPHALITIS COMPLICATED BY ENCEPHALOMALACIA - CASE PRESENTATION (Abstract): Enteroviral encephalitis is a rare neuroinfection more often diagnosed in children within the context of enteroviral epidemic outbreaks. It has pleomorphic clinical features, variable severity and a definite potential to cause neuropsychological sequelae especially in infants. Some subtypes are extremely severe with a mortality rate of up to 25% by affecting the brain stem. These subtypes usually come with highly specific neuroimagistic findings. We are presenting a paediatric case of diffuse encephalitis most likely enteroviral in nature, with some particular features concerning the severe clinical form, the neuroimagistic aspects and the neuropsychological sequelae due to the rarely described evolution towards encephalomalacia. **Key words**: ENTEROVIRUS, ENCEPHALITIS, ENCEPHALOMALACIA.

Acute encephalitis is a rare manifestation of infection with enteroviruses, causing less than 5% of all encephalitis (1). The most widespread form is that of common, diffuse encephalitis, with sudden onset within 3 days after prodrome, with hyperpyrexia syndrome of intracranial hypertension, seizures impaired sensorimotor leading to apathy, lethargy, and coma. The focal forms occur before the age of 18 months, associated with partial seizures, hemichorea, ataxia and they generally have a minimal correspondent imaging (2,3). Severe damage to the brainstem by some strains, to neurogenic pulmonary edema, is described as being associated with certain epidemic outbreaks, with a mortality of 25% (4). Accurate diagnosis is conferred by virus isolation from cerebrospinal fluid (CSF), brain tissue, or possibly from other sites, accepting the serological diagnosis as well. Ultrasound examination is extremely valuable in locating specific lesions (5).

CASE PRESENTATION

A 3-year old patient without a significant pathological history, properly vaccinated for her age, having a sudden onset of the disease 48 hours before admission, with a temperature of 38°C, accompanied by an episode of tonicoclonical seizures which lasted for 2 minutes. The pediatrician sets the diagnosis, that of herpangina, and recommends treatment at home, and further neuropsychiatric evaluation. After 24 hours, the patient presents vomiting, has a seizure episode lasting for 30 minutes, accompanied by emission of urine, which is slowly cupped with intravenous Diazepam. She is admitted to the Infectious Disease

Clinic on the 29th of May 2011, suspected with acute meningoencephalitis.Admission examination. Influenced malaise, low grade fever 37.8°C, drowsy (after administration of Diazepam), active attitude, stature-weight hypotrophy, pale facies, erythematous pharynx with microvesicles and ulcer on the posterior wall, stethacoustic pulmonary vesicular murmur, pulse rate (PR) 104 beats/ min, liver palpable at 1 cm below the right costal part, absent meningeal irritation syndrome, unreleased abdominal skin reflexes on the right, more reduced osteotendinous reflexes (OTR) on the level of the right lower limb. Laboratory analysis revealed no biological inflammatory syndrome, the absence of haematologic, liver or kidney disorders, repeated negative serology for HIV, herpes simplex virus and Borrelia burgdorferi. Anti-enterovirus IgM antibodies showed a positive value in the second week of hospitalization 12.65 U, with values rising after another week (13.30 U).

Laboratory investigations

Fundus examination (FE) on admission shows papilla with vaguely defined borders, an aspect that is maintained for six weeks. Chest X-ray: emphasised broncho-vascular hilo-basal scanning on the right side. Lingual secretion: *Candida albicans* sensitive to fluconazole.

Initial examination by cranial computer tomography (CT) (29th of May 2011) does not reveal heterodense intracerebral lesions. Examination by multiplanar magnetic resonance imaging (cranial MRI) (31st of May 2011) in dual weight discretely reveals a T2 hypersignal and a T2- flair and a diffuse T1 hyposignal, by deleting the left temporal lobe and left parietal plot, suggesting the onset of encephalitis; symmetrical ventricular system, in the midline, basal cisterns without pathological modifications of signal (fig. 1).

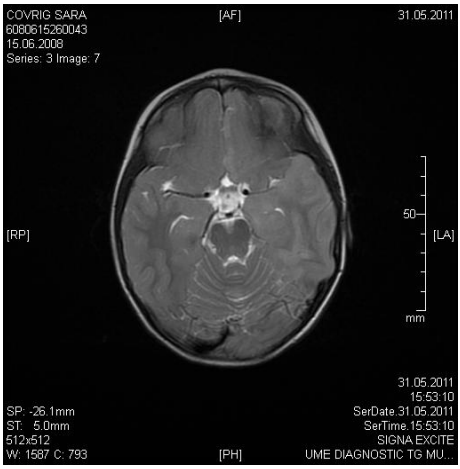

Fig. 1. IRM cranian 31.05.2011

Cranial CT (24th of June 2011): large areas of digitiform hypodensity, with net, hyperdense contours, data given by the affected gyri (cortical) located on the basal front and left parietal part. No spontaneous hyperdense accumulation on the level of the brain substance and subarachnoid space was present. Cerebral atrophy, increase in the size of the basal cisterns (fig. 2).

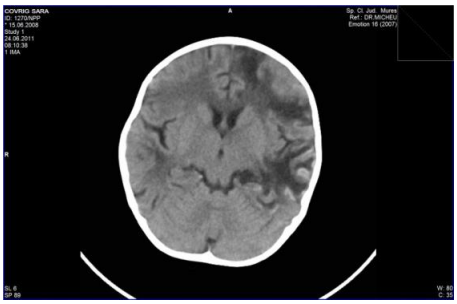

Fig. 2. CT cranian 24.06.2011

Cranial MRI (21st of July 2011): extensive damage in T2 hypersignal and T1 hyposignal detected in both cerebral hemispheres, suggesting an encephalomalacia in the context of meningoencephalitis. Poste-

rior fossa structures and basal ganglia seem less affected (fig. 3).

Fig. 3. IRM craniu 21.07.2011

Other investigations

Pediatric neuropsychiatric examination (PNE) (31st of May 2011): Superficial comatose status, medication. Suspected encephalitis. Secondary generalized focal seizures. Re-evaluation of the PNE (17th of June 2011): severe, acute meningoencephalitis. Convulsive syndrome, with secondary generalised focal motor seizures. Motor aphasia. Flaccid quadriparesis with predominant right hemiparesis; 1st of July 2011: severe acute meningoencephalitis. Flaccid right hemiparesis. Papilloedema. Motor aphasia. Right facial palsy of central type.

Electroencephalographic examination (EEG) (the 6th of July 2011): Diffuse hypovolted route for that age, a delta-theta background, with regular pseudorhythmic discharges of Radermecker-like complex.

Positive diagnosis

Severe acute encephalitis, probably having the aetiology of an enterovirus, complicated by encephalomalacia. Flaccid quadricparesis with predominant hemiparesis on the right. Right facial palsy of central type. Mixed aphasia. Symptomatic focal epilepsy with secondary generalized seizures. Severe neuropsychomotor setback. Herpangina. Oral candidiasis. Positive aetiologic diagnosis is difficult to be sustained, without CSF examination, which was contraindicated because of the FO results, and which would have allowed in the acute phase of the illness, its accurate assertion, by identifying the ideal viral RNA, or that of acute phase anti-Enterovirus antibodies, and also the evaluation in order to determine the presence of meningina and the constant inflammatory changes. Differentiation required by the major focal manifestations of herpetic aetiology, with much higher incidence in these forms, but without a medical history, suggestive clinical data and specific positive serology was denied. Enterovirus aetiology is supported by epidemiological data - regional epidemic context represented by suggestive clinical diagnosis (hand-foot-mouth disease, herpangina, diarrhoeal diseases) in summer, by clinical data - herpangina concomitance, and Ig M positive serology, with frowing values.

Treatment

Steroidal anti-inflammatory treatment with Solu-Medrol, Dexamethasone, Hydrocortisone hemisuccinate, depletion with Mannitol, Furosemide, Spironolactone, glycerol, Acetazolamide, intravenous Immunoglobulin (Humaglobin, Flebogamma) 10 days; antiviral treatment with Virolex for 14 days and antibiotics in the first 10 days; anticonvulsant therapy with Diazepam during seizures, with dose reduction in parallel with the introduction of Depakine, Phenobarbital. Anxiolytic therapy during the severe periods of turmoil and antisecretory neuroroborants.

Evolution

The immediate evolution was rapidly unfavourable by secondary generalised

seizures of the limbs, followed by temporary hemiparesis on the right and right facial paresis of central type, profound coma GCS 3/15 for 72 hours.

On awakening, the patient showed signs of increased focus, mixed aphasia, apathy, lack of communication. Neuropsychological decline was continuous, with progressive installation of other signs of brainstem involvement (nystagmus, divergent strabismus, significant problems with swallowing), ataxia, myoclonus, intentional tremor, choreoathetosis movements on the right hemibody, ocular deviation to the right, lethargy together with the progression of brain lesions visible on the ultrasound images, evolutionary aspect to encephalomalacia up to 5 weeks after onset, during which the patient remained feverish. The patient is hospitalized in a pediatric neurology clinic for neuropsychomotor recovery; the neuropsychomotor level was initially evaluated to 5-6 months, the psychic to 9-10 months, the 10-12 weeks of illness scoring the peak of the neuropsychomotor regression. On a long-term basis the evolution was slow but favorable, with improved muscle tone, eye contact, active motility resumption, independent movement, individual feeding with solid food, the utterance of a few words. The current neuropsychomotor level is of 18-20 months, the psyche of 15-16 months. The ophthalmologic evaluation (October 2011) revealed bilateral elongated visual potential, with normal amplitude, suggesting bilateral affection of the optical paths. The last EEG shows a theta background, hypovolted on the bilateral OP leads and left central part, large and front (F) right part irritative with continuous CVU discharges, poliVU, confounding with the current slow CVU/ poliVU and F right side as paroxysmal discharges, and hypervolted theta waves - worse aspect than the previous one.

MRI examination (April 2012) describes multiple porencephalitic bihemispherical areas, predominantly on the left side, showing a T2 hypersignal, FLAIR and T1 hyposignal, minimal perilesional residual edema, right frontal hypersignal cortical areas and occipital bilateral, a moderate ventricular system/ severely asymmetrical, the left ventricle being more dilated (fig. 4).

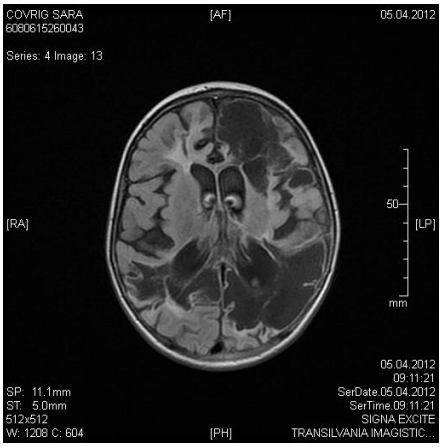

Fig. 4. MRI cranial 05.04.2012

In April 2012 it was possible to perform lumbar puncture and to collect CSF, in which, the examination for Plex-ID virus was negative.

DISCUSSION

The severity of the enteroviral encephalitis is variable, potentially causing neurological sequelae in infants, but with full psychomotor restitution after this age (1). The standard in establishing the etiological diagnosis is to isolate the virus through polymerase chain reaction (PCR) from the brain tissue or CSF (1.6).

The neuro-imaging appearance caused by some serotypes, the latest one being the EV71, led some of the authors to take into consideration the damage of the posterior

structures of the bulb, bridge, mid-brain and dentate cerebellar nuclei in severe cases and anterior horns of the spinal cord and that of the supratentorial nuclei (5) .

The evolution to cavitation and the development of encephalomalacia has been sporadically described, with little reference: marrow and brain stem encephalomalacia after severe rhombencephalitis in the case of two children, two newborns with wide cystic lesions, and with fronto-parieto-occipital distribution respectively (5.7).

On the other hand, there are reports of atypical localization of the inflammatory process in the hippocampus, imposing the differentiation of herpetic etiology (8). The aspect of the MRI was meticulous through the localization of the initial, unilateral and temporal inflammatory modifications, with cavitary degeneration and progressive, bilateral, fronto-occipital extension.

Persistence at 3 months of the lesions with T2 hypersignal and T1 hyposignal confirm their irreversibility data signals, which are associated with particular forms of severity (9). The negative CSF virological result after 11 months is normal. The possibility of a negative PCR test of CSF, when there is a lack of meningeal disturbance, is highly possible according to some reports. (4.7).

CONCLUSIONS

The patient had acute severe diffuse encephalitis affecting the brainstem as well, the etiology of which was likely enteroviral. The accurate diagnosis by confirming the presence of the viral RNA by PCR was inaccessible at that time, the method being very expensive. Clinical manifestations, although the symptoms described are identical to those presented in the literature, are astonishing because of their severity, extension and malevolent development to encephalomalacia and disabling neuropsychiatric screen, atypical after infancy. Although rare, enteroviruses are able to induce progression to encephalomalacia with diffuse localization, requiring differentiation of herpetic etiology. The contribution of diagnostic imaging is essential in monitoring the severe cases of neuroinfections.

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REZULTATE IMEDIATE ÎN MANAGEMENTUL CHIRURGICAL AL TUMORILOR STROMALE GASTROINTESTINALE PRIMARE (GIST) – EXPERIENȚA CLINICII A III-A CHIRURGICALE

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EARLY RESULTS OF THE SURGICAL MANAGEMENT OF GASTROINTESTINAL STROMAL TUMORS (GIST) – THE EXPERIENCE OF THE 3rd SURGICAL UNIT. (Abstract): This study provides a clinical and paraclinical algorithm of assessment of gastrointestinal stromal tumors (GIST), with the possibilities and limits of their current management. It is focused on defining an optimal imaging and anatomoclinic diagnosis protocol. In the absence of specific algorithm of surgical management, the study proposes an evaluation of possible histologic diagnosis and the indication for surgery, and the assessing the immediate results of thesurgical treatment. **Materials and methods.** A retrospective analysis was carried out over a period of 8 years (2004-2011), including patients with pathologic results of resection specimens revealing the stromal nature. There were analyzed the tumor characteristics, the association with other digestive tumors, the biologic behavior, clinical manifestations and the comorbidities of the patient, the indication for surgery, and also, the surgical morbidity and mortality. From 2004 to 2011 there have been operated 24 cases of GIST diagnosed histologically by common stains. Among them, a total of 16 patients (beginning with 2007) were confirmed immunohistochemicaly. **Results.** During the study, each clinical suspicion of GIST was an opportunity for thorough assessment of the clinical picture, imaging studies or anatomoclinical aspects (intraoperative macroscopical aspects, pathological and immunhistochemical examination). We have tried a standardization of diagnostic and therapeutic algorithm. Positive diagnosis of GIST was clinicaly suspected in patients with digestive tract tumors (22 cases out of 24) without mucosal involvement, or based on intraoperative macroscopic appearance. The lesions were confirmed by the pathologist. The surgical morbidity (4 cases - 16.6%), was to the surgery (a single case of anastomotic leakage) or due to patient's comorbidities (diabetus, chronic renal failure, etc), and has been framed to acceptable extent. No deaths occurred in relation to the surgery. **Conclusions.** The diagnostic difficulties are overcome in terms of a positive laboratory features revealed by immunhistochemical examination. The surgical approach of GIST is different from that of a carcinoma. The postoperative immunhistochemical confirmation of GIST , leads the patient to a specific adjuvant treatment. **Key words:** STROMAL TUMOR, SURGICAL MANAGEMENT, IMUNOHISTOCHEMISTRY

Tumorile stromale gastrointestinale (GIST) sunt cele mai frecvente tumori mezenchimale ale tractului digestiv. Se dezvoltă din celule precursoare intestinale (celule Cajal), recunoscute ca pacemaker al peristaltismului intestinal. Majoritatea tumorilor stromale sunt descoperite incidental, cu ocazia laparotomiilor/laparoscopiilor pentru diferite afecțiuni chirurgicale. Caracterele clinicopatologice ale acestor tumori sunt neclare. Modalitățile de diagnostic clinic şi paraclinic (imagistic, laborator) sunt la latitudinea clinicianului, deoarece nu sunt elaborate protocoale (algoritmuri) specifice. Diagnosticul anatomopatologic convențional (pozitiv şi diferențial) este de asemenea dificil şi nu constituie un argument suficient de diferențiere de alte tumori de origine conjunctivă. Fiecare localizare tumorală prezintă particularități de indicație operatorie. Rezultatele tratamentului sunt variabile.

Obiectivul acestui studiu este de a evidenția dificultățile de diagnostic pozitiv şi diferențial al tumorilor stromale, de a confirma fezabilitatea unui act terapeutic chirurgical radical, cu particularități pentru GIST şi de a demonstra importanța unui diagnostic de certitudine pentru a putea orienta ulterior pacientul către un tratament adjuvant specific.

MATERIAL ŞI METODE

În perioada 2004-2011 au fost operate 24 cazuri de tumori stromale gastrointestinale (GIST) diagnosticate histologic prin colorații uzuale; dintre acestea, un număr de 16 cazuri (începând cu anul 2007, de când kit-urile de imunhistochimie au devenit disponibile) au fost confirmate imunohistochimic. Incidența tumorilor stromale a fost mai mare la sexul masculin (F/B=8/16). Vârsta pacienților a fost cuprinsă între 43 şi 81 ani, cu o medie de 61 ani, cu proveniența majoritar rurală (rural/urban= 16/8). Înrolarea pacienților în studiu a fost făcută retrospectiv, utilizînd datele din foile de observație şi din buletinele anatomopatologice în care figura diagnosticul de tumoră stromală gastrointestinală.

REZULTATE

Simptomatologia a fost dominată de scăderea ponderală, sindromul anemic şi durerile abdominale. În afara acestei triade clinice, au mai fost notate şi alte semne şi simptome precum sindromul dispeptic intestinal, ocluzia intestinală, mai rar hemoragia digestivă, sau o tumoră abdominală palpabilă. Un număr de trei cazuri au fost asimptomatice, descoperirea tumorii fiind întîmplătoare, cu ocazia unei laparotomii pentru o altă patologie abdominală. La un singur pacient s-a descoperit asociat unei tumori stromale gastrice un hemoperitoneu în cantitate medie prin ruptura spontană a tumorii (tab. I).

TABELUL I

Simptomatologia clinică a pacientilor cu GIST

Simptomatologie	Nr. cazuri
Scadere ponderală	13
Sindrom dureros abdominal	10
Sindrom anemic	8
Tumoră abdominală palpabilă	6
Hemoragie digestivă	4
Ocluzie intestinală	3
Asimptomatice – descoperire intraoperatorie	3
Sindrom dispeptic biliopancreatic	2
Hemoperitoneu	1

Explorările imagistice au precizat detalii de topografie, dimensiuni, rapoarte anatomice, elemente care au fost coroborate cu

datele clinice şi de laborator, pentru un management chirurgical adecvat, particularizat fiecărui caz în parte. Ecografia, explorare aplicată la toți pacienții, a sugerat la 13 din 24 de cazuri diagnosticul probabil de tumora stromală, în timp ce tomografia computerizată (TC) cu aplicație mai restrînsă, a stabilit un diagnostic probabil de GIST la toți cei patru pacienți care au beneficiat de aportul acesteia (tab. II).

TABELUL II
Investigații imagistice

Procedura	Nr total	Rezultate pozitive	Procent
Ecografie	24	13	54,2%
Endoscopie digestiva	18	7	38,8
Irigografie	7	1	14,2
Tranzit baritat	6	3	50%
Tomografie computerizată	4	4	100%
Rx abdominală simplă	3	1	33,3%
Videocapsula	1	0	0%

Caracterul predominant expansiv al tumorii, absența adenopatiilor locale sau regionale au fost principalele elemente precizate la explorările imagistice. Endoscopia digestivă superioară sau inferioară au evidențiat tumori de perete gastric sau colonic, acoperite de mucoasă integră. La tumorile de mari dimensiuni, producând îngustarea de diferite grade a lumenului digestiv, mucoasa prezenta ulcerație. Aspectele endoscopice au fost confirmate de radioscopiile baritate. Pentru localizările la nivelul intestinului subțire au fost utilizate tranzitul baritat şi videocapsula.

Aspectul macroscopic al tumorilor a fost particular: în general se observă o tumoră cu suprafața lobulată (fig. 1.a), lucioasă, galben-brun, netedă, ce bombează seroasa organului digestiv, iar la nivelul mucoasei, şterge pliurile. La toate tumorile în stadii avansate (14 cazuri) organele vecine sau marele epiploon erau aderente la tumora. Tumorile stromale descoperite au fost în general unice, cu dimensiuni variabile. La un singur caz au fost evidențiate tumori multiple jejunale, de dimensiuni mici (fig. 1.b), iar in alt caz, tumora stromală colon cu invaginație (fig 1.c).

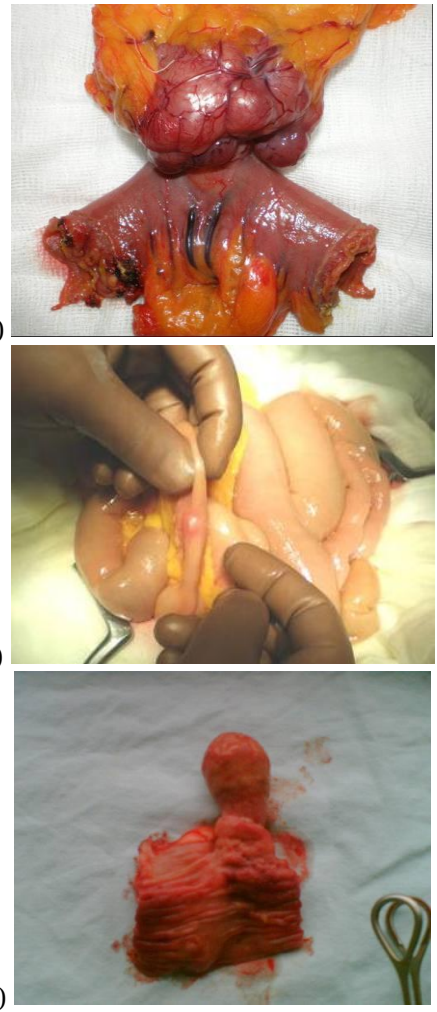

Fig 1 a) tumoră stromală jejun proximal, b) tumoră stromală ileon, c) tumoră stromală colon sigmoid, cu invaginație.

Examinarea microscopică în colorație hematoxilină-eozină a descris o proliferare tumorală de tip conjunctiv ce ocupă submucoasa şi care, în majoritatea cazurilor, se insinuează şi disociază stratul muscular neted, în distribuție compactă sau fasciculat. Orice tumoră conjunctivă a tubului digestiv poate ascunde o tumoră stromală gastrointestinală. În colorațiile obişnuite o tumoră stromală poate mima o tumoră musculară, o tumoră nervoasă sau chiar una epitelială. Pentru confirmarea tumorilor stromale a fost utilizată o baterie standard de teste imunhistochimice: Vimentin (atestă originea conjunctivă a tumorii) şi CD117 (c Kit - marker specific tumorilor stromale). Sunt aplicate şi teste complementare: CD 34 (alt marker specific tumorilor stromale, pozitiv în majoritatea cazurilor), αSMA (actina de muşchi neted- pozitivă în 30-40% din cazuri), proteina S100 (atestă originea nervoasă a tumorii - utilizat mai ales pentru diagnostic diferențial), şi Ki67 (defineşte comportamentul biologic malign sau benign, în funcție de pozitivitate). Rezultatele marcajului imunologic complet la 8 din cei 24 pacienți, sunt prezentate în tabelul III.

Diagnosticul pozitiv anatomopatologic presupune identificarea celulelor Cajal. Importanța marcajului pentru CD 117 şi CD 34 rezultă din faptul că intensitatea imunomarcajului pentru CD117 este corelată direct proporțional cu răspunsul la terapia cu Glivec (Imatinib).

Un alt element anatomopatologic cu valoare prognostica este marcajul Ki67 (>10% pozitiv denotă tumora malignă). De asemenea, dimensiunea tumorii la momentul diagnosticului pozitiv are valoare prognostică prin aceea că o tumoră pînă la 5 cm este probabil benignă, între 5 şi 10 cm are un caracter malign incert, iar peste 10 cm este o tumoră malignă certă.

Comportamentul biologic este etichetat şi de numărul de mitoze (> 5 mitoze/50HPF indică un grad înalt de malignitate).

TABELUL III

Rezultatele marcajului imunhistochimic.

	Localizare	AP uzuală/ Nr mitoze	IMUNOMARCAJ				
			CD 117	CD 34	SMA	S100	Ki67
1 (AM)	ileon	Prolif conj / < 5m / % hpf	+++	++ difuz	++ zonal	+ focal	12–13%
2 (VA)	Jejun + ADK gastric	Prolif conj fasciculata	++ difuz	+ difuz	+ difuz	+ focal	< 1 %
3 (BI)	jejun	Prolif conj fasciculata	++ difuz		+ zonal		2%
4 (MMP)	stomac	Prolif sarc. 30m / 10 hpf	+++	++	+ zonal	-	10 % variabil
5 (LM)	stomac	Prolif conj Rare mitoze	++ difuz	++ difuz	+ focal	+ focal	11-12%
6 (TV)	stomac	Prolif conj	+++ difuz	+ difuz	+ zonal		15%
7 (TF)	sigmoid	Prolif conj	++	+	+/-		2%
8 (SG)	sigmoid	Prolif conj f. rare mitoze	++	+	+		< 1%

Diagnosticul diferențial anatomopatologic a avut în vedere tumorile sistemului nervos autonom gastrointestinal (GANT), leiomioamele, leiomiosarcoamele, schwanoamele, lipoamele, tumorile cu celule granulare.

În total s-au confirmat 11 tumorri stromale maligne şi 13 tumori stromale benigne. Localizarea acestor tumori în tot lungul tubului digestiv este redată în tabelul IV.

Cele mai multe au fost localizate la nivelul stomacului, celelalte localizări fiind în ordine descrescătoare pe jejun, colon stang, ileon şi rect. Un număr de 5 pacienți au fost descoperiți având asocieri de tumori stromale cu alte tumori abdominale: GIST jejunală + adenocarcinom gastric, GIST ileală + chist ovarian bilateral, GIST gastrică + sarcom retroperitoneal, GIST gastrică + hemangiom hepatic, GIST gastrică + chist seros hepatic.

TABELUL IV

Localizările tumorilor stromale: GT(Gastrectomie totala), GST (Gastrectomie subtotala), RC((Rezectie cuneiforma), IH (imunhistochimie); M/B (malign/benign)

	Stomac	**jejun**	**ileon**	**Colon stg**	**rect**
Nr cazuri	15 cazuri	5	1	2	1
M/B	6 / 9	3 / 2	0 / 1	0/2	1 / 0
IH	3	3		2	
Operația	GT 4 GST 7 RC 4	RS 5	RS 1	HC Stg - 1 OH- 1	RAP 1

IH - confirmare prin Imunhistochimie; GT- gastreectomie totala; GST- gastrectomie subtotala; RC- rezectie cuneiforma; RS-rezectie segmentara; HCStg- hemicolectomie stanga; OH- oparatia Hartmann; RAP- rezectie abdominoperineala.

Vîrsta înaintată şi comorbiditațile existente la momentul diagnosticului (diabet, obezitate, litiaza veziculară) au influențat în mod variabil indicația operatorie.

Evoluția postoperatorie a înregistrat complicații la 4 pacienți (17%) din totalul de 24:

- fistula eso-jejunală după gastrectomie totală pentru GIST gastric;
- complicații septice pulmonare la un pacient vîrstnic cu bronhopneumopatie obstructivă cronică, remise sub tratament;
- insuficiență renală acută, reversibilă sub tratament;
- decompensare diabet, reechilibrat prin terapie insulinică.

Nu s-au înregistrat decese la lotul luat în studiu în perioada primelor 60 de zile, până la luarea în evidență în rețeaua oncologică şi începerea terapiei adjuvante.

DISCUȚII

Identificarea celulelor interstițiale Cajal, celule pacemaker ale activității motorii autonome gastrointestinale, ca celule de origine a unei bune părți a tumorilor mezenchimale ale tubului digestiv (aproximativ 80%), a impus în primă instanță rafinarea diagnosticului pozitiv şi diferențial, odată cu statuarea termenului de tumori stromale gastrointestinale (GIST), de către Mazur şi Clark în anul 1983 (7). În fapt, acest grup de tumori, considerate pînă

atunci, în marea lor majoritate, leiomiosarcoame (care, în bună parte, răspundeau la chimioterapie), se dovedeau a fi non-responsive. Imunhistochimia, prin utilizarea anticorpilor monoclonali, este cea care a tranşat diagnosticul, atît pozitiv şi diferenţial, al acestora.

Prezenţa în procentaj ridicat, la nivelul membranelor celulelor Cajal a receptorilor c-Kit (CD 117, SCFR)-85-90% (atît pentru tumorile mici cît şi pentru cele metastazate), a receptorilor CD34 (60-80%), (în 75% din cazuri ei se întîlnesc concomitent la aceeaşi tumora), ca şi, mai rar, a PDGFR (5%), certifică diagnosticul de tumora GIST. În fapt, este vorba de modificări apărute predominant la nivelul porţiunii intracelulare a receptorului c-Kit (consecutive unor mutaţii la nivelul genei c-Kit, situate pe cromozomul 4 la om) . Mai nou, chiar şi pentru tumorile fără răspuns pozitiv la cei doi receptori, un nou receptor a intrat în setul de diagnostic pozitiv: DOG-1 (discovered on GIST-1).

În faţa unei tumori confirmate GIST, o a doua dilema apare: cât de "malignă" este o astfel de tumoră? Există diferenţe între agresivitatea unei tumori gastrice şi a unei tumori intestinale, sau cu altă localizare pe tubul digestiv? Se consideră actual că nici o tumoră GIST nu poate fi considerată, *a priori*, "benignă" (8). Experienţa acumulată în cele două decenii de când se vorbeşte de tumori GIST a permis identificarea câtorva markeri prognostici:

1. *Mărimea* tumorii: astfel, pentru tumorile pînă la 5 cm diametru, riscul de transformare malignă este scăzut (tumori între 2-5 cm), sau foarte scăzut (0-2 cm).

2. *Indexul mitotic*, definit prin numărul de mitoze pe câmpul microscopic de înaltă rezoluţie (HPF), care indică o tumoră cu o proliferare marcată în cazul unui index de peste 5 mitoze/50 cîmpuri studiate. Tot aici putem include şi celulele tumorale al căror procentaj de celule Ki 67 + depăşeşte 70% din numărul total de celule (malignitate înaltă) (9).

3.*Localizarea* tumorii poate orienta asupra potenţialului malign: astfel, se consideră că tumorile gastrice au un prognostic mai bun faţă de cele intestinale.

Într-un amplu studiu pe 1684 pacienţi, Miettinen et al. (10), au corelat primii doi markeri prognostici menţionaţi (mărimea tumorii şi indexul mitotic) cu localizarea acesteia: astfel, ei au arătat că GIST gastrice au un risc crescut (>50%) de evoluţie nefavorabilă (inclusiv progresia bolii sub tratamentul cu Imatinib), atunci cînd dimensiunea tumorii e mai mare de 5 cm, iar indexul mitotic >5/50 HPF, pe cînd tumorile GIST intestinale prezintă acelaşi risc ridicat chiar atunci când dimensiunea tumorii e <2 cm, dar cu indexul mitotic >5/50 HPF. În plus, alte două caracteristici pot orienta către o apreciere a modului de evoluţie a tumorii: astfel, cele cu celularitate crescută şi care se prezintă cu o necroză scăzută, au un potenţial evolutiv mai bun.

Atitudinea terapeutică în cazul unei tumori GIST a cunoscut, în aceste două decenii, un reper calendaristic extrem de important. Astfel, până în anul 2000, intervenţia chirurgicală era considerată prima secvenţă, cea mai importantă, din arsenalul terapeutic adresat pacientului cu tumoră GIST:

- rezecţia completă, „*en bloc*" a tumorii, ori de câte ori acest lucru era posibil, cu evitarea rupturii tumorii.
- evitarea limfadenectomiei (tumorile au tendinţa de a interesa structura parietală în întregime, cu invazia organele învecinate, metastazând rar pe cale limfatică - doar 10% din cazuri;

- peritonectomie largã, atunci când există diseminare peritoneală.

S-a constatat că o rezecție curativă R0 este urmată în 50 % din cazuri de dezvoltarea recidivei locale (de multe ori consecutiva rupturii intraoperatorii a tumorii), sau a metastazei la distanță. Supraviețuirea la 5 ani este cuprinsă între 32 – 78 % (13), iar supraviețuirea la 10 ani variază între 19 – 63 %.

Literatura arată o variabilitate mare a supraviețuirii. Această constatare a intrigat unii autori şi a condus, ulterior, la studiul profilului genetic al pacientului: s-a constatat astfel că receptorul c-kit prezintă diferite modificări în structură, cu sedii diferite, în funcție de mutațiile ce apar la nivelul protooncogenei c-kit. Rapoartele cu privire la polichimioterapia postoperatorie (Doxorubicina, Dacarbazina, Epirubicina, Paclitaxel, s.a.), s-a corelat cu o rată de răspuns parțial (PRR) dezamăgitoare, de doar 8%. În acelaşi timp, radioterapia postoperatorie a fost adresată cazurilor R1- R2, (cu reziduu tumoral), cu rezultate incerte.

O schimbare majoră în tratamentul GIST a constituit-o introducerea, în premieră pentru o tumoră malignă digestivă, a unei terapii țintite la nivelul celulei tumorale. Utilizarea Imatinibului cu varianta sa, Imatinib mesylate, (folosit inițial în tratamentul leucemiilor mieloide cronice), a permis un abord direct al celulei tumorale. Blocarea receptorului tirozin-kinazic c-Kit (CD117, SCFR) de la nivelul membranei celulei Cajal, duce la o blocare a procesului de autofosforilare a acestui receptor, (apărută în condițiile unor mutații la diferite nivele ale protooncogenei c-kit), şi, implicit, la o diminuare a ratei multiplicării celulelor tumorale. În plus, Imatinib blochează şi un alt receptor cu funcție kinazica, Akt1, o serin/treonin kinază. În acest fel, Imatinib creşte semnificativ rata distrugerii celulelor tumorale prin apoptoza. O serie de alte mecanisme care duc la blocarea multiplicării celulelor tumorale după 24-48 ore de la administrarea Imatinib sunt în studiu.

În prezent, se consideră că dacă GIST este rezecabilă şi nu este metastazată (ficat, cel mai des, epiploon sau peritoneu), primul pas rămâne intervenția chirurgicală cu intenție R0. În ultimii ani s-a perfecționat şi abordul laparoscopic al acestora, în special pentru formațiunile mici, fără necroză evidentă (care ar creşte riscul diseminării intraperitoneale) (11, 12). Tratamentul cu Imatinib la aceşti pacienți se începe în adjuvanță pentru o perioadă de 12 luni postoperator, pentru a preveni recidiva. S-a constatat că riscul recidivei este de 6% pentru cei tratați cu Imatinib, față de 17% care nu urmează tratament. Dacă diagnosticul de GIST a fost pus în preoperator, prin certificare imunhistochimica, şi tumora e metastazată sau potențial nerezecabilă, planul terapeutic se modifică, tratamentul începând, de această dată, cu Imatinib. Dacă, sub tratament cu Imatinib boala se stabilizează sau se remite (criterii clinice, biologice, imagistice care atestă dispariția formațiunii) se indică tratamentul chirurgical (13,14). În situațiile în care boala progresează sub tratament, tratamentul chirurgical nu este recomandat.

Rata supravieturirii la pacienții cu tumori stromale nerezecabile sau metastazate, în urma tratamentului cu Imatinib, Sunitinib (17, 18) sau Nilotinib (19, 20) a ajuns de 75-80% la 2 ani (14). Tratamentul chirurgical în cazul formelor avansate, pe lângă îndepărtarea tumorii, are avantatajul de a preveni dezvoltarea rezistenței secundare la tratamentul ulterior cu Imatinib şi, în plus, prelungeşte semnificativ atît intervalul liber de boală, cât şi supraviețuirea

globală (15).

În situația când tumora nu a fost confirmată ca GIST în preoperator şi este local avansată, pretându-se frecvent la confuzia cu un adenocarcinom local avansat, devine obligatorie cel puțin o biopsie prin laparotomie/laparoscopie pentru certificarea diagnosticului şi instituirea tratamentului cu Imatinib (aşa cum a fost cazul cu o parte din cazuistica prezentată).

CONCLUZII

Diagnosticul clinic şi imagistic poate releva unele aspecte ce sugereaza GIST; cu tote acestea, tumorile stromale pot constitui o descoperire surpriză la examenul anatomopatologic microscopic.

Cea mai frecventă localizare este cea gastrică, incidența tumorilor stromale scăzând spre capătul distal al tubului digestiv; de asemenea, stomacul este sediul celei mai frecvente localizari de tumoră stromală malignă.

Abordul chirurgical primar al tumorilor nemetastazate constituie cea mai bună opțiune terapeutică; tehnica chirurgicală impune rezecție largă dar nu şi limfadenectomii extinse ca în cazul carcinoamelor.

Confirmarea GIST prin imunomarcaj este necesară pentru încadrarea fenotipică, definirea agresivității şi aprecierea indicației de terapie adjuvantă.

Diagnosticul imunhistochimic orientează pacientul către un tratament oncologic specific cu anticorpi monoclonali (Imatinib sau Nilotinib) care poate preveni recidiva neoplazică şi îmbunătățeşte prognosticul pacienților.

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NOUTĂȚI

NIVEL CRESCUT AL COFEINEI ÎN SÂNGE ASOCIAT CU RISC SCĂZUT AL PROGRESIEI SPRE DEMENȚĂ

Deşi studiile epidemiologice efectuate la om şi animale evidențiază rolul protector al cofeinei împotriva bolii Alzheimer, nu există dovezi directe în acest sens. În studiul efectuat asupra a 124 de persoane (65-88 ani) a fost utilizat drept marker biologic nivelul cofeinei în plasmă, li s-a stabilit nivelul cognitiv şi au fost monitorizați timp de 2-4 ani. Nivelul plasmatic al cofeinei inițial a fost scăzut în deprecierile cognitive medii, respectiv la pacienții ce au progresat către demență (grupul I), comparativ cu nivelul pacienților cu deprecieri cognitive medii stabili (grupul II). Niciunul dintre pacienții grupului I nu a atins nivelul critic al cafeinei de 1200ng/ml, în timp ce jumătate dintre cei din grupul II-lea au prezentat nivele peste 1200 ng/ml. Astfel că nivele peste 1200 ng/ml (≈ 6 μM) sunt considerate a împiedica evoluția spre demență. Dintre 11 citokine determinate plasmatic, doar 3 (FSCG,IL-10,IL-6) au scăzut şi numai la pacienții grupului I. Cafeua a fost singura sursă de cofeină pentru pacienții grupului II. Studiul dovedeşte corelația directă a consumului de cafeină cu riscul scăzut al evoluției spre demență sau întîrzierea acesteia la pacienții care au deprecieri cognitive medii (Chuanhai Cao, David A. Loewenstein, Xiaoyang Lin et al. High Blood Caffeine Levels in MCI Linked to Lack of Progression to Dementia. *Journal of Alzheimer's Disease*, 2012,30(3):550-572).i

Şt. Pânzaru

IMMUNOTHERAPY IN ADVANCED HEPATOCELLULAR CANCER

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IMMUNOTHERAPY IN ADVANCED HEPATOCELLULAR CANCER (Abstract): Hepatocellular carcinoma (HCC) is the commonest primary cancer of the liver, with a poor prognosis in advanced setting. Current treatment for advanced HCC includes mainly sorafenib and more recently approved regorafenib and nivolumab, as chemotherapy has shown little benefit. However, the modest benefit and the low response rate from systemic treatments suggest an increased need for new strategies. Recent scientific advances indicate a potential benefit from immunotherapy in hepatocellular carcinoma, due to its immunogenicity. Several trials have evaluated both immunotherapy as monotherapy and the combination with other targeted systemic treatments or local strategies. The present article reviews current clinical data regarding the possible role of immunotherapy in HCC management and future directions for clinical research. **Keywords**: IMMUNOTHERAPY, HEPATO-CELLULAR CANCER, CLINICAL TRIALS, LIVER.

Hepatocellular carcinoma (HCC) is one of the leading causes of cancer mortality worldwide (1), being the most frequent primary malignancy of the liver. Although 60-80% patients with HCC have liver cirrhosis (2), it can also arise without any underlying disease. Viral infections with B hepatitis, C hepatitis are the most prevalent factors, but other factors such as alcohol, metabolic alterations (hemochromatosis, Wilson disease, α-1 antitrypsin deficiency) non-alcoholic fatty liver disease, genetic and environmental factors have been considered implicated in HCC (3).

Clinical features and diagnosis of primary hepatocellular carcinoma

Patients who develop HHC usually have no symptoms other than those related to their chronic liver disease. Suspicion for HHC should be heightened in patients with previously compensated cirrhosis who develop decompensation such as ascites, encephalopathy, jaundice or variceal bleeding. Hypoglycemia is a well-known paraneoplastic manifestation of hepatocellular carcinoma usually occurring in the terminal stages of the disease. However, during initial presentation this manifestation is uncommon (4). Differential diagnosis of hypoglycemia in hepatocarcinoma can be done primarily with pancreatic insulinomas. Insulinomas are the most common cause of hypoglycemia related to endogenous hyperinsulinism. The episodic nature of the hypoglycemic attack is due to the intermittent secretion of insulin by the tumor (5).

Although loco-regional therapies have provided a benefit for patients diagnosed with early disease, 60% of HCC are diag-

nosed in the advanced setting. Current treatments for advanced HCC include sorafenib in the first line setting, a tyrosine-kinase inhibitor which provide a survival benefit of 2.8 months compared to placebo 10.7 months vs. 7.9 months; hazard ratio in the sorafenib group, 0.69 (95% confidence interval [CI], 0.55 to 0.87; P<0.001) according to the SHARP trial (6).

Regorafenib was recently approved in second line setting, after the RESORCE trial (7) has shown an improved overall survival (HR= 0.63, 95% CI 0·50-0·79; one-sided p<0·0001, median overall survival 10.6 months vs. 7.8 months) compared to placebo in patients progressing to sorafenib.

Food and drug administration (FDA) recently approved nivolumab after progression to sorafenib, based on CheckMate 040 trial (8), a phase I/II open-label, dose escalation and expansion trial (8) that evaluated the efficacy of nivolumab, a fully IgG4 monoclonal antibody targeting PD-1, in first or second-line treatment in patients with advanced HCC with or without hepatitis C or B infection. 262 patients were included in the trial (48 patients in the dose escalation phase and 214 in the dose expansion phase), receiving 3 mg/kg b.w. every 2 weeks in the dose expansion phase. 77% of the patients completed treatment with a manageable safety profile. In terms of response, the 9-month survival rate was 70% and the median survival was 15 months (CI 95% 9.6-20.2 months). Tumor responses were durable, lasting for a median of 17 months. Both patients who were sorafenib naïve or who progressed after sorafenib benefited from nivolumab. PD-L1 expression was also assessed and only 20% of the patients were PD-L1 positive. Tumor responses were seen in 26% of PD-L1 positive patients and in 19% of PD-L1 negative patients.

The Cancer Genome project (9) characterized the main genomic alterations involved in HCC pathogenesis, as well as potential therapeutic targets. The unfavorable prognosis of patients diagnosed with advanced disease or who progress after loco-regional therapies has encouraged the study of various targeted therapies in clinical trial, such as antiangiogenic therapy, c-MET inhibitors, mTOR inhibitors and various multi-kinase inhibitors.

Immunotherapy has shown a prolonged survival in a variety of tumors and settings, such as non-small cell lung cancer, advanced urothelial cancer, metastatic locally advanced melanoma and head and neck squamous cell carcinoma. In HCC, chronic inflammation and liver injury lead to various genetic and epigenetic alterations. As a result, overexpression of oncofetal antigens recognizable by the immune system leads to a potential antitumor immune response. Evidence supports the fact that HCC has a high antigenity (10) and that it also express a high lymphocytic infiltrate (11), suggesting possible benefit from immunotherapy.

Background

Immune checkpoints represent surface glycoproteins expressed on various cell types involved in the immune response, such as B and T cells, dendritic cells, natural killer cells (NK), tumor associated macrophages, monocytes and myeloid-derived suppressor cells (12). Although in physiological condition this process prevents the over-activation of the T cells against pathogens and limit the peripheral damage, in cancer patients this process leads to tumor immune tolerance, as they are responsible for inhibitory signals delivered to T cells or natural killer cells, leading to their inactivation.

Cytotoxic T-lymphocyte protein 4 (CTLA-4) plays an important role in the

activation of CD4+ T cells. Expressed on the activated T cells and on regulatory T cells (Treg), leads to immunosuppression by antagonizing CD28, enhancing Treg activity and interfering with the function of dendritic cells (13). Treg inhibit the immune response by secreting immunosuppressive factors (TGF-β, IL-10) and depleting IL-2.

The activated CD8+ and CD4+ T cells, B cells, NK, Treg, dendritic cells and monocytes express PD-1. Various hematopoietic cells, antigen presenting cells or parenchymal cells express ligands for PD-1 (PD-L1 and PD-L2). Overexpression of these ligands is induced by cytokines, leading upon binding to PD-1 to increased secretion of IL-10 and, consequently, to CD8+ and CD4+ T cell inactivation. One of the reasons cancer cells escape immune surveillance is by expressing these ligands on their surface.

There is a physiological interaction between different cells (Kupffer cells, hepatic stellate cells, immune cells), providing various mechanisms aimed to limit the inflammatory response to constant antigens. This suggests that the liver offers a more immunotolerant microenvironment compared to other organs. Gene expression analysis (9) of these tumors suggests a high expression of the checkpoint genes, such as CTLA-4, PD-1, PD-L1, without any association with viral infection. Moreover, the presence of lymphocytic infiltration was associated with an improved outcome in patients who underwent surgery for HCC (14).

Clinical trials with checkpoint inhibitors in HCC

Current clinical trials exploring the role of checkpoint inhibitors in HCC have shown encouraging results. Tremelimumab, a fully human IgG2 monoclonal antibody that blocks the CTLA-4, is the first agent evaluated in HCC. In a phase II, non-controlled, multicenter study (15), 21 patients with advanced HCC who progressed to previous therapies were enrolled, 42.9% having a Child-Pugh stage B. They received 15 mg/kg b.w. every 90 days, which now is considered a suboptimal dose of the drug. The disease control rate was 76.4%, with a median time to progression of 6.48 months (CI 95% 3.95-9.14 months) and median overall survival of 8.2 months (CI 95% 4.64-21.34 months). The study also assessed the antiviral effect of tremelimumab, observing a decrease in median viral load in most patients, followed for at least 3 months and observed in 75% of the patients with an immune response. In terms of safety the grade 3 adverse effects were rash (65%), elevated ASAT (70%) and diarrhea (30%), however none of the patients received systemic steroids.

A phase I/II trial (16) evaluated the efficacy of an increased dose of tremelimumab (3.5 to 10 mg/kg b.w. every 4 weeks x 6 cycles and then every 3 months) in combination with local therapy (radiofrequency ablation (RFA) or transarterial chemoembolization (TACE). 32 patients with advanced HCC (75% with BCLC stage C) were included in the trial. 75% had viral hepatitis as cause of liver cirrhosis. In 45% of the patients the stable disease lasted longer than 6 months, the median time to progression being 7.4 months (CI 95% 4.7-9.4 months) and overall survival was 12.3 months (CI 95% 9.3-15.4 months). The safety profile showed a reduced incidence of grade 3 side effects like rash (15%), pruritus (9.3%), diarrhea (6.2%) and elevated ASAT (34%). Blood levels of CD4+ and CD8+ T cells increased after tremelimumab administration.

Future directions

The clinical development of checkpoint

inhibitors in HCC shows promising results, considering the disappointing results in drug development for patients with advanced disease in the last decade.

A randomized, phase III trial (NCT 02576509) of nivolumab versus sorafenib is currently ongoing, with the aim to better explore the role of immunotherapy in HCC. The activity of pembrolizumab (NCT 02702401), another PD-1 blocking drug, against supportive care in patients previously treated with sorafenib is currently ongoing. Other ongoing trials are exploring the use of immunotherapy as monotherapy in both first and second-line setting, such as pembrolizumab (NCT 02702414) or nivolumab (NCT 01658878).

Considering the benefit of double blockade in other setting such as melanoma, blocking both PD-L1 and CTLA-4 seems to be an attractive approach. One ongoing trial (NCT 02519348) is comparing durvalumab in combination with tremelimumab *vs.* each agent as monotherapy in first and second-line setting HCC. Moreover, it has been shown that intrahepatic CD8+ T cells in patients with chronic hepatitis C infection seem to be refractory to PD-1/PD-L1 blockade, despite the increased PD-1 expression (17). However, the combined blockade of CTLA-4 and PD-1 seem to overcome this resistance (18) and have efficacy in HCC (15).

TGF-β plays a unique role in the pathogenesis of HCC, as it can act as a tumor suppressor in early stage cancer development. In preclinical models, TGF-β increased the PD-L1 expression in dendritic cells and induces the apoptosis of T cells, increasing the percentage of regulatory T cells (19,20). An ongoing trial (NCT 02423343) is currently exploring the efficacy of combination nivolumab and galunisertib, a TGF-β inhibitor.

Other pathways targeted in combination with immunotherapy in HCC are c-met and VEGF. Ongoing trials with a c-met inhibitor, capmatinib (NCT 02795429) and VEGFR2 inhibitor (NCT 02572687) have been reported.

Due to the already proven benefit of various local therapies for patients in earlier settings and the encouraging data on checkpoint inhibitors, the combination of immunotherapy with local treatment could help patients with HCC. Adding checkpoint inhibitors to TACE or liver ablation seems a feasible combination, as both local treatments seem to increase T cell infiltration (21), especially CD8+ cells. The combination between TACE and tremelimumab (16) has been explored in a small study; more trials are needed to support the suggested data. As radioembolization is increasingly used in HCC and there seem to be a potentially synergistic effect between radiotherapy and CTLA-4 blockade (22), clinical trials are trying to exploit this possibility with nivolumab and Y90 radioembolization (NCT 03033446 and NCT02837029) in HHC patients.

Primary resistance to immunotherapy has been observed in CheckMate 040 study (8), where objective remissions were seen in only 26% of PD-L1 positive patients, suggesting a need for predictive biomarkers and alternatives for these patients. As PD-L1 expression seem to be correlated with various markers of tumor aggressiveness, such as AFP levels, microvascular invasion, poorer histological differentiation (23), more trials that include biomarker evaluation are needed.

CONCLUSIONS

Sorafenib remains the only approved first line treatment in advanced HCC and levantibib is being considered for approval

in first line setting by FDA based on the reported results of REFLECT trial (NCT 01761266). In patients progressing to sorafenib, new drugs such as regorafebib and nivolumab have already been approved. However, there are still unmet needs that must be addressed. The role of combination systemic and local treatment, as well as combined systemic treatment is still unknown.

Immunotherapy is a novel approach in the treatment of advanced HCC, alongside with the development of other agents targeting the main pathways involved in HCC development. Early preclinical and clinical data are encouraging, although further clinical trials exploring monotherapy or combination treatment strategies are needed. There is a need to further define the patients that may benefit most from this approach, as well as its role in combination with other known treatment modalities.

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NEWS

THE VETERAN NASAL CANNULA - STILL A CARD UP THE ANAESTHESIOLOGIST'S SLEEVE?

High-flow nasal oxygenation (Hi) has already shown its benefits in awake, sedated or asleep with obstructive sleep apnea spontaneous breathing patients. The use of SponTaneous Respiration using IntraVEnous anaesthesia and Hi (STRIVE Hi) technique, applying a FiO_2 =1 during the induction phase was studied in thirty cases of adult patients who undergone elective laringotracheal surgery, 87% of which with significant airway and/or respiratory compromise. None experienced apnea or complete airway obstruction during induction, with the lowest median oxygen saturation of 100%. If the technique continued during maintenance, the median duration of spontaneous ventilation was of 44 minutes and the median $ETCO_2$ at the end of spontaneous ventilation of 6.8 kPa. Hi's increase in oxygenation, ventilation and upper airway patency are the advantages that help manage difficult airways, while maintaining normoxia (Booth AWG, Vidhani K, P.K. Lee, and Thomsett C-M. SponTaneous Respiration using IntraVEnous anaesthesia and Hi-flow nasal oxygen (STRIVE Hi) maintains oxygenation and airway patency during management of the obstructed airway: an observational study. *British Journal of Anaesthesia*. 2017 Mar; 118(3): 444–451).

Olguța Diaconu

AVANTAJELE OPERAȚIILOR TENSION-FREE CU MATERIALE PROSTETICE ÎN CURA HERNIEI INGHINALE

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THE BENEFITS OF TENSION-FREE OPERATIONS WITH PROSTHETIC MATERIALS IN THE REPAIR OF INGUINAL HERNIA (Abstract): Recurrences after inguinal hernia repair represent a significant problem for both surgeons and patients. **Material and methods**. This study tries to evaluate operations that were performed in Clinical Emergency Hospital Oradea, II-nd Surgical Department, between September 2007 and December 2009, using polypropylene meshes. The operations were performed using the Lichtenstein technique in loco-regional or general anesthesia. The follow up was performed at one month after surgery and then at 3, 6, 12 months with a compliance rate of 80%. **Results.** were similar to the results found in literature. **Conclusions**. The technique is easy to learn and to perform and the benefits for patients are represented by the low recurrence rates, comfort due to low levels of postoperative pain, early discharge and early socio-professional reintegration. **Key words**: INGUINAL HERNIA, LICHTENSTEIN OPERATION, RECURENCE

Recurențele după repararea herniei inghinale reprezintă o problemă de larg interes pentru chirurg şi pacient (1). Evidențele arată că există o perturbare a metabolismului implicată în patogeneza herniei inghinale la adult care determină modificări în funcțiile dependente de *fascia transversalis*. Rezultă că utilizarea unui astfel de țesut cu rezistența scazută este problematică în reparația herniei inghinale (2). Într-o încercare de a reduce incidența recurenței şi de a întări reconstrucția plastică au fost utilizate variate tehnici cu țesut autolog sau biomateriale. În urmă cu peste un secol Usher (cit. 1) a propus utilizarea polietilenei cu densitate mare pentru repararea defectelor tisulare la nivelul toracelui şi peretelui abdominal. De atunci s-a observat o tendință spre o largă utilizare a materialelor sintetice (3).

MATERIAL ŞI METODE

Studiul de față îşi propune o evaluare a intervențiilor chirurgicale în care s-au utilizat plase de polipropilenă în Clinica Chirurgie II a Spitalului clinic de Urgență din Oradea, în perioada Septembrie 2007 – Decembrie 2009. S-au efectuat 73 de intervenții chirurgicale folosind tehnica Lichtenstein (4) din care 26 au fost indicate pentru cura bilaterală. Hernia inghinală a fost indirectă în 47 de cazuri, directă în 15 şi mixtă în 11. Vârsta pacienților, toți de sex masculin, a fost cuprinsă între 18 şi 79 cu o medie de 52,3 ani, toți pacienții fiind de sex masculin (tab. I, fig. 1). Operațiile s-au efectuat în anestezie loco-regională sau generală.

TABELUL I

Distribuția pe grupuri de vârstă a pacienților incluşi in studiu

Vârsta (ani)	Nr.	% *
<30	1	1,4
31-40	8	11,0
41-50	19	26,0
51-60	27	37,0
61-70	15	20,5
>70	3	4,1
Total	**73**	**100,0**
Vârsta medie	**52,3±7,8 ani**	

* Pentru valorile mai mici de 10, proporțiile sunt orientative

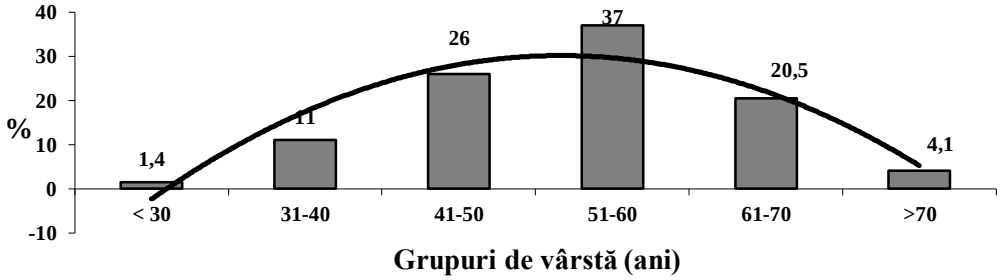

Fig. 1. Distribuția pe grupuri de vârstă a lotului de 73 pacienți incluşi în studiu

Distribuția pe medii de proveniență a indicat diferențe nesemnificative între urban şi rural (54,8%-45,2%) (p≥0,05) (tab. II, fig.2)

TABELUL II

Distribuția cazurilor în relație cu mediul de proveniență

Mediu	Nr.	%
Rural	33	45,2
Urban	40	54,8
Total	**73**	**100,0**

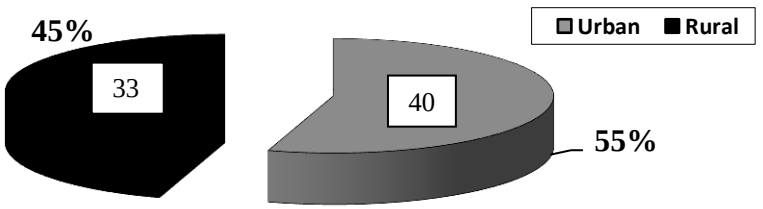

Fig. 2. Raportarea pe medii a lotului de 73 de pacienți luați în studiu

TABELUL III

Distribuția cazurilor în relație cu tipul herniei inghinale

Tipul herniei inghinale	Nr.	%
indirectă	47	64,4
directă	15	20,5
mixtă	11	15,1
Total	73	100,0

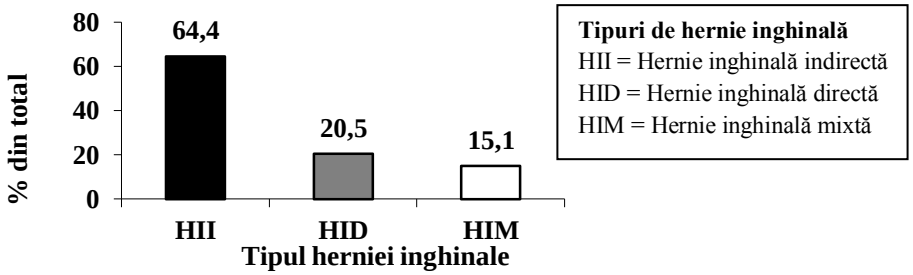

Fig. 3. Repartiția pacienților pe trei categorii de hernie inghinală

Hernia inghinală pentru care pacienții au solicitat asistența în clinica a II-a Chirurgie a fost de trei tipuri: direct, indirect şi mixtă (tab.III, fig.3)

TABELUL IV

Distribuția cazurilor în relație cu tipul anesteziei

	Nr.	% *
Loco-regională	48	65,8
Generală	18	24,7
Loco-regională completată cu generală	2	2,7

* Pentru valorile mai mari de 10, proporțiile sunt orientative

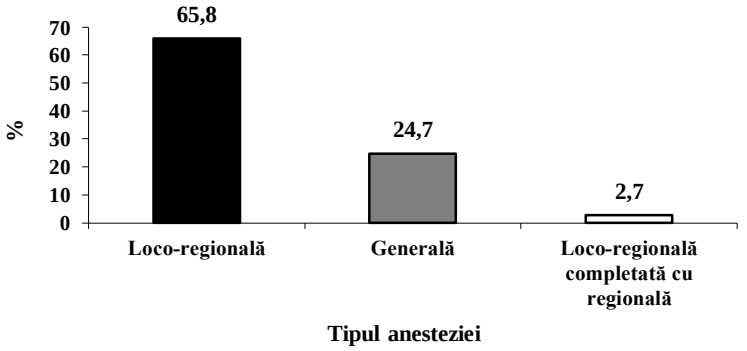

Fig. 4. Repartiția pacienților în relație cu tipul de anestezic folosit la tratamentul chirurgical

Tehnica operatorie. Intervențiile chirurgicale s-au realizat sub anestezie locoregională sau generală (tab. IV, fig. 4), pacientul aflat în clinostatism, cu regiunea inghinală pregătită, iar înainte de incizie s-a administrat, intravenos, o doză de cefalosporină de generația a IIa. După incizia pielii, țesutului subcutanat şi a aponevrozei muşchiului oblic extern cordonul spermatic s-a ridicat de pe peretele posterior al canalului inghinal şi s-a evidențiat traiectul nervilor ilio-hipogastric, ilio-inghinal şi genital.

În herniile indirecte sacul herniar a fost identificat, disecat de inelul profund şi deschis pentru a permite examinarea conținutului, sacul herniar ligaturat la bază cu rezecția porțiunii distale.

Plasa de polipropilenă a fost ajustată pentru a se potrivi dimensiunilor canalului inghinal urmată de fixarea la vârf de tuberculul pubic. Apoi s-a suturat cu fire separate marginea inferioară a plasei la ligamentul inghinal după practicarea unei deschideri prin marginea inferioară a plasei pentru acomodarea funiculului spermatic până la nivelul crestei iliace anterioare. De asemenea, sunt suturate cele două margini rezultate prin tăierea plasei în jurul cordonului spermatic. Colțul infero-medial al plasei a fost suturat astfel încât să acopere tuberculul pubic, iar medial plasa a fost suturată la tendonul conjunct şi s-a practicat controlul hemostazei. În cazul unor hernii voluminoase s-a plasat un tub de dren sub aponevroza oblicului extern suturată cu fire separate şi, în final, s-a închis plaga chirurgicală (5) (fig.5)

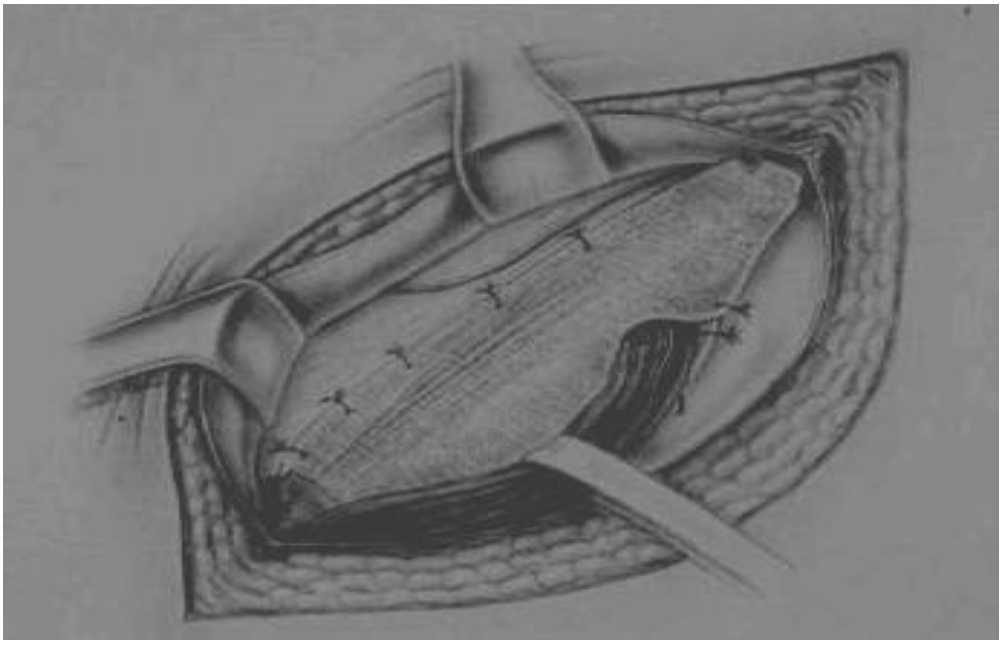

Fig. 5. Poziționarea plasei pe peretele posterior al canalului inghinal

Postoperator au fost administrate antibiotice pe durata a aproximativ 48-72 de ore. De asemenea, la pacienții cu risc crescut s-a administrat heparină cu greutate

moleculară mică pentru prevenția trombozei venoase profunde, cu 12 ore preoperator şi postoperator, pe toată durata spitalizării (6). Analgezia postoperatorie a fost asigurată prin administrarea de antialgice minore. Durata medie de spitalizare a fost de 3 zile.

REZULTATE

Durerea postoperatorie a fost controlată cu ajutorul analgezicelor minore (7). În perioada imediat postoperatorie s-au înregistrat complicații; 2 hematoame, 3 seroame (morbiditate = 6,84%) şi 3 cazuri cu edem testicular care a cedt la administrarea de AINS şi drenaj postural. Nu s-au semnalat abcese sau infecții (tab. V, fig. 6, 7).

Urmărirea pacienților a fost efectuată la 1, 3, 6 şi 12 luni de la externare cu o complianță de 59 de pacienți (80%), prin examinare clinică. Nu s-a înregistrat nici o recidivă herniară.

Nevralgia severă nu s-a înregistrat la nici un caz (8), dar în 5 a fost prezentă durerea postoperatorie pe o durată de peste 2 săptămâni, care a cedat, progresiv, sub administrare de antiinflamatoare nesteroidiene (9).

TABELUL V

Distribuția cazurilor în relație cu prezența unor complicații postoperatorii imediate

	Nr.	**% ***
Hematom	2	2,7
Serom	3	4,1
Total	**5**	**6,8**

* Pentru valori mai mici de 10, procentele sunt orientative

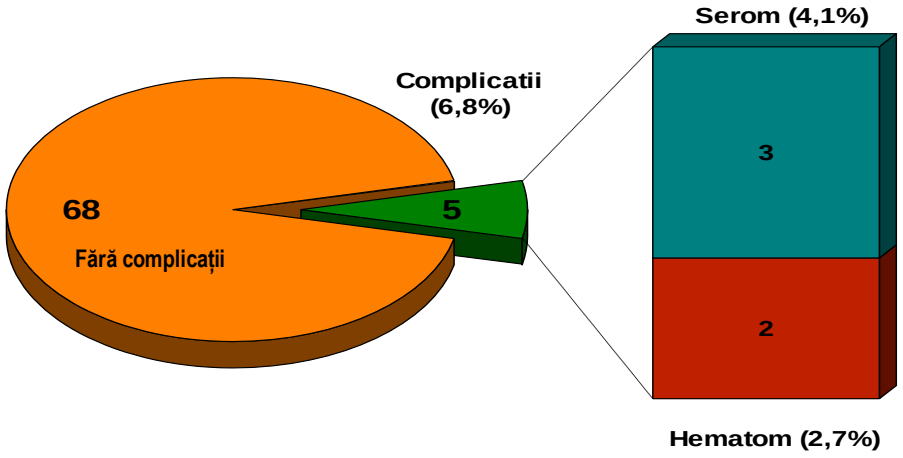

Fig.6. Complicațiile postoperatorii înregistrate la lotul de 73 pacienți

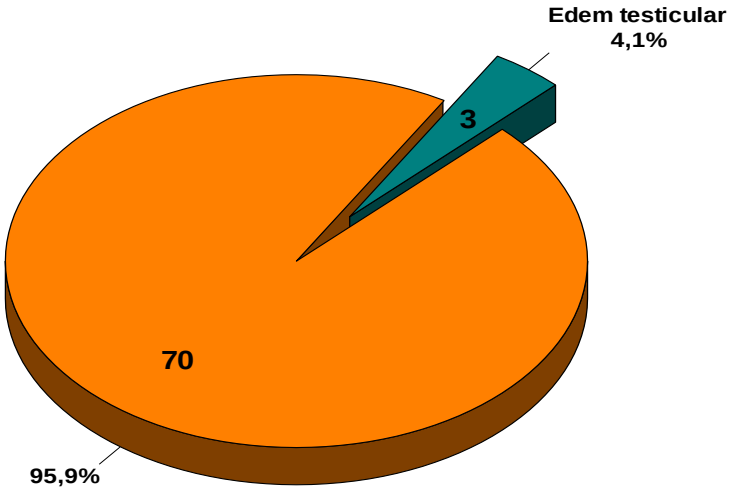

Fig. 7. Incidența cazurilor cu edem testicular postoperator

DISCUȚII

Descrierea tehnicii Lichtenstein fără tensiune, în urmă cu peste doua decenii, a deschis o nouă eră în chirurgia herniară. Metoda este simplă, eficientă, durerea postoperatorie este minimă datorită lipsei de tensiune iar rata de recurență este extrem de joasă(variază între 0 şi 2% în literatură) operația poate fi efectuată sub anestezie locală sau loco-regională. Din aceste motive această tehnică este cea mai preferată pentru reconstrucția plastică a canalului inghinal (10).

În prezent, chirurgii beneficiază de existența unei largi varietați de plase având calitatea de a fi inerte, rezistente la infecție, cu permeabilitate moleculară, pliabilitate, transparență, integritate mecanică şi biocompatibilitate. În schimb plasele absorbabile nu se mențin suficient timp pentru ca depozitele de colagen să fie adecvate iar cele multifilament predispun la infecții. Plasele monofilament sunt cele mai solicitate, fiind prezentate în variate tipuri de polipropilenă. Utilizarea plaselor poroase determină o suprafață largă de creştere în porii plasei a țesutului de conectivitate pemițând o fixare permanentă a protezei în peretele abdominal. Poziționarea intraparietală a plasei permite integrarea într-un țesut bine vascularizat şi o bună acoperire. Temerile legate de implantarea plasei s-au dovedit nefondate. Drenajul aspirativ s-a dovedit util în cazul herniilor inghinale mari pentru a minimaliza formarea hematoamelor şi a seroamelor. Durata menținerii drenajului şi a utilizării prevenției cu antibiotice este evaluată în mod diferit (11, 12).

Pentru a reduce incidența recidivei herniare plasa trebuie să depăşească cu 2-4 cm. marginile triunghiului Hesselbach. Poziționarea plasei sub aponevroza muşchiului oblic extern este necesară deoarece presiunea intraabdominală acționează în favoarea reparației, aponevroza muşchiului oblic extern fixează plasa în poziție acționând ca un suport extern când presiunea intraabdominală creşte. Plasa trebuie fixată cu atenție pentru a preveni plicaturarea,

şifonarea sau răsucirea acesteia în jurul cordonului spermatic.

Metoda poate fi aplicată şi de chirurgii care nu practică frecvent chirurgia herniară şi este eficientă în prevenţia recidivei care nu depăşeşte 0 – 2%. De asemenea, metoda prezintă o bună evoluţie postoperatorie, cu un control accesibil al durerii şi reintegrare socio-ocupaţională rapidă.

CONCLUZII

Metoda Lichtenstein este accesibilă, cu posibilitatea de a fi aplicată şi de chirurgii care nu prezintă un interes deosebit faţă de chirurgia herniară.

Tehnica nu a fost grevată de complicaţii majore, iar materialul prostetic utilizat nu a favorizat apariţia infecţiei postoperatorii sau a recidivei herniare.

Evoluţia postoperatorie a fost confortabilă cu un control al durerii uşor de aplicat, durată de spitalizare redusă, reintegrarea socio-ocupaţională realizată pe termen scurt.

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ELEMENTE DE STATISTICĂ A TRAUMATISMELOR CRANIO-CEREBRALE CU IMPLICAREA SINUSURILOR AERICE FRONTALE ÎN SPITALUL CLINIC DE URGENȚĂ „PROF. DR. NICOLAE OBLU" IAŞI

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STATISTICAL DATA CONCERNING HEAD TRAUMA WITH FRONTAL SINUS INVOLVEMENT IN CLINICAL HOSPITAL "PROF. DR. NICOLAE OBLU", IASI (Abstract): We present here some interesting points of view concerning head and brain injury with frontal sinus involvement. **Material and methods:** In general neurosurgical practice the frontal sinus traumatic involvement is about 3-5 per cent from all head trauma; we proposed to realise a extensive 5 years study to finnaly sumarise the characteristical elements for our geographical area. The amount of patients hospitalized in our clinic was about 80 000 in five years. **Results:** Concerning our statistical data, there are 677 patients with frontal sinus injury, 490 from this were complicated with brain pathology. **Conclusions:** We concluded there are differences between our series and the other studies from developed countries, in our region the domestical (agricultural works) causes are about 10% higher. **Key words:** FRONTAL SINUS, SKULL FRACTURE, HEAD TRAUMA

MATERIAL ŞI METODE

Studiul a inclus 80891 pacienti asistați în perioada 2002-2007 în Clinicile de Neurochirurgie ale Spitalului Clinic de Urgență „Prof. Dr. Nicolae Oblu" din Iaşi. Din totalul pacienților, 3658 (4%) prezentau leziuni considerate a fi corelate cu etajul anterior al bazei craniului. Leziunile la nivelul fosei craniene anterioare au fost decelate la 1295 pacienți (1,6%). Aşadar ne referim la un segment foarte strâns cazuistic, aspect întâlnit şi în literatura de specialitate internațională (1).

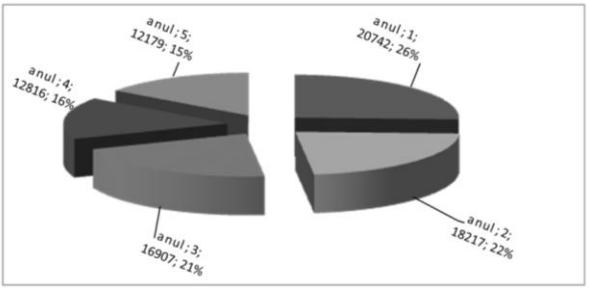

Fig. 1. Distribuția cazurilor incluse in studiu in 2002-2007

Toți pacienții au fost evaluați preoperator prin examen CT (prin secțiuni axiale şi coronale). Reconstrucția coronală a fost utilizată doar la pacienții ce nu se puteau poziționa adecvat scanării coronale. Parametrii utilizați pentru clasificarea pacienților au fost: vârstă, sex, etiologie, loc al traumei, prezența fracturilor cranio-faciale, a leziunilor asociate şi prezența complicațiilor, aceste criterii fiind cel mai des urmărite în majoritatea studiilor similare (2).

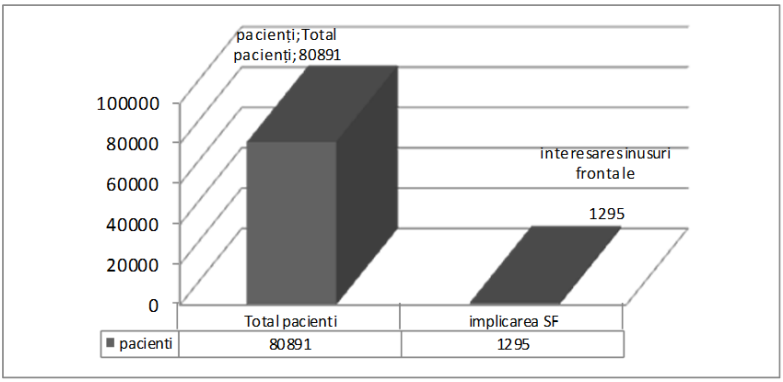

Fig. 2. Situația pacienților cu leziuni ce implică sinusurile frontale

În studiu s-au luat în considerație următoarele tipuri de leziuni:

- congenitale – 16;
- traumatice - 677;
- inflamatorii - 127;
- neoplazice cu origine la acest nivel sau prin extensie de vecinătate - 353;
- vasculare – 122.

Pe elemente de categorie lezională predomină traumatismele cranio-cerebrale, apoi patologia inflamatorie, tumorală şi leziunile vasculare şi congenitale.

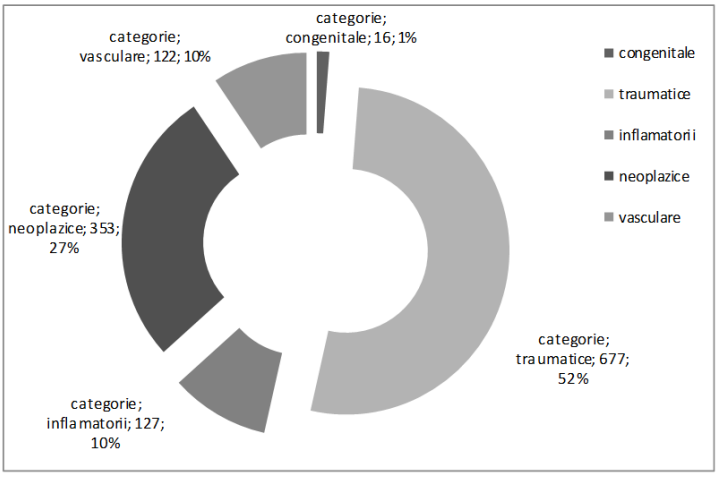

Fig. 3. Grupuri patologice studiate

Pacienții au fost evaluati pe grupuri de vârstă, tipuri de leziune, mediu de proveniență modul de rezolvare (chirurgical/conservator).

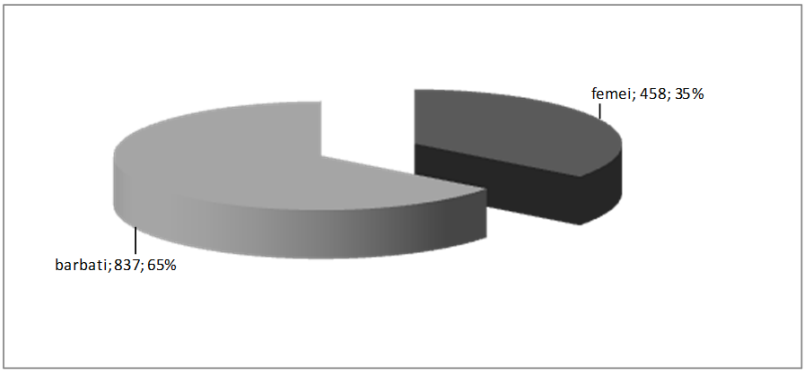

Fig. 4. Repartiția pe sex a pacienților cu leziuni ce interesează etajul anterior al bazei craniului

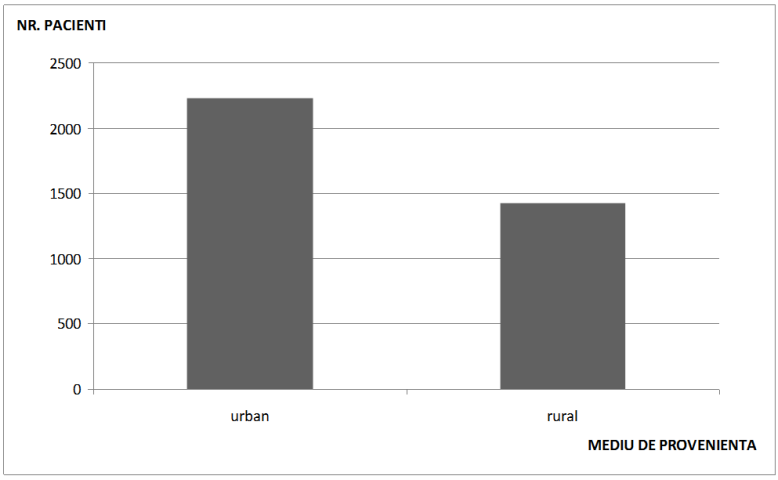

Fig. 5. Distribuția pe mediu de proveniență

REZULTATE

Pe grupuri de mediu rural/urban au predominat elementele patologice corelate cu accidentele de trafic rutier, dar corelate, de asemenea, şi cu o rată de diagnosticare mai ridicată, datorită creşterii numărului de centre specializate în politraumă la nivelul oraşelor. Din datele analizate reiese că implicarea sinusurilor aerice frontale apare în 677 (52,3%) din traumatismele cerebrale luate în studiu, iar din acestea 490 (72,4%) sunt expuse diverselor complicații; majoritatea dintre acestea au apărut în perioada acută, fapt concordant şi cu alte studii din literatura de specialitate (2).

Cea mai mare incidență se remarcă la nivelul grupelor de vârstă active, între 20-50 de ani; incidența este foarte redusă sub vârsta de 20ani datorită, pneumatizării limitate.

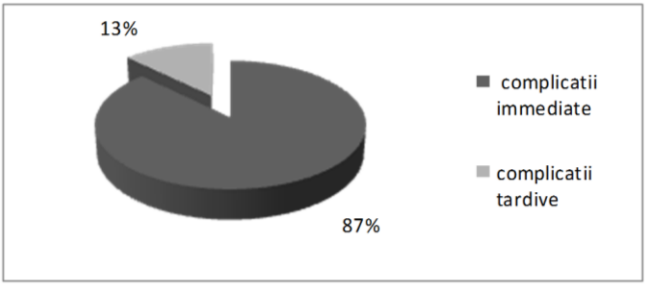

Fig. 6. Pacienți cu interesare traumatică la nivelul sinusului aeric frontal

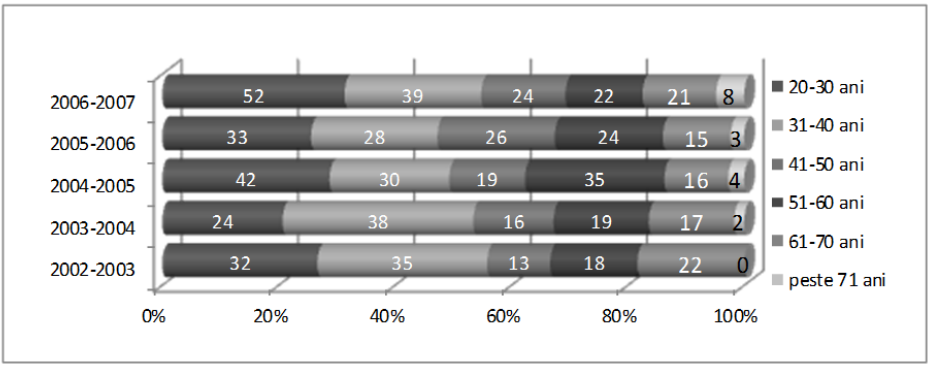

Fig. 7. Repartiția cazurilor pe ani de studiu şi intervale de vârstă

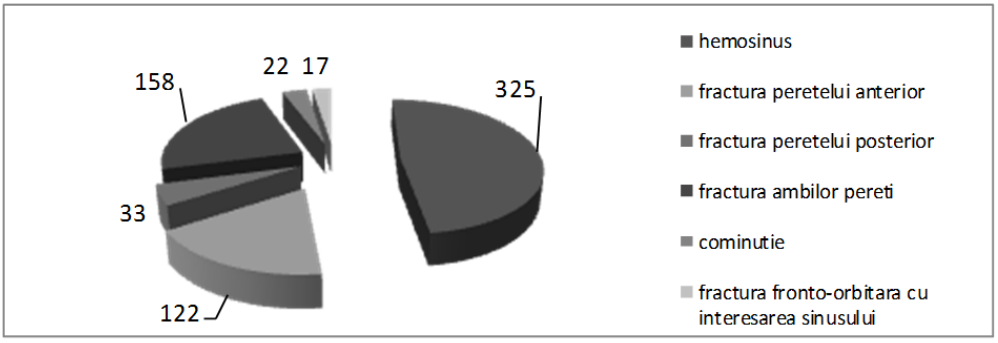

Fig. 8. Tipuri de interesări traumatice ale sinusurilor aerice frontale

Cea mai comună leziune este hemosinusul frontal (de multe ori asociat cu pan hemosinus), fiind urmată de fracturile pereților sinusurilor aerice frontale.

Din patologia craniocerebrală studiată, aproximativ 4% a fost cu implicarea etajului anterior al bazei craniului şi aproximativ 1,5% reprezintă interesarea fosei craniene anterioare, din care patologia majoritară este cea traumatică, cu preponderență masculină pentru ambele medii de proveniență (3). Evoluția pe luni în frecvența de producere a leziunilor traumatice ale sinusurilor frontale şi în general a traumatismelor craniocerebra-

le a evidențiat incidența scăzută în martie (2,8%) şi aprilie (2,5%). Începând cu luna iunie are loc o creştere aproape constantă a frecvenței de apariție a incidentelor traumatice cranio-cerebrale, până la un maximum atins în luna iulie (17,2%). După o uşoară scădere în luna august (9.9%) se înregistrează o ascensiune în lunile septembrie şi octombrie (corespunzătoare intensificării activităților agricole), după care s-a diminuat accentuat în octombrie, urmată de o creştere în lunile de iarnă.

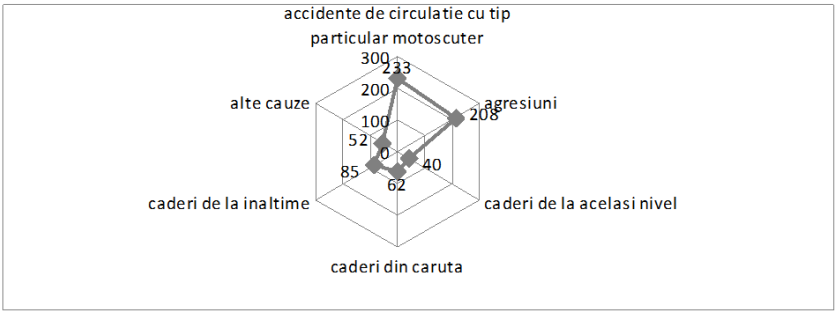

Fig. 10. Repartiția cazurilor pe tipuri etiologice

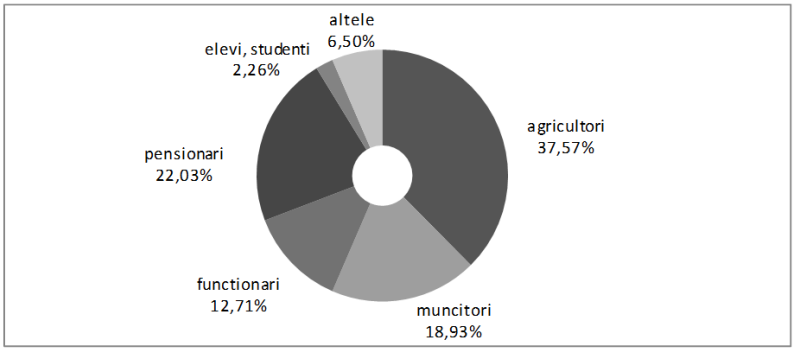

Fig. 11. Distributia cazurilor în relație cu situația ocupațională

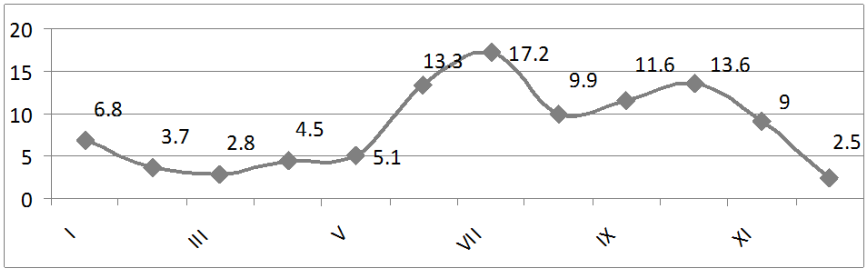

Fig. 12. Distribuția medie lunară a cazurilor cu implicarea traumatică a sinusurilor frontale

DISCUȚII

Analiza statistica a datelor arată o tendință uşoară de creştere a traumatismelor cranio-faciale cu implicare sinusală datorită creşterii numărului de autovehicule, mai ales a motoscuterelor, la care impactul ex-

tremității cefalice este foarte violent (4). Sexul masculin este interesat în proporție de peste 65 % iar ca mediu predomină mediul urban. În ceea ce privește ocupația, sunt interesate toate categoriile, dar mai frecvent muncitorii si agricultorii; mecanismele de producere principale sunt accidentele de trafic şi agresiunile, urmate de căderi accidentale pe fond de etilism. Studiindu-se traumatologia cranio-facială, s-a concluzionat că forța de impact necesar producerii unei fracturi sinusale este între 360-990 kg, ceea ce este suficient pentru a produce şi alte injurii craniene (5). În funcție de intensitatea forței, pot fi injurii ale tăbliilor, anterioară sau posterioară, ultima asociind frecvent şi injurii cerebrale, orbitare sau ale celulelor etmoidale: Wright şi col. au raportat că 76% din pacienții cu injurii ale pereților sinusali au avut alterări ale stării de conştiență şi 93 % au avut multiple fracturi faciale şi craniale (5).

De menționat, în ceea ce privește statistica noastră, ar fi că în comparație cu alte studii realizate în țări cu nivel de dezvoltare mai avansat, apar diferențe semnificative, în regiunea noastră accidentele domestice (în special cele referitoare la lucrările agricole sezoniere) sunt ca procent cu aproximativ 10% mai ridicate (6).

CONCLUZII

Sinusul frontal este o structură deosebit de variabilă în funcție de vârstă, sex, distribuție geografică. Importanța sa în dinamica extremității cefalice, fonație, apărarea antimicrobiană este dovedită. Deşi, în termeni fizici vorbind, structura piramidală a pereților săi îi conferă o elasticitate şi o rezistență deosebite, expunerea anterioară fac din sinusurile frontale o barieră de apărare eficientă. Abordarea statistică a problemelor de neurotraumatologie capătă o importanță deosebită, atât din punct de vedere clinic pentru stabilirea unor protocoale de explorare şi tratament, cât şi din punct de vedere economic, deoarece în problemele de traumă metodele de explorare în vederea tratamentului precoce sunt eficientizate (7).

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THE PROGNOSIS OF POSTERIOR URETHRAL VALVES, ASSOCIATED TO VESICOURETERAL REFLUX IN CHILDREN

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THE PROGNOSIS OF POSTERIOR URETHRAL VALVES ASSOCIATED TO VESICO-URETERAL REFLUX IN CHILDREN (Abstract): Posterior urethral valves represent a congenital barrier at the level of the posterior urethra, which opposes miction. They are located near the prostatic urethra, originating at the *verumontanum* level, affecting male patients. The ureters are inconstantly dilated; vesicoureteral reflux is met in 2/3 of the cases. The reflux can be secondary to the sub-bladder barrier, but it can also be considered primitive, determined by the intra bladder ectopy of the ureter. The presence of the reflux is interpreted as a severe associated factor. Semiology is rather unusual, positive diagnosis is based on early discovery or antenatally of the impairment, by fetal echography starting from the 28th week of pregnancy, postnatal echography followed by miction cystography, retrograde urethrography and creatinine dosage complete the diagnosis. The treatment is surgical: endoscopic ablation of the valves (in the absence of renal failure), percutaneous pyelostomy, high lateral ureterostomy, and in severe cases vesicostomy and renal transplant. Prognosis depends on how early the impairment is detected, on the degree of pulmonary hypoplasia, on the presence of the vesicoureteral reflux and the possibility of recovering renal function; 1/3 of the newborns develop in time Chronic Renal Failure which requires renal transplant. The studied group comprised male children diagnosed with vesicoureteral reflux their ages ranking between 0 -18 years, admitted to the Pediatric Clinic, Tg. Mures during the last 10 years and children diagnosed and operated with posterior urethra valves at the Surgical Clinic of the "*M. S. Curie"* Hospital, Bucharest during the last 20 years. Our results show that out of the total number of studied children 25 presented posterior urethra valves and 9 children presented vesicoureteral reflux. We can conclude that the presence of the vesicoureteral reflux is an unfavorable prognosis regarding the degree of renal failure. **Key words**: ANTENATAL ECHOGRAPHY, URETHRAL VALVE ABLATION, PROGNOSIS.

Posterior urethral valves (PUV) represent a congenital barrier at the level of the posterior urethra which opposes miction. These are the most frequent obstructive congenital urethral lesions and affect only male patients, being located in the distal section of the prostatic urethra (1). The incidence of posterior urethral valves is estimated to be 1: 5.000-8.000 (2).

The obstruction determines dilation of the prostatic urethra upstream, hypertrophy of the bladder detrusor muscle, which obstructs the urethrobladder junction.

The vesicoureteral reflux (VUR) is met in 2/3 of the cases, half of these being bilateral. The reflux can be secondary to the

sub-bladder barrier, but it can also be considered primitive, determined by the intrabladder ectopy of the ureter. The presence of the reflux is interpreted as a severe associated factor (3).

It presents an unusual symptomatology: unremitting fever, haematuria, sepsis, dehydration syndrome, urinary infections, dysuria, reduction of urinary jet, urinary incontinence, vesical globus, ureterohydronephrosis, renal failure (4).

Positive diagnosis is established by considering two aspects: antenatal diagnosis and postnatal diagnosis.

Antenatal diagnosis is based on detection of the disease by fetal echography starting from the 28th week of pregnancy, when only a high echo sound can be distinguished: uretero-hydronephrosis (5).

The postnatal diagnosis is based on the echographical examination during the neonatal or infant age, which highlights bilateral hydronephrosis with narrowing of the parenchyma, associated with renal dysplasia and megaureter due to uni- or bilateral reflux, relaxed urinary bladder with thick wall and dilated prostatic urethra (6, 7).

Miction cystography, retrograde urethrography, dosing seric creatinine completes the diagnosis (8, 9) and are considered to be the most important means of diagnosis.

Treatment is surgical intervention: endoscopic ablation of the valves in the absence of chronic renal failure, percutaneous pyelostomy, high lateral ureterostomy, and in severe cases vesicostomy (10) renal transplant (11, 12, 13). Early and correct treatment will avoid renal failure; can stop growth problems and the impairment of sexual function and fertility.

The prognosis depends on how early the impairment is detected, when VUR or chronic renal failure (CRF) are present, which is present in 1/3 of cases (14).

The main purpose of this article is to analyze the degree of the renal function impairment in case of vesicoureteral reflux associated or determined by PUV.

MATERIAL AND METHODS

Studied group: children diagnosed with vesicoureteral reflux between the ages of 0-18 years, of male sex, admitted in the Pediatric Clinic in Tg. Mures during the last 10 years.

Children diagnosed and operated with posterior urethra valves in the Surgical Clinic of the "*M. S. Curie"* Hospital from Bucharest in the last 20 years.

Data has been collected by analyzing medical records of children diagnosed with ureterohydronephrosis, vesicoureteral reflux and posterior urethral valves at the Department of Nephrology, II Pediatric Clinic Tg. Mures and in collaboration with Surgical Clinic from "*M. S. Curie"* Hospital from Bucharest.

Criteria for inclusion: children aged between 0 -18 years; certainty diagnosis of VUR and PUV

Criteria for exclusion:

- children aged between 0 -18 years with medical history of renal impairment
- errors in urodynamic studies
- children aged between 0 -18 years with sepsis, acute dehydration syndrome, unremitting fever having an etiology different from the renal one

Target population: 170 patients with VUR and possible PUV diagnosed at the II Pediatric Clinic in Tg. Mures and 20 children operated on at the Department of General Surgery, "*M. S. Curie"* Hospital, Bucharest.

Available population: investigations began with all those children who presented VUR, uretero-hydronephrosis discovered ante- and postnatally, associated or determined by posterior urethral valves.

Type of study: transversal, analytical, experimental.

Used methods were retrospective study, serial cases, statistical method (descriptive statistics).

Since no pictures were used which could lead to the identification of a patient, there is no informed consent.

RESULTS

This study presents the cases of five children out of 170 children presenting vesicoureteral reflux, were diagnosed with posterior urethral valve, and the cases of 20 children with PUV operated at the surgical clinic M. S. Curie Hospital from Bucharest.

Case number 1

3 years old child diagnosed with: Bilateral ureterohydrone, Operated posterior urethral valve, Relapsing high type urinary tract infection, Deficient anemia.

Discovered postnatally at the age of 4 months.

Treatment: ablation of the valves when he was 5 months old.

Evolution: favorable with repeated urinary infections and deficient anemia.

Case number 2

One year and 4 months old child diagnosed with bilateral ureterohydronephrosis: 1st degree left vesico-ureteral reflux, Posterior urethral valve, Deficient anemia. Diagnosed antenatally by fetal echography at 30 weeks highlighted bilateral hydronephrosis, then exploratory cystography highlighted urethral stenosis, posterior urethral valve and left vesicoureteral reflux.

Treatment: percutaneous cystostomy then valve ablation. Evolution: favorable

Case number 3

9 years old child diagnosed with: 5 degree ureterohydronephrosis by sub-bladder barrier, Posterior urethral valve, 5 degree right vesicoureteral reflux, Chronic renal failure, Bilateral cutaneous ureterostomy, Repeated urinary tract infections. Detected antenatally at 32 weeks of pregnancy, fetal echography visualising bilateral uretero-hydronephrosis

Treatment: bilateral cutaneous ureterostomy, then bilateral ureteral reimplantation and valve resection. Evolution: with 5th degree vesicoureteral reflux and chronic renal failure presently with peritoneal dialysis.

Case number 4

3 years old child diagnosed with: bilateral megadolicoureter, 5th degree bilateral vesicoureteral reflux, Posterior urethral valve, 3rd degree chronic renal failure, Bilateral cutaneous ureterostomy.

Detected postnatally at 5 weeks beginning with signs of 3rd degree acute dehydration syndrome (ADS), urinary tract infection, echography highlighted renal dysplasia; cystography and urography highlighted massive bilateral vesicoureteral reflux.

Treatment: bilateral ureterostomy at age of 10 months.

Evolution: unfavorable with 3rd degree chronic renal failure and peritoneal dialysis.

Case number 5

One year and 5 months old child diagnosed with: Posterior urethral valve,

Congenital hydronephrosis, Megaureter, Prolonged neonatal jaundice, Ostium secundum type atrial septal defect. Immediately detected perinatally with hydronephrosis and megaureter.

Treatment: fitted with a catheter at the Pediatric Clinic without results, programmed endoscopic resection of valves.

Case number 6

22 years child diagnosed with: Chronic urinal retention, PUV, Secondary bilateral uretero-hydronephrosis.

Child hospitalized in 1991 when underwent: Bladder Neck Plasy (Young), cystostomy (Pezzer), Foley *a demeure*, bladder drainage. Rehospitalized in 1992 and underwent surgery at the age of 2 and 3 with valve resection. Evolution: with repeated urinary infection.

Case number 7

11 years old child diagnosed with: PUV, VUR with bilateral hyronephrosis, Hypospadiasis, Micropenis with bifid gland. Detected postnatally, hospitalized at age of 9 months for bifid gland and urethra with hypospadias: it was not operated.

Case number 8

9 years old child diagnosed with: PUV, Bilateral ureterohydronephrosis, urinary tract infection.

Detected perinatally at one month after Foley probe, then at age of 2 month when 5th degree VUR, when undergoing endoscopic resection of valves appears, followed in time II after appreciatively 20 days by terminal cutaneous ureterostomy. Evolution: favorable.

Case number 9

10 years old child diagnosed with: PUV, Bilateral ureterohydronephrosis.

Detected at 10 months subsequently undergoing surgery by endoscopic resection of valves.

Case number 10

22 years old child diagnosed with: PUV, Bilateral ureterohyronephrosis, Bilateral VUR.

Detected postnatally, initially tried to mount a Foley probe, then rehospitalized in 1990 at the age of 2, subsequently undergoing transpubian resection of the valves, after 8 months presenting a chronic fistulizing orchiepididimitis, subsequently undergoing orchidectomy. At age of 5 years old he presented a chronic fistula at the left hemiscrotum and at the age of 12 years old he developed a chronic renal failure, needing peritoneal dialysis.

Case number 11

18 years old child diagnosed with: PUV subsequently undergoing surgery, Bilateral VUR.

Rehospitalized and tide correction in 1999, control after 5 year. Evolution: favorable.

Case number 12

10 years old child diagnosed with PUV. Detected in infant age, surgery at infant age.

Treatment: endoscopic resection of valves, control after 2 years. Evolution: favorable.

Case number 13

10 years old diagnosed with: PUV, Bilateral ureterohydronephrosis.

Detected by echography immediately after birth, at the age of 8 days old subsequently undergoing endoscopic resection of valves. Evolution: favorable.

Case number 14

9 years old child diagnosed with: PUV, Bilateral ureterohyronephrosis, right VUR, left juxta-vesical stenosis. Detected postnatally, hospitalized at age of 2 years old, and subsequently undergoing bilateral ureterostomy.

Case number 15

7 years old child diagnosed with: PUV, Bilateral ureterohydronephrosis, and urinary tract infection.

The disease was detected by echography after birth, hospitalized at the age of 9 months old subsequently undergoing an incision of valves "cold knifes", Foley probe *a demeure*, and then rehospitalized after 2 month subsequently undergoing endoscopic resection of valves.

Evolution: with repeated urinary infections.

Case number 16

7 years old child diagnosed with: PUV, Bladder diverticulum.

Detected after birth, surgery at the age of 2 years and a half, subsequently undergoing endoscopic resection of valves, and after 2 years iterative resection. Evolution with urinary infections and deficient anemia.

Case number 17

4 years old child diagnosed with: PUV type III, Bilateral ureterohydronephrosis, right VUR

Detected antenatally, hospitalized at the age of 3 months subsequently undergoing right ureterostomy, control after 4 months. Evolution: favorable.

Case number 18

6 years old child diagnosed with PUV type III, Bilateral uretherohydronephrosis, hypospadias and meatum stenosis. Ureterohydronephrosis detected postnatally by echography, besides hypospadisis detected at birth. Subsequently undergoing meatoplasty.

Case number 19

9 years old child diagnosed with: PUV type I, Bilateral ureterohydronephrosis.

Hospitalized at the age of 3, subsequently undergoing endoscopic resection of valves, after one year undergoing Bolmers iterative resection.

Case number 20

6 years old child diagnosed with: PUV type I, Bilateral ureterohydronephrosis, Bilateral ureterostomia. Hydronephrosis was revealed antenatally by a fetal echography.

Treatment: bilateral ureterostomya, then at the age of 2 rehospitalized subsequently undergoing initial endoscopic resection of valves and after a month iterative resection of valves.

Case number 21

10 years old child diagnosed with: PUV type I.

Detected postnatally, hospitalized in the surgical clinic at the age of 7 years old for weak urinary stream; subsequently undergoing endoscopic resection of valves.

Case number 22

5 years old child diagnosed with: PUV type I, Bilateral ureterohydrophenosis.

Detected antenatally when fetal echography revealed hydronephrosis, hospitalized at the age of one month, when subsequently undergoing partial endoscopic resection of valves, after 2 months endo-

scopic resection of valves. Evolution: favorable.

Case number 23

5 years old child diagnosed with: PUV type I, Bilateral ureterohydrophenosis, Pyelonephritis.

Diseases detected antenatally by fetal echography, when hospitalized at the age of 12 days, subsequently undergoing surgery by percutaneous cystostomy. Treatment continued at the age of 11 years old when it was practiced vesicostomy. At age of 1 and 2 years old the child underwent endoscopic resection of valves and after a month iterative resection, at the age of 1 and 4 years old endoscopic resection of diaphragm, at the age of 1 and 8 years old finally undergoing vesicostome.

Case number 24

23 years old child diagnosed with: PUV, Bilateral ureterohydronephrosis, Left ureterostomia, left VUR.Rehospitalized at the age of 2 years old, subsequently undergoing left Leadbetter-Poliano reimplantation after which appeared left VUR and at the age of 4 presented left cord cyst for which the child underwent cyst cure. There are no further details about the child's condition after becoming an adult.

Case number 25

22 years old child diagnosed with: PUV, Bilateral ureterohydronephrosis, Bilateral VUR, urinary tract infection. Detected postnatally, subsequently undergoing surgery: peritoneal ureterostomia and also cystostomy at the age of 6 months. Treatment was followed by transpubian, transvesical resection at the age of one year old. It was rehospitalized at the age of 8 years old with urinary incontinence subsequently undergoing right orchidopexy and control after 2 years. There are no further details about the child's condition after becoming an adult.

DISCUSSION

The 25 studied cases coming from the above mentioned two clinics, one of them with a surgical profile (from where most cases come) results revealed that although a number of 6 children were antenatally detected with renal diseases in the last decade, when the ultrasound technique and hospital equipment made more accessible positive diagnosis, neither of the fetus' undergoing surgery "*in uterus*" (Table I).

TABLE I
Detected diseases

	Antenatal	**Postnatal**
Number of patients	6	19
Percentage (%)	24	76

Correlating the results with recent literature data (15) we can assert that in the majority of the cases the diagnosis is set after the newborn age, most frequently at infant age when surgical intervention is performed, but positive diagnosis is set more easily in small children (1-3 years) when diagnosing methods are more permissive.

Four of the studied cases appeared in the neonatal period (between 10 days-1 month), at infant age, when they underwent surgical intervention in 9 of the cases, the other children (10 in number) were diagnosed between 1-3 year old and older, they also received surgical treatment, cystostomy, valve ablation etc. (2 children have not been operated yet) (Table II).

TABLE II
Moment of the surgical intervention

Age	Number of patients	%
0 – 12 months	4	17.39
0 -1 years	9	39.13
1 – 3 years	10	43.48

If in literature, the debut at infant age is quoted with a 30-36% frequency, between 1-5 year old with a 50-55% frequency, from the investigated casuistry apparently the number of diagnosed and operated cases appear at the infant age and it is increasing (13 infants).

At most of the infants were performed endoscopic valves ablations, a smaller initial number of cystomy, followed then in time II by valves resection. Their postoperative evolution was favorable. A child needed vesicostomy and a transpubian valve resection. No children of the studied group needed nefrostomy.

Although 3 children developed over time chronic renal failure, requiring peritoneal dialysis, none of them had to undergo a kidney transplant.

Nine children out of the studied group presented VUR, out of which being 5 bilateral. Regarding these results there is a similarity with the studied reference data. Other 4 children presented left or right vesicoureteral reflux of different types (Fig. 1).

In all 9 cases the treatment was performed surgically, from the ablation valves to cutaneous bilateral ureterostomies, vesicostomy and other subsequent corrections, the majority evolving propitiously with repeated urinal infections, although the diagnosis of posterior urethra valves in time still remain questionable.

This congenital malformation culminates in its evolution with renal failure (16) observed facts in cases 3, 4, 10, where the installation of uni- or bilateral VUR and repeated urinal infections relatively aggravated the evolution of the disease.

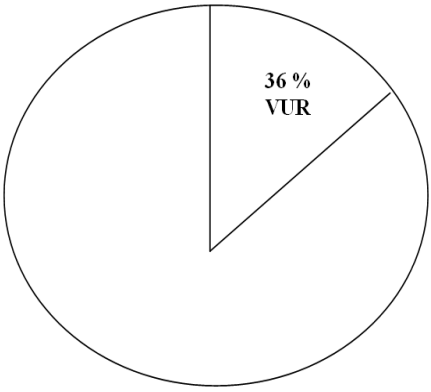

Fig. 1. Percentage of children discovered with VUR from the total number of children diagnosed with PUV

VUR may be considered as an associated gravity factor, 3 children required peritoneal dialysis and in the future probably undergoing renal transplant (17).

PUV are rare malformations of the posterior urethra, but their existence can lead to serious changes in the upper urinary system, culminating with chronic renal failure, despite the early surgical solution of the obstructed urethral, thus lowering the quality of life for children suffering from this diseases.

CONCLUSIONS

The majority of the studied cases presented favorable evolution and chronic renal failure in three cases, requiring dialysis and finally these patients possible undergoing renal transplant.

Vesicoureteral reflux, which can be secondary to the barrier, is interpreted as a

severe associated factor.

The diagnosis of PUV represents a challenge in the sense of detecting the impairment as early as possible and choosing the right therapeutic procedure in accordance with the degree of the renal impairment. Also it may be adversely influenced by: urinary infection, hypertension, bladder noncompliance. The diagnosis is more favorable when the reflux is unilateral, the heterolateral kidney being protected.

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CONSIDERAȚII ASUPRA ETIOLOGIEI SINDROMULUI DISFAGIC LA PACIENȚII LARINGECTOMIZAȚI

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ETIOLOGYCAL CONSIDERATIONS OF POSTLARYNGECTOMY DYSPHAGIA (Abstract): Dysphagia following laryngeal surgery is a common and severe symptom. Two life-threatening complications can compromise the patiens quality of life: pneumonia and malnutrition. Diagnostic procedures of swallowing disorders are represented by videofluoroscopic evaluation, CT scan and fiberoptics endoscopy. Material and methods: A retrospective evaluation was performed in a sample of 261 patients undergoing laryngectomy, hospitalized in the E.N.T Clinic of ,,St. Spiridon" Hospital, between 2006 and 2010. Postlaryngectomy dysphagia was diagnosed in 74 patients and was predominant in males (97,3%) aged between 50 and 60 years (37,8%). Results: Main causes of dysphagia were pharingocutaneous fistulas, tumor recurrences, postoperative cervical infections and hipopharyngeal stenosis. Conclusions: This study reveled the predominance of dyphagia in rural male patients undergoing total laryngectomy. Gastroesophageal reflux can represents a favoring factor of this type of dysphagia. **Key words**: DYSPHAGIA, LARYNGECTOMY, POSTOPERATIVE ANATOMICAL AND FUNCTIONAL CHANGES

Laringele are două roluri majore în cadrul procesului de deglutiție: asigură protecția căilor respiratorii (prin închiderea valvei trietajate alcătuită din epiglotă, benzile ventriculare şi corzile vocale) şi facilitează deschiderea sfincterului crico-faringian pentru trecerea bolului alimentar (prin ascensiune şi deplasare anterioară) (1). Dată fiind contribuția laringelui la buna desfăşurare a deglutiției, disfagia este un simptom comun rezultat în urma chirurgiei laringiene (2), disfuncțiile fiind mai severe şi frecvente, în comparație cu cele rezultate în urma extirpării tumorilor maligne cu alte localizări cervicale (1).

Disfagia se datorează în principal modificărilor anatomice şi funcționale apărute în urma extirpării totale sau parțiale a laringelui şi a reconstrucției faringelui, hipofaringele, esofagul superior şi laringele restant putând fi afectate major (1,3).

Cauzele disfagiei diferă în funcție de tipul de laringectomie.

În cazul laringectomiei totale, disfagia se poate datora spasmului muşchilor constrictori faringieni, spasmului şi deschiderii întârziate a sfincterului esofagian superior, stenozelor şi pseudodiverticulilor hipofaringieni, limitări mişcărilor bazei limbii, fistulelor faringo-cutanate, cât şi unor deficite ale nervilor cranieni. La acestea se pot adăuga lipsa coordonării musculaturii faringo-esofagiene la trecerea bolului alimentar şi îngroşarea sau proeminența peretelui

posterior faringian. (1,2,3,4,5). Frecvența disfagiei postlaringectomie este de 17-70% (2) sau 10-58% (4) din cazuri.

În laringectomiile verticale şi cele fronto-laterale, disfagia este severă şi greu tratabilă, putând avea drept cauză aspirația traheală, presiunea crescută din tractul faringo-esofagian, ascensiunea redusă a laringelui restant în timpul deglutiției şi incoordonarea mușchilor constrictori faringieni cu mușchiul crico-faringian (1,2,4,6).

Laringectomiile supraglotice produc disfagie mai frecvent decât laringectomiile totale (7).

Cordectomia produce disfagie minoră şi tranzitorie (1).

O complicație redutabilă şi frecventă a laringectomiilor, constant generatoare de disfagie, este fistula faringo-cutanată. Aceasta afectează 13% din pacienții laringectomizați (8). Studiile recente corelează apariția fistulei cu extensia tumorală la nivelul versantului laringian al sinusului piriform, impregnația tumorală a faringelui şi infecțiile locale postoperatorii (8,9).

Diagnosticul sindromului disfagic postlaringectomie necesită investigații diagnostice complexe: scintigrafia, videofluoroscopia, computertomografia, fibroendoscopia, microlaringoscopia, etc (1,4,5,10).

Tratamentul disfagie post-laringectomie este multimodal şi constă în tratament dietetic şi foniatric (1), medical şi chirurgical, fiind particularizat în funcție de cauza disfagiei. Stenoze hipofaringiene pot beneficia de dilatații sau faringoplastii (2,11). Fistulele faringo-cutanate pot fi închise prin sutură, faringoplastie cu grefe cutanate libere vascularizate (12) sau lambouri cutanate şi musculo-cutanate (13).

MATERIAL ŞI METODĂ

Studiul a avut drept scop identificarea cauzelor disfagiei la pacienții cu neoplasm laringian, internați şi operați în Clinica O.R.L a Spitalului „Sfântul Spiridon” din Iaşi în perioada 2006-2010.

Studiul a fost efectuat retrospectiv pe un eşantion reprezentativ statistic de 261 de pacienți cu neoplasm laringian operat.

Metodele de culegere şi interpretare a datelor au fost matematice şi statistice, realizându-se cu ajutorul unor echipamente şi programe informatice adecvate.

REZULTATE ŞI DISCUȚII

Lotul de studiu a fost alcătuit din 261 de pacienți cu neoplasm laringian operat : 13 femei (4,98%) şi 248 de bărbați (95,02%), 114 pacienți fiind din mediul urban (43,68%) iar 147 din mediul rural (56,32%).

În ce privește tipul de laringectomie:

168 de pacienți au suferit laringectomii totale (64,37%); 1 laringectomie totală cu faringectomie parțială (0,38%); 85 cordectomii prin tirotomie mediană (32,57%); 7 diverse tipuri de laringectomii parțiale: hemilaringectomie verticală, epiglotectomie, laringectomie frontală anterioară cu cordectomie lărgită bilaterală, laringectomie parțială bicordocomisurală (2,69%).

Distribuția cazurilor pe grupe de vârstă a fost următoarea: 4 pacienți au avut vârste cuprinse între 30 şi 40 de ani (1,53%); 36 pacienți între 40 şi 50 de ani (13,79%); 109 pacienți între 50 şi 60 de ani (41,76%); 72 pacienți între 60 şi 70 de ani (25,59%); 34 pacienți între 70 şi 80 de ani (13,03%) şi 6 pacienți între 80 şi 90 de ani (2,3%).

Preponderența neoplasmului laringian corespunde cu datele din literatură, aceasta fiind observată la sexul masculin (95,02%) şi la vârste cuprinse între 50 şi 70 de ani (67,35%).

Prin studiul lotului descris s-au identificat 74 de pacienți cu sindrom disfagic (28,35%): 72 de bărbați (97,3%) şi 2 femei (2,7%), 28 provenind din mediul urban (37,83%) şi 46 din mediul rural (62,17%).

Se observă preponderența sindromului disfagic la sexul masculin, corespondentă cu cea a neoplasmului laringian şi la pacienții din mediul rural, acesta putându-se datora adresabilității şi accesibilității mai reduse la serviciile medicale, condițiilor de igienă precare şi de educației sanitare deficitare.

Vârsta pacienților cu sindrom disfagic postlaringectomie a fost următoarea: 2 pacienți au avut vârste cuprinse între 30 şi 40 de ani (2,70%); 13 pacienți între 40 şi 50 de ani (17,56%); 28 pacienți între 50 şi 60 de ani (37,83%); 18 pacienți între 60 şi 70 de ani (24,32%); 12 pacienți între 70 şi 80 de ani (16,21%) şi 1 pacient între 80 şi 90 de ani (1,35%), comparabilă cu distribuția pe vârste a lotului studiat. Se observă preponderența netă a disfagiei datorată laringectomiilor la grupa de vârstă 50-60 de ani.

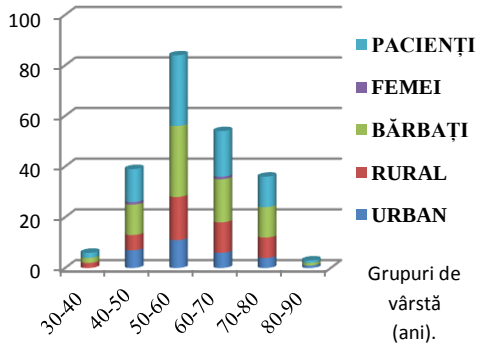

Fig. 1. Distribuția pacienților disfagici în funcție de vârstă, sex, mediu de domiciliu.

Extensia tumorală locală a fost T1-T2 la 20 de pacienți (27%) şi T3-T4 la 54 de pacienți (73%), reprezentând factorul principal în stabilirea tipului de intervenție chirurgicală. Astfel, au fost efectuate laringectomii totale la 52 de pacienți (70,3%), cordectomii la 18 pacienți (24,32%) şi alte tipuri de laringectomii parțiale la 4 pacienți (5,4%).

Studiind cauzele disfagiei în funcție de tipul de laringectomie au rezultat următoarele date :

În cazul celor 52 de pacienți cu laringectomii totale, cauzele sindromului disfagic au fost: fistulele faringo-cutanate la 23 de pacienți, recidivele tumorale sau continuările evoluției tumorale la 8 pacienți, supurațiile cervicale anterioare de etiologii diverse (serom cervical suprainfectat, granulom de fir abcedat) la 8 pacienți, dehiscența plăgii operatorii la 2 pacienți, fistula traheo-esofagiană la 1 pacient şi stenoza hipofaringiană la 2 pacienți. La 7 pacienți, sindromul disfagic a avut cauze multiple, combinate (fistulele faringo-cutanate, recidivele tumorale sau continuări de evoluției tumorale, supurațiile cervicale anterioare).

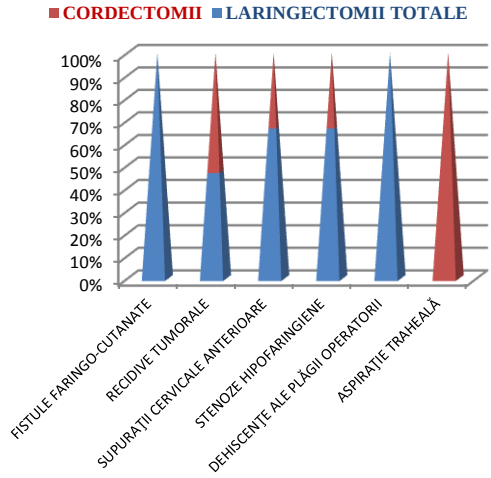

Fig. 2. Tipuri de laringectomii efectuate la pacienții disfagici.

Cordectomiile prin tirotomie mediană au fost urmate de sindrom disfagic datorat în special recidivelor tumorale sau continuărilor evoluției tumorale în 9 cazuri, supurațiilor cervicale anterioare în 4 cazuri, dehiscenței plăgii operatorii într-un caz, stenozei hipofaringiene într-un caz şi fără cauză aparentă/aspirație traheală în 3 cazuri.

Celelalte tipuri de laringectomii parțiale au fost urmate de recidive sau continuări ale evoluției tumorale ce au necesitat totalizarea laringectomiei. Au fost urmate de fistulele faringo-cutanate recidivante şi ulterior de stenoze hipofaringiene.

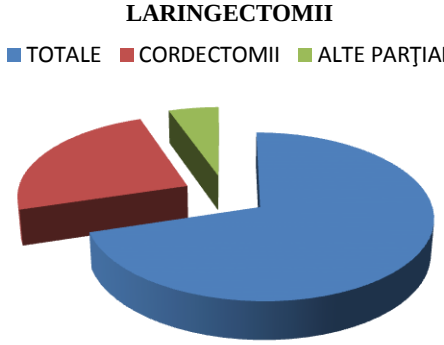

Fig. 3. Cauzele disfagiei în funcție de tipul laringectomiei.

Diagnosticul cauzal al sindromului disfagic la pacienții laringectomizați a fost stabilit prin examinare clinică şi explorări paraclinice, în special computertomografie cu substanță de contrast şi endoscopie.

Tratamentul a fost complex, fiind orientat în funcție de cauza disfagiei. În cazul fistulelor faringo-cutanate şi dehiscențelor plăgii operatorii s-a practicat sutura simplă per secundam sau faringoplastia cu lambouri cutanate şi musculo-cutanate cât şi cu grefe libere, intervenții susținute de alimentație parenterală şi vitaminoterapie. Pentru recidive şi continuări ale evoluției tumorale s-a practicat totalizarea laringectomiei, urmată de radio şi chimioterapie. Supurațiile cervicale au necesitat incizie şi drenaj asociate cu antibioterapie.

CONCLUZII

Studiul realizat pe un lot de 261 de pacienți cu neoplasm laringian operat în ultimii 6 ani, relevă predominența sindromului disfagic rezultat în urma laringectomiei la pacienții de sex masculin: (97,3%) proveniți din mediul rural; (62,17%) cu vârste cuprinse între 50 şi 80 de ani (79,7%).

Disfagia apare mai frecvent în urma laringectomiilor totale (70,27%), primele două cauze fiind reprezentate de fistulele faringo-cutanate şi recidivele sau continuările de evoluție tumorală. Între aceste două cauze a fost stabilită o corelație, fistulele faringo-cutanate fiind frecvente în cazul ablației incomplete a tumorii, urmată firesc de recidivă.

Alte cauze posibil generatoare de fistule faringo-cutanate şi implicit disfagie ar putea fi refluxul gastro-esofagian, hipersalivația şi deglutiția frecventă.

Cantonarea microbiană în special cu pseudomonas şi candida, generatoare de aciditate se pare că ar favoriza apariția fistulei faringo-cutanate.

Pentru a preveni apariția fistulelor faringo-cutanate, în Clinica O.R.L. a Spitalului „Sf. Spiridon", pacienții laringectomizați sunt monitorizați atent în vederea depistării precoce a acestor factori favorizanți.

Tratamentul preventiv constă în administrarea inhibitorilor pompei de protoni (omeprazol, pantoprazol, esomeprazol) în cazul refluxului gastro-esofagian, a antibioticelor şi antimicoticelor, în conformitate cu antibiograma sau antifungigrama în cazul infecțiilor şi a anticolinergicelor (atropina) pentru a reduce secreția salivară.

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THE IMPACT OF RENAL DENERVATION ON THE BAROREFLEX CONTROL OF HEART RATE IN AN EXPERIMENTAL MODEL OF REDUCED RENAL MASS, SALT SENSITIVE HYPERTENSION

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THE IMPACT OF RENAL DENERVATION ON THE BAROREFLEX CONTROL OF HEART RATE IN AN EXPERIMENTAL MODEL OF REDUCED RENAL MASS, SALT SENSITIVE VOLUME-OVERLOAD HYPERTENSION (Abstract): **Background**. Beyond lowering arterial blood pressure in hypertensive patients, renal denervation (RDNx) has also been shown to improve the cardiac autonomic nervous system, particularly in sympathetically mediated cardiovascular disorders. Nevertheless, little is known about the efficiency of renal nerve ablation in non-mediated sympathetically diseases. Therefore, our aim was to investigate the influence of renal denervation on the cardiac autonomic nervous system in an experimental rat model of salt sensitive, volume-overload hypertension developed after surgical reduction of renal mass (RRM) by 75-80% and salt loading. **Material and methods:** The dynamic time-dependent cardiac autonomic system analyses such as the baroreflex sensitivity (BRS), the power of the fluctuations of the heart rate in high frequency range (0.75-3Hz, HF power) and both short and long-term beat-to-beat variability were calculated from continuously recorded cardiac cycles before and after surgical RRM by 80%, including here RDNx and central sympathoinhibition with clonidine. **Results:** The BRS decreased significantly throughout the entire 2 weeks of the development of salt-sensitive, volume overload hypertension induced after surgical RRM and high salt diet. After RDNx, the HF power decreased during the first 7 days, paralleling the alterations of both long and short-term heart rate variability. Global sympathoinhibition after clonidine administration significantly improved the cardiac autonomic nervous system as reflected by the substantial enhancement of the BRS, the HF power and both short and long-term heart rate variability. **Conclusions:** The present results indicate that in salt-sensitive, volume overload hypertension, the cardiac autonomic nervous system is impaired, and these alterations are not improved over the long term by RDNx. On contrary, central sympathoinhibition after clonidine administration following RDNx significantly improved BRS, the HF power and both short- and long-term beat-to-beat variability. **Keywords**: HYPERTENSION, RENAL DENERVATION, CARDIAC AUTONOMIC NERVOUS SYSTEM, BAROREFLEX, HEART RATE VARIABILITY.

The arterial baroreflex plays a crucial role in maintaining the homeostasis of the cardiovascular system by reflexively modulating the balance between sympathetic and parasympathetic activity (1-3). Baroreflex dysfunction has been associated to many cardiovascular diseases that lead to autonomic imbalance and thus likely contribute to the progression of disease. Impairment of the baroreflex control of the heart rate has been found in patients with heart failure, myocardial infarction, coronary artery disease and essential hypertension (2, 4-7). While baroreflex dysfunction *per se* is correlated with a high mortality risk (8-10), reductions in heart rate variability (HRV), likely due to impaired baroreflex control of the cardiac autonomic outflow, has also been recognized as a predictor and a risk factor for developing life-threating atrial and ventricular tachyarrhythmia (11-16). Furthermore, since dysfunction of the baroreflex system has also been described in patients with treatment resistant hypertension (RHT) (17) these patients are likely exposed to arrhythmic risk (18).

Recent clinical and experimental studies indicate that ablation of the renal nerves may improve both baroreflex sensitivity (BRS) and HRV in patients with RHT (19, 20). Importantly, these effects occur in parallel to the lowering of blood pressure, thus indicating a putative beneficial cardioprotective effect. Overactivation of sympathetic efferent renal nerves increases blood pressure by stimulating renin secretion and renal tubular sodium reabsorption and interruption of these pathways by renal denervation (RDNx) is considered to underlie most of the antihypertensive effects of this non-pharmacological approach (21). However, RDNx also interrupts sympathoexcitatory afferent signaling from the kidneys, which may contribute to global sympathoinhibition, in addition to the renal-specific effects. An improvement in the baroreflex control of sympathetic outflow or suppression of the central sympathetic outflow via hypothalamic pathways has been suggested to mediate these latter effects (22, 23). Although the lowering of blood pressure and the improvement of autonomic function following RDNx appear to be efficient in conditions associated with sympathetic overactivation, it remains unclear whether these beneficial effects are also manifest in the absence of an increased sympathetic outflow.

The remnant kidney model is mainly used to investigate the pathophysiology of the RHT related to non-ischemic nephron loss and has the major advantage to closely replicate many of the abnormalities of this clinical condition (24). In this model of salt-sensitive, volume overload hypertension following surgical reduction of 75-80% of the renal mass, we have previously shown that the global sympathetic nervous system activity is actually reduced and RDNx does not further lower sympathetic activity, thus lacking sustained antihypertensive effects (25). However, it is unclear whether baroreflex function and HRV are altered during the development of salt-sensitive hypertension and whether RDNx may have beneficial effects on the baroreflex control of heart rate in the absence of a sustained antihypertensive and global sympatholytic effect. Considering the recent observations regarding the anti-arrhythmic effects in patients undergoing RDNx (19, 26-29), we hypothesized that renal nerve ablation may improve cardiac BRS and HRV in this clinically relevant model of human RHT. This evaluation in this experimental model which shares many of char-

acteristics of RHT may be important clinically because many patients with RHT are at high risk for detrimental cardiac arrhythmias (17, 18).

To test this hypothesis, spontaneous oscillations of blood pressure and heart rate were continuously analyzed in the time and frequency domains, to determine the daily time course of cardiovascular dynamics under different salt-loading conditions during normal renal function and throughout the progression of salt-sensitive hypertension following reductions in renal function. Furthermore, the ability of the renal denervation to improve cardiac baroreflex, and ultimately the HRV was evaluated. Finally, the response of the cardiovascular dynamics to global suppression of sympathetic outflow with clonidine was assessed.

MATERIAL AND METHODS

All experiments were performed according to the European Directive 2010/63/EU on the Protection of Animals Used for Scientific Purposes and were approved by the Research Ethics Committee of the "Grigore T. Popa" University of Medicine and Pharmacy Iași. As previously describe (25), eight adult male Sprague Dawley rats (28-32 weeks of age) were used in this study. The animals were obtained from the National Research Institute "*Cantacuzino*" (Bucharest, Romania). Rats were housed in a temperature (21-23°C) and humidity-controlled environment with a 12 hours light/dark cycle, with *ad libitum* access to food and water. Animals were fed a standard rodent diet containing either 0.8 % NaCl (Normal salt, NS), 0.1 % NaCl (Low Salt, LS) or 4 % NaCl (High Salt, HS) (National Research Institute "Cantacuzino" Bucharest, Romania). The long-term cardiovascular responses (24 hours averages) data from this study have been presented in a previous publication (25). The present study was designed to investigate the detailed time-dependent dynamic changes of baroreflex control of heart rate, as well as HRV.

Animal preparation

All surgical procedures aimed for continuous acquisition of blood pressure, surgical reduction of renal mass and renal denervation of the remnant kidney have been previously described (25).

Data acquisition and analysis

Arterial blood pressure was continuously recorded using implantable radio telemetry system. For signal acquisition and wireless charging of the implanted telemeters rats were placed on TR181 smart pads (*Millar, Inc., Houston, TX*)(25). The individual 24-h BP waveforms were acquired continuously at a sampling frequency of 2000 Hz using a PowerLab 16/35 data acquisition system (*ADInstruments, New South Wales, Australia*). All individual cardiac cycles were recorded continuously (on every day between 7.00 AM to 6.00 AM recordings, excluding the one hour necessary for daily animal care). The dynamic time-dependent cardiac autonomic system analyses were performed under both normal and reduced renal mass conditions, with the associated phases of the developing and established volume-overload hypertension, including here the renal denervation and the central sympathoinhibition with clonidine (in doses of 300 and 150 μg/kg/day in the drinking water with daily adjustment of the concentration per fluid intake to attain desired drug intakes). For subsequent time- and frequency-domain analyses, daily time series of beat-to-beat

systolic blood pressure (SBP) and pulse intervals (PI) were generated from artifact-free 23 hours long blood pressure waveforms with LabChart Pro v8.1.8 (*ADInstruments, New South Wales, Australia*) and peak detection algorithms.

Estimation of baroreflex control of heart rate

BRS was computed using frequency domain analyses as previously described. Briefly, a spectral analysis of the daily beat-to-beat data based on the Fast Fourier Transformation (FFT) was performed using MATLAB routines (*R14; MathWorks, Natick, MA*) using algorithms we have previously described (16, 30). The BRS was estimated as the magnitude of the transfer function between SBP and PI within 0.05 - 0.5 Hz frequency range.

Heart rate variability measurements

HRV measurements were performed using both time and frequency domain analyses. Long-term heart rate variability was obtained from time domain analyses entailing in determination of the standard deviation (SD) of all pulse intervals during a 23-hours. period. Short-term variability was computed as the means of the standard deviation calculated for all 5-min segments for the entire 23 hours period (SDNN) (31). The power of the heart rate oscillations within 0.75-3 Hz band (HF power) from the daily R-R interval variations, reflecting the parasympathetic control of the heart rate, was computed using the non-parametric lomb periodogram algorithm implemented into LabChart module (*LabChart Pro v8.1.8*).

Experimental design

Normal renal function and associated salt resistant hypertension

1. After implantation of the telemeters, the rats were allowed to recover for 2 weeks until the circadian rhythmicity was fully restored. Cardiac autonomic nervous system responses from control period were recorded while the animals were fed with normal salt (NS) diet. To assess the cardiac autonomic nervous system regulation during normal renal function and associated salt resistant hypertension, the salt intake varied as follows: for 7 days the rats were fed with LS, followed by another 7 days of HS diet. At the end of the second week, the LS diet was initiated and maintained until the mean arterial blood pressure and heart rate values plateaued around the levels from control period.

Reduced renal mass and associated salt sensitive hypertension

Cardiac autonomic nervous system responses were assessed continuously throughout the entire experimental periods as described below. Surgical RRM was performed as previously (25). Following the post-surgical recovery period, the HS diet was initiated and maintained for the next 6 weeks. After two weeks of HS diet, when arterial blood pressure significantly increased and plateaued at hypertensive levels, RDNx of the remnant left kidney was performed as previously described (25). Two weeks after RDNx, the cardiac autonomic nervous system responses to centrally sympatholytic clonidine were assessed. Finally, in the last week of the experiment, the rats were subjected again on LS diet.

Statistical analysis

Results are expressed as Mean ± SE. One-way, repeated measures ANOVA followed by Dunnett's multiple comparison test were used (*Prism 6.01, GraphPad Software*) to compare the experimental

periods mentioned here below and the differences were considered statistically significant for $p<0.05$.

Normal renal function

→ daily cardiac autonomic nervous system regulation during:

1) LS (days 5-11; 19-21) to NS control period (days 2-3);

2) HS (days 12-18) to NS control period (days 2-3);

Reduced renal mass and associated salt sensitive hypertension

→ daily cardiac autonomic nervous system regulation during:

1) HS (days 40-53) to LS (days 38-39);

2) HS + RDNx (days 54-67) to HS (days 52-53);

3) HS + RDNx + clonidine 300 μg/kg/day (days 68-73); + clonidine 150 μg/kg/day (days 74-76); + clonidine washout (days 77-81) to HS + RDNx (days 66-67);

4) LS (days 82-87) to HS + RDNx (days 80-81).

RESULTS

Responses during normal renal function and associated salt resistant hypertension.

As shown in the figure 1A, the estimated BRS as the magnitude of the transfer function between systolic blood pressure and pulse interval did not change during both LS and HS. While the HF power (fig. 1B) increased in the last day of the LS, it decreased significantly throughout the entire HS period. Although long term beat-to-beat variability did not suffer any alterations (fig. 2A), short term beat-to-beat variability decreases during both LS and HS periods (fig. 2B), paralleling the HF power.

Figure 1

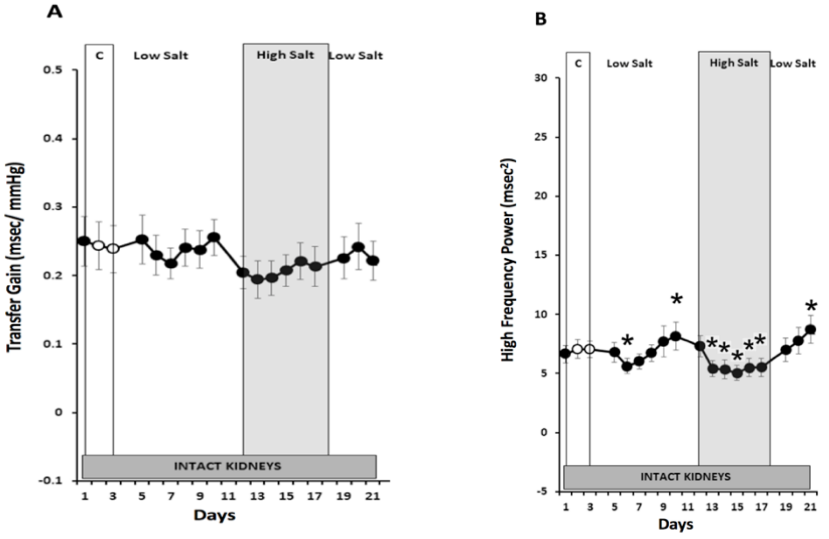

Fig. 1. Baroreflex sensitivity (**Panel A**) and the power of the fluctuations of the heart rate in high frequency range - HF power - **(Panel B)** responses to varying salt intake during normal renal function. Values are men ± SEM. Normal renal function: * $p < 0.05$ vs. NS Control (days 2-3). Respective baseline days are depicted as (○).

Figure 2

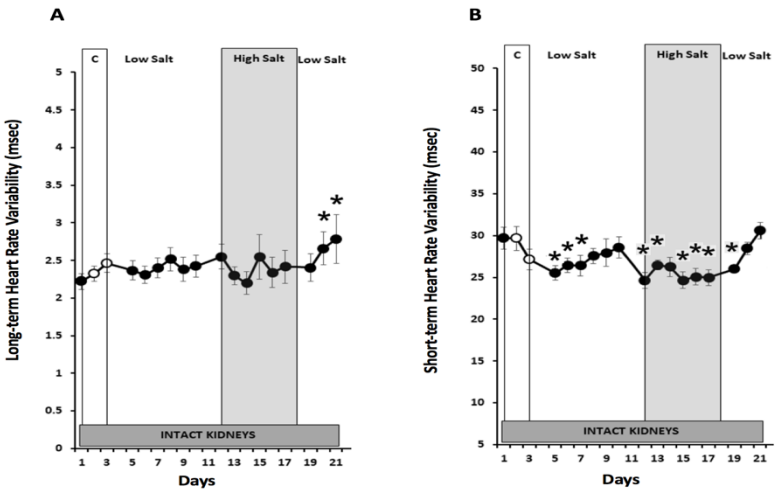

Fig. 2. Long (**Panel A**) and short-term (**Panel B**) beat-to-beat heart rate variability responses to varying salt intake during normal renal function. Values are men ± SEM. *Normal renal function*: * $p < 0.05$ vs. NS Control (days 2-3). Respective baseline days are depicted as (○).

Responses to renal denervation during established salt-sensitive, volume overload hypertension

Compared to the last 2 days of previous period of HS loading, the estimated BRS slightly increased at the end of the 2 weeks after renal denervation (fig. 3). As shown in figure 4, the HF power decreased during the first week after renal denervation, paralleling the alterations of the long- and short-term beat-to-beat variability (fig.5 and fig. 6). These changes induced by renal denervation were only transient, as all variables gradually returned towards pre-denervation levels within a week and were stable subsequently, except estimated baroreflex sensitivity which remained at higher levels than control period.

Responses to central sympathoinhibition during established salt-sensitive, volume overload hypertension

As depicted in figure 3, global sympathoinhibition induced by clonidine administration in doses of 300μg/ kg significantly increased the BRS. Moreover, the HF power significantly increased (fig. 4) and all these changes were accompanied by a marked increase of both long- and short-term beat-to-beat variability (fig. 5 and fig. 6). Then, during the clonidine dose tapering (150 μg/ kg) and the following washout period, all the variables gradually returned to post-denervation values.

Responses to salt restriction after reduced renal mass

No further changes of the BRS occurred along the last week of the experiment when the animals were on LS diet. The HF power slightly increased at the end of the last week (fig. 4), accompanied by the same variation of the short-term heart rate variability (fig. 6).

Figure 3

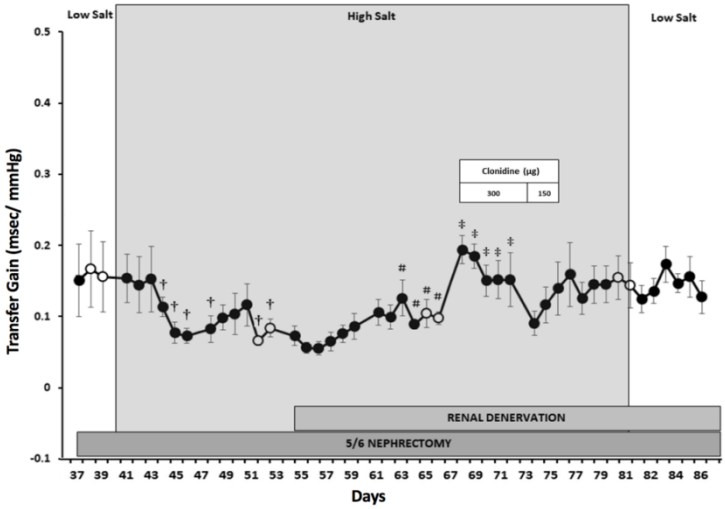

Fig. 3. Baroreflex sensitivity responses to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: † $p < 0.05$ vs. baseline LS (days 37-38); # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); Respective baseline days are depicted as (○).

Figure 4

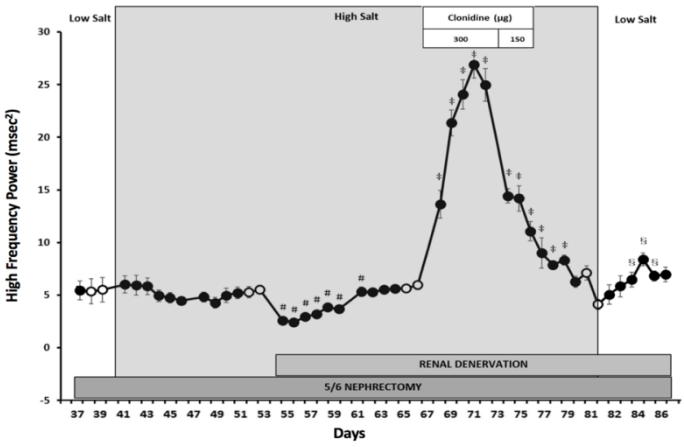

Fig. 4. Responses of the power of the heart rate fluctuations within high frequency range to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); § $p < 0.05$ vs. baseline Clonidine washout (days 79-80). Respective baseline days are depicted as (○).

Figure 5

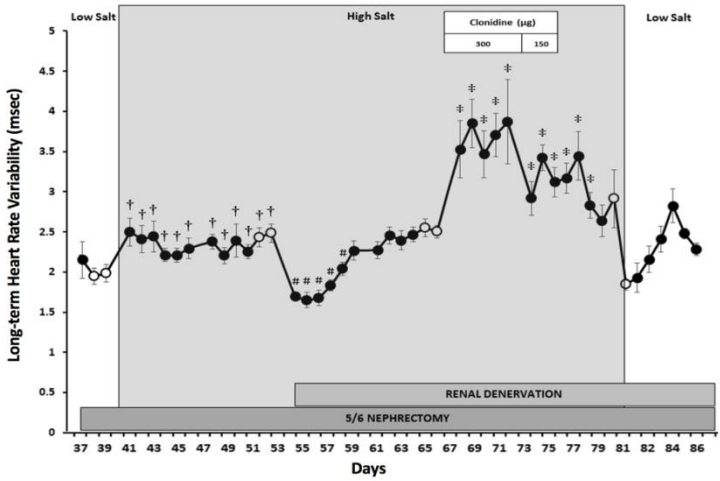

Fig. 5. Long term beat-to-beat heart rate variability responses to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: † $p < 0.05$ vs. baseline LS (days 37-38); # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); Respective baseline days are depicted as (○).

Figure 6

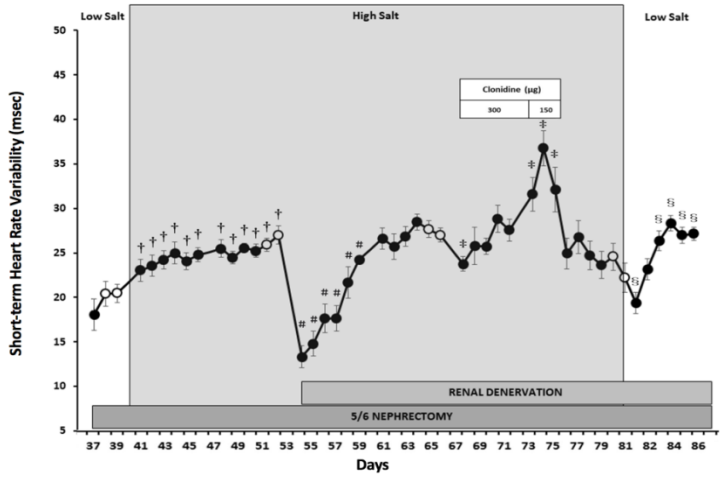

Fig. 6. Short term beat-to-beat heart rate variability responses to salt loading during reduced renal mass and responses to renal denervation and central sympathoinhibition during salt-sensitive, volume overload hypertension. Values are men ± SEM. *Reduced renal mass*: † $p < 0.05$ vs. baseline LS (days 37-38); # $p < 0.05$ vs. baseline HS (days 51-52); ‡ $p < 0.05$ vs. baseline HS + RDNx (days 64-65); § $p < 0.05$ vs. baseline Clonidine washout (days 79-80); Respective baseline days are depicted as (○).

DISCUSSION

A significant reduction of the estimated BRS during the development of salt-sensitive, volume overload hypertension following non-ischemic reduction of the renal mass and HS diet was found in this experimental study. Our results are in line with previously published data from experimental and clinical studies that show a blunted baroreflex sensitivity in both non-ischemic (32) and ischemic renovascular hypertension (33, 34). Although in the ischemic forms of the renovascular hypertension the impairment of the BRS is associated with the activation of the sympathetic nervous system (34, 35), little is known about the mechanisms responsible for the cardiac autonomic nervous system dysregulation in the non-ischemic renovascular arterial hypertension. While BRS decreased during high salt diet and associated progression to salt-sensitive, volume overload hypertension, both long and short-term heart rate variability did not paralleled the baroreflex evolution, possible due to involvement of the low-pressure cardiopulmonary mechanoreceptors in the heart rate control triggered by fluid overload (36). Moreover, we also found that the estimated BRS tended to be decreased during salt loading with normal kidney function although this effect did not reach statistical significance. During this time, both time- and frequency domain measures of heart rate variability were lower, as compared to normal salt intake. These findings are consistent with clinical (37) and experimental studies (38, 39) indicating that the sensitivity of the baroreflex control of heart rate is depressed during high salt diet in normotensives as a consequence to reduced sensitivity of the mechanoreceptors, possibly due to local alterations in the ionic milieu in the carotid sinus region (40-42). Tonic increases in central sympathetic outflow may also be responsible for the reduced variability of heart rate during exposure to high salt levels. Indeed, we have previously shown that during salt loading on normal kidney function, the sympathetic activity estimated from the power of the blood pressure oscillations in the low frequency range was increased (25). Furthermore, in normotensive rats, the magnitude of the sympathetic nervous system activation is directly correlated with the level of dietary sodium intake because of a NaCl-induced sensitization of the central autonomic pathways (43), likely involving neurons within rostral ventrolateral medulla (44-46). Although the activation of the sympathetic nervous system mediated through central mechanisms in response to salt loading could underlie the impairment of the cardiac autonomic modulation during salt-resistant hypertension, other mechanisms are likely responsible for the impairment of the BRS during salt-sensitive hypertension since the global sympathetic activity is actually decreased as we have previously described (25).

Recent experimental and clinical investigations suggest that beyond antihypertensive effects, renal denervation is also effective in reducing the incidence of arrhythmias particularly in cardiovascular diseases associating high levels of sympathetic activation such as chronic heart failure (47-51). In contrast, in this model of salt sensitive hypertension, in the absence of overt sympathoexcitation, we found that renal denervation did not improve baroreflex sensitivity, cardiac parasympathetic tone or short- and long-term heart rate variability. Furthermore, during the first week after renal nerve ablation these parameters decreased significantly and returned to pre-denervation levels only during the second week after denervation. Together with our previous findings indicating the lack of

sustained antihypertensive effects of renal denervation (25), our current data suggest that the failure of renal denervation to lower global sympathetic activity in this model of salt sensitive hypertension and non-ischemic reduction of renal function may also be responsible for its inability to improve the cardiac autonomic regulation.

The above contention is further supported by the responses to central sympathoinhibition with clonidine. In parallel with lowering sympathetic activity (25), Clonidine also significantly improved the sensitivity of the baroreflex system, the parasympathetic tone to the heart and both short and long term beat-to-beat heart rate variability. The improvement of the baroreflex sensitivity reported in our study is in line with the other results showing that by exerting a direct effect on baroreceptors themselves (52) or by stimulating the alpha-2 adrenoceptors from the *vagus nuclei* (53), clonidine is able to increase the cardiac parasympathetic tone and therefore the baroreflex sensitivity and heart rate variability.

CONCLUSIONS

The major findings of this study are: 1) during salt-sensitive, volume overload hypertension developed after non-ischemic reduction of renal mass, the BRS is reduced, 2) in this phenotype of non-sympathetically mediated arterial hypertension RDNx does not improve over the long term the BRS and 3) central sympathoinhibition after clonidine administration following RDNx led to a significant improvement of the BRS, parasympathetic modulation of the heart rate and both short and long term HRV.

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GIANT LIPOMATOUS TUMORS OF THE UPPER EXTREMITIES – A SERIES OF 8 CONSECUTIVE CASES

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GIANT LIPOMATOUS TUMORS OF THE UPPER EXTREMITIES – A SERIES OF 8 CONSECUTIVE CASES (Abstract): We present a series of eight consecutive patients evaluated in our department from 2002 to 2007 with giant lipomatous tumors (as defined in medical studies as greater than 5 cm) of upper extremities. There were three male and five female, ranged in age from 17 to 77 years (mean age of 55). The tumor's size ranged from 5 to 34 cm. All patients underwent total excision of the tumors with free margins. All specimens were sent to pathology: seven patients had benign tumors and one patient had liposarcoma. There were no recurrences of the tumors. Appropriate preoperative evaluation and complete surgical excision are mandatory for successful treatment of these tumors. **Key words**: GIANT LIPOMA, LIPOMATOUS TUMORS, UPPER EXTREMITY

Lipomatous tumors, the most common mesenchymal tumor, represent nearly half of all benign lesions. They can occur anywhere in the body, but are rarely found in the upper extremities. Most common locations of the lipomas are represented by the subcutaneous tissue of the head, neck, shoulders and back. However, lipomas can develop in deep locations (intermuscularly, intramuscularly, perineural, interosseously) or at a site of prior trauma or having unusual features (angiolipoma, fibrolipoma, chondroid lipoma, lipoma with hibernoma, spindle cell lipoma, myelolipoma, pleomorphic lipoma).

In their typical form, the diagnostic is not a problem for the pathologist, but in unusual cases these tumors can be confused with liposarcoma. Lipomas are slow-growing soft tissue tumors that rarely reach a size larger than 2 cm. Previous studies defined giant lipomas of the upper extremity the lesions larger than 5 cm in any one of their dimensions (1). Their painless slow growth can lead to large size masses at presentation, especially when are located in deep subfascial planes. Clinically, they appear as well-circumscribed, encapsulated masses with a doughy consistency and that are free mobile on the superficial and deep planes.

Cosmetic deformity or compressive symptoms usually bring the patient with lipomatous masses of the upper extremity to doctor earlier than rapidly growing masses in other locations of the body. Giant lipomas of the upper extremity are rare, but when they occur, an appropriate work-up must be done because a tumor of this size warrants a work-up for malignancy (1). This should be followed by adequate open surgical excision with free margins. These patients require repeated examinations over time to monitor the recurrence.

Fig. 1. Preoperatory aspect

Fig. 2. Intraoperatory stage after partial resection of deltoid muscle

Fig. 3. Final stage of the tumor resection

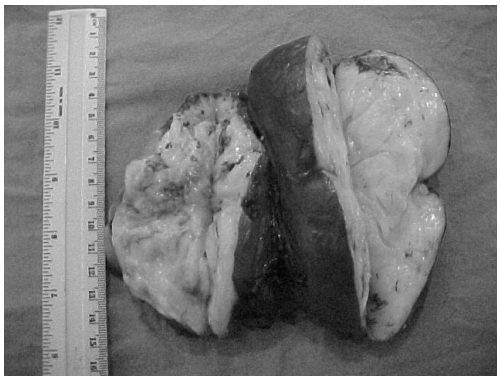

Fig. 4. Sections through the tumor

CASES DESCRIPTION

We present a series of eight patients evaluated in our department from 2002 to 2007 with giant lipomatous tumors of upper extremities (Table 1). There were three male and five female, ranged in age from 17 to 77 years (mean age of 55). The tumor's size ranged from 5 to 34 cm. All patients underwent total excision of the tumors with free margins. All specimens were sent to pathology : seven patients had benign tumors and one patient had liposarcoma. There were no recurrences of the tumors. We present three cases in more detail.

Case 1 (patient 1)

Patient H.C., a 61 year-old man was admitted in our department with a large mass in his right deltoid region. The patient reported appearance of the tumor with 6 months before admission. During physical examination was founded a painless mass having about 10/6 cm in size, well-defined, with high consistency, reduced mobility and no overlying skin changes. Ultrasound examination showed in right deltoid area a large tissue mass with heterogeneous appearance, relatively well-defined, poorly vascularized. The lesion disrupts muscle tissue. MRI revealed an expansive process relatively bulky (17/13/7 cm), well-defined, showing homogeneous fatty signal. Lesion is located in the upper third of the arm and develops into the inner part of deltoid muscle penetrating between its threads and dividing it. The mass develops in close proximity to the humerus, but not interested in

Fig. 5. Tumor in lateral region of the right arm

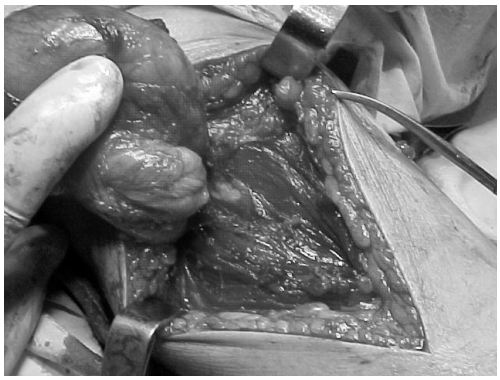

Fig. 6. Deep origin of the tumor between the triceps muscle bundles

its components. MRI appearance is suggestive of lipoma of the right shoulder. A radical excision of the lesion was performed with primary closure. Particularity consists in deep, intramuscularly location of the lipoma in the posterior deltoid beam as the existence of a lipomatous extension going near the humerus into the radial nerve groove. For excision of this extension was necessary to cut back beam of the deltoid muscle. Postoperatively, the patient recovered well with no functional deficits and was discharged after 6 days. Final pathology revealed lipoma (fig. 1-4).

Case 2 (patient 6)

Patient I.A., a 41 year-old woman, presented with a mass on her right arm that appeared about 2 years ago before admission. The patient reported a slow growth of the mass. On physical examination was observed a mass in the lateral region of the right arm measuring about 8/7 cm in size, well-circumscribed, with high consistency, relatively mobile on superficial and deep plans; the overlying skin presented a normal aspect. Sensitive and motor functions were intact throughout his right hand and forearm. Patient presented no other co morbidities and usual analysis was in normal range. Ultrasound revealed a mass within the right triceps consistent with a low-grade lipomatous lesion, likely an intramuscular lipoma. No vascular signal at Doppler examination was found. Plain radiography showed increased dimensions of the soft tissue in lateral region of the right arm; no bone modifications were recorded. The patient underwent complete excision of the lesion, which was found to be of 8/7 cm in size. Postoperative evolution was simple and the patient was discharged 3 days postoperatively. Final pathology revealed lipoma. The particular aspect of this case consisted in deep location of the mass within the triceps muscle bundles that had to be dissociated to address the tumor (fig. 5-6).

Case 3 (patient 2)

A 74 year-old woman was transferred from the department of cardiology because of a giant mass in the left axillary region. The patient presented with congestive heart failure, chronic atrial fibrillation, varicose ulcers of the lower limbs and obesity. She reported a history of 30 years of painless slow growth of this mass. Clinical examination revealed a bulky axillary mass having 30/20 cm, pasty consistency, with visible vascularization on surface. Overlying skin had areas of ulceration. Ultrasound showed a well-defined giant mass with heterogeneous fatty signal and a weak color Doppler signal. Radical excision of a 34/25/6.5 cm

TABLE I
Detailed report of the eight giant lipomatous tumors

P	G	Age	Location	Size (cm)	Pathology	R
1	M	61	Right deltoid region	17 / 13 / 7	Lipoma	No
2	F	74	Left axilla	34 / 25 / 6.5	Fibrolipoma	No
3	F	63	Left arm	12 / 7	Liposarcoma	No
4	M	53	Right arm	5 / 2.5 / 3	Fibrolipoma	No
5	F	54	Right arm	7 / 6 / 4	Angiolipoma	No
6	F	41	Right arm	8 / 7	Lipoma	No
7	M	17	Left forearm	8 / 7	Angiolipoma	No
8	F	77	Scapulohumeral region	10 / 7	Lipoma	No

Abbreviations: P = patient; G = gender; R = recurrence

mass with overlying skin was achieved. Primary closure was performed. Final pathology revealed diagnosis of fibrolipoma. The patient healed well and was discharged without complications.

DISCUSSION

Most lipomatous tumors are benign and can develop alone or in multiple locations. Lipomas occur more frequently in female patients, probably due to their tendency to accumulate more adipose tissue. Solitary lipomas are more frequent in women and the multiple lipomas are more common in men. Usually, lipomas grow with a very slow rate. The cause of a rapid growth to giant lipomas is still a matter of debate. It has been suggested that blunt trauma can cause rupture of the fibrous septa and anchorage connections between the skin and deep fascia, allowing the adipose tissue to proliferate (2). Whatever the mechanism of rapid growth is, when evaluating a giant lipoma of the upper extremity, the main issue is to exclude malignancy. Recent rapid growth, size larger than 5 cm, intramuscular location, have been reported as risk factors for malignancy (1, 3, 4).

Preoperative evaluation of a large mass in the upper extremity must include imaging evaluation. Among imaging techniques, ultrasound seems an excellent test for screening for soft tissue masses because of its safety, low cost, sensitivity and the ability to provide dynamic examination. On ultrasound, lipomas appear usually as a well-defined, hyperechoic solid mass in the subcutaneous layer without a color Doppler signal (5). However, MRI is the best diagnostic tools available. MRI is the method which allows an easy distinction of lipomas from well-differentiated liposarcomas due to increased vascularization seen in septal structures within the lesion (6). On MRI, lipoma appears as a well-circumscribed mass surrounded by a pseudocapsule and has homogenous fat signal. The role of imaging is essential either to confirm the benign or malignant nature of the mass or to give information for a diagnostic biopsy.

The proper management of giant lipomas is represented by surgical excision. Usually, lipomas are well encapsulated tumors allowing relatively easy complete removal. Intramuscular location makes removal more technically difficult and may require removal of some surrounding muscle to en-

sure adequate margins (7). Extensive excision can lead to significant dissections and mobilizations of neurovascular structures. However if a lipoma is incompletely excised it might redevelop. Liposarcomas are notorious for local recurrence, and even with attempted total resection, local recurrence rates can rise up to 50% (1). Pathological examination of the specimen is mandatory to determine final diagnosis and to establish the possible need for further treatment. Surgical excision can be followed by radiation and chemotherapy if malignancy is confirmed.

CONCLUSIONS

Lipomas may develop in single or multiple sites in both superficial and deep intermuscular fat; that might raise issues of dissection, isolation and excision. Preoperatively, imaging diagnosis (MRI is recommended) is important to delineate the extent of the lesion and to assist in operative planning. The indicated treatment is complete surgical excision that might require significant dissection and mobilization of neurovascular structures. It is imperative to discuss preoperatively with patients regarding potential loss of function.

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A COMPARATIVE ULTRASTRUCTURAL STUDY BETWEEN COLORECTAL CANCER AND GASTRIC CANCER

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A COMPARATIVE ULTRASTRUCTURAL STUDY BETWEEN COLORECTAL CANCER AND GASTRIC CANCER (Abstract): The large intestine is often the site of a tumor development, colorectal cancer being on the 3rd place as frequency, and having also a high mortality rate. Another malignancy with a strong impact over medicine is the gastric cancer; Even if it`s incidence has a descending course, it still remains one of the main neoplasia with a high mortality, occupying the 3rd place worldwide. Considering the fact that in the specialized literature exists insufficient evidence and often contradictions regarding the morphologic and molecular characteristics found in the early stages of malignization and regarding the limits imposed by the usual photonic microscopy techniques, we considered necessary approaching a comparative ultrastructural study between the two types of cancer mentioned above. **Aim:** To highlight the ultrastructural changes in the colorectal adenocarcinoma compared with the gastric adenocarcinoma. **Material and methods:** The study included a number of 21 patients diagnosed with colorectal and gastric cancer from which we gathered biopsies that were processed and analyzed using a Transmission Electron Microscope Philips CM100. **Results:** The ultrastructural study identifies common characteristics of the malign transformation of both colorectal cancer and gastric cancer and also a series of particularities for colorectal cancer inaccessible for common microscopy techniques. **Conclusions:** New information about the aggressivity and the metastatic potential are accessible through TEM examination, which can improve the therapeutic management of these cases. **Keywords:** COLORECTAL CANCER, GASTRIC CANCER, ULTRASTRUCTURE, ELECTRON MICROSCOPY.

In the XXI century, one of the fundamental issues of medicine is represented by cancer, with its multiple sites of localization, with its multiple cell types and manifestations. Regarding these aspects, the colon is often the site of tumors development, colorectal cancer being on the 3rd place as frequency (along with gastric cancer) and hav-

ing also a high mortality rate (1). The incidence of these pathologies could be decreased, by improving the general population awareness regarding the risk factors, by encouraging the specialty consults at the first symptoms and by convincing the patients to accept the endoscopic examinations as screening tests (2, 3, 4); moreover, the understanding of the mechanisms and reactions that are taking place at the molecular level and puts the tumorigenesis process in motion represents the new approaching in the fight against cancer (2, 3, 4).

In the majority cases of tumors, the cause of death is not represented by the effects of the primary tumor, but by the tumor's cells capacity to migrate and to be sown as secondary tumors, often with a more aggressive form (5). This reaction is made possible through alterations of the cell adhesion mechanisms, abnormal growing factor synthesis, aberrant expression of some proteins involved in maintaining of the cell environment within normal limits (including the cell shape and the cellular motility) (5).

Despite the fact that gastric cancer`s incidence in the last decades has a descending trend (probably because of the lifestyle changes, the fight over *H. Pylori* infection and the screening tests), it still remains one of the main neoplasia with a high mortality rate, being situated on the 3rd place worldwide (6, 7).

The usual photonic microscopy techniques have as a major purpose the establishment of a certainty diagnostic in neoplasia suspicions but are limited in detecting the early changes during tumorigenesis or regarding premalignant lesions; for example, the presence of inflammatory cells is common to family intestinal polyposis syndrome, ulcerative colitis and cancer. The light microscopy allows the identification of the basal cells, but it is not specific for early changes (8, 9). Thereby, the purpose of this research is to bring about a comparative ultrastructural study of colorectal towards gastric cancer, for early identification of changes in localization of colorectal tumors.

MATERIAL AND METHODS

Twenty-one patients suspected of having colorectal neoplasia have been enrolled in this study, with the age between 49 and 75, who came at the Regional Oncology Institute from Iași to confirm their diagnosis through endoscopy and biopsy, subsequently following the establishment of the medical management. Informed consent was obtained from each patient prior to the endoscopic biopsy.

Sixteen of these patients (76.19%) clinically and imagistically suspected of having colorectal cancer asked for a specialized physical examination due to the following symptoms: rectal bleeding, diffuse abdominal pain and transit changes. Other five patients (23.81%) were suspected of having gastric cancer.

All of the patients denied having neoplastic heredo-collateral antecedents, or any other personal important antecedents, except for cardiovascular disease excepting the chronic treatment which could be involved in the carcinogenesis.

Of great significance is the fact that none (0.0%) of the patients underwent chemotherapy, radiotherapy or any other cancer treatment prior to the sample collection.

The tumoral bodies have been spotted due to the colonoscopy and upper gastrointestinal endoscopy and both tumoral and normal tissue have been sampled to be

tested. Some fragments obtained from the gathered tissue samples were processed through the immersion in paraffin technique, sectioned and colored with hematoxylin-eosin (HE), for a diagnosis within the Pathological Anatomy and Morgue Service of the Regional Oncology Institute, Iași and examined with the photonic Olympus BX40 microscope with the Olympus E330 camera attached.

For electron microscopy examination, the other fragments were fixed on ice and there were added few drops of 2% glutaraldehyde, in furtherance to prevent any type of tissue deterioration. Following that, certain pieces were gathered to be processed through the standard technique of the electron transmission microscopy (TEM): double fixing, dehydration using 30% alcohol, at 4°C, for 15 minutes; colored all-together – 2% uranyl acetate 2%, in 30 alcohol, at 4°C, for 45 minutes; dehydration – successive baths of alcohol with increasing concentrations, preceded by absolute acetone, at room temperature, for 20 minutes.

Equal quantity of EPON 812 and absolute acetone was used during overnight time, at room temperature for the impregnation stage. Then, EPON 812 was used for 2 hours at room temperature, and last, the fragments followed the immersion in EPON 812, in gelatin capsules, polymerization and cutting. The development of the sample`s processing technique was staged in the electron Microscopy Laboratory within the Cellular and Molecular Biology Department ("Grigore T. Popa" University of Medicine and Pharmacy Iasi) later steps being carried on within the Electron Microscopy Laboratory of the Life Sciences Institute, "Vasile Goldis" West University (Arad). The examination of the samples was screened with a Philips CM100 electron transmission microscope equipped with a digital video camera with dedicated sensor and photo-taking system.

RESULTS

The examination of hematoxylin eosin (HE) stained samples in the case of tumors with gastric localization revealed characteristic elements based of which in current medical practice the histopathological diagnostic is established: significant desmoplastic reaction, muscular invasion, glandular outbreaks of different shapes and sizes, with muscular layer invasion. In addition, there were depicted lymphatic slits with tumor embolisms, large areas of necrosis and lymphoplasmocitary infiltrate (fig. 1).

Regarding the specimens collected from the tumor formations located in the sigmoid colon, there were identified: irregularly shaped glands, sometimes fused with a high, dysplastic, stratified epithelium, sometimes with imprecisely limited boundaries from the varying thickened stromal lax; cells with heterogeneous appearance, with large, hyper chromatic, large-shaped nuclei, sometimes with secreting vacuoles, glandular tubules invading the sub mucosal area. The hyperchromatic nuclei reverse in their favor the nucleo-cytoplasmic ratio. The stroma is dense, hypercellular, with a rich lymphoplasmocitary infiltrate, dilated vessels, full of red blood cells (fig. 2).

At once, the examination of these samples also revealed anaplastic tumors with tubular atypical glands, which invade the muscle patch, stromal desmoplastic reaction, with the invasion of the subserous. In these areas, the glandular cells exhibit nuclear pleiomorphism and loss of polarity. There were mentioned tumor embolisms in blood and lymph vessels and small mu-

cosecreeting, interstitial focuses (fig. 2).

With reference to the samples collected from the formations in the rectum, it was observed an inconsistent thickened epithelium with nuclear hyperchromatism, with elongated, oval or irregular nuclei, giving the impression of overlapping in certain areas (fig. 3).

The TEM examination in the case of patients diagnosed with gastric cancer, at the level of larger nuclei, it showed a small quantity of nuclear chromatin disposed in an irregular manner, which is characteristic for the malignant transformations; the widening of the nuclear pores followed by the appearance and the presence of some discontinuities of the nuclear envelope.

Along with these, it was detailed a disorganization of the morphological structure of the membrane and the cellular coupling zones - the impermeable junctions did not exhibit a normal architectonic, with a vacuolar structure and enlarged intercellular spaces observed in some areas.

In addition, there are obvious changes in the basal membrane showing disorganized structure and discontinuities, with the presence of the residual material in lamina propria and mitochondrial degeneration of different grades. The same elements are present in the case of peripherally located cells within which nucleoli are observed with changes in both morphological and numerical appearance: either there is only one modified enlarged nucleolus, or two nucleoli with the characteristic appearance of malignant cells (fig. 4, 5, 6, 7, 8).

As regards to patients with localized neoplasia in the sigmoid colon, the examination in the electron transmission microscopy highlights: the presence of hypertrophic nuclei with edentulous appearance and dilated perinuclear cisterns; nucleoli with a disorganized structure, located peripherally to the internal lamina, and in some cells, also located peripherally, towards the inner lamella, two nucleoli were disposed in “glasses” shape - characteristic of the advanced malignant transformation.

Likewise, it is noted perinuclear cytoplasmic vacuolization with the disorganization of the organelles structure - advanced mitochondrial degeneration.

The basal membrane also presents changes as: discontinuities and variations in thickness (fig. 9, 10, 11, 12).

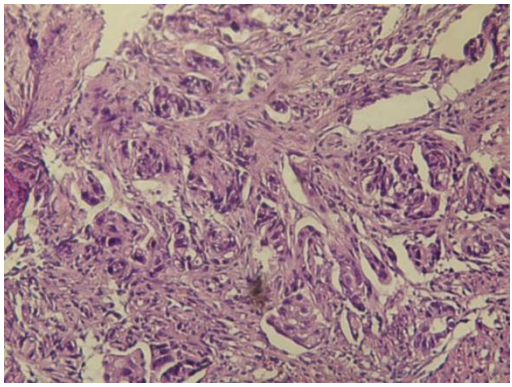

Fig. 1. Gastric infiltrative adenocarcinoma (H&E stain, 10X)

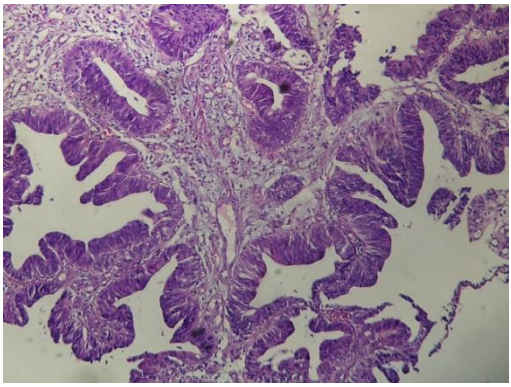

Fig. 2. Moderately differentiated sigmoidian adenocarcinoma (H&E stain, 10X)

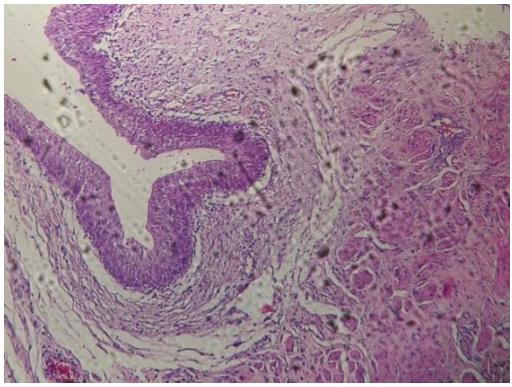

Fig. 3. Fragment of rectal mucosa showing severe dysplasia (H&E stain, 20X)

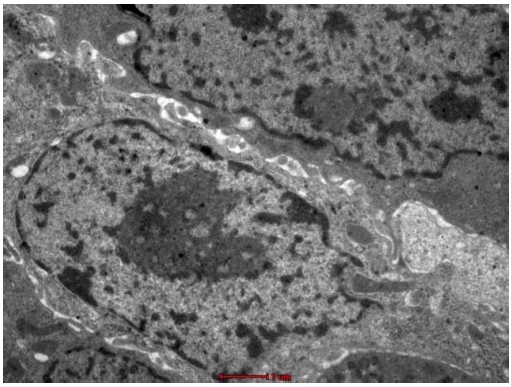

Fig. 4. Gastric adenocarcinoma (TEM)

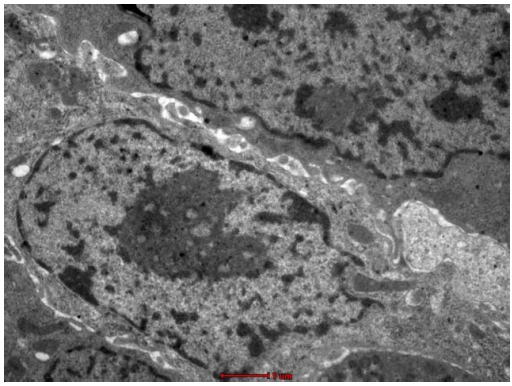

Fig. 5. Gastric adenocarcinoma (TEM)

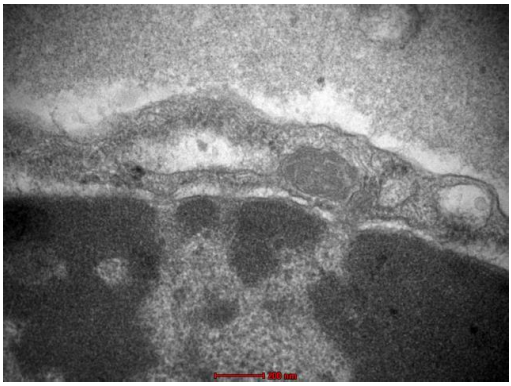

Fig. 6. Gastric adenocarcinoma (TEM)

Fig. 7. Gastric adenocarcinoma (TEM)

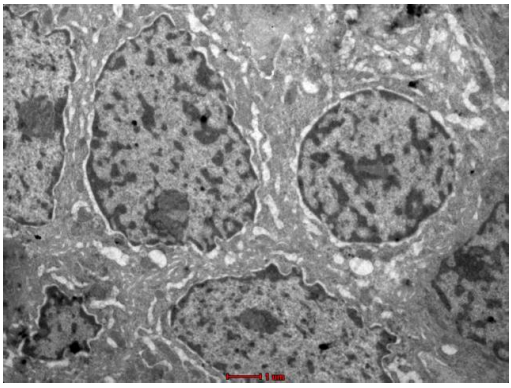

Fig. 8. Gastric adenocarcinoma (TEM)

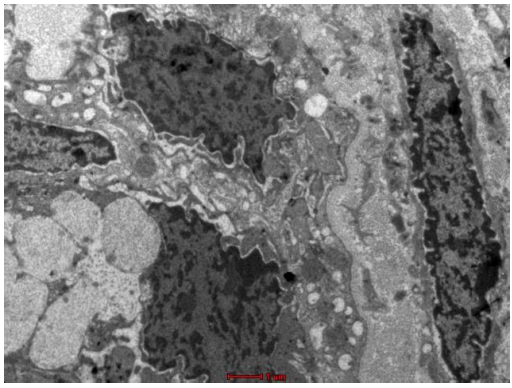

Fig. 9. Colon adenocarcinoma (TEM)

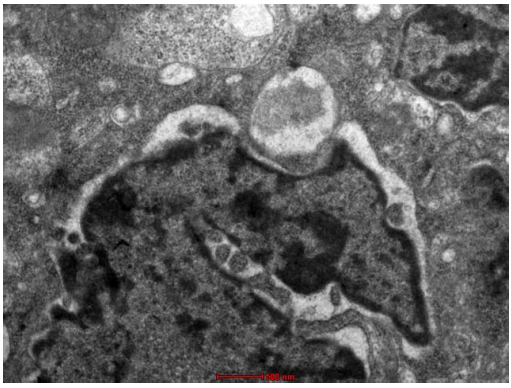

Fig. 10. Colon adenocarcinoma (TEM)

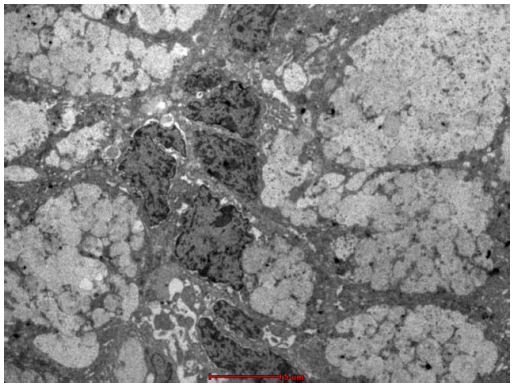

Fig. 11. Colon adenocarcinoma (TEM)

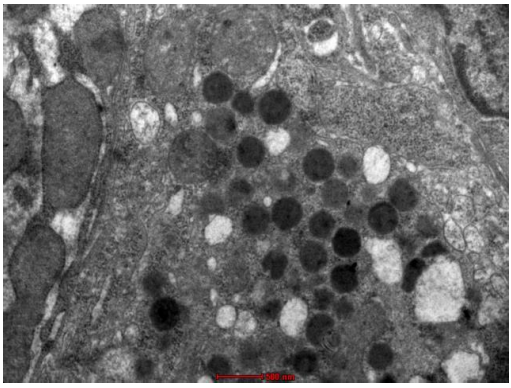

Fig. 12. Colon adenocarcinoma (TEM)

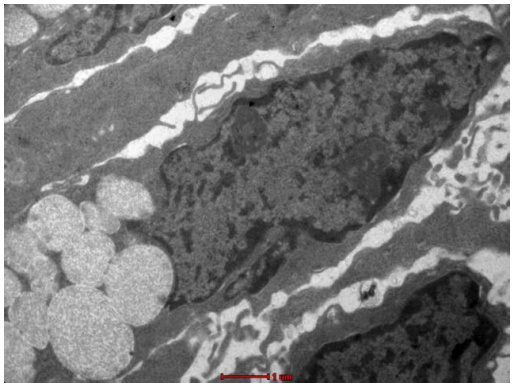

Fig. 13. Rectal adenocarcinoma (TEM)

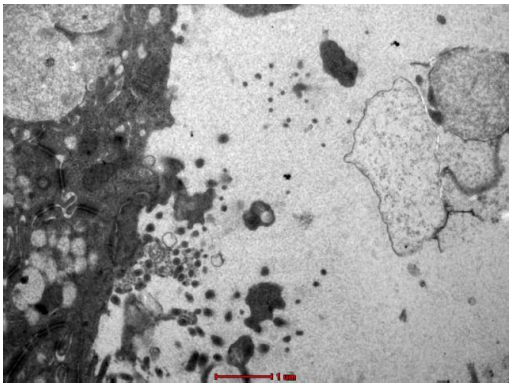

Fig. 14. Rectal adenocarcinoma (TEM)

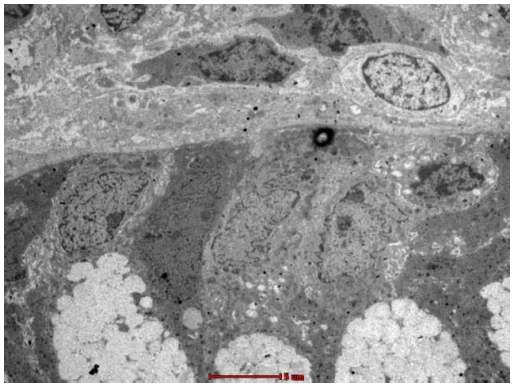

Fig. 15. Rectal adenocarcinoma (TEM)

DISCUSSION

TEM examination completes the data obtained by HE-stained samples examination and reveals changes of the cellular morphologic aspects and the subcellular structures important for establishing the prognosis and the proper therapeutically approach, explaining in the same time the evolution of each particular case.

HE-stained samples examined under the photonic microscope revealed the morphological changes characteristic for malignant transformation, confirming the clinically and imagistic diagnosis of gastric and colorectal adenocarcinoma: in gastric adenocarcinoma, an important desmoplastic reaction, with the invasion of the muscular layer, with glandular outbreaks of different shapes and sizes, invading the characteristic muscular (fig. 1).

In addition to these aspects, the electron microscopy revealed the reversal of the nucleocytoplasmic ratio, reduced quantity and irregular distribution of the chromatin, nuclear envelope with edentulous aspect, elements that completes the histopathological aspects and have particular significance to malignant transformation (fig. 4, 5, 6, 7, 8).

Furthermore, modified cellular coupling areas (vacuolar aspect and disorganization of tight junction architectures) indicate an increased potential for metastasis. These morphological aspects displayed by the examination of the samples in the electron transmission microscopy also suggest changes in the signaling pathways at the cellular level, disruption of the nucleocytoplasmic exchanges and of mechanism of the cellular synthesis responsible for functional perturbation at this level.

So, if the standard histopathological examination misses some specific elements to a malignant transformation, examination in the electron transmission microscopy bring elements to underline the severity of dysplasia, and the changes within the subcellular levels relevant for tumoral staging.

The study of colon samples presents severe cellular lesions in both photon microscopy, but especially in electron microscopy. In addition to the aspects observed in the examination of HE-stained - high, dysplastic, stratified epithelium, sometimes with imprecisely defined boundaries, with large, hyperchromic nuclei, with shape changes; anaplastic tumors with tubular atypical glands, glandular cells with nuclear pleiomorphism and the loss of polarity (fig. 2)-electronomicroscopic changes have been highlighted at both nuclear level and cellular organelles: the presence of hypertrophic nuclei, edentulous and highly dilated perinuclear cisterns; the nucleoli with a disorganized structure, located peripherally to the internal lamina and in some cells, two nucleoli disposed in “glasses” shape, also located peripherally, to the internal lamella – which are characteristic aspects of advanced malignant transformation that could not be revealed by photon microscopy (fig. 9, 10, 11, 12).

Besides, perinuclear cytoplasmic vacuolization was observed the disorganization

of the organelles structure, with particular emphasis on advanced mitochondrial degeneration, changes at the basal membrane level (their detachment from the epithelial cells, the presence of the residual material in their own lamina). These morphological alterations revealed by the TEM also indicate the existence of important molecular changes: disturbances of intercellular communication pathways, ATP synthesis deficiency with significant functional repercussions. Photonic microscopy images have showed a secretion penetrating the intestinal wall layers, which favors the extension of the malignant process and at the same time, explains the severity of the disruption in the electron microscopic study.

The studies support the link between the ultrastructural aspects of different cellular components and the secondary phenomena of the tumorigenesis process. Thus, Mitmaker and colleagues argue in a study that the ratio of nucleus to cytoplasm may suggest aggression of neoplasia and an unfavorable prognosis (16). Also, elliptic nuclei are found in the mucosa of a well-differentiated gastric carcinoma, whereas spherical nuclei in the mildly differentiated mucosa (17).

It is known that in malignant transformation, nuclei undergo important changes, visible both by photon microscopy and especially by electron microscopy (18). Nuclear chromatin in tumor cells undergoes changes in aggregation and mood, being denser and asymmetrically aggregated independent of cell cycle progression – there aspects are identified as well in our case studies. Also, studies conducted by Dakubo *et al*. (19) and Damania *et al*. (20) describe nuclear changes in patients diagnosed with colorectal adenocarcinoma if compared to cell nuclei present in normal mucosa. They describe the so-called phenomenon of carcinogenesis which represents the concept of modified genetic or epigenetic microclimate that creates fertile ground and allows the development of focal tumors. Thus, colorectal cancer is one of the focal points in which in vivo cancer inoculums have to be studied as well as the study of cells at different stages of evolution are crucial in order to identify modifications at the earliest stages.

The electronomicroscopic study of the tumors reveals the increased prevalence of important tumor-specific ultrastructural changes, thus highlighting their severity and major potential for dissemination. It also highlights the severity of morphological changes in the subcellular compartments in case of colon adenocarcinoma compared to the gastric and rectal levels in the studied cases. This change represents the morphological substrate of the characteristic molecular disruptions of these tumoral types.

CONCLUSIONS

Our study emphasizes the ultrastructural details provided by the electron transmission microscopy for the lesions taken under consideration and completes the information provided by the photon microscopy technique and highlights the value of the electron microscopic examination that is often underestimated or ignored. At the same time, the results of the present comparative study between gastric and colorectal adenocarcinoma, complements the data from the few ultrastructural studies in the literature on this subject, it contributes to a complete and relevant image of the specificity of the morphological context in this tumors which accompany the molecular perturbations and provides important in-

formation for their clinical and therapeutic management for these types of injuries that are a real challenge to global public health systems.

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STRESUL TRAUMATIC SECUNDAR, EFECTUL ADVERS AL EMPATIEI CLINICE

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SECONDARY TRAUMATIC STRESS, THE SIDE EFFECT OF CLINICAL EMPATHY (Abstract) : There is growing clinical evidence that a second category of trauma victims can result from working with traumatized populations. This article introduces the concept of secondary traumatic stress (STS) and analysis its impact and relevance in the particular situation of medical staff. In the second part we discuss medical workers' empathy as an important factor associated with clinical competence and patient outcomes on one side, and as a recognized risk factor for STS on the other side. Finally, we conclude that the paradox of the clinical relationship needs further attention and study, to find a mutual beneficial solution. Assessment has to be connected to prevention and intervention efforts to ensure effective management of STS among medical workers. **Key words** : SECONDARY TRAUMATIC STRESS, EMPATHY, MEDICAL STAFF

Stresul ocupaţional este o problemă recunoscută printre specialiştii din sănătate. Personalul medical este considerat a reprezenta un risc semnificativ, înregistrând rate crescute de *burnout*, epuizare emoţională, depresie (1,2,3). Mai ales în unele specializări, angajaţii se confruntă cu provocări caracteristice cum ar fi : o cantitate de muncă imprevizibilă, resurse limitate, expunerea repetată la evenimente traumatogene, evenimente violente posibile, şi luarea de decizii critice având adesea la bază doar informaţii incomplete. Contextul economic naţional adaugă deficienţe grave în infrastructură, salarizare şi resurse umane. Personalul medical se confruntă zilnic cu dramele şi traumele a numeroşi pacienţi. Este de aşteptat ca particularităţile îngrijirii medicale în spaţiul Românesc să fie caracterizate de manifestări specifice ale stresului ocupaţional. În acest sens, articolul de faţă îşi propune să evidenţieze vulnerabilitatea cadrelor medicale faţă de stresul traumatic secundar. Această tulburarea afectează global viaţa specialistului, periclitând în acelaşi timp relaţia clinică pacient – cadru medical, precum şi demersul terapeutic.

DE LA TRAUMĂ PRIMARĂ LA STRES TRAUMATIC SECUNDAR

În societatea actuală trauma îşi multiplică ipostazele prin războaie, atacuri teroriste, dezastre naturale şi violenţe de tot felul. Expunerea la evenimente copleşitoare şi traumatice este răspândită în rândurile populaţiei la nivel global. Statisticile arată că în jur de 90% din cetăţenii Statelor Unite sunt supuşi la măcar un eveniment traumatic ce le-a pus existenţa în pericol, de-a lungul vieţii (4). Manualul de diagnostic şi statistică a tulburărilor mentale (DSM) susţine că sindromul de stres posttraumatic se poate instala ca urmare a unei situaţii în care persoana a trăit sau a asistat la un eveniment ce a reprezentat un pericol pentru viaţa sau securitate sa (sau pentru viaţa sau securita-

tea celor din jur) şi a resimţit sentimente de frică intensă, de neputinţă şi oroare (5). Meichenbaum consideră că repercusiunile evenimentului traumatogen pot fi diferenţiate pe patru planuri : consecinţe în plan afectiv : şoc, teroare, culpabilitate, anxietate, ostilitate, depresie ; în plan cognitiv : slăbirea sau diminuarea capacităţilor ; în plan somatic : perturbări ale somnului, coşmaruri, simptome psihosomatice ; consecinţe comportamentale : evitarea, retragerea socială, stresul interpersonal, abuzul de substanţe (6). Situaţiile traumatogene pot fi multiple : un accident rutier sau alt tip de accident grav, o agresiune fizică sau sexuală, războiul sau tortura, catastrofe naturale, cutremure, incendii, inundaţii. Grabmeier arată că şi diagnosticarea unei maladii grave, ca de exemplu cancerul, poate provoca un sindrom de stres posttraumatic. Criteriile de diagnostic oferite de DSM nu explică în totalitate varietatea de răspunsuri umane la traumă. Van der Kolk şi McFarlane atenţionează că „în ciuda eforturilor de a surprinde esenţa răspunsului indivizilor la traumă, diagnosticul sindromului de stres postraumatic nu reuşeşte să descrie complexitatea felului în care oamenii reacţionează în faţa evenimentelor copleşitoare”(7).

McFarlane şi Girolama sugerează că având în vedere varietatea solicitărilor ce cuprind o persoană în funcţie de traumă, şi diversitatea capacităţilor de a pune în practică diverse comportamente adaptative între diferite persoane precum şi în cazul aceleiaşi persoane în momente diferite, reacţiile de stres posttraumatic sunt mult mai complexe decât criteriile DSM o arată (8). Evenimentele traumatogene pun sub semnul întrebării relaţiile umane de bază şi pot rupe ataşamentele din familie, prietenie, dragoste şi comunitate. Zguduie constructul sinelui care s-a format şi este susţinut în relaţie cu ceilalţi. Ele slăbesc sistemele de credinţe ce oferă înţeles experienţei umane. Violează credinţa persoanei într-o ordine divină sau naturală şi izolează victima într-o stare de criză existenţială (9). Unul dintre efectele cele mai profunde în a trăi o traumă îl reprezintă distrugerea credinţelor de bază despre sine şi lume. Janoff-Bluman susţine că expunerea la o experienţă traumatică anulează iluzia de invulnerabilitate pe care oamenii o dezvoltă pentru a se proteja de stres şi anxietate, credinţa lor într-o lume sigură, dreaptă şi plină de sens este pusă sub semnul întrebării (10). În plus, victimele unei traume se întreabă despre rostul şi valoarea lor ca oameni cât şi despre încrederea pe care o pot acorda celorlalţi (11).

Ultimii ani au adus cu sine dezvoltarea domeniului traumatologiei iar conceptul de stres traumatic s-a bucurat de o atenţie sporită. În acest context studiile ce se concentrau firesc pe reacţiile victimei în urma evenimentului traumatic, au descoperit treptat că momentul de criză nu afectează doar pe cei ce sunt de faţă iar victimele se pot multiplica în timp, chiar mult după ce evenimentul marcant a luat sfârşit. Manualul de diagnostic şi statistică a tulburărilor mentale marchează această schimbare de perspectivă adaptând criteriile de diagnostic pentru stresul posttraumatic în cea de-a patra ediţie. Acestea includ acum condiţia în care persoana se confruntă cu un eveniment sau evenimente traumatice care au implicat ameninţări cu moartea, moartea efectivă, vătămarea serioasă sau ameninţarea integrităţii corporale proprii ori a altora (5). Având în vedere că unele categorii profesionale se confruntă sistematic cu experienţele traumatice ale altora, nu este surprinzător că trăiesc simptome ale stresului posttraumatic. Prin urmare nu doar victima primară este expusă riscului de a dezvolta sindromul de stres posttraumatic ci şi martorii direcţi la eveniment precum şi persoanele apropiate victimei care află despre ceea ce i s-a întâmplat. Această precizare a complicat şi mai mult încercările de a delimita cât mai clar diverse concepte ce începuseră deja a se

forma pe această temă. Faptul că stresul posttraumatic poate fi contagios este o chestiune discutată şi cercetată de mai bine de două decenii. În 1988 Figley observă cum familia victimei este afectată de suferinţa acesteia şi adună simptomele constatate sub numele de „victimizare secundară" (12). În ciuda actualizării criteriilor de diagnostic, sindromul de stres posttraumatic aşa cum este el conceput de Asociaţia psihiatrică americană (5), pare a nu acoperi exhaustiv posibilităţile de propagare a simptomelor descrise. Munca sistematică în slujba pacienţilor ce au suferit traume riscă să afecteze în timp pe unii dintre profesionişti. Courtois vorbeşte în acest sens despre „victimizare de contact" (13) iar Mollica, în acelaşi an sugerează că specialiştii se "infectează" cu deznădejdea celor pe care încearcă să îi ajute (14). În ultimii ani etichete cum ar fi epuizare empatică (compassion fatigue), traumatizare vicariantă sau stres traumatic secundar încearcă să împartă şi să clarifice diversitatea simptomelor ce pot apărea în cazul celor expuşi interacţiunii cu victime ale unor evenimente traumatice. Termenul de epuizare empatică a fost introdus pentru prima dată de Joinson in anul 1992 în articolul său despre sindromul de *burnout* la asistente medicale (15). Doi ani mai târziu Figley introduce conceptul de stres traumatic secundar pe care îl descrie ca fiind sinonim cu epuizare empatica. Stresul traumatic secundar apare când un individ este indirect expus la traumă prin intermediul unei descrieri personale sau prin povestirea unei experienţe traumatice (16). Figley defineşte conceptul ca emoţiile şi comportamentele ce rezultă natural atunci când individul află dintr-o descriere personală sau printr-o povestire despre un eveniment traumatic trăit de o persoană semnificativă (17). În continuare Figley susţine că anumite categorii profesionale, cum ar fi de exemplu terapeuţii ce lucrează cu victime, specialiştii ce intervin în situaţii de urgenţă, poliţiştii etc. prezintă un risc crescut de a dezvolta acest tip de stres pentru că ei intră regulat în contact cu istorisiri despre evenimente traumatice. El arată că indivizii pot fi traumatizaţi fără a fi răniţi fizic sau ameninţaţi în mod direct. Atât retrăirea realistă a traumei de către victimă, cât şi interpretarea cognitivă sau emoţională a evenimentului realizată apoi de specialist, pot provoca simptome similare celor din sindromul de stres posttraumatic (hiperexcitaţie, simptome intruzive, evitare, anxietate). În acest caz, persoana afectată nu este rudă sau prieten apropiat cu victima, dar încercarea de a ajuta şi deschiderea empatică o expun aceloraşi simptome. O'Halloran susţine că persoanele care se confruntă cu material traumatic sau lucrează cu persoane traumatizate pot fi afectate profund de experienţele trăite la nivel cognitiv prin capacitatea redusă de concentrare, emoţional prin stări de distres sau spiritual prin revoltă împotriva divinităţii (18). Sindromul de stres traumatic secundar este identic în simptomatologie cu stresul posttraumatic cu excepţia faptului ca în primul caz evenimentul traumatic nu este trăit direct de persoana afectată, ci aceasta află despre ceea ce s-a întâmplat din povestirile unei alte persoane. Simptomele trăite vor fi de evitare, intruziune şi hiperexcitaţie. La fel ca în stresul posttraumatic, în cazul simptomelor intruzive specialistul va retrăi la nivel imaginar evenimentul traumatic ce a afectat victima. Stresul traumatic secundar se află în strânsă legătură cu evoluţia persoanei suferind de stres posttraumatic, pentru că ameliorarea victimei primare va ajuta ameliorarea suferindului secundar (17). Factorii de risc identificaţi de Figley sunt : răspunsul empatic, istoricul traumatic personal, reactualizarea problemelor personale nerezolvate prin ascultarea traumei victimei, lucrul cu populaţii vulnerabile. Dincolo de simptomatologia specifică, persoanele afectate pot resimţi modificări ale credinţelor de bază, ale aşteptărilor şi atitudinilor faţă

de viaţă. Dutton şi Rubinstein susţin că specialiştii pot resimţi sentimente de vină atunci când se surprind bucurându-se de viaţă, gândindu-se la dificultăţile pacienţilor în lupta lor cu trauma (19).

MODELE TEORETICE ALE STRESULUI TRAUMATIC SECUNDAR

Având în vedere similaritatea dintre simptomatologia stresului traumatic secundar şi cea a stresului posttraumatic, şi modelele explicative pentru cele doua patologii vor fi în parte similare. La fel ca şi cei suferind de stres posttraumatic, indivizii care sunt expuşi secundar la stresori traumatici, pot suporta episoade de gândire intruzivă şi etape de evitare, apatie sau excitaţie persistentă. Conform lui Yassen stresul traumatic secundar poate afecta persoana : cognitiv (concentrare diminuată, stimă de sine scăzută, imagerie legată de traumă), emoţional (apatie, depresie, hipersensibilitate), comportamental (tulburări de somn, răspuns exagerat de tresărire, abuz de alcool sau alte substanţe), spiritual (furie împotriva divinităţii, pierdere de semnificaţie şi sens, disperare persistentă), interpersonal (interes scăzut în intimitate, izolare faţă de prieteni, lipsă de încredere), fizic (reacţii somatice, sistem imunitar afectat, dureri) (20).

Modelul neurobiologic

Din punct de vedere neurobiologic, asistând la trauma trăită de pacienţii lor, specialiştii în cauză se confruntă cu varietatea de posibilităţi de boală, ameninţare şi vătămare pe care oamenii o pot întâlni. Când ascultă despre răul ce a survenit altora, este un proces natural ca persoana să evalueze periculozitatea ameninţării pentru propria persoană. Pentru că sarcina principală a creierului uman este de a promova supravieţuirea (21), creierul este setat biologic să caute şi să îşi amintească datele de supravieţuire. Informaţia venită de la pacient, percepută ca fiind potenţial ameninţătoare, nu poate fi ignorată de procesele de căutare de informaţie din creier. Cu alte cuvinte, sistemul limbic al specialistului poate fi activat prin simpla interacţiune cu pacientul. Creierul este de asemeni setat să reţină mai bine acele amintiri cărora le este ataşată semnificaţie emoţională. Astfel, de-a lungul timpului, specialistul poate dezvolta o serie întreagă de amintiri ameninţătoare semnificative emoţional, care pot de asemeni contribui la a activa sistemul limbic când informaţia primită este asociată cu o ameninţare precedentă. În acest fel, expunerea cronică la stresori traumatizanţi poate deteriora sistemul imunitar al persoanei, rezultând într-o varietate de simptome fizice serioase (22).

Modelul de transmitere a traumei

Figley a dezvoltat un model de transmitere a traumei constând din două componente : stresul empatic (compassion stress) şi epuizarea empatică (compassion fatigue) care îşi propune să explice procesul de transmitere a traumei şi oferă o explicaţie plauzibilă pentru motivele pentru care unii indivizi dezvoltă epuizarea empatică iar alţii nu. Acest model susţine că specialiştii încearcă să înţeleagă experienţele pacienţilor identificându-se cu aceştia şi în acest proces specialistul însuşi ajunge să trăiască dificultăţi similare cu pacientul. Prima componentă a modelului descrie instalarea stresului empatic ca o funcţie a interacţiunii dintre şase variabile. Aspectul cheie al acestui model este empatia şi Figley împarte empatia în trei tipuri : abilitate empatică, grijă empatică şi răspuns empatic. Abilitatea empatică se referă la capacitatea specialistului de a recunoaşte durerea celorlalţi, de a oferi necondiţionat o imagine pozitivă şi autentică. Astfel conceptul de empatie este legat de cel de grijă care constituie motivaţia principală de a răspunde pacientului. Literatura de specialitate susţine că abilităţile empatice ale cadrelor medicale reprezintă calităţi cheie pentru ca ei sa poată avea o minte deschisă faţă de pacienţii lor. Fără această

calitate abordarea clinică ar putea avea de suferit semnificativ. Răspunsul empatic este combinaţia dintre abilitatea empatică a specialistului şi grija empatică. Măsoară nivelul de efort investit de specialist pentru a asista pacientul în gestionarea traumei (17). Contagiunea emoţională pe care Figley o defineşte ca fiind trăirea sentimentelor suferindului ca o funcţie a expunerii la suferind, este conectată la abilitatea empatică şi astfel determină stresul empatic. Totuşi dacă specialistul se poate dezangaja din relaţia sa cu victima, el/ea va resimţi un sentiment de satisfacţie prin relaţia de ajutorare, în loc să trăiască efectele stresante ale muncii cu materialul traumatic al clientului. În partea a doua a modelului de transmitere a traumei, Figley susţine că dezvoltarea stresului traumatic secundar este rezultatul interacţiunii a patru variabile : nivelul stresului empatic, expunerea prelungită la victimă, amintirile traumatice şi nivelul de tulburare a propriei vieţi. Figley arată ca expunerea prelungită la materialul traumatic apare ca urmare a sentimentului specialistului că trebuie sa aibă grijă neîntreruptă de pacient şi că este responsabil pentru acesta. Specialistul se simte unicul responsabil pentru pacient şi nu reuşeşte a-şi diminua stresul empatic. În plus, amintirile provoacă simptome secundare şi alte asemenea răspunsuri din partea specialistului. Figley susţine că epuizarea empatică devine un fenomen natural când există aceste circumstanţe.

Un cadru ecologic al traumei

Dutton şi Rubinstein încearcă de asemenea să ofere un cadru teoretic pentru stresul traumatic secundar. Modelul lor, un cadru ecologic al traumei, integrează aspecte ale modelului de transmisie a traumei propus de Figley (17). Conform lui Dutton şi Rubinstein gama de reacţii pe care specialiştii o pot trăi ca urmare a interacţiunii lor cu victime ale unor evenimente traumatice poate fi împărţită în trei arii. Prima categorie se referă la simptomele detresei psihologice, cum ar fi comportamentul de evitare, imageria intruzivă, acuzele somatice sau apatia psihologică. O a doua categorie de reacţii se referă la schimbări în credinţe şi impresii despre lume (10). Indivizii îşi situează presupunerile şi credinţele la un nivel preconştient, ceea ce le permite să îşi stabilească obiective, să plănuiască activităţi şi să îşi ghideze comportamentul. Se crede că aceste credinţe sunt alterate prin expunerea directă sau indirectă la traumă, ceea ce determină stres psihologic şi formarea simptomelor. Ultima categorie care este perturbarea relaţională, poate apărea ca urmare a expunerii secundare, relaţiile specialistului (personale si profesionale) putând avea de suferit. Sensibilitatea lor la întâmplări similare în viaţa personală poate cauza dificultăţi în relaţiile lor şi se pot izola la locul de munca (19). Astfel modelul lui Dutton şi Rubinstein pentru stresul traumatic secundar a propus patru componente : evenimentul traumatic la care specialistul este expus ; strategiile de *coping* ale specialistului ; reacţiile posttraumatice ; factorii personali şi de mediu. Autorii susţin că expunerea la materialul traumatic este unică pentru fiecare specialist în parte şi oferă cinci motive pentru aceasta : materialul traumatic diferă în grad de severitate de la un pacient la altul ; specialistul nu este expus doar materialului traumatic, dar şi emoţiilor pe care clientul le resimte în legătură cu evenimentul ; specialistul este expus revictimizării clientului care poate interveni ca urmare a sistemelor sociale ; specialistul este expus evidenţei faptului că acel tip de traumă există ceea ce îi poate pune sub semnul întrebării credinţele cognitive, şi în ultimul rând specialistul poate avea a face faţă traumei anterioare a clientului ce ar putea reapărea. Pentru că natura expunerii este unică fiecărui specialist, acest model direcţionează cercetarea către toate aceste particularităţi ale recepţionării materialului traumatic la care specialistul are acces. A

doua componentă se referă la strategiile de *coping* despre care autorii susţin că afectează dezvoltarea şi parcursul stresului traumatic secundar. Există două tipuri de strategii de *coping*, personale şi profesionale şi aceste strategii sunt legate de reţeaua socială de suport a persoanei. Autorii arată în continuare că factorii individuali şi de mediu pot fi mediatori ai stresului traumatic secundar. Factorii individuali includ forţa internă a specialistului, resursele sale, vulnerabilităţile şi nivelul de satisfacţie cu viaţa sa personală şi profesională. Dutton şi Rubinstein consideră că factorii de mediu cum sunt suportul social, locul de muncă, factorii culturali, sociali şi profesionali sunt importanţi în influenţarea reacţiilor la materialul traumatic.

EMPATIA, RISC NECESAR ÎN RELAŢIA CLINICĂ

„În mod ironic (…) cei mai eficienţi specialişti sunt cei mai vulnerabili la efectul de oglindire sau contagiune. Cei care dispun de capacităţi enorme de a simţi şi de a exprima empatie tind să fie mai expuşi stresului traumatic secundar”(17). Numeroase studii confirmă importanţa empatiei în profesiile care implică interacţiunea cu oameni. Capacitatea de a-l înţelege pe cel care are nevoie de ajutor este esenţială pentru un bun profesionist. Aceeaşi capacitate însa poate expune persoana unor consecinţe nocive asupra sănătăţii sale fizice şi psihice. Carl Rogers se află printre primii care a vorbit despre importanţa empatiei în lucrul cu oamenii aflaţi în suferinţă. El propune empatia ca mod de a fi, ca formă de comunicare ce implică a îngriji de celălalt, a asculta, a observa, a înţelege, şi a răspunde îngrijorării sale cu respect profund şi autenticitate (23). Cea mai mare parte din comunicare, din ceea ce transmitem celorlalţi se face pe cale nonverbală. Empatia presupune a fi conştient de celălalt şi de ceea ce vrea sa spună prin contact vizual, prin limbajul corporal, prin tăcerile sale, tonul vocii, gesturi, expresii faciale, folosirea spaţiului fizic şi prin multe alte metode. De la Rogers până în zilele noastre abordările terapeutice şi perspectiva asupra interacţiunii cu pacientul s-au modificat, devenind mai complexe, mai diverse, dar importanţa empatiei în profesiile sociale nu a fost niciodată contestată. Este instrumentul principal în construirea unei relaţii de încredere autentică şi durabilă care să garanteze aderarea pacientului la tratament, încrederea lui în reuşita demersului întreprins şi continuarea vizitelor la specialist cu frecvenţa necesară. Una dintre cele mai discutate operaţionalizări ale empatiei, îi atribuie trei dimensiuni. Contagiune emoţională se referă la capacitatea de a împărtăşi suferinţa unei alte persoane. De a trăi alături de cel îndurerat sentimente de tristeţe asemănătoare. Grija empatică arată preocuparea pentru starea celuilalt, îngrijorarea pentru cum va evolua şi dorinţa de a-l sprijini. A treia dimensiune este disponibilitatea de a comunica. Abilitatea de a comunica eficient cu ceilalţi despre subiecte sensibile şi emoţionale (24). Toate cele trei variabile au fost studiate în timp şi s-au arătat a fi benefice pentru interlocutor, dar dintre ele se pare că o importanţă deosebită o are contagiunea emoţională care determină mai multe răspunsuri pozitive. A simţi alături de celălalt, a-i împărtăşi durerea, îl ajută pe cel aflat în suferinţă să se simtă înţeles. Stările emoţionale îi sunt validate de răspunsurile celeilalte persoane şi nu se mai simte însingurat. Empatia este adesea greşit înţeleasă, pentru că a comunica empatic nu înseamnă doar a compătimi pe cel care îşi povesteşte neliniştile ci a-l înţelege şi a-i arăta susţinere. Partenerul empatic trebuie să fie activ în interacţiune şi conectat deplin cu interlocutorul său. A asculta cu atenţie şi a simţi nevoile şi dorinţele celeilalte persoane constituie elementul central al oricărei relaţii centrate pe client.

Hojat, Gonnella, Nasca şi colab. raportează despre importanţa empatiei pentru medici în particular subliniind efectele ei benefice în relaţia cu pacientul şi în rezultatele clinice. Susţin că în ciuda recunoaşterii ei ca element de bază, prea puţine studii s-au ocupat de acest subiect (25). Există însă şi o parte întunecată a implicării empatice pe măsură ce specialistul pătrunde cu înţelegere şi implicare în universul interior al unei persoane tulburate sau traumatizate. Unele dintre experienţele şi emoţiile pacientului pot fi distorsionate pe măsură ce sunt asimilate de către interlocutor şi potrivite în schemele cognitive şi credinţele sale despre lume. În acest sens, Miller şi colaboratorii au investigat rolul empatiei ca precursor al *burnout*-ului asupra unui eşantion de 417 cadre medicale. Rezultatele obţinute arătau cum grija empatică favorizează disponibilitatea comunicaţională, dar contagiunea emoţională duce la o scăderea a acesteia. Disponibilitatea de a comunica pe de altă parte s-a dovedit a fi un predictor important pentru toate cele trei dimensiuni ale *burnout*-ului propuse de Maslach (depersonalizare, reducerea împlinirii personale şi epuizare emoţională) (26). Omdahl şi O'Donnell au realizat un studiu similar centrându-se doar pe situaţia asistenţilor medicali. Autorii au analizat rolul variabilelor ce ţin de empatie în stresul profesional şi în angajamentul ocupaţional la asistenţi medicali. Stresul a fost exprimat prin modelul tridimensional al *burnout*-ului propus de Maslach. Rezultatele au arătat că cele trei variabile ale empatiei explică în proporţie semnificativă varianţa tuturor celor trei dimensiuni ale stresului. Lipsa grijii empatice şi a disponibilităţii comunicaţionale explică mare parte din varianţa factorului depersonalizare, în timp ce lipsa grijii empatice, lipsa disponibilităţii comunicaţionale şi contagiunea emoţională ridicată contribuie semnificativ la reducerea sentimentului de realizare personală. Contagiunea emoţională pare a fi o cauză importantă a epuizării emoţionale, fiind implicată şi în scăderea angajamentului ocupaţional (24). În afara tuturor acestor efecte nefaste ale implicării empatice, Figley, identifica empatia ca factor de risc asociat în sindromul de stres traumatic secundar. El sugera că profesioniştii implicaţi în servicii de ajutorare a celor aflaţi în suferinţă, trebuie sa rămână obiectivi şi să păstreze o anumită distanţă faţă de client atunci când încearcă să îi vină în ajutor. Consemnul este de a asculta empatic, făcând însă abstracţie de trăirile şi părerile personale pentru garantarea unei analize obiective, pentru alegerea tratamentului potrivit şi pentru desfăşurarea intervenţiei în cele mai bune condiţii. Abordarea clinică se plasează cumva într-un context paradoxal care stabileşte compasiunea şi empatia ca fiind esenţiale pentru o relaţie terapeutică oportună, cerând în acelaşi timp detaşare şi obiectivism (27).

CONCLUZII

Există studii care să evalueze nivelul de stres ocupaţional, satisfacţia în muncă, epuizarea emoţională, *burnout*-ul, dar dincolo de stresul resimţit în forma lui generală, personalul medical este zi de zi martor la mari suferinţe, drame şi traume. Stresul traumatic secundar ar putea fi o potenţialitate sumbră în acest caz. Dăruirea, promptitudinea şi eficienţa absolută sunt exigenţe constante pentru personalul medical. Mai mult decât în alte profesii ezitările şi greşelile pot fi ireparabile. Empatia este un element necesar pentru garantarea de servicii medicale competente, pentru o bună relaţie cu pacienţii, pentru obţinerea de rezultate pozitive. În acelaşi timp empatia vulnerabilizează cadrele medicale în faţa stresului în formele sale particulare, ceea ce ameninţă tocmai relaţia cu pacienţii şi calitatea serviciilor oferite. Este dificil de stabilit calea optimă şi studii suplimentare sunt

necesare în această direcţie. Din această cauză evaluarea şi prevenirea unei simptomatologii atât de marcante psihic cum este cea din stresul traumatic secundar, sunt o necesitate.

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NOUTĂŢI

Efectele SUBSTITUIENŢILOR SALIVARI asupra statusului oral la pacienţii cu diabet zaharat de tip 2

Un grup de cercetători italieni a evaluat statusul oral la un lot de pacienţii cu diabet zaharat tip 2, înainte şi după terapia cu substituienţi salivari şi la un lot de pacienţi diabetici care nu a urmat terapie cu înlocuitori de salivă. În studiu au fost incluşi 134 de pacienţi cu diabet zaharat de tip 2, având vârsta medie de 47,9 $\pm$ 2,9 ani, cărora li s-a determinat rata fluxului salivar. Media ratei fluxului salivar a fost semnificativ mai scăzută în comparaţie cu lotul martor. Lotul de 134 de pacienţi a fost împărţit aleator în două grupuri a câte 67 de persoane fiecare. Un grup a primit substituienţi salivari imunologic activi timp de 6 luni, celălalt grup neavând nimic administrat. Fiecare pacient din cele două grupuri a fost supus unui examen dentar si parodontal la începutul studiului şi 6 luni mai târziu. Rezultatele studiului arată că, în ceea ce priveşte leziunile carioase şi pierderea dinţilor, nu au existat diferenţe statistice între primul grup, după 6 luni de tratament cu substituienţi salivari şi grupul de control ($P > 0,01$). Înlocuitorii salivari nu au redus în mod semnificativ boala parodontală ($P > 0,01$). În grupul tratat cu substituienţi salivari, după 6 luni de tratament, media indicelui de placă dentară a scăzut de la 2,3 $\pm$ 0,73 la 1,6 $\pm$ 0,56, la pacienţii cu gingivită a scăzut de la 66% la 43%, iar la pacienţii cu o infestare fungică semnificativă, numărul a scăzut de la 60% la 37%. Aceste diferenţe au fost semnificative statistic ($P < 0.01$). În concluzie, la pacienţii cu diabet zaharat de tip 2, în caz de hiposalivaţie, tratamentul cu substituienţi salivari imunologic activi poate fi util în reducerea cantităţii de placă, gingivitei şi infestării fungice (Montaldo L, Montaldo P, Papa A, Caramico N, Toro G. Effects of saliva substitutes on oral status in patients with type 2 diabetes. *Diabet Med* 2010 ; 27 (11) : 1280–1283).

Irina Grădinaru

A COMPARATIVE STUDY OF PERSONALITY PROFILES BETWEEN TWO POPULATIONS OF STUDENTS FROM TIMISOARA, ROMANIA AND CHISINAU, REPUBLIC OF MOLDOVA

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A COMPARATIVE STUDY OF PERSONALITY PROFILES BETWEEN TWO POPULATIONS OF STUDENTS FROM TIMISOARA, ROMANIA AND CHISINAU, REPUBLIC OF MOLDOVA (Abstract): The aim of this study was to compare personality profiles between two populations of students from Timisoara, Romania and Chisinau, Republic of Moldova. The study was performed on two samples of students at Medicine in the 2nd year of study: 172 students from Timisoara (72.67% male, 27.33% female, aged 19-22 years), and 418 from Chisinau (75.84% girls and 24.16 boys, aged 19-22 years). The study conducted was a cross sectional one and it consisted in applying a Freiburg Personality Inventory (FPI) (212 items, Cronbach`s α index 0.753 for Timisoara and 0.695 for Chisinau samples). Individual FPI profiles (12 personality traits with 9 ranks of intensity) were built using an Excel 2003 Program. FPI profiles (median) of the samples indicated the same level of intensity in both samples for 6 personality traits (Aggressiveness, Sociability, Calm, Sincerity, Extraversion, Masculinity) and different levels for the other 6 (Nervousness rank 7, Depression, Excitability, Domination, Inhibition, Emotional lability). Personality profiles of female-students were similar with personality profiles of entire samples (except Depression in Timisoara, and Calm in Chisinau). Personality profiles of students aged 20 years were similar with personality profiles of entire sample (except Domination in Timisoara). Only Nervousness (rank 7) was outside etalon interval (ranks 4-6) in the Chisinau sample and it was higher than in the Timisoara sample with 1 rank for all personality profiles. In conclusion personality profiles of both samples presented equally similarities and differences and are specific for female-students aged 20 years in the 2nd year of Medicine school. **Keywords:** PERSONALITY PROFILES, STUDENTS, TIMISOARA AND CHISINAU, CROSS SECTIONAL STUDY.

Personality traits of individuals from different countries have become a subject of growing interest (1). The Banat with the capital in Timisoara and Bessarabia with the capital in Chisinau were in the past (1918-1940) two historical provinces of Romania. In the meantime (1940-2018) they became two regions in two different countries (Romania and Republic of Moldova). Personality traits found in certain populations indicate heritage or/and environmental influence (2). Personality profiles of cultures can be operationalized as the mean trait levels of the members of a

culture (3). The aim of this study is to compare personality traits (prevalence and profiles) between two populations of students in the 2nd year of study at the Medicine Faculty, from Timisoara and Chisinau.

MATERIAL AND METHODS

A cross sectional study was performed on two samples of students in the 2nd year of study at Medicine Faculty from Timisoara (Romania) and Chisinau (Republic of Moldova). It consisted in a Freiburg Personality Inventory (FPI, 212 items) applying (4). Sample structure, statistic and reliability of method for Timisoara and Chisinau are presented in first table.

The questionnaire, standardized for Romanian population, and measuring 12 personality traits (Nervousness (Nrv), Aggressiveness (Agr), Depression (D), Excitability (Exc), Sociability (Soc), Calm (Clm), Domination (Do), Inhibition (Inh), Sincerity (Snc), Extraversion (Exv), Emotional lability (Lbl) and Masculinity (M)) was applied individually and anonymously, in a one-hour session. The ethical approval of the Ethical Committees of both Universities and the informed consent of each student to participate were asked for and obtained. FPI individual profiles were built using an Excel 2003 Program. Statistical work was performed using a SPSS Statistics 20. Prevalence (%) and personality profiles (median trait levels) of both samples were performed. Samples size, gender and age were carefully considered.

TABLE I

Samples structure and reliability of the method

	Timisoara[1]						Chisinau[2]					
	All sample		Females		Males		All sample		Female		Males	
Age	N	%	N	%	N	%	N	%	N	%	N	%
19 years old	14	8.1	10	8.5	4	8.5	89	21.3	73	23	16	15.9
20 years old	83	48.3	58	53.2	25	53.2	244	58.4	185	58.4	59	58.4
21 years old	56	32.6	43	27.7	13	27.7	52	11	35	11	17	16.8
22 years old	19	11	14	10.6	5	10.6	33	7.6	24	7.6	9	8.9
Total	172	100	125	100	47	100	418	100	317	100	101	100
Mean Age	20.47		20.49		20.4		20.07		20.03		20.19	
Median Age	20		20		20		20		20		20	
SD Age	0.798		0.799		0.798		0.806		0.803		0.809	
Reliability of applied questionnaire (FPI)												
Cronbach's α	0.753		0.765		0.734		0.695		0.682		0.756	

Note: [1] - 26 excluded students from the original sample;

[2] – 42 excluded students from the original sample; N-number of students; % - percent.

RESULTS

In comparing the Timisoara and the Chisinau entire samples, the highest students` prevalence was at the same levels of intensity for 7 personality traits (Nrv, Agr, D, Clm, Inh, Snc, Exv) and at the different

intensity levels for 5 traits (Exc, Soc, Do, Lbl and M) (tab. II). Personality profiles (median level of the traits intensity) of the entire samples indicated 6 personality traits (Agr, Soc, Clm, Snc, Exv, M) at the same level of intensity and 6 traits (Nrv, D, Exc, Do, Inh, and Lbl) at different levels of intensity (fig. 1).

TABLE II

Students' prevalence (%) for personality treats considering intensity ranks (1 to 9) for Timisoara (T) and Chisinau (C).

		1	2	3	4	5	6	7	8	9	Total
Nvr	T	2.4	1.2	8.1	8.7	15.1	19.2	**45.3**	0	0	100
	C	0	0	0.2	1	13.4	24.4	**61**	0	0	100
Agr	T	0.7	3.5	14.5	**20.9**	**20.9**	15.1	18	4.7	f	100
	C	1.7	2.9	13.4	15.8	**20.1**	19.1	16.5	6	4.5	100
D	T	1.2	3.5	12.9	11.7	23.4	**25.7**	14.6	2.9	4.1	100
	C	0.5	5	6.5	12.7	22.2	**25.1**	16.3	5.7	6	100
Exc	T	4.7	4.7	9.9	15.8	18.1	**23.4**	18.1	4.7	0.6	100
	C	1.2	1.9	7.9	12.7	**23.2**	18.9	25.8	6	2.4	100
Soc	T	1.7	1.7	11.6	20.9	19.8	**22.7**	9.9	7	4.7	100
	C	2.9	5.7	14.6	**19.9**	15.3	19.6	12.4	6.5	3.1	100
Clm	T	6.3	4.1	16.4	18.7	**32.2**	7	12.9	0.6	1.8	100
	C	7.8	5.5	15.6	19.9	**24.6**	10.8	12.2	2.9	0.7	100
Do	T	7	12.2	12.2	**23.3**	15.7	14.5	11	3.5	0.6	100
	C	3.8	8.4	5.7	18.7	**23.9**	13.4	18.2	5.5	2.4	100
Inh	T	3.4	3.5	9.3	20.3	16.9	**23.3**	13.4	7.6	2.3	100
	C			3.6	14.1	23.7	**38**	15.8	3.8	1	100
Snc	T	2.9	9.9	15.1	12.8	25	**25.6**	6.4	2.3	0	100
	C	2.7	5	11.7	12.9	27.5	**29.7**	6.9	3.6		100
Exv	T	3.4	7.6	1.8	16.4	18.7	**36.3**	7	4.7	4.1	100
	C	5.9	8.4	1.7	9.3	12.7	**46.4**	6.2	6.5	2.9	100
Lbl	T	1.1	5.3	8.2	17	**24.6**	24	16.4	3.4	0	100
	C	1	6.2	6.9	8.6	19.4	**22.5**	17.7	14.8	2.9	100
M	T	12.3	**21.6**	21.1	15.2	19.9	5.8	3.5	0.6		100
	C	16.5	13.6	**23.7**	21.1	13.9	6.7	3.3	0.7	0.5	100

Note: T – Timisoara; C –Chisinau; 1…9 – ranks of intensity for each personality dimension.

The median age for both samples was 20 years (tab. I). Personality profiles of students aged 20 were identical to with personality profiles of the entire sample (except Do in Timisoara). From the entire sample 72.67% (125) in Timisoara and 75.84% (317) in Chisinau were female-students (tab. I). The female students` profiles were identical to (except D in Timisoara and Clm in Chisinau) with the entire samples profiles. The male-students' profiles were different from the entire sample, students aged 20, and the female-students' profiles. Nevertheless, the similarities between Timisoara and Chisinau were higher (8 personality traits at the same rank) than all the others (fig. 1). Nervousness (rank 7) was outside etalon interval (ranks 4-6) for all analyzed profiles in Chisinau sample.

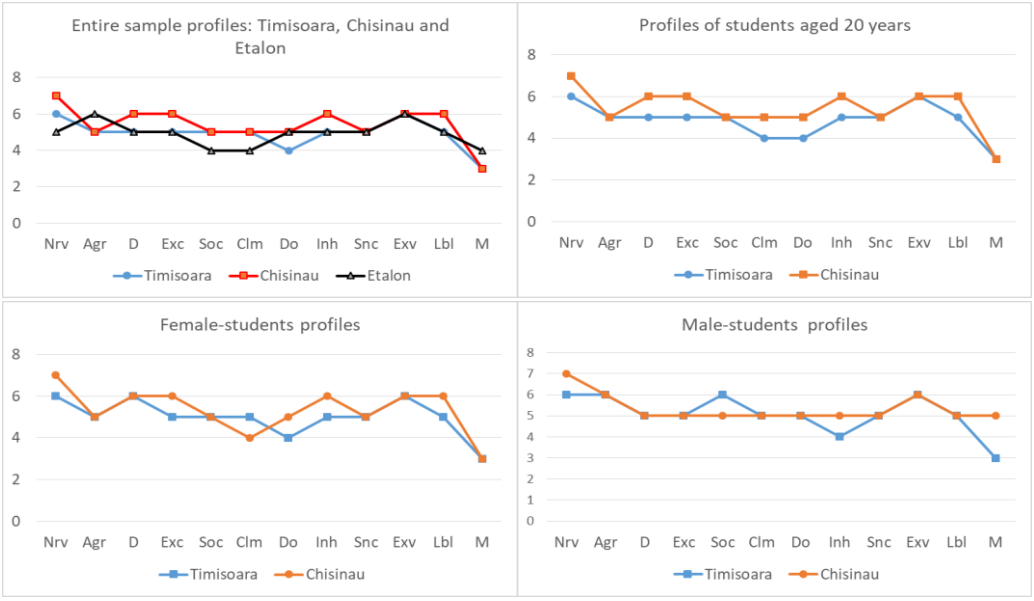

Fig. 1. Personality profiles built for entire samples, students aged 20, female- and male-students samples in Timisoara and Chisinau

DISCUSSION

Real differences in personality traits across countries found in past research and the difficulty in establishing a relationship between personality traits and culture in present studies (1) let the question "Is it a relation between personality profile and culture?" still open. In the performed study we found similarities and differences of personality profiles between the two cities, and a powerful influence of gender and age on personality profiles of students in the 2nd year of study at Medicine from Chisinau and Timisoara. Aggregate personality profiles provide insight into cultural differences (3) and similarities between cultures into segregated cultures. FPI tool offered us the possibility to pinpoint interesting cultural, gender, age and professional contribution (5, 6) to personality profiles in 2 populations with common cultural elements. A limit of this study is that it is an

observational one and future investigation needs to be done.

CONCLUSIONS

Personality profiles of both samples (Timisoara and Chisinau) present equally, similarities and differences and are specific for female-students aged 20 years in the 2nd year at Medicine Faculty. Nervousness (rank 7) is outside etalon interval in the Chisinau sample and is higher, with 1 rank, than in the Timisoara sample for all personality profiles.

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NEWS

LIQUID BIOPSIES COULD REVOLUTIONISE CANCER MEDICINE

Tumor biopsies are the current standard for cancer diagnosis and the preferred method for molecular testing to guide the selection of precision therapies. Liquid biopsies, mostly those involving cell-free DNA (cfDNA) from plasma, are rapidly emerging as an important and minimally alternative approach. Liquid biopsy is becoming a valuable tool for molecular testing for new insights into tumor heterogeneity, and for cancer detection and monitoring. Although liquid biopsy has most often referred to the analysis of cfDNA from peripheral blood, this term also refers to the isolation and analysis of tumor derived material from blood or other bodily fluid like cerebrospinal fluid, saliva, pleural fluid, ascites, stool and urine. Although more prospective studies are needed liquid biopsies have the potential to revolutionize cancer medicine (Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. *N Engl J Med* 2018; 379(18): 1754-1765).

THE ROLE OF DYSFUNCTIONS AND PARAFUNCTION HABITS IN THE DEVELOPMENT OF MALOCCLUSIONS

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THE ROLE OF DYSFUNCTIONS AND PARAFUNCTION HABITS IN THE DEVELOPMENT OF MALOCCLUSIONS (Abstract): The aim of this study was to find any correlations between dysfunctions and parafunction habits in mixed dentition children and malocclusions prevalence. **Material and methods**: A number of 50 children were included in this study, 27 girls and 23 boys, aged between 6 and 8 years old. They were examined by an orthodontist regarding the presence of malocclusions: overjet, overbite, anterior or posterior crossbite, anterior open bite and canine and first molar relationship. A statistical test was used to find the correlation strength between bad habits and malocclusions. **Results:** An increased overjet was correlated with long time artificial feeding and a deep overbite was associated with baby pacifier habits. Canine and molar class II were associated with thumb sucking habits and artificial feeding. **Conclusions:** The presence of bad habits was directly associated with the presence of malocclusions and the malocclusion prevalence was higher at children with bad habits. **Keywords**: DYSFUNCTION, PARAFUNCTION, HABITS, MIXED DENTITION, MALOCCLUSION.

Malocclusion is a development deviation from the orofacial equilibrium having consequences on dental health and psychological comfort of the patient.

Malocclusions prevalence depends on the interaction of different factors: genetic, environmental, neuromuscular and pathological changes in primary or early mixed dentition (1). The etiology of malocclusion is numerous, and the risk factors can be divided in three categories: general causes (heredity, endocrine disorders, harmful habits and disorder of physiological functions), local causes (number, form or position abnormalities, premature loss of primary teeth), dento-alveolar disharmony (crowding) (2).

Another author divides the dento-maxillary pathological functions in two groups:

- Dysfunction habits: artificial feeding, oral breathing, tongue-thrust habit, bad phonation;
- Parafunction habits: sucking habits, tics and bruxism (3).

Artificial feeding has been associated with malocclusions especially in primary dentition. Some studies revealed that 53% of artificial-fed children developed myofunc-

tional disorders with tongue resting on the mandibular arch or between dental arches, because of muscle hypotonicity (4). Alteration of oral muscles caused by artificial feeding can affect many functions: deglutition, mastication, breathing and speech-sound pronouncement (5). Several authors affirmed that bottle-fed children can easily develop a nonnutritive-sucking habit (6, 7).

Both types of sucking habits are associated with an atypical swallowing pattern, with tongue thrusting, which can be related to the development of posterior crossbite malocclusion (8).

Also, oral breathing, which is frequently found during the night, is a risk factor for malocclusion developing, especially open bite or class II/1 with an increased overjet. Mouth-breathing is often associated with the presence of enlarged adenoids and nasopharyngeal airway obstruction. Several studies associated these dysfunctions with the development of skeletal and dento-alveolar malocclusions (9, 10).

Tongue thrust or immature deglutition is a harmful habit which can favor the appearance of some dento-maxillary abnormalities. Kydd *et al.* found that patients with tongue thrust put more pressure on the upper anterior teeth than those with normal deglutition (11). Neff and Kydd established that deglutition frequency is double on those with normal deglutition than children with tongue thrust (12). Whitman *et al.* showed the lack of pharyngeal reflex on those with immature deglutition (13).

Thumb sucking behavior was recognized by many authors as affecting occlusion and characteristics of dento-alveolar arches. Farsi and Salama affirmed that thumb sucking children have frequently dental class II on molars and canines and an increased overjet (14).

The aim of the present study is to assess the relationship between different dysfunction habits and the prevalence of malocclusions in children with mixed dentition.

MATERIAL AND METHODS

This prospective study was performed on 50 children from a public school from Tirgu-Mures, 27 girls and 23 boys, aged between 6-8 years old. The children were examined regarding the presence of malocclusions: overjet and overbite measurement, presence or absence of anterior or posterior crossbite, presence or absence of anterior open bite and canine and first molar relationship. Examination was made in the school medical office by an orthodontist using single use examination kits keeping the intercuspation relationship (fig. 1). The information about history and duration of habits has been taken from parents, who answered to a questionnaire and signed a consent for children examination.

The habits that were analyzed were: thumb sucking, oral breathing, tongue thrust, artificial feeding and baby pacifier. The collected data were statistically analyzed using Spearman's test, detecting the correlation between multiple habits and the presence of malocclusions.

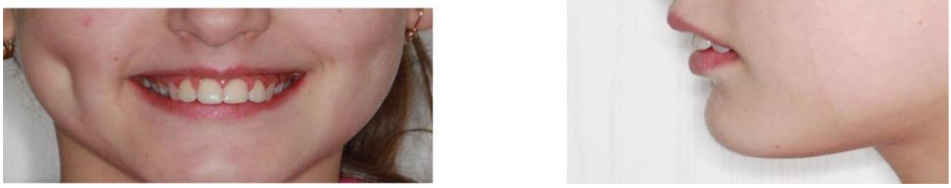

Fig. 1. Female patient; Class II/1 malocclusion; thumb sucking habit followed by oral breathing

RESULTS

An increased overjet can be caused by a long-time artificial feeding habit, a weak correlation has been found between those two. An increased overbite is weakly correlated with baby pacifier habits, but also with natural feeding, and a decreased overbite is weakly correlated with thumb sucking habit. A canine and molar class II can be associated with a thumb sucking habit, with a moderate correlation, and also with artificial feeding, but having a weak correlation. On the other hand, Class III relations were found on children with pacifier sucking habit. A crossbite occlusion can be associated with a long-time pacifier sucking habit with a moderate correlation between them. None of dysfunction habits has been correlated with crowding or spacing incongruence (tab. I).

TABLE I

Statistical correlation between occlusal variables and dysfunction habits using Spearman`s test. Correlations

		Natural Feeding	Artificial Feeding	Using Baby Pacifier	Thumb sucking	Oral Breathing
Spearman's Overjet Rho	Correlation	-.233	.306	-.054	.243	.262
	Coefficient Sig. (2-tailed)	.252	.128	.793	.232	.196
	N	26	26	26	26	26
Overbite	Correlation	-.399*	.140	.322	-.349	.181
	Coefficient Sig. (2-tailed)	.043	.495	.109	.081	.377
	N	26	26	26	26	26
Crossbite	Correlation	-.318	.123	.432*	-.140	-.123
	Coefficient Sig. (2-tailed)	.113	.551	.027	.495	.551
	N	26	26	26	26	26
Molar Relation	Correlation	.328	-.306	.282	-.536**	-.259
	Coefficient Sig. (2-tailed)	.102	.128	.163	.005	,201
	N	26	26	26	26	26
Canine Relation	Correlation	.430*	-.273	.296	-.387	-.259
	Coefficient Sig. (2-tailed)	.028	.178	.142	.051	.201
	N	26	26	26	26	26
Crowding	Correlation	-.065	-.022	.089	-.169	.109
	Coefficient Sig. (2-tailed)	.751	.914	.666	.410	.596
	N	26	26	26	26	26
Spacing	Correlation	.161	.221	.241	.237	-.207
	Coefficient Sig. (2-tailed)	.431	.278	.235	.244	.310
	N	26	26	26	26	26

*. Correlation is significant at the 0.05 level (2-tailed). **. Correlation is significant at the 0.01 level (2-tailed).

The prevalence of malocclusions was: 8 children presented an increased overjet; 15 children presented a deep overbite, 11 children had edge to edge anterior bite and only 2 children had an open anterior bite. The posterior crossbite was found in only 3 cases. The canine and molar relations were: dental class II was the most frequent on canine and molar, both present on 7 cases and 6 cases having only the canine in class II. Crowding and spacing incongruences were found: 13 crowding cases and 15 spacing cases.

DISCUSSION

The study design is similar with other studies in the literature being based on cause-effect relation. It was observed that a long time of bad habits increase malocclusions. Also, in the Elton *et al.*, study has been showed that children with a prolonged pacifier-sucking habit after 2 years of age had greater chances of malocclusion than normal children (15).

Also, other authors, like Farsi and Salama, found that nonnutritive sucking habits determine distal molar and canine relationships and larger overjet (14). Warren and Bishara study were similar with our study regarding an increased overjet on children with artificial feeding habit. They also found a reduced overbite on this patient, but our results are no significant regarding this (16).

A Slovenian study demonstrated that thumb or pacifier sucking habit and artificial feeding practiced after the age of 5 are correlated with an atypical deglutition at the age of 6-9 years and with the presence of a malocclusion after 12 years (17).

Suzely *et al.* study showed that pacifier sucking habit practiced for 12-30 months is associated with an increased overjet and open bite but practiced for more than 30 months can be associated with an overbite (18). In our study the pacifier sucking habit is weakly correlated with an increased overjet and moderate correlated with crossbite malocclusion.

The prevalence of malocclusion from our study is 19.6 %, lower than other studies: 32.5% in Brazil on a 381 children sample or 70% from an 80 children sample (6).

The highest percentage among dysfunctions was the artificial feeding more than 12 months- 40 %, followed by pacifier-sucking more than 2 years - 20 %, which are similar with other studies percentages: artificial feeding - 90% and pacifier sucking - 38% (19).

According to World Health Organization, breast feeding should be exclusive till the age of 6 months and additional till the age of 2. In this study 8% of children haven't ever been breast fed and 24% have been fed more than 12 months. The insufficiency of natural alimentation can be correlated with an undeveloped facial muscles, atypical deglutition, bad sucking habits and oral breath.

Premature loss of temporary teeth can also be the reason of underdeveloped bones and secondary open bite, which can cause deviation from normal function. The use of space maintainers after premature loss of the second upper temporary molar is a last solution in preventing tridimensional lesions in the dental arch and occlusion (19).

The limit of our study is the small number of patients, especially those diagnosed with oral breathing and atypical deglutition, the reason of the absence of correlations between these dysfunctions and malocclusions development.

CONCLUSIONS

The presence of bad habits was directly associated with the presence of malocclusions.

The malocclusion prevalence is increased on children with dysfunctions and

parafunctions.

Children artificial fed had greater chances to an increased overjet and molar class II.

Thumb sucking habits at children were weakly correlated with a decreased overbite and an increased overjet and moderate correlated with molar and canine class II.

The high frequency of bad habits seems to be a real problem for the development of dento-maxillary system, and it needs more attention from dentists and also from parents.

Using the baby pacifier for a long time can increase the overbite and the prevalence of crossbite malocclusion.

It is a bit difficult to find statistical correlations between dysfunctions and malocclusions given a smaller number of children, but the cause-effect relationship is clearly demonstrated in literature.

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STUDY ON CORRELATION BETWEEN CERVICAL VERTEBRAE MATURATION, DENTAL AGE AND ACTUAL AGE OF CHILDREN

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STUDY ON CORRELATION BETWEEN CERVICAL VERTEBRAE MATURATION, DENTAL AGE AND ACTUAL AGE OF CHILDREN (Abstract): The study **aim** was to provide a version of the cervical vertebral maturation index (CVM) method for evaluating maxillary and mandibular skeletal maturation for each patient in the study group. **Material and methods:** The morphology of the three cervical vertebrae, the presence of concavities on the lower vertebral border and the form of the vertebral body at CS 2, CS 3 and CS 4 was evaluated by visual inspection of lateral cephalograms by two evaluators. The percentage of concordance among examiners was 96.7% For the study of the correlation the 2 evaluators examined 60 panoramic radiographies and analyzed the dental age using the Demirjian Index. **Results**: The descriptive indicators of the study group show an average age of 12.65 years. The average CVM score awarded in the study group is 3.133. The most frequently score is CVSM 2 ,anatomical feature that identifies the stage immediately ahead of the mandibular growth peak. For the correlation the dental age was determined with the better known Demirjian Index using scores calculated on the reference teeth 33, 34, 37 on the panoramic radiographies in the study group. **Conclusions**: The results of visual cephalometric analysis required a relatively short time and are consistent with the CVM method in published studies, the reproducibility of CVM steps being equally high. **Keywords:** VERTEBRAE, FORM, CEPHALOGRAMS, CVM SCORE, DEMIRJIAN INDEX, DENTAL AGE.

The fundamental concept of dentofacial orthopedics is to achieve maximum, three-dimensional effects in the optimum mandibular growth period. Treating inaccurately before puberty gives the impression that functional appliances do not work although they are only used in a biologically unfavorable moment .

There are few studies in our country that include information on skeletal maturity in patients treated with functional appliances, and for the most part, the biological growth indicator is the supplementary analysis of hand-wrist X-ray or Demirjian analysis on panoramic radiographs (1, 2).

The Baccetti CVM method considers that maximal mandibular growth time is associated with CVM 3 - CVM 4 stage not in temporary or mixed dentition but prepuberty (3, 4, 5, 6, 7) Recently, cervical vertebral maturation index CVM has gained maximum attention in the literature as an alternative to evaluate skeletal maturity in the office, replacing an additional radiological exam (hand-wrist radiograph) in the conditions where conventional cephalogram is recommended for diagnosis, and the majority of orthodontists are trained and familiar with its interpretation.

AIM

In this study we intended to provide a version of the cervical vertebral maturation index CVM method for evaluating maxillary and mandibular skeletal maturation for each patient, we made the analysis of the morphological characteristics of C2, C3 and C4 cervical vertebrae on lateral cephalograms and determined the correlation with the values of the Demirjian Index evaluated on panoramic radiographs.

MATERIAL AND METHOD

Visual analysis - 2 evaluators examined the morphology of the three cervical vertebrae by visual inspection on lateral cephalograms. The proportion of concordance among examiners was 96.7%.

Visual analysis - 2 evaluators examined the panoramic radiographs and the Demirjian Index was determined. The percentage of concordance among examiners was 96.7%;

60 lateral cephalograms and panoramic radiographs were realized with the same FONA radiological equipment was selected for the study: 29 girls and 31 boys were selected for the study. The distribution was almost equal - 47.5% girls and 50.8% boys. The age distribution of the study group is heterogeneous: 12-year-old children predominate 26.2%, followed by 11-year-olds and 13-year-olds, the smallest 15-year-old (3.3%) (tab. I).

TABLE I

Distribution of the study group by age

	Frequency	Percentage	Valid percentage	Cumulative percentage
10.00	8	13.1	13.3	13.3
11.00	11	18.0	18.3	31.7
12.00	16	26.2	26.7	58.3
13.00	10	16.4	16.7	75.0
14.00	3	4.9	5.0	80.0
15.00	2	3.3	3.3	83.3
16.00	10	16.4	16.4	75.0
TOTAL	**60**	**100.0**		

The statistical descriptive parameters of the study group show an average age of 12.65 years, average Demirjian Index of 7.43 for the lower canine, 7.35 for the lower first premolar, 6.88 for the lower second premolar, 9 for the lower second molar.

The average cervical vertebral maturation index CVM score in the study group is 3.133 with a standard deviation of 1.52 (tab. II).

TABLE II

Statistical descriptive parameters in the study group

	N	Minimum	Maximum	Medium value	Standard Deviation
Age	60	10.00	16.00	12.6500	2.08160
DI 33	60	6.00	8.00	7.4333	0.67313
DI 34	60	5.00	8.00	7.3500	0.75521
DI 35	60	6.00	8.00	6.8833	0.82527
DI 37	60	5.00	8.00	6.9000	0.72952
CVM	60	1.00	6.00	3.1333	1.52345

RESULTS AND DISCUSSION

The first examiner gave cervical vertebral maturation index CVM scores between 1 and 6 with an average of 3. The second observer gave cervical vertebral maturation index CVM close but less expressive CVM with a mean greater than 3.5, bringing the patients to the maximum value of the CVM score (tab. III).

The first examiner gave cervical vertebral maturation index CVM 1 scores in 8% cases; CVM 2 in 40% cases; CVM 3 score in 18%;CVM 4 score in 16% cases; CVM 5 score in 14% cases and CVM 6 score in 4% of cases. The second examiner gave CVM 1 scores in 12% cases; CVM 2 in 28% cases; CVM 3 score in 24% cases; CVM 4 score in 16% cases; CVM 5 score in 12% cases and CVM score 6 in 4% cases.

TABLE III

The CVM scores calculated in the study group

Age	N	Medium value	Standard Dev.	Medium Error
	60	12.65	2.08160	0.84728
CVM scores Observer 1	60	3.0000	1.35526	0.19166
CVM scores Observer 2	60	3.5400	4.47765	0.63324

In the study group, cervical vertebral maturation index CVM 1 scores were seen in 13.1%. lateral cephalograms; CVM 2 scores were seen in 27% examinations.; CVM 3 scores were seen in 19.7% examinations; CVM 4 scores were seen in 18% examinations and CVM scores 5 and 6 were seen in 10% examinations (tab. IV).

The cervical vertebral maturation index CVM score most often accorded is CVM 2 which has the highest percentage distribution as in Howell's studies (8). In this stage of skeletal development, vertebrae C2 and C3 are trapezoidal or rectangular in shape and have concavities on the lower edge of the vertebral body.

General tendency is that the skeletal age is lower than the chronological age, which leads to the conclusion that these children tend to late mature as the Arab children in the study of Alhadlaq (9), the Turkish children in the study of Uysal (10, 11) and different from Thai children with early maturation from Kumar's study (12).

TABLE IV

CVM scores in the study group

CVM				
	Frequency	Percentage	Valid Percentage	Cumulative percentage
1.00	8	13.1	13.3	13.3
2.00	17	27.9	28.3	41.7
3.00	12	19.7	20.0	61.7
4.00	11	18.0	18.3	80.0
5.00	6	9.8	10.0	90.0
6.00	6	9.8	10.0	100.0
TOTAL	60	100.0		

For the correlation of age identified using the cervical vertebral maturation index CVM stages on the lateral cephalograms Demirjian scores on the reference teeth 33, 34, 37 were calculated on panoramic radiographs of the patients in the study group (13, 14).

The score for the lower canine was 6 for 9.8% of patients; 7 for 36.1% and 8 for half of the patients in the study group, the lower canine being in stage G or H of dental development after Demirjian. This stage is reached 0.4 years before the pubertal growth peak in girls, and in boys 1.3 years earlier.

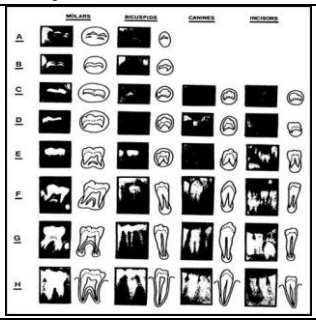
The Demirjian score for the first lower premolar was 5 for 3.3% of patients; 6 for 6.6%, 7 for 41%; and 8 for 41% of the patients in the study group, the lower canine being in stage G or H of development after Demirjian.

This stage is reached 0.4 years before puberty growth peak in girls and in boys with 1.3 years previously. The score for the lower second molar was 5 for 3.3% of patients, 6 for 21.3%, **7** for 55.7% and 8 for 18% of the patients in the study group, the lower molar being in stage F, G or H dental development after Demirjian Index. Stage F marks the onset of pubertal growth peak in girls (tab. V) (15).

TABLE V

Values of the Demirjian Index on panoramic radiographies at 33, 34 and 37 in the study group. Imaging with the Demirjian Index by Maniar *et al.*

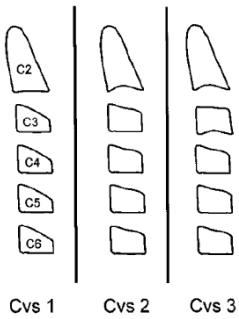
		Demirjian Index 33	Demirjian Index 34	Demirjian Index 37
		Frequency	Frequency	Frequency
F	5.00	6	2	2
G	6.00	22	4	4
G	7.00	32	25	25
H	8.00	61	29	29
TOTAL		60	60	60



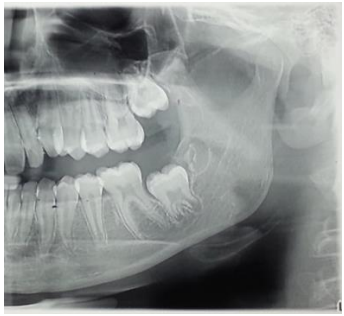
For the 11-year-old CE diagnosed with Class II / 1 Angle Malocclusion, lateral cephalogram and panoramic radiography were analyzed, a maximum increase at 11 years old was identified and a functional appliance treatment was initiated with a Klammt elastic activator that corrected the overjet and class II molar relation in 6 months (figs. 1, 2, 3).

Fig. 1. Initial intraoral aspects

Fig. 2. Intraoral image after 6 months treatment with Klammt elastic activator-normal overjet and molar occlusion



Stage CVM 2 Lower margins of vertebrae C2, C3 have a visible concavity on the lower edge, vertebrae C3 and C4 are rectangular in shape and the mandible will have a maximum increase in one year.



Stage G of the Demirjian Index, in which the limiting walls of the 33,34 apex are parallel, and the distal roots of the molars, especially the distal one is open

The Klammt activator used for patient CE in the optimal CVM 2 stage who corrected the occlusion in 6 months

Fig. 3. CVM score, Demirjian Index and the functional appliance for the patient CE

CONCLUSIONS

Changes in the size and shape of cervical vertebrae seen in growing children are of major concern as individual skeletal maturation indicators.

The results of visual cephalometric analysis require a relatively short time and are consistent with the CVM method in published studies, the reproducibility of CVM steps being equally large.

The appearance of a concavity on the C3 cervical vertebrae represents the anatomical characteristic that identifies the stage immediately before the peak of mandibular growth.

Correspondence between the records of the two examiners is maximum.

For correlation of age identified with CVM stages on cephalograms Demirjian scores on reference teeth 33, 34, 37 were

calculated on panoramic radiographies of patients in the study group. The score for the lower canine was 6 for 9.8% of patients, 7 for 36.1% and 8 for half of the patients in the study group, the lower canine being in stage G or H of dental development after Demirjian. This stage is reached 0.4 years before the pubertal growth peak in girls, and 1.3 years earlier in boys .

CVM score more often granted is CVSM 2 which has the highest percentage distribution.

The general tendency is that the skeletal age is lower than the chronological one, which leads to the conclusion that these children tend to late mature as the Arab children, Turkish children and different from Thai children with early maturation.

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BOALA KOSTMANN LA COPIL

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KOSTMANN DISEASE IN CHILDREN (Abstract) : Kostmann's disease (KD) is a severe congenital neutropenia, a rare autosomal recessive disorder of neutrophil number. Complete blood count with differential reveals an ANC less than 500/mm^{3,} as seen in classic cases, monocytosis and eosinophilia. Bone marrow aspiration or biopsy reveals an arrest of neutrophil precursor maturation at the promielocyte or myelocyte level. KD results in an increased susceptibility to frequent bacterial infections. In the absence of medical intervention with granulocyte colony-stimulating factor (G-CSF), bone marrow transplantation, the mortality rate is 70% within the first year of life. We present three cases to illustrate this rare entity, the difficulty of diagnosis, but also to underline the importance of correct treatment. **Key words** : SEVERE CONGENITAL NEUTROPENIA, KOSTMANN DISEASE

Boala Kostmann (KD) sau neutropenia congenitală severă este o entitate rară (1-2 cazuri/1 milion de persoane) (1), cu transmitere autosomal recesivă (4), având drept caracteristică scăderea numărului absolut de neutrofile (ANC) $< 500/mm^3$(3), ce se reflectă clinic în creşterea susceptibilităţii la infecţii bacteriene în special, apărute la scurt timp după naştere. Biopsia sau aspiratul de măduvă osoasă (MO) relevă oprirea în maturare a precursorilor neutrofilici în stadiul de promielocit sau mielocit (4).

Tratamentul medical vizează antibioterapia în infecţiile bacteriene şi stimularea producţiei de neutrofile (cu factor de creştere a coloniilor granulocitare G-CSF în administrare zilnică, pe termen lung pentru a obţine beneficiu clinic) (4,5), iar cel chirurgical implică drenarea eventualelor abcese. Splenectomia nu este eficientă (4).

În absenţa intervenţiei medicale cu factor de creştere a coloniilor granulocitare (G-CSF), rata mortalităţii este mare (70% în primul an), rămânând de luat în considerare transplantul de MO (2) la pacienţii nonresponsivi la G-CSF sau la cei cu transformare leucemică (KD fiind apreciat ca stare preleucemică) (4,5) .

Vă prezentăm o suită de 3 cazuri, două surori (P.C.E. şi P.P.M.) şi un băieţel (T.V.D.), diagnosticate, îngrijite şi monitorizate în serviciul nostru.

CAZUL I

Pacienta P.C.E., în vârstă de 7 ani, din mediul rural, se internează pentru cura chirurgicală a unor multiple flegmoane la nivelul ambelor coapse şi faţa externă a genunchiului drept, colecţii tratate cu oxacilină 5 zile şi care au fistulizat spontan. Din ante-

TABELUL I
Aspirat de măduvă osoasă

	Mieloblast	Promielocit	Mielocit N	Mielocit E	Metamielocit	N nesegm
02.09.04	2%	49%			23%	3%
14.09.04	3%	19%	18%	17%	6%	2%
02.10.04	1%	31%	12%	5%	14%	2%

cedente se remarcă numeroase infecţii respiratorii şi cutanate, spitalizate. La examenul clinic se remarcă cicatrici multiple la nivelul întregului corp, markeri ai infecţiilor cutanate numeroase din antecedente. Biologic se constată: neutropenie (NP) (GA $=5.000/mm^3$, PN=6%, ANC=$300/mm^3$), limfomonocitoză şi eozinofilie, sindrom anemic important, hipocrom microcitar (Hb =7,4 g/dL), reactanţi de fază acută marcat crescuţi. Din puroiul recoltat s-a izolat Stafilococ coagulazo-negativ. Prezenţa bicitopeniei a impus efectuarea aspiratului de MO: măduvă polimorfă, bogat celularizată, granulopoieză cu blocaj maturativ şi cu asincronisme de maturaţie, eritropoieză cu semne morfologice de carenţă mixtă, mega-cariopoieză plachetogenă, eozinofilie 40%, plasmocitoză 10%, macrofagie. LDH în limite normale, electroforeza proteinelor serice: α – 2globulinele $\uparrow$, γ – globulinele $\uparrow$. Testul HIV negativ, imunograma a relevat Ig A $\uparrow\uparrow$ şi Ig M $\uparrow$, cu Ig G normale. Sub triplă combinaţie de antibiotice, transfuzii de sânge, evoluţia a fost lent favorabilă. Investigaţiile vizând NP au fost aprofundate în secţia Hemato-Oncologie. Evolutiv (tab. I, fig. 3.) NP s-a menţinut severă ($<500/mm^3$, în repetate rânduri chiar până la valoarea 0), timp de 8 săptămâni, cu limfomonocitoză >60%, eozinofilie > 20%, liniile GR şi ale TR fiind normale. Aspiratul de MO, în mod repetat a evidenţiat blocaj maturativ la nivel de metamielocit.

S-a iniţiat urgent tratament cu Neupogen (G-CSF), având în vedere infecţia severă cu risc de diseminare septicemică.

Diagnosticul de Boală Kostmann a fost pus prin coroborarea datelor anamnestice (antecedente de infecţii tegumentare şi respiratorii, fără istoric de boli infecto-contagioase recente, sau de consum de medicamente în ultimele 7-10 zile, cu potenţial de a provoca reacţii idiosincrazice), a datelor clinice (evoluţie lentă a unei infecţii tegumentare, corect tratată medical şi chirurgical), şi a datelor paraclinice - NP severă ($<500/mm^3$), obiectivată pe o perioadă de >14 zile, fără pancitopenie, cu aspect al MO de blocaj maturativ la nivel de promielocit, cu hiperplazie pe linia eozinofilelor, monocitelor şi prezenţa în număr $\uparrow$a plasmocitelor.

Au fost excluse în cadrul diagnosticului diferenţial NP de cauză extrinsecă (medicamentoase, postinfecţioase, cele secundare infiltrării medulare), NP benignă familială (dar mama şi tatăl au avut ANC în limite normale), NP ciclică (dar nu s-au înregistrat oscilaţii ale PN timp de 8 săptămâni şi răspunsul a fost dificil la administrarea de G-CSF), mielokathexis (dar morfologia PN şi aspectul MO au fost normale), NP intrinsecă din cadrul sindromului Schwachmann, diskeratoza congenitală, sindromul Chediak-Higashi; NP extrinsecă autoimună (exclusă pe baza AAN, celule lupice şi FR negativi) şi NP benignă cronică (sindromul „leucocitelor leneşe"). Pentru realizarea acestui ultim deziderat, ar fi fost necesară determinarea Ac anti neutrofilici (NA1, NA2, NB1, ND1, NE1), care nu a fost posibilă însă. S-au efectuat evaluări paraclinice indirecte şi anume testul la dexametazonă şi testul la epinefrină (ambele fiind negative).

Tratamentul a fost efectuat cu Neupogen (G-CSF), 7 $\rightarrow$ 60 μg/kg/zi, (cu răspuns pozitiv obţinut după 8 săptămâni), antibioterapie şi tratament chirurgical local în continuare.

Particularitatea cazului: s-a crezut iniţial că este o formă particulară de boală cu

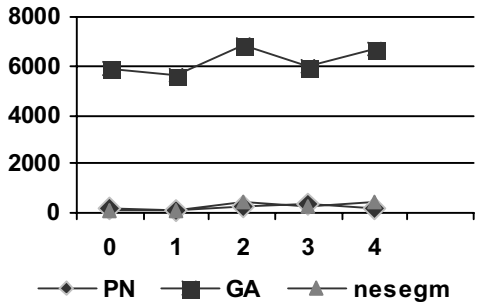

Fig. 1. Testul la epinefrină

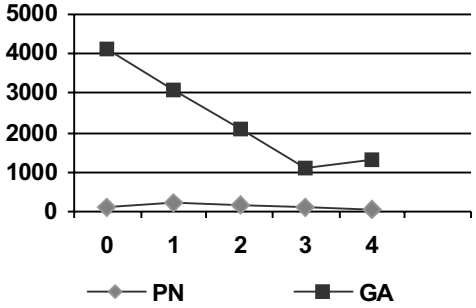

Fig. 2. Testul la dexametazonă

receptor mutant pentru G-CSF, datorită răspunsului negativ în prima săptămână de tratament şi, de asemenea, supravieţuirii până la vârsta de 7 ani, deoarece apărarea umorală, celulară şi macrofagele tisulare au jucat un rol critic.

CAZUL II

Pacienta P.P.M., în vârstă de 9 luni este investigată şi ea având în vedere antecedentele heredocolaterale (sora sa P.C.E., diagnosticată cu boală Kostmann) şi anamneza cu foarte frecvente infecţii (digestive, cutanate, respiratorii, sferă ORL- otite medii repetate) şi diagnosticată cu KD. Sub tratament cu Neupogen si multiple asocieri de antibiotice s-a obţinut vindecarea infecţiilor, unele dintre ele de gravitate mare dat fiind ANC $<200/mm^3$ şi riscul de diseminare septicemică.

CAZUL III

Pacientul T.V.D., în vârstă de 10 ani, din mediul rural, care se internează pentru conduita chirurgicală a unui flegmon situat în spaţiul popliteu drept. Din antecedente se remarcă, încă de la naştere, numeroase infecţii cu piogeni (adenoflegmoane operate, angine, abcese dentare), hipoacuzia bilaterală de gravitate medie, secundară multiplelor infecţii otice, pneumonie cu pleurezie, duplicitate pielo-caliceală bilaterală .Sub tratament cu asociere de antibiotice evoluţia a fost favorabilă şi copilul a fost transferat în Secţia de Hemato-Oncologie pentru aprofundarea investigaţiilor şi conduită terapeutică adecvată. Examenul obiectiv la internare releva stare generală influenţată, hipotrofie staturo-ponderală, numeroase cicatrici postinfecţioase cutanate, flegmon la nivelul 1/3 inferioare faţa posterioară a coapsei drepte şi spaţiului popliteu drept (fig. 4.), cu impotenţă funcţională la nivelul articulaţiei genunchiului drept, micropoliadenopatie generalizată, hipotrofie musculară, dentiţie întârziată, hipoacuzie bilaterală.

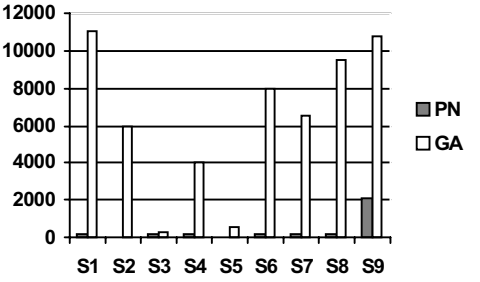

Fig. 3. Supraveghere clinică – curba GA, PN/G-CSF

Paraclinic, prezenta HLG cu GA normale / ↓, dar cu PN în FL de 3%, cu limfomonocitoză şi eozinofilie, seriile GR şi TR normale. Aspectul aspiratului de MO a fost de granulopoieză uşor hiperplazică, cu predominenţa stadiilor de promielocit, mielocit şi metamielocit şi rare elemente neutrofile finale, hipereozinofilie, plasmocitoză importantă, celelalte serii normale.

S-a efectuat tratament chirurgical, antibioterapie conform anti-biogramei şi tratament

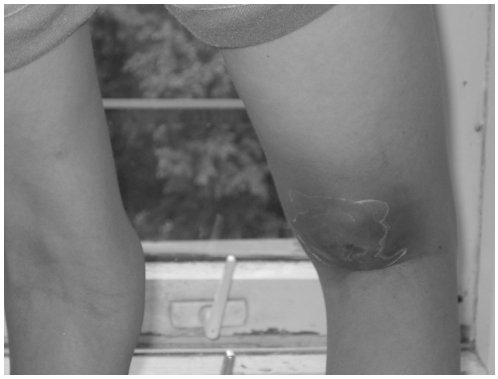

Fig. 4. Flegmon 1/3 inferioară faţa posterioară a coapsei

cu Neupogen, cu evoluţie lent favorabilă.

Cei trei copii cu KD sunt în viaţă la momentul redactării acestui material, cu stare generală şi calitate a vieţii bună - indice Karnovski 100%, fără complicaţii ale tratamentului cu Neupogen şi fără o maladie secundară.

DISCUŢII

Prognosticul în KD este rezervat. Introducerea tratamentului cu G-CSF produce o creştere semnificativă şi susţinută a ANC, rezultând în infecţii febrile de scurtă durată .Altă complicaţie redutabilă este LAM, care se poate dezvolta la 5-7% din bolnavi, KD fiind recunoscută ca stare preleucemică. Complicaţiile pe termen lung cu G-CSF nu sunt de neglijat : durerile osoase şi fracturile (la 50% din bolnavi – se datorează ↑ resorbţiei osoase), osteopenie, fibroza MO. În caz de epuizare a efectului G-CSF, singura resursă terapeutică rămâne transplantul de MO.

CONCLUZII

KD este o boala rară, care atrage atenţia prin frecvenţa infecţiilor, în special bacteriene. Tratamentul cu G-CSF prelungeşte evoluţia şi ameliorează prognosticul bolnavului.

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FACTORS INFLUENCING DIETARY HABITS AMONG MEDICAL STUDENTS AND DOCTORS. A THEORETICAL APPROACH

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FACTORS INFLUENCING DIETARY HABITS AMONG MEDICAL STUDENTS AND DOCTORS. A THEORETICAL APPROACH (Abstract): Dietary habits are strong related to physical and psychological health. During academic years, students are more prone to experience unhealthy food and to develop unhealthy and risky behaviors like smoking, eating fast food, skipping meals, unbalance nutritional dinners, skipping lunches and breakfasts and eating disorders due to stress. Later in their life as doctors, these dietary habits are more and more influenced by professional life, job-related factors like swifts, long working hours with great impact on health. The paper presents risk factors related to dietary habits among medical students and doctors, and their consequences on physical and psychological health. **Keywords**: PHYSICIANS, DIETARY HABITS, MEDICAL STUDENTS, EATING DISORDERS, STRESS.

The World Health Organization defines health as a state of complete physical, mental and social well-being and not just the absence of disease or infirmity (1). A healthy lifestyle that includes regular physical activity, a balanced diet and avoiding smoking as well as excessive alcohol consumption has been shown to reduce the alarming overall mortality caused by non-communicable diseases (NCDs), which annually, represents 36 million deaths worldwide (2). Moreover, healthy eating has an influence on motor concentration (3), memory and performance (4), mood and fatigue (5) - the latter being associated with cognitive and motor disorders, injuries and mistakes (6).

Medicine, which involves long hours of work, emotional stress and high volume of tasks, is an example of demanding activity, both physically and mentally, which requires high levels of cognitive and motor performance (7). The pre-existing patterns of unhealthy behavior established during the student's years of medical training can continue to develop during adult life, transforming into behaviors that will shape the future related to the health of the young doctor (8-10). Thus, it is important to first explore the lifestyle (nutrition, exercise, alcohol and tobacco use) and certain aspects of medical students' mental health, and then to see what impact the medical profession has on the dietary behavior of physicians.

Unhealthy eating habits among students

The transition in university education is significant because during this period young

adults experience greater freedom to make choices regarding food behavior and lifestyle (11). Rapid changes in mental growth and development place these young adults in a group of nutritionally vulnerable people with poor eating habits who are unable to meet their dietary requirements. The most common unhealthy food choices among students seem to be omitting the main meals, frequent meals in the city, increased consumption of snacks and fast food (12). According to studies conducted at US universities, significant increases in fat intake and alcohol intake have been reported in these students, so that unhealthy eating and excessive alcohol consumption make a significant contribution to energy intake and, therefore, may facilitates the student's weight gain. Also, the same pattern of weight gain in students is under development in Europe (13).

In European countries, the consumption of snacks is very widespread, proving that the most common contexts for serving snacks among young people are after courses (4,6 times a week), while watching TV (3.5 times a week) and while staying with friends (2.4 times a week) (14). Also, environmental factors (through the emergence of malls, shops, vending machines and fast food markets) contribute to the adoption of unhealthy eating habits among students. They have a lack of nutritional knowledge and tend to make their own food choices based on the cost of food and the availability of fast food, and this can negatively affect their nutritional status (12).

Excessive alcohol consumption among students is a widespread problem in several university campuses, with studies indicating an increased prevalence of a desire to drink among students (drinking five or more alcoholic drinks at a single meeting). Alcohol consumption among students was also associated with other unhealthy behaviors including smoking, risky sexual behaviors, contact with multiple sexual partners, injuries, and failure to use the seat belt in the car. Smoking is also widespread among students worldwide given the fact that a quarter of all or even more US students smoke and 75% of them continue to smoke into adulthood, placing future adults at greater risk of developing lung cancer and cardiovascular disease (15). In addition, students often fail to meet dietary recommendations and physical activity requirements, the highest rate of physical activity decline being recorded in the early maturity period, between 18 and 24 years old (16).

Students from later university years show a higher prevalence of drinking, smoking, and their consumption of fruit and vegetables decreases with each completed semester (17, 18). The academic environment can affect students' mental health; for example, academic overload can lead to stress, anxiety and depression, and in addition, contact with patients with poor health can be a unique stressor in medical school (10).

Medical students were supposed to practice healthier eating habits compared to non-medical students, but some studies have found the opposite. A previous study in China revealed that medical students presented risk factors for early chronic disease due to poor eating habits. It was found that although medical students had enough knowledge of healthy eating habits, they failed to apply this knowledge in practice (12). Also, another study conducted in Poland shows that medical students are aware of the benefits of a balanced diet and an active lifestyle on health, but nevertheless fail to turn theory into practice (19). Therefore, the fact that many medical stu-

dents apparently do not meet the criteria for a healthy lifestyle, despite knowing in theory these notions unlike non-medical students, is a very serious health problem. Thus, raising awareness of this issue among students should become a high priority for medical universities.

A study conducted in China shows that the prevalence of regular breakfast consumption (71.1%) is lower compared to the prevalence recently reported among medical students in Western countries (80% in the United States, 90% in France and 72,5% in Australia for example) but is higher compared to Lebanon (52.7%) (20). In Croatia, it seems like students from Zagreb School of Medicine from 1-6 years have an unhealthy diet and lifestyle, and studies in other countries have also reported that young people have developed irregular dietary patterns like skipping meals, snacking between meals and often eating away from home. The reasons for these unhealthy behaviors may be their busy schedules, lectures and different places provided by lectures, lack of experience and time for organizing an economical, fast and healthy meal and more (21).

High prevalence of overweight (24%, N = 36) and obesity (9.3%, N = 14 - out of a total of 150 students) associated with poor dietary practices and low levels of physical activity among medical students included in a study in India is a matter of serious concern. Factors such as fruit intake, duration of physical activity, sleep and sedentary behavior had a significant association with nutritional status. A higher proportion of overweight (47.2%) and obese (50%) had lower fruit intake compared to those with normal BMI (25.9%), and average fruit and vegetable consumption was lower than recommended. Most participants reported the consumption of fruits and vegetables only once or twice a week. These observations show that medical students, despite adequate knowledge, are not sufficiently motivated to change their lifestyle. Students should be encouraged to adopt healthy eating practices and to undertake various outdoor sports activities (22).

Factors that affect eating habits

The stress of university life could be one of the major factors that negatively influence the diet of young adults. It has been found that students in the first years of college are particularly vulnerable to stress (23). Excessive fatigue is often a result of the different challenges of university life (compliance with academic requirements and university standards, weakening family ties, meeting new people) that have an impact on both physical and mental health. In a study in Australia, fatigue proved to be a common health problem among more than half of the participants, being negatively associated with academic behavior (absenteeism or even university dropout). Fatigue has been shown to be associated with a number of health-risk behaviors, especially around alcohol consumption, as well as unhealthy weight loss behaviors. These findings may reflect two types of fatigue in the student population: fatigue related to sleep deprivation (assuming alcohol consumption was associated with less sleep) and fatigue related to illness-most commonly found in this study (24).

Additionally, what causes students to eat unhealthy food is not only the choice of food, but there are structural forces that make it difficult for students to consume healthy foods such as: financial incomes, meals' costs, unhealthy food environment,

nutritional education and health beliefs. Besides these, there are many factors that affect the eating habits among students, among which: the differences of social class, the cost of food, the knowledge of food, the time of food preparation, the family structure, culture and so on, some of these factors can even be multiplied, considering lives of many students (25).

More and more evidence show that a fairly large proportion of young people are experiencing poor physical health and the prevalence of psychological disorders is higher in students than in the general population (non-students) (24). The incidence of mental health problems among medical students can be up to 30%, but most students do not ask for help (26).

Particularities among medical students - eating disorders and nutritional knowledge

Eating disorders are another problem among students. Studies have shown that the estimated prevalence of eating disorders among them has increased from 8% to 20.5%. Alarming is the fact that a significant portion of those who show signs of eating disorders have not been diagnosed and do not want treatment (27). In Romania, previous studies conducted on medical students showed that they had high levels of academic distress which affect sleep and food behaviors and this academic level of distress is correlated with a high score for depression (28).

Sleep is another important component of the lifestyle, having consequences on both physical and mental health. The freshmen students from dental medicine sleep, on average, 6.34 hours per night (29). Thus, shorter sleep duration was associated with higher physical and mental stress, lower academic outcomes, suicide risk, mental and eating disorders (30). Also, high scores of body dissatisfaction were associated with the practice of following a weight loss diet and eating during nights (29). Another study showed that complaints about sleep problems are common in young medical students, the quality of student sleep being assessed as good in less than half of them (40%), excellence at only one third (29%) and satisfactory to less than a quarter (24%). Sleep quality is associated with academic progress and living conditions and daytime sleepiness is a significant problem for students and is associated with both sleep disorders and work while studying (31).

Another aspect that medical students face is nutritional knowledge. Historically, nutrition education has been underrepresented in many medical schools and residency programs. Surveys showed that most medical schools in the United States do not yet provided adequate nutrition education and do not produced graduates with the necessary nutritional skills in medical practice (32). It is noted that students considered that nutrition information is poorly integrated into the curriculum, and more than that, students attended few nutrition counselors during their residency years and the nutrition information that was provided was often outdated or incorrect (33). Improving students' nutrition knowledge is important because this allows medical students to learn about the lifestyle and challenges that patients face, in addition to aspects of the disease (34).

Although the National Academy of Sciences has recommended a minimum of 25 hours of nutrition education, most medical schools offer medical students very little nutrition education or do not offer at all

during university years (33). Physicians, residents and medical students do not feel comfortable, confident or adequately prepared to provide nutritional counseling and clearly need more training and further education in nutrition assessment and intervention (32).

Impact of the medical profession on the dietary behavior of physicians

The culture of medicine promotes the belief that not only students, but also doctors are never sick. Traditionally, doctors have neglected their own health in favor of numerous professional and personal obligations and often consider that attention to their own needs is a sign of weakness. Fortunately, attitudes have changed and nowadays, more attention is paid to the doctor's health. Medical associations are beginning to recognize the potential risks of the health care profession, and many have created programs to meet the health care needs of physicians (35). Furthermore, it is recognized that the health of physicians has a direct impact on the health of the population because numerous studies have established a link between the health behaviors of physicians and their interactions with patients (36).

Physicians have the privilege and responsibility to play a leading role in promoting healthy habits because they consult their patients daily, and studies confirm that physicians' lifestyle tips induce and maintain positive behavioral changes in patients. Physicians' life habits are of particular importance both because they influence the physician's own health and because these habits influence patient consultations, one of the strongest predictors of physicians offering lifestyle advice is the physician's own health behavior. Healthy practitioners are more likely to discuss preventive measures with their patients being credible, while the advice of physicians with unfavorable life habits is forgotten because they do not follow themselves (2).

Most of the researches in the health of the doctor focused on three areas: stress related to work and burning; mental health disorders such as depression and suicide; and substance abuse. In contrast, there was very little research into lifestyle and unhealthy eating habits among physicians. Since not all physicians become depressed or addicted to certain substances, but absolutely all face the challenge of incorporating healthy habits into their busy lives, there should be an increased focus on healthy daily life among physicians who might be able to help to prevent progression to serious health problems, including mental health problems and addiction (35).

Poor living habits among both physicians and the general population of Bahrain reflect rooted cultural patterns present in the population of the Middle East, which can be exaggerated among physicians due to excessive workload and long shifts. In terms of eating habits, less than half of the population eats all three main meals each day, with breakfast being the most commonly overlooked meal during an average week. More than half of the doctors (53.3%, N = 80) reported that they did not eat fast meals during the week, while one third (28.9%) eat fast food once a week. The average daily intake of water was 1.7 L, and carbonated soft drinks are consumed daily by about half of the physicians (47%). Most doctors (86.2%) consume at least one caffeinated beverage daily, and 47% of them followed a diet to reduce weight during the last 6 months, while 6.6% took medicines to help them helps to

lose weight (2). In another study, Canadian physician reports an average daily intake of 4.8 servings of fruits and vegetables, and over half of physicians consume at least five recommended servings (37). Doctors reported reasonably healthy behaviors in relation to alcohol consumption, smoking, diet and exercise. However, daily water consumption, weekly breakfast and weekly aerobic exercises were reduced (7).

Doctors, like the rest of the population, do not consistently meet the recommended recommendations for physical activity (35). In a study conducted in Bahrain, only one-third (29.6%) of physicians reported performing more than 30 minutes of continuous physical activity over a whole week. Of those who do weekly sports, the majority (81%) practiced physical activity for 3 days a week or less, the most frequent activities being walking and swimming. Physicians who were physically active during the week were less stressed and consumed less fat food during the week (2).

While statistics between physicians may simply reflect a larger cultural problem, there may be factors specific to medicines that discourage physical activity. A study of medical students showed that weekly physical exercises decrease during medical training, most likely due to increased workloads and long hospital changes during the clinical years (38). Increasing activity among physicians has the potential to benefit physicians, patients and the overall health care system. British Columbia's "Walk With Your Doc" initiative, involving over 100 physicians and 2000 patients in the province, is an excellent example of a simple program that encourages regular exercise for both physicians and patients and allows physicians to serve as healthy role models in their community (35).

In several European countries (such as Germany, Finland, Estonia) and in the USA, the prevalence of smoking among physicians is now less than 20%. In these countries, the population seems to be well informed about the dangers of smoking, and doctors seem to respond by giving up smoking more frequently than other people which leads to this significant drop in prevalence. An inverse model was found for alcohol consumption, Swiss doctors consuming alcohol more than the general population and only 4% were abstinent (compared to 22% in the general population) (39). Some authors suggest that the higher prevalence of alcohol use among physicians could be explained by their higher socio-economic status (40), while other studies claim that health institutions, medical schools and hospitals do not have effective health promotion programs for prevent alcohol use and abuse among physicians (41). Finally, higher alcohol consumption among physicians may be related to the fact that drinking a small amount of alcohol can protect against cardiovascular disease and is therefore not a risky behavior for health (42).

Sleep deprivation has been shown to affect clinician performance and cognitive scores (43). Although primary care physicians in Bahrain enjoy a more relaxed time frame than most other specialties, the average sleep time reported for physicians in our study is 5.9 hours, with most physicians (86.3%) who reports less than 8 hours of sleep per night. Doctors who sleep longer than 6 hours are less likely to be smokers and are more likely to eat breakfast most days of the week. On average, the level of perceived stress was 6.4/10, and women were significantly more likely to report severe stress levels than men (2). Among

Canadian general practitioners, half of them report difficulty sleeping and almost two thirds complain of exhaustion or drowsiness at least 3 days a week. Chronic sleep deprivation for physicians and residents is alarming, as it has been repeatedly correlated with decreased cognitive performance, increased likelihood of medical error, and higher cases of self-injury, such as prickling with a contaminated needle (44). Other studies have shown that moderate sleep deprivation - the equivalent of about 18 hours without sleep - may be more disabling than being legally drunk (6). Some research has begun to investigate ways to minimize sleep deprivation to the doctor. Nelson (45) focused on emergency department physicians, a group that is typically vulnerable because of work instead, and suggests scheduling models that would reduce sleep deprivation, allowing each doctor at least 4 hours of constant "anchor sleep" per night, a technique that has been shown to maintain a constant sleep and wake cycle and reduce fatigue (46).

The most commonly cited obstacle to following a healthy diet by physicians was the lack of time due to excessive workloads and frequent meeting at noon. Many doctors have mentioned the lack of access to healthy foods, especially those who work outside regular working hours (when the hospital cafeteria is closed, for example). Inadequate food storage, lack of healthy choices and lack of prioritization of physician well-being were also identified as problems (35).

Another study shows that many doctors have considered that their employer and work environment are not compatible with healthy eating. Doctors noticed this because of the lack of breaks, inadequate selection of food and the opening time of the canteen. Most physicians consider that a healthy diet has an important influence on their work performance. Respondents' dissatisfaction with the provision of healthy and vegetarian options suggests that the canteen delivery does not reflect the views of physicians or nutritional expectations. Most hospital canteens are shared by doctors and visitors to the hospital, providing hospital kitchens and catering services for patients. Therefore, poor delivery of canteens and dissatisfaction with canteen services may have a greater impact and relevance than for physicians (7).

Considering the importance of a healthy diet, all the doctors questioned in a study reported that inadequate nutrition had an impact on them at work. Most mentioned emotional symptoms, such as irritability and frustration. Many also mentioned physical symptoms (ease, tremor, nausea) and cognitive effects (difficulty concentrating, making slow / slow decisions) as consequences of inadequate diet. These findings are alarming, given the ubiquity of inadequate nutrition among physicians, which can lead to decreased patient care quality and medical errors (35).

Also, doctors may feel pressure from both patients and their colleagues to seem healthy, as their own physical health is considered an indicator of their medical competence (47). Studies named doctors "notoriously bad patients" considering that one third of Australian residents do not have a family doctor and a large proportion of young Irish doctors have not been in the spotlight for a doctor in the last 5 years which indicates that most physicians engage in self-treatment (48, 49). Furthermore, a significant number of physicians admit to self-prescribing drugs, a practice that is considered unethical by all medical

associations and has been banned by legislation in certain jurisdictions (50). Very often, doctors do not seek help, even though they may realize that they need it; a study showed that only 2% of Canadian physicians who were identified as depressed sought treatment. Therefore, all physicians should have their own family doctor who can provide regular, continuous and effective care; this would avoid the problem of self-prescribing and ensure that physicians take the recommended preventive screening measures (35).

Numerous studies have found that many medical staff are experiencing burnout, a syndrome resulting from work stress, characterized by emotional exhaustion, feelings of cynicism and detachment from patients (depersonalization) and a low sense of personal achievement (51-54).Workplace stressors deplete employees' mental and physical resources and lead to exhaustion and health problems. Thus, burnout was significantly positively associated with higher fast food intake, infrequent exercise, higher alcohol use, and more frequent use of painkillers, regardless of country of residence. Transnational comparisons showed significant differences in exhaustion and health behaviors, with health professionals in Turkey, Greece and Bulgaria reporting the most unfavorable experiences.

Behaviors and health risks among health professionals are important both in the context of the health and well-being of medical professionals, as well as factors contributing to medical errors and inadequate patient safety (55). Other studies found that the level of higher education, work experience and higher status decrease burnout, while working at night on shifts, difficulties in care of the children and in the accomplishment of the household tasks, health problems of the nurse or of their children, the economic difficulties and the difficulties encountered in transport, increases the level of burnout among medical doctors (56-59).

CONCLUSIONS

Studies show significantly higher rates of obesity, poor physical activity and other unfavorable life habits among physicians, which could have a negative impact on physician-patient interaction. A multitude of factors interact to influence the health of physicians in the workplace, including diet, workload, stress, mental health and lifestyle. Other studies show that many doctors do not consider the work environment conducive to healthy eating, which is due to the lack of breaks, the lack of selection of the canteen and the opening hours of the canteen. Let's not forget that medical students are a key demographic for interventions that promote health and well-being. A four-year intervention study showed that medical students who received curricular and extracurricular education in diet, exercise, alcohol and tobacco improved personal health practices compared to controls. Students were more likely to advise patients on healthy living habits.

It is necessary to continuously promote the health of the doctor, to avoid placing their patients' needs before their needs and to dispel the myth that doctors never get sick. The medical culture must move away from its highly competitive, individualistic focus, excessive workloads and extreme self-sacrifice and embrace changes that promote optimal physician well-being to improve patient care. But until such a change occurs, improving the physician's health will be an ongoing upward struggle.

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NEWS

GREAT STEP IN WIN THE BATTLE AGAINST CANCER: PTPN2 GENE

A group of researchers from the Blavatnik Institute at Harvard Medical have shown that disrupting a key immune regulator, a gene called PTPN2, boosts anti-tumor immunity and enables cancer clearance in animal models. The work, published in Nature Immunology, demonstrates that deleting the gene from the immune system of mice harboring cancer stimulates the production and fitness of cancer-and-infection-fighting immune cells known as T-killer cells. In one experiment, deleting the PTPN2 gene from the immune systems of mice eradicated colon cancer in all animals. In another, the approach combined with PD-1 checkpoint blockade successfully eliminated a particularly aggressive and treatment-resistant form of melanoma in one-fourth of the mice with this form of cancer. Taken together, these findings identify a promising new target for cancer therapy, but further testing of the approach will be needed (LaFleur MW, Nguyen TH, Coxe MA. *et al*. PTPN2 regulates the generation of exhausted $CD8^+$ T cell subpopulations and restrains tumor immunity. *Nat Immunol* 2019; 20: 1335-1347)

MINIMALLY INVASIVE BRIDGE INLAY-RETAINED - AN OPTION FOR FIXED DENTAL PROSTHESIS. CASE REPORT

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MINIMALLY INVASIVE BRIDGE INLAY-RETAINED - AN OPTION FOR FIXED DENTAL PROSTHESIS. CASE REPORT (Abstract): The minimally invasive bridges are considered a common substitute for conventional fixed prosthesis because require a minimal teeth preparation, protection of the pulp vitality and less periodontal irritation. Modern materials currently available on the domain - ceramic systems or zirconium dioxide-allow the realization of such prosthetic constructions, ensuring sufficient strength and longevity over time. The purpose of this article is to describe clinical procedures related to the placement of minimally invasive bridge inlay-retained with a zirconia framework, subsequently veneered with ceramic. Digital CAD-CAM technology greatly reduces working time and allows a high-precision prosthetic device. All these advantages make our therapeutic solution a viable option for restoring the continuity of the dental arch in the posterior area. **Keywords:** MINIMALLY INVASIVE BRIDGE, INLAYS.

Now days different indirect restorations used to replace a single missing tooth in the posterior area, such as traditional full-coverage fixed dental prostheses, implant-supported crowns, and inlay-retained fixed dental prosthesis are available in dentistry (1). The minimally invasive bridges are considered a common substitute for conventional fixed prosthesis, to avoid full crowns applied on the non-affected abutments. This treatment is an efficient method to provide satisfactory aesthetics with minimal loss of dental tissue. The design of these devices is a viable option for treating a single tooth edentation.

Currently, there is a wide range of materials and technologies to solve a reduced partial edentation in the posterior area of the dental arches. When implant therapy is contraindicated or is not possible to be applied, minimally invasive devices becomes an optimal treatment option, because allow a higher preservation of healthy dental structure and the protection of periodontal tissue. There are some advantages of this reconstruction compared to a fixed prosthetic bridge, as the minimal teeth preparation, the protection of the vitality of the dental pulp, less periodontal irritation. In order to ensure an optimal adaptation and a longevity to these prosthetic constructions, a careful evaluation of the clinical situation is necessary. Inlay-retained bridges are indicated for patients with good oral hygiene and low caries susceptibility, with a minimum coronal tooth height of 5

mm, and parallel abutments. Contraindications include extensive crown defects, tooth mobility, parafunctions, the absence of enamel on the preparation margins (2, 3).

Modern materials currently available on the market such as ceramic systems or zirconium dioxide, allow the realization of such prosthetic constructions, with sufficient strength and longevity over time. The precision of these minimally invasive bridges and the variability of the materials and technologies that can be used, make patients to accept them as a treatment solution for reduced edentulous situations (4).

The purpose of this article is to describe clinical procedures related to the placement of minimally invasive bridge inlay-retained with a zirconia framework, subsequently veneered with ceramic.

Prosthetic restorations made of zirconia have a low weight, very good mechanical strength, excellent biocompatibility, appropriate color, close to dental tissues, low thermal conductivity, smooth surface, which is not favorable to adhesion of biofilm. CAD-CAM technology greatly reduces working time and allows a high-precision prosthetic appliance. All these advantages make our therapeutic solution a viable option for restoring the continuity of the dental arch in the posterior area (5).

CASE REPORT

A 32 year old female patient came to the dental clinic with esthetic problems, consequently to second upper premolar loss. After the clinical (fig. 1) and complementary examinations because the patient refused the implant solution, we chose as therapeutic strategy a minimally invasive bridge with inlay crown on the abutment 1.4 and 1.6 and pontic for 1.5.

Fig. 1. Clinical examination of the patient

On the very first stage, a zirconia framework was made, in order to ensure the optimal resistance of the bridge; subsequently ceramic veneered, to restore the aesthetic appearance.

We prepared the inlay cavities- mesio-occlusal, and disto-occlusal, on the abutment teeth and the impressions was registered for the upper and lower arches (fig. 2).

Fig. 2. Upper and lower arches impression registration

The impressions were sent to the dental laboratory, and the technician poured the

working casts. These are properly prepared and introduced into the scanning room. The first step consisted in a highly accuracy optical impression, registered by scanning, in order to gain a digital model (fig. 3). The design of the framework was realized using the software- design component and analyzed from the point of view of the occlusion relationships and of the restoration of morphological elements.

Fig. 3. Working stone casts and scanning

The information were transmitted to the CAM component, in order to manufacture the framework, by milling the zirconia blocks (fig. 4). For the framework VITA YZ HT blanks were used, a solution for precise, efficient and true-to-shade zirconia reconstructions. The prosthetic piece was finished, sandblasted for 10 sec at a pressure of 2.7 bar, with 50μ alumina powder. Then the framework was checked on the model and into the oral cavity and because no adjustments were necessary, the dental technician did the ceramic veneering (fig. 5).

Fig. 4. Milling the framework

Fig. 5. Zirconia framework after finishing

The opperation started with an oxidation in order to clean the framework and to provide a good chemical and mechanical bond of the components. Then the layers for dentin and enamel were applied, and sintered in vacuum (fig. 6). For veneering we used Vita VM 9 (Vita), a highly-esthetic, fine-structure feldspar ceramic, that is perfectly adapted for zirconia framework.

After processing and checking on the working cast and in to the oral cavity, the superficial ceramic layer, the glaze, was applied and sintered in normal atmospheric

conditions (fig. 7).
The final bridge is sent to the dental office for the final adaptation and cementation in to the oral cavity (fig. 8).

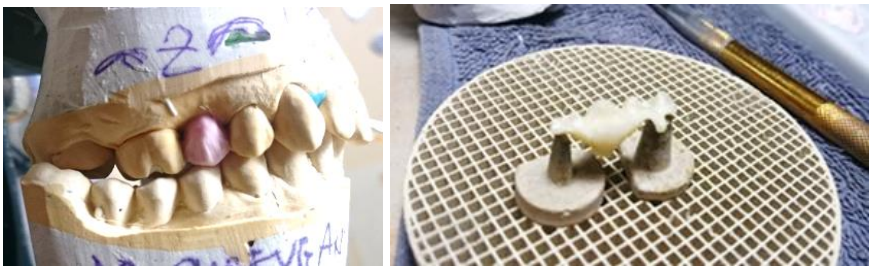

Fig. 6. Application and sintering of ceramic layers

Fig. 7. Checking ang glaze application

Fig. 8. Final adaptation and cementation in to the oral cavity

DISCUSSION

Minimally invasive bridges inlay-retained are considered a conservative therapeutic option, because the use of inlays as retainer element on the abutment teeth allows a minimum sacrifice of dental tissue. Preserving tooth structure is adequate to the overall health of the tooth and periodontal tissues. The use of minimally invasive bonded restoration induce less trauma and superior prognosis (5, 6) In the last years, the demand for esthetics and biocompatibility led to the use of zirconia CAD/CAM materials for fixed prosthodontics (7). New materials, with a very good mechanical behavior, currently available, allow the use of this type of prosthetic restoration on premolars and molars, in mesio-occlusal and disto-occlusal cavities. The realization of these bridges using CAD-CAM technologies has many advantages: the registration of a digital im-

pression and ordering the restoration online, quickly and easily maximum precision for all results combined with easy handling, extremely wide range of applications and material versatility, high flexibility and future security due to open CAD/CAM interfaces, a diversity of possibilities of integration. Minimizing the treatment time and the number of appointments is favorable for the patient, and also precludes the complications and it is possible to achieve the preferred treatment outcomes with expedition of the whole procedure. Another advantage is represented by the leading of an excellent intraoral fit of the prosthetic appliance (8).

CONCLUSIONS

The technique presented in this case report describes a conservative method of rehabilitation of a single-tooth edentation, as an alternative to an implant-supported crown. The mechanical and aesthetic advantages of this type of restoration make our treatment alternative an extremely viable option. Briefly, the advantages of a minimally invasive bridge are conservative, highly aesthetic, strong bonded to teeth support, no discoloration of dental tissues, insulation of the teeth, good suited for large cavities, long lasting (9, 10). Surveying the mechanical properties of materials, their behavior over time, the advantages and disadvantages of the techniques, the causes that lead to failures, as well as the requirements of each patient, we can establish the optimal therapeutic solution for each clinical case.

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DIAGNOSTIC VALUE OF A NEGATIVE SINGLE COLOR DUPLEX ULTRASOUND IN DEEP VEIN THROMBOSIS SUSPICION DURING PREGNANCY

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DIAGNOSTIC VALUE OF A NEGATIVE SINGLE COLOR DUPLEX ULTRASOUND IN DEEP VEIN THROMBOSIS SUSPICION DURING PREGNANCY (Abstract): Objective: to determine whether a negative single complete ultrasound (US) of the lower limbs veins is a safe and accurate diagnostic method to rule out deep venous thrombosis (DVT) in pregnancy. **Material and methods**: We performed a prospective study on 87 consecutive pregnant women with clinical suspicion of DVT in "Dr. D. Popescu" Maternity, Timişoara, between January 2007 and December 2009. All cases were investigated by color duplex ultrasound. Those who were negative but with high clinical probability of DVT underwent MR venography, the rest with negative US findings did not received anticoagulant therapy and US was repeated after seven days. All were instructed about the signs and symptoms of DVT and asked to return at six weeks postpartum when they were questioned if presented any. **Results**: from the 87 women 30 (34.48%) were found positive and two (2.29%) had a high clinical suspicion of DVT. 55 (63.21%) with negative findings repeated the US exam after 7 days which remained negative. Only 43 (78.18%) returned for the 6 weeks postpartum visit and none of them experienced any thromboembolic event during follow-up. **Conclusions**: A single complete color duplex ultrasonography of lower limbs veins appears to be a safe method to rule out the diagnosis of deep venous thrombosis in pregnant women and if negative is safe to withhold heparin therapy. **Key words**: DEEP VEIN THROMBOSIS, PREGNANCY, VENOUS ULTRASOUND

BIOPHARMACEUTICAL CHARACTERISTICS AND *IN VITRO* EVALUATION OF THE REDUCING ANTIOXIDANT POWER OF SOME DICLOFENAC DERIVATIVES

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BIOPHARMACEUTICAL CHARACTERISTICS AND *IN VITRO* EVALUATION OF THE REDUCING ANTIOXIDANT POWER OF SOME DICLOFENAC DERIVATIVES (Abstract): **Aim**: Biopharmaceutical characterization using the prediction program MedChemDesigner 5.5 and the evaluation of the reducing antioxidant power of some diclofenac derivatives. **Material and methods**: The synthesized compounds (4a-s) were characterized *in silico*, by estimation of octanol/water partition coefficient (OWPC), octanol/water distribution coefficient (OWDC), absorptive capacity at digestive level, and topological polar surface area. The evaluation of antioxidant power was performed using *in vitro* methods: ferric and phosphomolybdenum reducing antioxidant power assays. **Results**: The biopharmaceutical characteristics suggest an optimal pharmacokinetic profile with a lipophilic character of the studied derivatives, which allows both the crossing of cell membranes and digestive tract absorption. In addition, several compounds have demonstrated notable antioxidant capacity, higher than that of diclofenac. The most active compounds were 4s (R=2-Br-3-OH-4-OCH_3) and 4h (R=H). **Conclusions**: The biopharmaceutical study revealed promising characteristics regarding the pharmacokinetic profile of the studied compounds. Moreover, it was noticed that the antioxidant effect increases with concentration and is influenced by the nature of the substituent in the aromatic ring. **Keywords**: BIOPHARMACEUTICAL CHARACTERIZATION, HYDRAZONES, ANTI-OXIDANT CAPACITY.

Lipophilicity is an important parameter in the design of drugs, having a relevant role in the pharmacokinetic profile, including distribution, transport and metabolism of the drugs. If lipophilicity is increased, meaning that the compounds are hydrophobic, they can accumulate in the body and cause possible side effects (1, 2). The lipophilic character of the compounds is assessed using the partition and distribution coefficients in two immiscible phases: water (hydrophilic phase) and n-octanol (hydrophobic phase), which mimics the biological membranes (3-5).

OWPC (log P) is the ratio of concentration of a compound in a mixture of two solvents, octanol and water. If OWPC is higher than 5, the compound is considered to be highly lipophilic, meaning an increased diffusion through the cell membranes, but a reduced intestinal absorption rate due to the lack of solubility in water.

An ideal value of the partition coefficient is considered to be in the range of 0-5. The partition coefficient generally refers to the concentration ratio of un-ionized species of the compound, whereas the distribution coefficient refers to the concentration ratio of all species of the compound (ionized and un-ionized). Compounds that do not undergo an ionization process have a log D (OWDC) value equal to OWPC (6, 7).

MATERIAL AND METHODS

Biopharmaceutical characterization

The diclofenac derivatives with hydrazone structure (**4a-s**) synthesized by our research group (8,9) were characterized *in silico*, using the MedChem Designer™ program by the estimation of OWPC (log P), OWDC (log D), oral absorptive capacity by checking the Lipinski's rule, molar mass (MM), number of acceptor groups and proton donors (M_NO), topological polar surface area (T_PSA), and the number of proton donor groups (HBDH).

Antioxidant assays

Ferric Reducing Antioxidant Power (FRAP) Assay

Stock solutions of 10 mg/mL were prepared by dissolving the tested compounds (**4a-s**) in DMSO. From the stock solution different volumes were measured and DMSO was added up to 0.5 mL to obtain different concentrations (0.045 mg/mL, 0.09 mg/mL, 0.18 mg/mL, and 0.36 mg/mL). To 0.5 mL of sample of each concentration, 0.5 mL of 0.2 M phosphate buffer (pH=6.6) was added. The reaction was initiated by adding 0.5 mL of 1% potassium ferricyanide, after which the samples were incubated at 50°C for 20 min in a water bath and then the reaction was stopped by adding of 0.5 mL of 10% trichloroacetic acid. To 1 mL of this solution, 1 mL double-distilled water and 0.2 mL of 0.1% ferric chloride were added. The resulting mixture was stirred for homogenization, left at room temperature for 5 min, and then the absorbance was measured at 700 nm using a control sample which was prepared similarly to the tested compound sample, using 0.5 mL of DMSO instead of 0.5 mL of tested compound sample (10,11). For each tested compound sample, the effective concentration (EC_{50}) was calculated by linear regression analysis. Diclofenac (0.045 mg/mL, 0.09 mg/mL, 0.18 mg/mL, 0.36 mg/mL) and ascorbic acid (AA) (0.015 mg/mL, 0.030 mg/mL, 0.045 mg/mL) were used as reference and positive control, respectively. All tests were performed in triplicate.

Phosphomolybdenum Reducing Antioxidant Power (PRAP) Assay

Stock solutions of 7.5 mg/mL were prepared by dissolving the tested compounds (**4a-s**) in DMSO. From stock solution, different volumes were measured and DMSO was added up to 200 µL in order to obtain different concentrations (0.09 mg/mL, 0.18 mg/mL, 0.37 mg/mL, and 0.75 mg/mL). To samples of different concentration, 2 mL of reagent solution (0.6 M sulfuric acid, 28 mM disodium phosphate and 4 mM ammonium molybdate) were added, and the solutions were incubated at 95°C for 90 minutes; after cooling to room temperature the absorbance of each sample was measured at 695 nm using a control sample prepared from 200 µL DMSO and 2 mL reagent solution (12, 13). For each tested compound sample, the effective concentration (EC_{50}) was calculated by linear regression analysis. Diclofenac (0.09 mg/mL, 0.18 mg/mL, 0.37 mg/mL and 0.75 mg/mL) and ascorbic acid (AA) (0.022 mg/mL, 0.045 mg/mL, 0.09 mg/mL) for were used as reference and positive control, respectively. All tests were

performed in triplicate.

RESULTS AND DISCUSSION

Biopharmaceutical characterization

The biopharmaceutical characteristics of diclofenac derivatives with hydrazone structure (**4a-s**) such as OWPC, S+log P, S+log D, oral absorptive capacity by checking Lipinski's rule, MM, M_NO, T_PSA and HBDB were predicted using the MedChemDesigner™ program. All these data are shown in first table.

TABLE I

Biopharmaceutical characteristics of diclofenac derivatives with hydrazone structure (4a-s)

Comp.	R	OWPC	S+ log P	S+ log D	Unsatisfied conditions of Lipinski's Rule	MM (g/mol)	M_NO	T_PSA	HBDB
4a	2-NO_2	4.290	3.788	3.776	0	442.06	6	87.28	1
4b	4-CN	3.913	3.992	3.988	0	423.30	4	65.25	1
4c	3-NO_2	4.290	3.853	3.848	0	442.06	6	87.28	1
4d	3-OCH_2CH_3-4-OH	3.945	4.378	4.360	0	458.34	5	70.92	2
4e	2-Br-4-NO_2	4.644	4.203	4.188	MM	522.18	6	87.28	1
4f	2-Cl-5-CF_3	5.369	5.187	5.176	log P, MM	500.73	3	41.46	1
4g	4-OCH_3	3.989	4.247	4.244	0	428.31	4	50.69	1
4h	-H	4.291	4.167	4.165	0	398.29	3	41.46	1
4i	4-CH_3	4.529	4.560	4.558	0	412.31	3	41.46	1
4j	3-OCF_3	5.143	4.778	4.773	log P	466.28	3	41.46	1
4k	3,4-diF	4.797	4.493	4.487	0	434.27	3	41.46	1
4l	3-Br-4-OH	4.106	4.564	4.331	0	493.18	4	61.69	2
4m	2,5-diBr	5.257	5.180	5.172	log P, MM	556.08	3	41.46	1
4n	2-Br-4-F	5.029	4.829	4.823	log P	495.17	3	41.46	1
4o	4-Br-2-F	5.029	4.804	4.799	log P	495.17	3	41.46	1
4p	3-F-4-CH_3	4.914	4.706	4.703	0	430.30	3	41.46	1
4q	4-F	4.679	4.405	4.401	0	416.28	3	41.46	1
4r	3-OCH_3-4-CH_3	4.221	4.490	4.488	0	442.34	4	50.69	1
4s	2-Br-3-OH-4-OCH_3	4.059	4.640	4.517	MM	523.21	5	70.92	2

Lipinski's rule is commonly used to predict the degree of digestive absorption of an active substance. This rule, known as "rule of five", imposes substances some limitations in order to be orally administered. In this respect, for a maximum oral absorption and bioavailability, the molecule compound has to meet the following conditions: OWPC value less than 5, molar mass less than 500 g/mol, HBDH less than 5, number M_NO less than 10. The T_PSA is calculated by summing the surfaces corresponding to the polar fragments of a compound. This value highlights the polar nature of a substance and, implicitly, the oral bioavailability. A T_PSA over 140 indicates reduced digestive absorption, while less than 60 shows absorption in the

digestive tract of more than 90% (14-16). The analysis of the obtained results (tab. I) revealed that the substitution of the aromatic ring with the radicals: *methoxy* (**4g**), *cyano* (**4b**), *methyl* (**4i**, **4r**) and *hydroxyl* (**4d**) results in increased cell membrane crossing. For these compounds, the OWPC and OWDC values ranged between 3.913 and 4.529. In case of *halogen* in the aromatic ring, the OWPC and OWDC values were higher than 5, meaning that the absorption rate was negatively influenced.

Antioxidant assays *Ferric Reducing Antioxidant Power (FRAP) Assay*

The results recorded for diclofenac derivatives (**4a-s**), expressed as absorbance values at different concentrations (0.045 mg/mL, 0.09 mg/mL, 0.18 mg/mL, 0.36 mg/mL), are shown in first figure.

Based on the absorbance values recorded at different concentrations, the EC_{50} for each compound was determined (tab. II), a lower EC_{50} value indicating a higher reducing power.

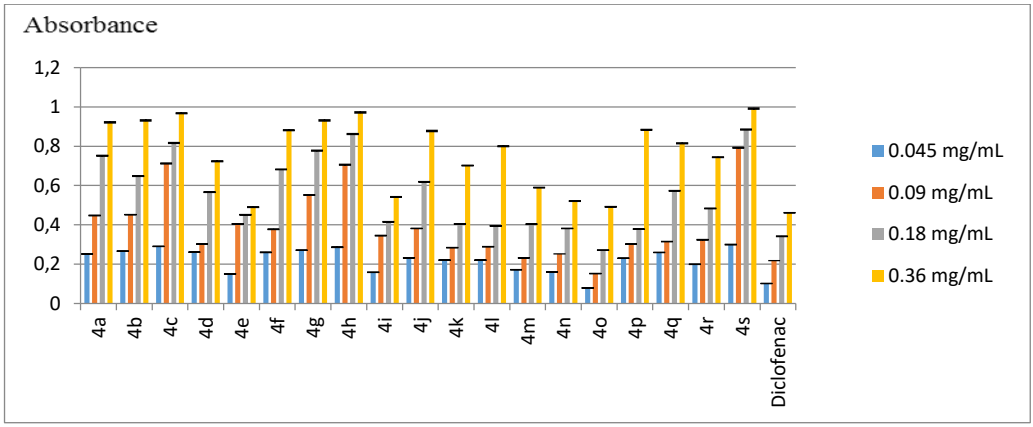

Fig 1. Absorbance values of diclofenac derivatives with hydrazone structure (4a-s) at different concentrations

TABLE II

The ferric reducing antioxidant power (EC_{50}, mg/mL) of diclofenac derivatives with hydrazone structure (4a-s)

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
Diclofenac	0.4351 ± 0.036	**4g**	0.0831 ± 0.005	**4n**	0.3460 ± 0.027
4a	0.1079 ± 0.008	**4h**	0.0687 ± 0.006	**4o**	0.3702 ± 0.028
4b	0.1157 ± 0.010	**4i**	0.3122 ± 0.027	**4p**	0.2211 ± 0.020
4c	0.0565 ± 0.005	**4j**	0.1378 ± 0.009	**4q**	0.1560 ± 0.011
4d	0.1607 ± 0.007	**4k**	0.1971 ± 0.013	**4r**	0.2082 ± 0.017
4e	0.3783 ± 0.024	**4l**	0.2209 ± 0.021	**4s**	0.0540 ± 0.008
4f	0.1284 ± 0.011	**4m**	0.3055 ± 0.027	**AA**	0.0162 ± 0.005

AA = ascorbic acid

It was observed that all tested compounds were more active than diclofenac but less than AA. The most active compounds were **4s**, (R=2-Br-3-OH-4-OCH_3, EC_{50}=0.054±0.008)

and **4c** (R=3-NO_2, EC_{50}=0.0565± 0.005), which were 8 and 7.7 times, respectively more active than diclofenac (EC_{50}=0.4351±0.036). An appreciable activity was also shown by **4h** (R=H, EC_{50}=0.0687±0.006), **4g** (R=4-OCH_3, EC_{50}=0.0831±0.005) and **4a** (R=2-NO_2, EC_{50}=0.1079±0.008) which were 6.30 times, 5.20 times and 4 times, respectively, more active than diclofenac.

Phosphomolybdenum Reducing Antioxidant Power (PRAP) Assay

The results recorded for diclofenac derivatives (**4a-s**), expressed as absorbance values at different concentrations (0.09 mg/mL, 0.18 mg/mL, 0.37 mg/mL and 0.75 mg/mL) are shown in figure 2.

Based on absorbance values recorded at different concentrations, the EC_{50} for each compound was determined (tab. III), a lower of EC_{50} value indicating a higher reducing power.

It was observed that most of the tested compounds were more active than diclofenac but less than AA. The most active compounds were **4h** (R=H, EC_{50}=0.0954 ±0.010), **4j** (R=3-CF_3, EC_{50}=0.0987±0.007), **4g** (R=4-OCH_3, EC_{50}=0.0988±0.009) and **4b** (R=4-CN, EC_{50}=0.1008±0.007), which are approximately 5 times more active than diclofenac (EC_{50}=0.4694±0.018). An appreciable activity was also shown by **4f** (R=2-Cl-5-CF_3, EC_{50}=0.1041±0.004) and **4e** (R=4-Br-2-NO_2, EC_{50}=0.1096±0.007) which were approximately 4.5 times more active than diclofenac.

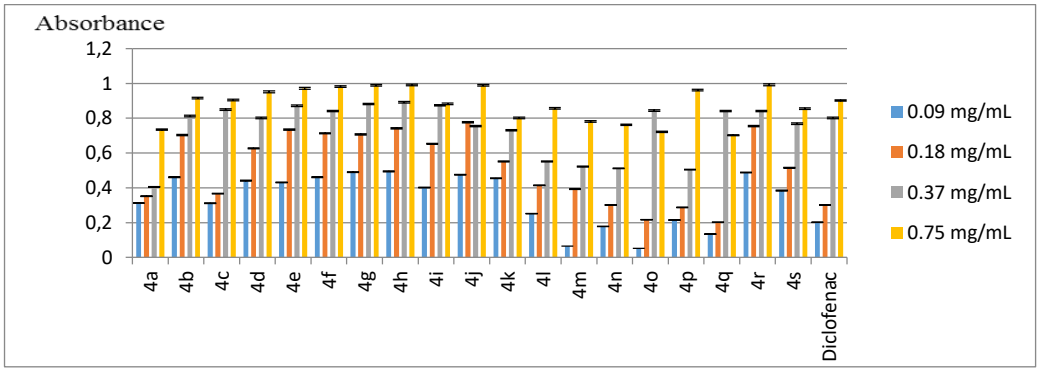

Fig 2. Absorbance values of diclofenac derivatives with hydrazone structure (4a-s) at different concentrations

TABLE III

The phosphomolybdenum reducing antioxidant power (EC_{50}, mg/mL) of diclofenac derivatives with hydrazone structure (4a-s)

Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL	Compound	EC_{50} mg/mL
Diclofenac	0.4694 ± 0.018	**4g**	0.0988 ± 0.009	**4n**	0.7173 ± 0.014
4a	0.4064 ± 0.025	**4h**	0.0954 ± 0.010	**4o**	0.7378 ± 0.018
4b	0.1008 ± 0.007	**4i**	0.1315 ± 0.012	**4p**	0.4441 ± 0.017
4c	0.2389 ± 0.014	**4j**	0.0987 ± 0.007	**4q**	0.2734 ± 0.021
4d	0.1245 ± 0.009	**4k**	0.2743 ± 0.026	**4r**	0.1930 ± 0.017
4e	0.1096 ± 0.007	**4l**	0.4024 ± 0.014	**4s**	0.3503 ± 0.023
4f	0.1041 ± 0.004	**4m**	0.6329 ± 0.024	**AA**	0.0242 ± 0.001

AA = ascorbic acid

CONCLUSIONS

In this paper we have presented the biopharmaceutical characteristics of some diclofenac derivatives with hydrazone structure (**4a-s**) were predicted using *in silico* computerized methods. Different parameters such as the OWPC, OWDC, the level of digestive tract absorption, and the topological area of the polar surface were determined. Based on the obtained results it can be concluded that the condensation of diclofenac hydrazide with different aromatic aldehydes is associated with increased lipophilic character of the synthesized derivatives, which is beneficial for diffusion through the lipid membranes, and indicates a good pharmacokinetic profile.

The antioxidant power of diclofenac derivatives was assessed using the ferric and phosphomolybdenum reducing antioxidant power assays. The results highlighted that the most active compounds were **4s** (R=2-Br-3-OH-4-OCH_3) and **4h** (R=H), obtained through the condensation of diclofenac hydrazide with 2-bromo-3-hydroxy-4-methoxybenzaldehyde and benzaldehyde, respectively.

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AN ATTENUATED TOTAL REFLECTANCE STUDY OF DIFFERENT ALGINATE IMPRESSION MATERIALS USED IN DENTAL MEDICINE

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AN ATTENUATED TOTAL REFLECTANCE STUDY OF DIFFERENT ALGINATE IMPRESSION MATERIALS USED IN DENTAL MEDICINE (Abstract): The aim of this study is to characterize and compare different alginate impression materials used in dental medicine. For this, we have prepared three different commercially available impression materials which have been characterized through the FTIR-ATR technique, thus highlighting rapidly and easily functionalized groups contained in these materials. This is possible as consequence of the absorption of electromagnetic radiation at certain frequencies that are associated with the vibrations specific to each type of chemical bond existing in the analyzed molecule. The materials used for this study were three impression materials commercially available (Kromopan and Alginor-ortho from Lascod, and Tropicalgin from Zhermack). For the spectral analysis a Bruker Vertex 70 spectrometer (Bruker Optics, Ettlingen, Germany) was used, and the spectra were obtained in the ATR (Attenuated Total Reflectance) mode, at room temperature in the range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1}. Spectral ranges results are shown for each material, as well as the deconvoluted spectra for the most representative ranges. The alginate composition for each of the materials has been calculated and based on the obtained results we can indicate the use of these materials for the impression step in indirect restorations, as study models and casts and for fabrication of primary casts.
Keywords: ALGINATE IMPRESSION MATERIALS, DENTISTRY, FTIR-ATR STUDY.

In dental restoration, impression taking represents the first step of the process by creating a form in which working analogues can be obtained, thus helping the clinician undergo diagnosis and part of the treatment (1).

The field of dental medicine is in a constant improvement mode as technology and characterization methods have progressed over the last years. The performance of a dental material still relies on its properties and qualities, a fact that makes knowing these characteristics a particularly important step in selecting and using such a material (2, 3).

Alginate impression materials are irreversible hydrocolloids commonly used in impression taking which change from a sol to a gel state without being able to reverse back to the sol state and are usually constituted of sodium and potassium salts of alginic acid (4, 5). Alginic acid is an anion-

ic polysaccharide that can be extracted from brown algae of the *Phaeophyceae* family (6). The setting phase can be controlled through temperature and water usage, parameters easy to work with (7). These materials are recommended as impression materials by their easy flow, minimal displacement, and swift setting time (8).

Alginate materials poses the quality of being hydrophilic which makes them able to take impressions even in the presence of saliva or blood (9). Thus, alginate impression materials are used for prosthodontic reasons in the fabrication of primary casts, for indirect restorations as final impression materials and for orthodontic reasons as study models and casts as duplication materials (10).

Specific frequencies of light correspond to the specific molecules' structure, this is the principle that is used in the spectral analysis of the Infrared (IR) studies. Attenuated total reflection (ATR) spectroscopy is the sampling technique that along with the IR property allows the direct examination, without elaborate preparation, of samples in solid or liquid state. ATR-FTIR, or attenuated total reflectance used with the Fourier transform IR spectrometers allows the study of materials that do not have the property of being transparent (11). This represents a very big advantage in the characterization of imprinting dental materials as such materials do not always have the transparency property, alginate materials being an example, and is a very useful tool to determine the functional groups as well as characteristic spectrum of substances contained in such a material that can be recognized and identified. Also, as water solutions (or water itself) are used as compounds for different biomaterials and impression materials are among these materials, the ATR technique is the relevant one and a development from FTIR alone since infrared spectroscopy and water are not compatible (12).

MATERIALS AND METHODS

Lascod's Kromopan and Alginor-ortho products are alginates, elastic impression materials with dimensional stability, promising high accuracy and quick setting time. For the easy use of these materials, the alginates have a chromatic indicator of phase, helping the impression work in convenient environmental conditions of temperature and wettability (13).

Tropicalgin is an alginate impression material, from Zhermack, also having the chromatic phase indicator, promising easy-working procedures and good thixotropy properties. Its main distinctive characteristic recommends it as 'user-friendly' with a fast type of setting (14). This material is used in the dental practice as an impression system in the preliminary impression stage as a universal alginate. In the first table are centralized some of the important characteristics for these materials for efficient comparison, properties that were given by the producers (13, 14).

The three impression materials were prepared accordingly to the producers instructions, mixing the powder with a liquid phase (distilled water), in the indicated proportions, first figure showing the obtained products after hardening.

As method of characterization, Attenuated Total Reflectance (ATR) mode of an Infrared (IR) Bruker Vertex 70 spectrometer (Bruker Optics, Ettlingen, Germany) was used, and the measurements were carried out at room temperature with a 4 cm^{-1} resolution and in the 4000-600 cm^{-1}

range. Figure 2 illustrates the ATR mode of the spectrometer with a probe.

The obtained spectra were shown by the *OPUS 6.5 software* connected to the device.

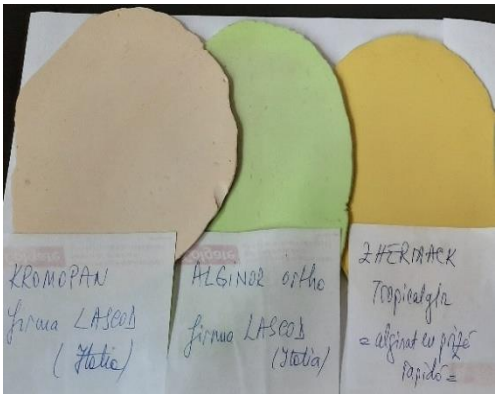

Fig. 1. The three commercially available impression materials used, after hardening

Fig. 2. The ATR mode of an IR spectrometer

TABLE I

Given characteristics of materials (12, 13)

Material	Working time (min: sec)	Biological contact time (min: sec)	Accuracy (μm)	Compressive strength resistance	Elastic recovery
Kromopan	1'45''	at least 30''	20	95.5%	95.5%
Alginor-ortho	1'45''	at least 30''	20	95.5%	95.5%
Tropicalgin	1'35''	1'	high	1.4MPa	99%

RESULTS AND DISCUSSION

ATR-FTIR analysis of the alginate (ALGINOR-ortho, KROMOPAN, TROPICALGIN) samples revealed the characteristic bands in the range 3800-3000 cm^{-1} assigned to –OH intramolecular and intermolecular stretches modes from alginate-based derivatives mixtures.

The most representative bands can be observed in the range 1800-600 cm^{-1}. The number and position of the peaks in this spectral region have been calculated by the second derivative of the spectra.

Based on the obtained maxima, the spectra have been deconvoluted by a curve-fitting method and the areas were calculated with a 50% Lorentzian and 50% Gaussian function by using OPUS 6.5 software. The procedure led to a best fit of the original curve with an error of less than 0.003. The specific peaks and area are presented in the second table.

In the 1800-1460 cm^{-1} spectral range the stretching vibrations assigned to the asymmetric carboxylate bands can be observed at 1636 cm^{-1} (ALGINOR), 1638 cm^{-1} (KROMOPAN) and 1641cm^{-1} (TROPICALGIN), while the symmetric stretching vibrations can be seen at 1416 cm^{-1}.

Based on the ration between area corresponding to the symmetric and asymmetric carboxylate stretches one can say that a lower content in alginate can be found in TROPICALGIN (fig. 3).

TABLE II

The peaks and the specific area calculated by curve-fitting method based on the FTIR spectra of alginate derivatives

Sample	*Spectral range*			
ALGINOR -ORTHO	**1800-1460 cm-1**	**area**	**1460-1180 cm-1**	**area**
	1746	1.05	1436	0.93
	1676	11.95	1416	1.16
	1636	25.88	1397	0.88
	1589	7.5	1358	0.13
	1543	5.01	1328	0.62
	1489	1.55	1284	0.19
			1244	0.09
			1204	0.35
	Spectral range			
	1180-800 cm-1	**area**	**800-600 cm-1**	**area**
	1122	6.55	787	2.22
	1077	17.68	768	2.47
	1032	0.63	734	3.14
	994	0.94	712	2.09
	944	0.37	688	3.82
	903	0.21	653	0.61
	875	0.11		
KROMOPAN	**Spectral range**			
	1800-1460 cm-1	**area**	**1460-1269 cm-1**	**area**
	1676	12.23	1427	0.54
	1638	26.48	1416	1.19
	1595	12.51	1402	0.37
			1332	0.05
			1316	0.07
			1284	0.01
	Spectral range			
	1269-860 cm-1	**area**	**860-600 cm-1**	**area**
	1202	1.15	790	4.09
	1164	1.94	770	4.07
	1114	22.44	724	6.09
	1068	33.75	679	2.96
	1029	4.86	618	0.79
	1006	4.41		
	938	0.32		
	900	0.22		

Sample	*Spectral range*			
TROPICALGIN				
	1800-1450 cm-1	**area**	**1450-1170 cm-1**	**area**
	1671	8.55	1443	0.04
	1641	20.88	1415	0.04
	1562	3.36	1397	0.17
	1475	0.36	1354	0.09
			1326	0.18
			1282	0.51
			1252	0.33
			1219	0.08
			1195	0.17
	Spectral range			
	1170-940 cm-1	**area**	**940-600 cm-1**	**area**
	1131	0.59	902	0.29
	1102	3.78	854	2.07
	1069	0.41	825	3.3
	1028	0.13	786	8.96
	991	0.16	735	11.32
	951	0.11	688	5.92
			656	1.59
			641	0.84

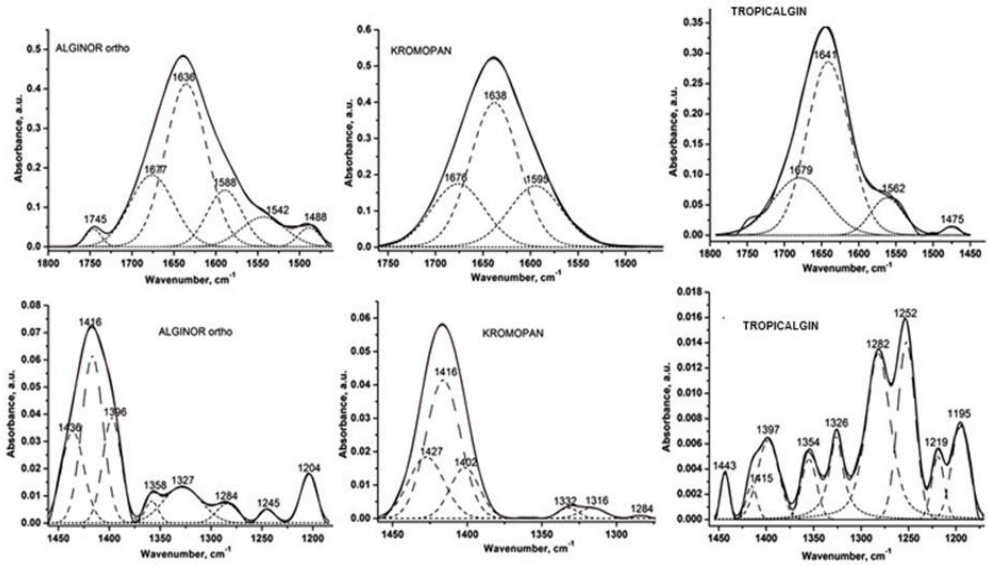

Fig. 3. ATR-FTIR deconvoluted spectra in the 1800-1170 cm^{-1} spectral range

The other bands in this spectral region are assigned to the alginic acid fragments (1677 cm^{-1}) and water (OH deformation vibrations conjugated with the C-O groups from carboxylate) at 1588, 1595 and 1562 cm^{-1}. In the 1470-1170 cm^{-1} spectral range a number of bands assigned to the different vibration modes of the C-H groups can be observed: C-H bending (1443-1427 cm^{-1}), C-H C-H bending (1399-1354 cm^{-1}) and C-H wagging (1326 cm^{-1}) (fig.3).

The bands at 1332-1200 cm^{-1} are characteristic for the asymmetric stretching vibrations of the sulphate groups.

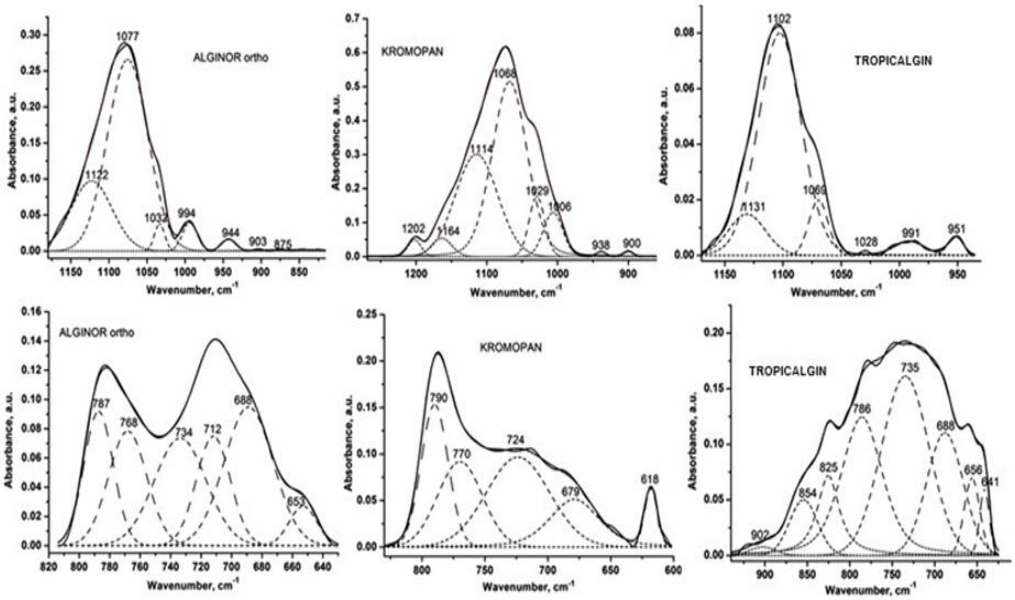

Fig. 4. ATR-FTIR deconvoluted spectra in the 1170-600 cm^{-1} spectral range

The strong bands of the ionic sulphates appeared in the spectral range 1200-1080 cm^{-1} (the symmetric stretching vibrations).

Based on the ratio between the symmetric and asymmetric stretches of the sulphate groups one can conclude that the higher content in sulphates is present in TROPICALGIN sample. The same sample presents the most bands in the 900-600 cm^{-1} spectral range, which are specific for the sulphate ion. The polysaccharide rings can be observed by the bands at 1077-1030 and 790-786 cm^{-1} (fig. 4).

CONCLUSIONS

Even through hydrocolloids were the first elastic materials used in the medical dental field, alginate impression materials are still used nowadays having multiple advantages.

Three alginate commercially available impression materials (Kromopan and Alginor-ortho from Lascod, and Tropicalgin from Zhermack) have been prepared as instructed by the producers and studied through Attenuated Total Reflectance-Fourier Transformed Infrared spectroscopy.

For the three alginate impression materials tested in our study we can say that based on the ratio between the area corresponding to the bands 1077/1030 and the area corresponding to the band at 787 cm^{-1},

one can determine the alginate composition, respectively the glucuronic acid and mannuronic acid contents.

Thus, the calculated glucuronic and mannuronic acids in ALGINOR sample is 92.66% and 7.33%, respectively for KROMOPAN sample is 62.91% and 37.08% and for TROPICALGIN is 75.93 % and 24.07%. In this case, the three alginate materials exhibit the appropriate properties for being used for the impression step in indirect restorations, as study models and casts as duplication materials, and for fabrication of primary casts.

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PSYCHO-EMOTIONAL FACTORS AND CRANIOMANDIBULAR DISORDER SEVERITY

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PSYCHO-EMOTIONAL FACTORS AND CRANIOMANDIBULAR DISORDER SEVERITY (Abstract): Craniomandibular disorders (CMD) are a series of clinical dysfunctions on the level of masticatory muscles, temporomandibular joint (TMJ) or both. The symptomatology is represented by: muscle pain in the head and neck region, noise and pain in the temporomandibular joint, limitation of the mandibular mobility. This study aims to analyze the interrelation between psycho-emotional status and the severity of cranio-mandibular disorder (CMD) in patients from NE Romania. **Materials and methods:** We conducted a cross-sectional study in a cohort of 106 consecutive volunteer patients who attended private praxis in Iasi during an eight-month period (October 2023- March 2024). Before the clinical evaluation, patients were required to answer a personalized merged version of the Temporomandibular Disorder-Pain Screener (TMD-Pain Screener) (ver. October 11, 2013) and Fonseca Anamnestic Index questionnaire used for screening of CMD. After this step the patients filled in the Patient Health Questionnaire-9 (PHQ-9) (ver. May 12, 2013), which consists of 9 closed-ended questions and is designed for screening, diagnosing, monitoring, measuring the severity of depression. This questionnaire is completed by the patient and can also be administered repeatedly, to reflect improvement or worsening of depression in response to treatment. **Results:** The analysis of the scores recorded by patients on the PHQ-9 questionnaire, revealed that most of subjects (85.9%) have minimal (43 subjects) or mild levels of depression (48 subjects), in almost equal proportions (p=0.183). Only 11.3% of patients were registered with moderate depression, while 2.8% of patients (3 cases) were classified as having moderately-to-severe depression. We noticed that the severity of depression increases among subjects with a demanding profession (economist, commerce, IT). **Conclusions:** The severity of CMDs is more common in women and at the young age. Stress has been established as one of the most significant risk factor for TMJ disorders apart from parafunctional habits (clenching and grinding) and pathologic occlusion. **Keywords:** CRANIOMANDIBULAR DISORDER, PSYCHOSOCIAL FACTORS, TEMPOROMANDIBULAR JOINT, STRESS.

Craniomandibular disorders (CMD) are a series of clinical dysfunctions on the level of masticatory muscles, temporomandibular joint (TMJ) or both; there is an important influence for other muscle-articular structures of the head and neck and for the postural stance (1, 2).

The symptomatology of CMD is widespread in adult population and an average value of 44% against 30% of the latter (1), but they are not just found in adult life, but also in children and teenagers.

The main symptomatology is muscle pain in the head and neck region, noise and pain in the temporomandibular joint, limitation of the mandibular mobility; and also, symptoms like: headache, facial and posterior neck pain, dental pain, tinnitus dysgeusia are present.

CMD has multifactorial etiology. The pain is determined by a complex interaction between mechanical, biological, cognitive, and psycho-emotional factors, also there are predisposing, instigating and perpetuating factors (3, 4).

The predisposing factors include 3 main categories: morpho-structural alterations (anatomical alterations), psycho-emotional alterations (stress, anxiety) and pathophysiological alterations (parafunctions).

The instigating factors include micro-traumas and macro-traumas. Perpetuating factors are represented by the psycho-emotional dimension of the patient (emotional and social factors) (3-5).

This study aims to analyze the interrelation between psycho-emotional status and the severity of cranio-mandibular disorder (CMD) in patients from NE Romania.

MATERIALS AND METHODS

We conducted a cross-sectional study in a cohort of 106 consecutive volunteer patients who attended private praxis in Iasi during an eight-month period (October 2023- March 2024). Before the clinical evaluation, patients were required to answer a personalized merged version of the Temporomandibular Disorder-Pain Screener (TMD-Pain Screener) (ver. October 11, 2013) and Fonseca Anamnestic Index questionnaire used for screening of CMD. Since the TMD-Pain Screener contains only 4 items, we decided to attach the rest of the items from the Fonseca Anamnestic Index to obtain statistically relevant results. After this step the patients filled in the Patient Health Questionnaire-9 (PHQ-9) (ver. May 12, 2013), which consists of 9 closed-ended questions and is designed for screening, diagnosing, monitoring, measuring the severity of depression. This questionnaire is completed by the patient and can also be administered repeatedly, to reflect improvement or worsening of depression in response to treatment.

Volunteers were fully informed on the research objectives, experimental procedures, potential risks, and benefits, and were instructed to select only one response for each item. They were also told that they could take as much time as needed to complete the questionnaire.

The study was approved by the Ethics Committee Board of “Grigore T. Popa” University of Medicine and Pharmacy of Iasi (Nr. 271/14.02.2023), and all subjects provided written informed consent before enrollment.

Subjects who had been previously diagnosed with CMD or were currently receiving CMD treatment were excluded from the study. The inclusion and exclusion criteria are presented in second table.

TABLE I.

PHQ-9 Questionnaire (6)

Q.I Abrev		N.A	S.D	M.H.D	N.E.D
Q I.1	Little interest or pleasure in doing things				
Q I.2	Feeling down, depressed, or hopeless				
Q I.3	Trouble falling or staying asleep, or sleeping too much				
Q I.4	Feeling tired or having little energy				
Q I.5	Poor appetite or overeating				
Q I.6	Feeling bad about yourself or that you are a failure or have let yourself or your family down				
Q I.7	Trouble concentrating on things, such as reading the newspaper or watching television				
Q I.8	Moving or speaking so slowly that other people could have noticed. Or the opposite being so fidgety or restless that you have been moving around a lot more than usual				
Q I.9	Thoughts that you would be better off dead, or of hurting yourself				

Interpretation of Total Score	Depression Severity
1-4	Minimal depression
5-9	Mild depression
10-14	Moderate depression
15-19	Moderately severe depression
20-27	Severe depression

N.A- Not at all; S.D- Several days; M.H.D- More than half the days; N.E.D- Nearly every day.

TABLE II.

Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
• patients who are at least 18 years old • patients who have provided completed informed consent forms • patients who have completed the questionnaire PHQ-9	• patients who refused to participate in the study. • uncooperative patients • patients with systemic joint disorders • patients with neurological diseases • patients with psychiatric diseases • patients with cervical spine alterations • patients with facial paralysis • patients with previously diagnosed temporomandibular joint (TMJ) pathology

Data analysis

The data were centralized in *SPSS 24* databases and processed with suitable statistical function. Both descriptive and analytical methods were used.

The analysis of the results entailed assessing the frequency distribution of responses to the questionnaire. We compared

average percentages between various genders and severity levels using the Pearson Chi-squared test, with a significance level of error set at 95%.

The Mann-Whitney Test is parametric statistical test used to compare two samples or groups with low numbers of individuals in each group which are not normally distributed, and where the data are continuous.

The Kruskal-Wallis test is a nonparametric test in which we compared three or more frequency distributions intergroupally.

RESULTS

We investigated a sample of 106 patients, mainly women (70.8%), generally of young age - with an average age of 39.51 ± 12.324 years; women are about 8 years older than men, with an average of 41.80 ± 11.856 years compared to 33.97 ± 11.834 years - the observed difference being statistically significant. The youngest patients were 18 years old, and the oldest is a 75-year-old man (tab. III).

We also made a division of patients by age groups of approximative 10 years; the majority of patients are of adult age, between 26 – 55 years (72.7%), the percentage of young people between 18-25 years being 18.9%, and that of adults over 55 years being of only 8.5% (tab. IV).

TABLE III.

The age of the investigated patients – descriptive statistics (global sample and comparatively by gender)

Gender	n	Age Average	Std. error of the mean	Std. deviation	Min	Max	Median	Mann-Whitney test
F	75	41.80	1.369	11.856	21	65	43.00	U = 699.500
M	31	33.97	2.125	11.834	18	75	29.00	p = 0.001**
Total	106	39.51	1.197	12.324	18	75	39.00	

TABLE IV.

The structure of the investigated sample by age groups

		n	%
Age group	18-25 yrs.	20	18.9
	26-35 yrs.	23	21.7
	36-45 yrs.	25	23.6
	46-55 yrs.	29	27.4
	over 55 yrs.	9	8.5
	Total	106	100.0

The sample's structure by occupations is quite heterogeneous: 40% of the interviewed patients are economists, to which is added a percentage of people working in

commerce (17.0%) or IT (10.4%) and also a percentage of almost 10% students; the other categories of occupations occur in relatively isolated cases (tab. V).

TABLE V.

The structure of the investigated sample by occupations

		n	%
Occupation	Commerce	18	17.0
	Economist	42	39.6
	Civil servant	7	6.6
	Engineer	4	3.8
	IT	11	10.4
	Mechanic	1	0.9
	Doctor	3	2.8
	Dentist	6	5.7
	Optician	1	0.9
	Retired	2	1.9
	Policeman	1	0.9
	Student	10	9.4
	Total	106	100.0

Patients were given a questionnaire consisting of 9 items, to which they had 4 options of answer, namely "not at all", "several days", "more than half the days" and "nearly every day". The global analysis of patients' responses to the questionnaire reveals that most responses (over 75%) fall into the "not at all" and "several days" categories. It can also be noted that none of the patients reported severe signs of depression, all responding with "not at all" to the item targeting them (Q I.9 – "Thinking that you would be better off dead or of hurting yourself in some way") (tab. VI).

TABLE VI.

Structure of patients' responses to the questionnaire – global characterization

	N.A		S.D		M.H.D		N.E.D		Total	
	n	%	n	%	n	%	n	%	n	%
Q I.1.	37	34.9	60	56.6	9	8.5			106	100.0
Q I.2.	51	48.1	48	45.3	7	6.6			106	100.0
Q I.3.	38	35.8	48	45.3	17	16.0	3	2.8	106	100.0
Q I.4.	20	18.9	58	54.7	24	22.6	4	3.8	106	100.0
Q I.5.	42	39.6	48	45.3	14	13.2	2	1.9	106	100.0
Q I.6.	69	65.1	27	25.5	10	9.4			106	100.0
Q I.7.	46	43.4	46	43.4	11	10.4	3	2.8	106	100.0
Q I.8.	64	60.4	33	31.1	8	7.5	1	0.9	106	100.0
Q I.9.	106	100.0							106	100.0
N.A- Not at all; S.D- Several days; M.H.D- More than half the days; N.E.D- Nearly every day.										

Consequently, based on the analysis of the scores recorded by patients on the questionnaire, it is found that most of them (85.9%) have minimal or mild levels of

depression, in almost equal proportions. Only 11.3% of patients were registered with moderate depression, while 2.8% of patients (3 cases) were classified as having moderately-to-severe depression (tab. VII).

We also analyzed comparatively by genders and age groups the patients' responses to the questionnaire.

The comparative study by genders reveals that generally, no statistically significant differences were found between women and men regarding their responses, with one exception, namely item Q I.4. (tab. VIII).

TABLE VII.

The structure of the investigated sample according to the degrees of depression identified by the questionnaire

		n	%
Interpretation	minimal depression	43	40.6
	mild depression	48	45.3
	moderate depression	12	11.3
	moderate-to-severe depression	3	2.8
	Total	106	100.0

TABLE VIII.

The questionnaire's responses comparatively study on genders

		Gender				Pearson Chi-squared test
		M		F		
		N	%	N	%	
Q I.1.	N.A	7	22.6%	30	40.0%	$Chi^2 = 2.940$
	S.D	21	67.7%	39	52.0%	p = 0.230
	M.H.D	3	9.7%	6	8.0%	
Q I.2	N.A	13	41.9%	38	50.7%	$Chi^2 = 1.068$
	S.D	15	48.4%	33	44.0%	p = 0.586
	M.H.D	3	9.7%	4	5.3%	
Q I.3.	N.A	8	25.8%	30	40.0%	$Chi^2 = 1.945$
	S.D	16	51.6%	32	42.7%	p = 0.584
	M.H.D	6	19.4%	11	14.7%	
	N.E.D	1	3.2%	2	2.7%	
Q I.4.	N.A	4	12.9%	16	21.3%	$Chi^2 = 10.414$
	S.D	14	45.2%	44	58.7%	p = 0.015*
	M.H.D	13	41.9%	11	14.7%	
	N.E.D			4	5.3%	
Q I.5.	N.A	12	38.7%	30	40.0%	$Chi^2 = 0.817$
	S.D	13	41.9%	35	46.7%	p = 0.845
	M.H.D	5	16.1%	9	12.0%	
	N.E.D	1	3.2%	1	1.3%	

		Gender				Pearson Chi-squared test
		M		F		
		N	%	N	%	
Q I.6.	N.A	19	61.3%	50	66.7%	$Chi^2 = 3.612$
	S.D	11	35.5%	16	21.3%	p = 0.164
	M.H.D	1	3.2%	9	12.0%	
Q I.7.	N.A	15	48.4%	31	41.3%	$Chi^2 = 0.948$
	S.D	13	41.9%	33	44.0%	p = 0.814
	M.H.D	2	6.5%	9	12.0%	
	N.E.D	1	3.2%	2	2.7%	
Q I.8.	N.A	19	61.3%	45	60.0%	$Chi^2 = 0.507$
	S.D	10	32.3%	23	30.7%	p = 0.917
	M.H.D	2	6.5%	6	8.0%	
	N.E.D			1	1.3%	
Q I.9.	N.A	31	100.0%	75	100.0%	-
Total		**31**	**100.0%**	**75**	**100.0%**	
N.A- Not at all; S.D- Several days; M.H.D- More than half the days; N.E.D- Nearly every day.						

The overall degree of depression assessed by the questionnaire is also different between genders. It can be noted that a percentage almost double of women compared to the similar percentage of men have a minimal degree of depression (45.3% vs. 29.0%); on the other hand, a significantly higher percentage of men compared to women have mild depression (61.3% vs. 38.7%) and again a double percentage of women compared to men have moderate depression (13.3% vs. 6.5%). Of the 3 patients with moderate-to-severe depression, one is male and two are female, corresponding to 3.2% of men and 2.7% of women, respectively (tab. IX).

TABLE IX.

The structure of the investigated sample according to the degrees of depression identified by questionnaire – comparatively by genders

		Gender				Pearson Chi-squared test
		M		F		
		N	%	N	%	
Interpretation	minimal depression	9	29.0%	34	45.3%	$Chi^2 = 4.858$
	mild depression	19	61.3%	29	38.7%	p = 0.183
	moderate depression	2	6.5%	10	13.3%	
	moderate-to-severe depression	1	3.2%	2	2.7%	
Total		31	100.0%	75	100.0%	

The comparative study by age groups reveals, in turn, the following (tab. X): generally, no statistically significant differences were found between the 5 identified age groups in terms of recorded responses, except for items Q I.1, Q I.6 and Q I.8.

TABLE X.

The questionnaire's responses comparatively study on age groups

		Age group										Pearson Chi-squared test
		18-25 yrs.		26-35 yrs.		36-45 yrs.		46-55 yrs.		over 55 yrs.		
		n	%	n	%	n	%	n	%	n	%	
Q I.1.	N.A	2	10.0%	12	52.2%	6	24.0%	12	41.4%	5	55.6%	$Chi^2 = 26.683$
	S.D	13	65.0%	7	30.4%	19	76.0%	17	58.6%	4	44.4%	$p < 0.001^{**}$
	M.H.D	5	25.0%	4	17.4%							
Q I.2.	N.A	5	25.0%	12	52.2%	15	60.0%	15	51.7%	4	44.4%	$Chi^2 = 11.742$
	S.D	12	60.0%	8	34.8%	9	36.0%	14	48.3%	5	55.6%	$p = 0.163$
	M.H.D	3	15.0%	3	13.0%	1	4.0%					
Q I.3.	N.A	8	40.0%	8	34.8%	7	28.0%	11	37.9%	4	44.4%	$Chi^2 = 15.481$
	S.D	8	40.0%	8	34.8%	16	64.0%	11	37.9%	5	55.6%	$p = 0.216$
	M.H.D	2	10.0%	7	30.4%	2	8.0%	6	20.7%			
	N.E.D	2	10.0%					1	3.4%			
Q I.4.	N.A	1	5.0%	4	17.4%	7	28.0%	7	24.1%	1	11.1%	$Chi^2 = 7.386$
	S.D	12	60.0%	12	52.2%	11	44.0%	16	55.2%	7	77.8%	$p = 0.831$
	M.H.D	6	30.0%	6	26.1%	6	24.0%	5	17.2%	1	11.1%	
	N.E.D	1	5.0%	1	4.3%	1	4.0%	1	3.4%			
Q I.5.	N.A	7	35.0%	8	34.8%	13	52.0%	11	37.9%	3	33.3%	$Chi^2 = 5.991$
	S.D	9	45.0%	12	52.2%	9	36.0%	14	48.3%	4	44.4%	$p = 0.917$
	M.H.D	3	15.0%	3	13.0%	2	8.0%	4	13.8%	2	22.2%	
	N.E.D	1	5.0%			1	4.0%					
Q I.6.	N.A	7	35.0%	15	65.2%	19	76.0%	24	82.8%	4	44.4%	$Chi^2 = 17.883$
	S.D	11	55.0%	5	21.7%	4	16.0%	4	13.8%	3	33.3%	$p = 0.022^{*}$
	M.H.D	2	10.0%	3	13.0%	2	8.0%	1	3.4%	2	22.2%	
Q I.7.	N.A	5	25.0%	11	47.8%	12	48.0%	13	44.8%	5	55.6%	$Chi^2 = 13.258$
	S.D	11	55.0%	8	34.8%	13	52.0%	11	37.9%	3	33.3%	$p = 0.351$
	M.H.D	3	15.0%	2	8.7%			5	17.2%	1	11.1%	
	N.E.D	1	5.0%	2	8.7%							
Q I.8.	N.A	6	30.0%	16	69.6%	18	72.0%	17	58.6%	7	77.8%	$Chi^2 = 20.609$
	S.D	13	65.0%	4	17.4%	7	28.0%	8	27.6%	1	11.1%	$p = 0.056$
	M.H.D	1	5.0%	3	13.0%			3	10.3%	1	11.1%	
	N.E.D							1	3.4%			
Q I.9.	N.A	20	100.0%	23	100.0%	25	100.0%	29	100.0%	9	100.0%	-
Total		20	100.0%	23	100.0%	25	100.0%	29	100.0%	9	100.0%	

N.A- Not at all; S.D- Several days; M.H.D- More than half the days; N.E.D- Nearly every day.

There are no statistically significant differences between age groups in which concerns the distribution of patients by degrees of depression (tab. XI). It can be noted, however, that the lowest percentage of cases with minimal depression (15.0%) was reported in the age group 18-25 years (while it tends to 50% in the other age groups), but also the highest percentages of cases with mild (60.0%), moderate (20.0%) and moderate-to-severe (5.0%) depression were reported again here.

TABLE XI.

The structure of the investigated group according to the degrees of depression identified by questionnaire – comparatively by age groups

		Age group										Pearson Chi-squared test
		18-25 yrs.		26-35 yrs.		36-45 yrs.		46-55 yrs.		over 55 yrs.		
		n	%	n	%	n	%	n	%	n	%	
Interpretation	minimal depression	3	15.0%	10	43.5%	12	48.0%	14	48.3%	4	44.4%	$Chi^2 = 9.727$
	mild depression	12	60.0%	9	39.1%	12	48.0%	11	37.9%	4	44.4%	p = 0.640
	moderate depression	4	20.0%	3	13.0%	1	4.0%	3	10.3%	1	11.1%	
	moderate-to-severe depression	1	5.0%	1	4.3%			1	3.4%			
Total		20	100.0%	23	100.0%	25	100.0%	29	100.0%	9	100.0%	

A numerical depression score was also calculated based on the questionnaire's responses. Men have a slightly higher score than women, with an average of 6.16 ± 3,407 versus 5.53 ± 3,923, and the highest depression score was recorded in patients between 18-25 years, with an average of 7.70 ± 3,827, while the lowest score was observed in the age group 36-45 years, with an average of 4.80 ± 3,149 (tab. XII).

TABLE XII.

The depression score calculated by the questionnaire – descriptive statistics, overall and comparatively by genders and age groups

		n	Average	Std. error of the mean	Std. deviation	Min	Max	Median	Mann-Whitney / Kruskal-Wallis test
Final score	total	106	5.72	0.367	3.774	0	18	5.00	
	M	31	6.16	0.612	3.407	0	15	6.00	U = 976.000
	F	75	5.53	0.453	3.923	0	18	5.00	p = 0.193
	18-25 yrs.	20	7.70	0.856	3.827	3	18	7.00	H = 7.726
	26-35 yrs.	23	5.83	0.864	4.141	0	15	5.00	p = 0.102
	36-45 yrs.	25	4.80	0.630	3.149	0	12	5.00	
	46-55 yrs.	29	5.24	0.717	3.861	0	16	5.00	
	over 55 yrs.	9	5.11	1.020	3.060	2	11	5.00	

DISCUSSION

Depressive and anxiety disorders are the two most common mental disorders, affecting 280 and 301 million people around the world, respectively (7).

Over the decades, many studies have demonstrated positive correlations between CMD and symptoms of anxiety and depression. In the systematic review of De La Torre Canales *et al*, a high prevalence of moderate–to–severe depression was observed to range from 21.4 to 60.1% in patients diagnosed with CMD (8).

The results of the current study suggest that the developed questionnaire is useful for the initial assessment of the psycho-emotional state of CMD patients and obtained information, can be an invaluable addition to the diagnosis of CMD patients and may constitute a critical element for effective treatment of the disorder. The results are significant and suggest that patients with CMD suffer from psycho-emotional problems and require more frequent consultation than healthy individuals (9). Sleep is paramount for managing depression, in line with medication and professional psychological support. Pop *et al.* claims in a study that sleep-related problems and stress were also considered a problem among medical students. Studies showed that medical students have more sleep-related problems compared to non-medical students (10), and in our opinion this could open a way for specifically applying our questionnaires to this category of subjects.

It can be observed in our study that exists a correlation between static style of life (Economists, IT worker), active style of life (Engineer, Mechanic) and the risk of TMJ diseases. Patients with static occupations are more likely to develop craniomandibular dysfunction. A special category for evaluation is represented by healthcare professionals. Iorga *et al.* affirmed that apart from burnout, depression is one of the most important psychological problems experienced by adults during their working time. Identifying and providing knowledge about depression and burnout among healthcare professionals is an important aspect of medical education (11).

We also analyzed comparatively by genders and age group the patients' responses to the questionnaire (tab. VIII). The only item for which statistically significant differences between genders were found is, as already mentioned, item Q I.4, which refers to the feeling of fatigue and lack of energy. The perceptions differ between genders, even if it is not possible to detect a systematic association between them and the patients' gender. Almost a quarter of women (21.3%) said they did not feel at all tired or having little energy, compared to only 12.9% of men. On the other hand, a higher percentage of women than the corresponding percentage of men report this feeling "several days" (58.7% compared to 45.2%), while a percentage almost 3 times higher of men than the corresponding percentage of women report this feeling "more than half the days" (41.9% compared to 14.3%); none of the men, however, find themselves feeling tired or lacking energy "nearly every day".

A significantly higher amount of men (67.7%) compared to the equivalent amount of women (52.0%) stated that "several days" they have little interest or pleasure in doing things (item I.1); also, the percentage of men claiming to have such feelings "more than half the days" is slightly higher than the similar percentage of women (9.7% vs. 8.0%).

Regarding the feeling of depression and hopelessness (item I.2), the percentages of women and men who report it "several days" are relatively close, although it can be seen in this case that men who have such feelings "more than half the days" are almost double in percentage compared to women (9.7% compared to 5.3%).

A percentage 40.0% of women said they have no sleep disturbances at all (neither insomnia nor tendency to sleep too much – item I.3), compared to only 25.8% of men. Of these, on the other hand, 51.6% complain about the presence of sleep disorders "several days" compared to 42.7% of women and 19.4% say they have such problems "more than half the days" compared to 14.7% of women.

In the case of appetite disorders (item I.5) the differences between genders are even smaller than in other items. A slightly higher percentage of women compared to the corresponding percentage of men report this feeling "several days" (46.7% vs. 41.9%), while a slightly higher percentage of men compared to the corresponding percentage of women report this feeling "more than half the days" (16.1% vs. 12.0%).

The negative feelings towards oneself, the feeling of failure and being disappointed (item I.6), are more acute in women than in men. Thus, although a higher percentage of men compared to the similar percentage of women accuse such feelings "several days" (35.5% compared to 21.3%), yet a percentage almost 4 times higher of women compared to the similar one of men accuse such feelings "more than half the days" (12.0% compared to 3.2%).

A relatively similar situation is observed in the case of difficulties of concentration on things (item I.7): the percentage of men who think that they do not have such a problem is higher than the similar percentage of women (48.4% compared to 41.3%), while the percentages of women who consider that they have this problem "several days", as well as "more than half the days" are higher than the similar percentages of men. There were 1 man and 2 women who said they had such difficulty of concentration "nearly every day".

The behavior disorders as hyperactivity or its opposite, slowness in reactions (item I.8), are reported in their intense forms slightly more frequently in women than in men. Thus, 8.0% of women compared to 6.5% of men said they had such problems "more than half the days", and in addition one woman (and no men) said they had this problem "nearly every day".

The comparative study by age groups reveals, in turn (tab. X), that none of the patients over 35 years of age complained of lack of interest or pleasure in doing things (item Q I.1), except at most "several days", all patients who complained of this feeling "more than half the days" belonging to the young age, between 18-35 years. Among patients under 25 years old, 65.0% complain of this feeling "several days" and 25.0% accuse it "more than half the days", a situation that improves significantly in the next age group. Thus, of patients between 26-35 years, half (52.2%) do not have this problem at all, 30.4% accuse it "several days" and only 17.4% accuse it "more than half the days". With advancing in age, the presence of this feeling is reported less and less – being reported only "several days", by 76.0% of patients between 36-45 years, 58.6% of those between 46-55 years and only 44.4% of those over 55 years.

Negative feelings towards oneself, feel-

ing of failure and being disappointed (item Q I.6), are instead significantly associated with the age of patients. They seem to be characteristic in both young ages and adults over 55 years old. Of the 18-25 age group, only 35.0% do not experience such feelings, with 55.0% reporting them "several days" and 10.0% reporting them "more than half the days". Among patients over 55, 33.3% report such feelings "several days" and another 22.2% report them "more than half the days". In the other age groups, although there are percentages of patients who complain about these feelings "more than half the days" ranging between 3 and 13%, the amount of those who do not experience such feelings increases over 65%.

Hyperactivity or slow reaction disorders (item Q I.8) are also most associated with young patients, with ages between 18-25 years, of whom 65.0% report them "several days" and another 5.0% report them "more than half the days". Their percentage decreases to about 30% in the age groups 26-35 years and 36-45 years, then increases to 41.4% in the age group 46-55 years, after which it decreases to 22.2% of those over 55 years. The level of significance associated with this item is 0.056, very close to the statistical significance threshold.

In their cross-sectional analysis, Fredricson *et al.* demonstrated a strong association between the occurrence of mental and behavioral disorders and CMD. Pain, the most common symptom of CMD, was associated with anxiety and stress-related disorders, which are modified in CMD (12).

Several studies emphasize the importance the assessment of psycho-emotional state performed by dentists and developing tools to refer patients for specialized consultation. Although the etiology of TMD is complex and still not clearly understood, it is generally believed to comprise of biological, psychological, and social factors, it is important to also consider the psychological symptoms during diagnosis of the disease (13).

In Romania many patients have confidence in the pharmacist opinion and ask for advice regarding suitable medication formulations for specific conditions (14). Probably this has to do with a reduced number of visits to dentists and or medical physicians compared to other Western EU countries (15), as a consequence of lower average income. We have to state that dental procedures are partially supported by the National Health Insurance House in Romania, and this can contribute to a lower addressability for the Romanian patient, who has to bear the remainder cost.

According to a Eurostat study conducted in 2019, Romania is the country with the lowest percentage of chronic depression at European level. The average is 1.5%, with an incidence of 1% among men and 2% among women (16). In our study the highest depression score was recorded in patients between 18-25 years, while the lowest score was observed in the age group 36-45 years, the results obtained are in line with the previously mentioned studies.

In other study, Seweryn *et at.* discovered a correlation between the intensity of pain associated with CMD and the quality of life and sleep. These parameters are influential in modifying CMD management (17).

The results of the study suggest that the questionnaire PHQ-9 can be used as a supplement to the examination for CMD diagnosis, so we find ourselves in agreement with similar studies (18, 19). Also, it

can help dentists in identifying potential psycho-emotional issues in patients and recommending therapeutic management (20, 21).

The implication of stress in CMD is apparently not enough researched at the present moment due to a scariness of result in PubMed Database. I am aware it is a difficult task to initiate type of study in EU country with the smallest occurrence of depression.

CONCLUSIONS

Based on the findings of this research, it can be noted, that the lowest percentage of cases with minimal depression was reported in the age group 18-25 years.

According to the results of this study, the severity of CMDs is more common in women and at the young age. Stress has been established as a significant risk factor for TMJ disorders apart from parafunctional habits (grinding, thumb sucking, or tongue thrusting) and pathologic occlusion.

Our study, aligned to the other studies with the same topic, demonstrated again that exist an interrelation between CMDs and psycho-emotional factors.

We can conclude that craniomandibular disorders (CMDs) is a significant concern that should be deemed a matter of significant importance in dental medicine and addressed accordingly.

The study advocates for an increased awareness in the medical community for the relationship between temporomandibular joint (TMJ) screening and the role of psycho-emotional factors.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest. This study received no funding. This study is part of the first author's research program at the Doctoral School.

INSTITUTIONAL REVIEW BOARD STATEMENT

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy of Iasi (271/14.02.2023)" for studies involving humans.

INFORMED CONSENT STATEMENT

Informed consent was obtained from all subjects involved in the study.

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ODONTOGENIC MAXILLARY SINUSITIS: CLINICAL AND PARACLINICAL STUDY ON ETIOPATHOGENY, DIAGNOSTIC AND THERAPEUTIC MANAGEMENT

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ODONTOGENIC MAXILLARY SINUSITIS: CLINICAL AND PARACLINICAL STUDY ON ETIOPATHOGENY, DIAGNOSTIC AND THERAPEUTIC MANAGEMENT (Abstract): Chronic sinusitis has an estimated prevalence rate of about 10.4% in Europe. Odontogenic sinusitis is defined by inflammation of the paranasal sinuses caused by dental conditions. Chronic maxillary sinusitis of dental cause is the common clinical form and can occur from the beginning in this form or may be the result of chronic acute maxillary sinusitis, the condition becomes chronic if it lasts for more than 3 months. **Materials and methods:** This study investigated 47 patients admitted to the ENT Clinic of the Iasi Clinical Recovery Hospital between January 2022 - June 2023 diagnosed with maxillary sinusitis. **Results:** The study revealed a maximum incidence of maxillary sinusitis in the decade between the ages of 41 and 50 years more frequently in the male sex, and with relatively low frequency of odontogenic cause (14.8 %), versus rhino genic sinusitis. **Discussion:** Odontogenic S is a pathology at the border of two specialties, ENT and OMF with an interdisciplinary treatment approach. The maxillary status is dependent on the correct and efficient treatment of the underlying cause which sprouts from the dental root and maxillary floor. Endoscopic approach of the rhino logical pathology is a good option after the resolution of the oromaxillary interface osteitis and has successfully replaced other more invasive procedures (like Caldwell-Luc operation). **Conclusions:** odontogenic sinus pathology is a growing but frequently undervalued and underdiagnosed condition whose dimensions and impact on patients' health require a multidisciplinary approach. Odontogenic maxillary sinusitis can be prevented by maintaining a rigorous oral hygiene, regular dental checkups, correct treatment of odontal and periodontal diseases, avoiding smoking. Maxillary sinus FESS is an efficient and comfortable treatment option for resolving rhinological accuses.
Keywords: ODONTOGENIC MAXILLARY SINUSITIS, ETIOPATHOGENY.

INTRODUCTION

Acute and chronic sinusitis is a significant public health problem, both because of its high prevalence and the economic im-

pact it generates.

Chronic sinusitis has an estimated prevalence rate of about 10.4% in Europe and about 14% in the U.S. (1). For acute sinusitis, prevalence estimates are more difficult to make because many patients do not require specialized medical care for this condition. The costs associated with the treatment and management of sinusitis are substantial. In the United States, direct and indirect annual costs are estimated to exceed $6 billion, plus approximately $2 billion for over-the-counter treatments (1). These costs include expenses for medical consultations, imaging investigations, pharmacological treatments and, in some cases, surgery.

Odontogenic sinusitis is a pathology distinct from rhinosinusitis in several features. It is an inflammatory and infectious condition of the maxillary sinus of dental origin or is a complication of dental procedures. Most often it appears unilaterally and is in close association with underlying dental conditions (2). The maxillary sinus is the largest of the four paranasal sinuses (maxillary, frontal, ethmoid, sphenoid) and, because of its close relationship with the underlying dentition, is often susceptible to inflammatory processes. Although odontogenic sinusitis may present symptoms similar to those of rhinosinusitis, such as facial pain, nasal congestion and nasal discharge, its origin and pathological mechanism are distinct (2).

Odontogenic sinusitis is defined as inflammation of the paranasal sinuses caused by dental disease. It always starts with the maxillary sinus, given its size and position in relation to the dental arches, but can evolve to pan-sinusitis (all sinuses affected on the affected side) or even produce complications (orbital, thrombosis, brain abscess, sepsis). As with other forms of sinusitis, the maxillary sinus is prone to inflammation and infection when there is pathological communication between the oral cavity and the sinus in question. This pathology occurs when a dental condition, arising from a lack of proper oral hygiene or from inappropriate dental interventions (extractions or dental implants), comes to affect the maxillary sinus (1).

The incidence of odontogenic sinusitis is about 10-12% of sinusitis cases and this percentage increases with age. Thus, in people over the age of 50 who suffer from sinus infections, there is an approximately 50% probability that they have a dental cause following pulpal, periapical, periodontal disease or as a result of infectious complications of upper wisdom molar or upper canine inclusion (1).

These conditions can also be caused by incorrect dental procedures such as: Overflow root canal fillings in a tooth with sinus involvement, in which the filling material will form a foreign body in the maxillary sinus; incomplete or missing root canal fillings in abutment teeth of old prosthetic work, accidents and complications of tooth extraction, unnoticed or incorrectly treated oro-sinus communication, perforation of the sub antral space by untimely curettage; pushing of a root into the maxillary sinus during dental extraction, pushing of the upper wisdom molar into the maxillary sinus during odontotomy, failures in oral implantology, insertion of improper end osseous implants or errors in surgical technique in sinus lifting (3, 4, 5).

The risk factors for dental maxillary sinusitis are related to pathology of the teeth with sinus relation and the alveolar bone of this level, including following accidents and complications of dental treatments such as chronic inflammation and can be aggravated by several rhinological conditions: allergic

diseases of the rhinosinusal mucosa, obstruction of the ostium in the middle nasal meatus, by inflammatory mechanism - mucosal edema or mechanically - sinus polyps, deviated septum, decreased ciliary motility, simultaneous with increased mucus secretion. Also, some general factors are important: decreased overall resistance of the body to infection, diabetes, liver and kidney pathology, smoking and exposure to a noxious environment (6).

The clinical signs of acute maxillary sinusitis are: unilateral pain, located in the mid-face, radiating to the orbital, frontotemporal or occipital region, exacerbated by head tilt; stuffy nose, purulent nasal discharge, anterior or posterior, most commonly unilateral. Fever, sensation of pressure in the genian region; digital pressure exerted on the anterolateral wall of the sinus is painful, subjective cacosmia, dental pain, sometimes pulsatile in character, swollen and congested genial teguments, painful on pressure; altered general condition, lack of appetite, halitosis, cough and fatigue (4, 7).

Chronic dental maxillary sinusitis is the most commonly encountered clinical form and may be present from the outset in this form or may be the result of chronicization of acute maxillary sinusitis, the condition becoming chronic if it lasts more than 3 months.

Maxillary odontogenic sinusitis can be prevented by maintaining rigorous oral hygiene, regular dental check-ups, correct treatment of dental and periodontal diseases, avoidance of smoking (9, 10).

Given the trend of increasing resistance to conventional treatment of odontogenic sinusitis, the need for an interdisciplinary consensus of odontogenic sinusitis is emphasized. The therapeutic algorithm usually includes two stages: the first stage is non-surgical treatment with antibiotic treatment for infection and resolution of dental lesions, and the second stage is sinus surgical treatment (8, 10).

MATERIALS AND METHODS

This study investigated 47 patients admitted to the ENT Clinic of the Iasi Rehabilitation Hospital between January 2022 and June 2023. Patients required medical care for common symptoms of maxillary sinusitis, which were also associated with signs and symptoms of other systemic or local conditions. The age range of patients included in the study was between 13 and 77 years. After treatment, which included drug, surgical or a combination of drug and surgical options, patients were discharged and follow-ups were planned for a specified period of time.

The study was conducted retrospectively, based on analysis of discharge notes of patients included in the initial selection. This method allowed to evaluate the efficacy of the treatment administered and to analyses their post-treatment evolution. Therefore, the study provides an important insight into the management and treatment outcomes of maxillary sinusitis in this institution.

The study included patients who presented with maxillary sinusitis objectified on computed tomography (CT) imaging and evaluated by nasal endoscopy. Cases were assessed according to several variables, including age, gender, duration of hospitalization, location of maxillary sinusitis (right/left/bilateral), predominant clinical symptoms, treatment modality (medical, medical + surgical; type of surgery), comorbidities, and preoperative and postoperative complications.

Cases with traumatic, tumoral or allergic rhinosinusal pathology, trigeminal neu-

ralgia, dental pathology without sinus involvement, fungal sinusitis, associated with polypoid formations (such as nasal polyposis, sinus-choanal polyp) or in ciliary immobility (e.g. Sjogren's) or allergic syndromes (Vidal syndrome) were excluded from the study.

These inclusion and exclusion criteria were applied to ensure an appropriate selection of cases that would meet the specific objectives of the study and provide relevant data for the evaluation of maxillary sinusitis in the study population.

Patient assessment included: ENT clinical examination, general clinical examination, ATI consultation, internal medicine consultation, infectious diseases consultation, ophthalmology consultation and dental consultation; ENT clinical examination included buco-pharingoscopy (BFS), anterior rhinoscopy, posterior rhinoscopy, indirect laryngoscopy, nasal endoscopy or fibroscopy, palpation of trigeminal points, percussion of anterior sinus wall and upper quadrant teeth (canine, premolar, molar); pituitary congestion and edema, status of the inferior and middle turbinate, appearance of the middle meatus, presence of mucous or purulent secretions in the middle meatus; purulent secretions were collected from the middle meatus when present on admission.

Paraclinical investigations performed are: computer tomography (CT) examination, recommended in all cases; sometimes additional MRI (nuclear magnetic resonance) examination; bacteriological and microbiological examination of secretions with antibiogram and antifungigram; dental X-rays - if a dental cause of sinusitis is suspected; chest X-ray, EKG and cardiological consultation, in case of surgery under general anesthesia.

Pre-operatively the patients underwent tests such as: hemolucogram, coagulogram, liver tests, kidney tests, blood glucose.

RESULTS

It is interesting to note that in this study, the highest incidence of maxillary sinusitis was recorded in the age decade between 41 and 50 years. This finding suggests that this age group may be more prone to the development of maxillary sinusitis compared to other age groups. The biggest prevalence was however between ages 31 and 60 with 33p from the total of 47 (70.21%).

In the following graph, the incidence of maxillary sinusitis has been plotted by gender, and as can be seen, of the 47 patients selected for this study, the highest incidence is identified in males.

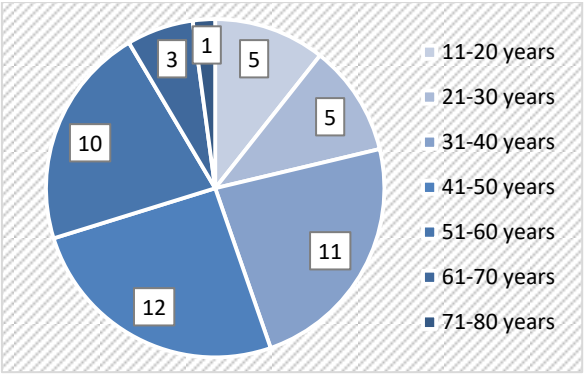

Fig. 1. Age of patients by decades diagnosed with maxillary sinusitis

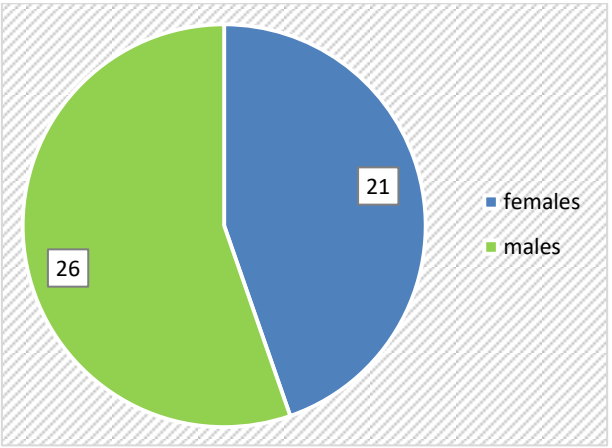

Fig. 2. Incidence of maxillary sinusitis by gender

In the diagram below, the incidence by location of maxillary sinusitis is shown. There is an increased incidence of bilaterally located sinusitis. Thus, we found 10 cases of left-sided maxillary sinusitis, of which 2 were odontogenic sinusitis, 16 cases of right-sided maxillary sinusitis, of which 5 were odontogenic sinusitis, and 21 cases of bilaterally located maxillary sinusitis, with no cases of odontogenic sinusitis.

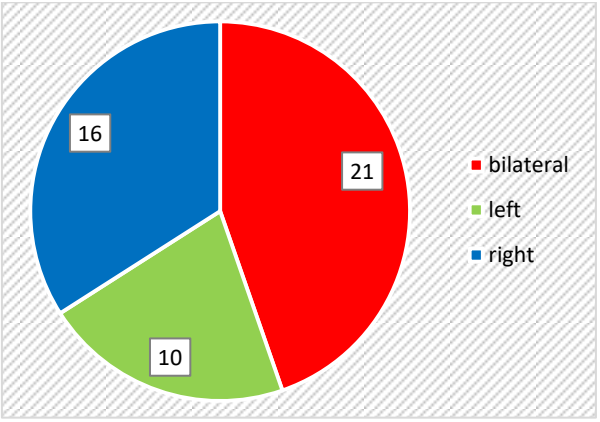

Fig. 3. Location of maxillary sinusitis

No statistically significant differences were found either between the sexes or between the 3 age groups in the localization of maxillary sinusitis (tab. I). However, it can be noted that sinusitis with bilateral localization was observed slightly more frequently in females than in males, with an incidence of 57.1%, and in most cases also in adult patients aged 31-60 years (71.4%). Left-sided sinus sinusitis was observed equally in females and males, and most frequently in adult patients (60.0%), while right-sided sinus sinusitis was observed predominantly in males (75.0% of cases) and also in adult patients (75.0%), being completely absent in elderly patients.

TABLE I.

Distribution of maxillary sinus sinusitis according to localization, by sex and age groups

		Location						
		bilateral		left		right		
		N	%	N	%	N	%	p-value†
Gender	male	9	42.9%	5	50.0%	12	75.0%	0.139
	female	12	57.1%	5	50.0%	4	25.0%	
Age group	<= 30 years	3	14.3%	3	30.0%	4	25.0%	0.499
	31-60 years	15	71.4%	6	60.0%	12	75.0%	
	> 60 years	3	14.3%	1	10.0%			
Total		21	100.0%	10	100.0%	16	100.0%	
†Pearson Chi-squared test								

Of the total patients included in the study, 7 patients (14,9%) were diagnosed with odontogenic sinusitis, which was unilateral (tab. II, fig. 4).

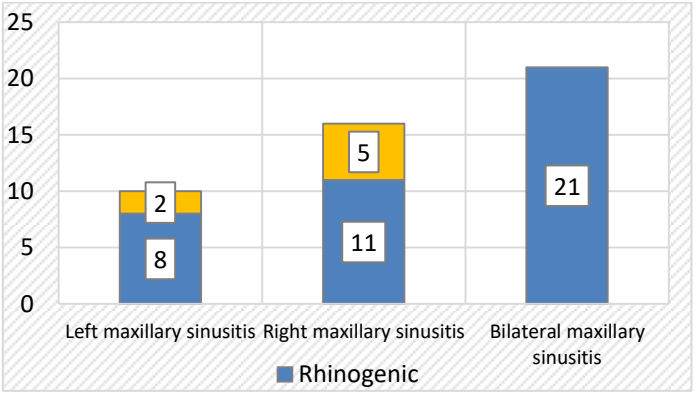

Fig. 4. Total number of patients with rhino genic sinusitis and odontogenic sinusitis

TABLE II.

Distribution of maxillary sinusitis by location and type

		Location						p-value†
		bilateral		left		right		
		N	%	N	%	N	%	
Type	Rhinogenic	21	100.0%	8	80.0%	11	68.8%	0.027*
	Odontogenic			2	20.0%	5	31.3%	
Total		21	100.0%	10	100.0%	16	100.0%	
†Pearson Chi-squared test; p<0.05* statistically significant								

The most pronounced clinical symptoms with which patients presented to the clinic were: nasal obstruction (right/left/ bilateral); anterior and/or posterior purulent rhinorrhea; facial pressure and pain; cacosmia, hyposmia, anosmia; intermittent

headache. Nasal obstruction syndrome is usually the first symptom, accompanied by anterior and/or posterior purulent rhinorrhea.

In the following diagram, we have represented the subjective symptoms of the selected patients. All patients had nasal obstruction and most of them (42) had also purulent rhinorrhea; we also found 13 patients with headaches, 8 patients presenting facial pressure sensation, 5 patients presenting hyposmia, 4 patients with facial pain, 2 patients presenting cacosmia, and 1 patient presenting anosmia (fig. 5).

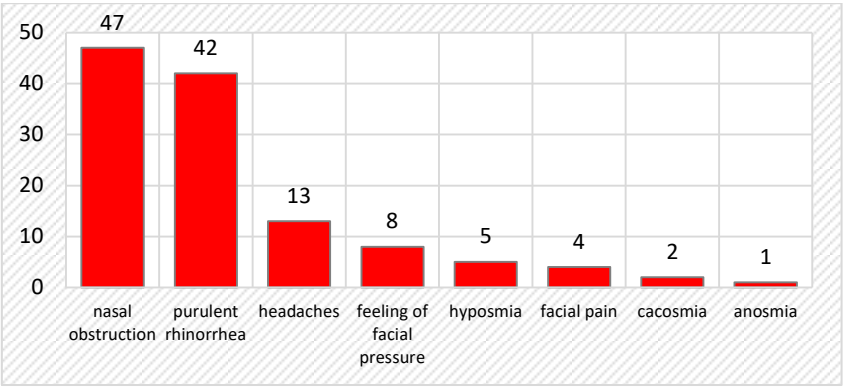

Fig. 5. Incidence of the most important subjective symptoms of patients in the study

We comparatively investigated the presence of symptoms according to the type of sinusitis, its localization, as well as the age group and gender of the patients, the results of which are presented in tables III-VI. There were no statistically significant differences in the incidence of symptoms between the listed categories.

TABLE III.

Incidence of symptoms compared by type of sinusitis

	Type				p-value†
	Rhinogenic		Odontogenic		
	N	%	N	%	
Nasal obstruction	40	100.0%	7	100.0%	
Purulent rhinorrhea	37	92.5%	5	71.4%	0.154
Headaches	10	25.0%	3	42.9%	0.377
Feeling of facial pressure	5	12.5%	3	42.9%	0.084
Facial pain	2	5.0%	2	28.6%	0.100
Hyposmia	5	12.5%			-
Cacosmia	2	5.0%			-
Anosmia	1	2.5%			-
Total	40	100.0%	7	100.0%	
†Pearson Chi-squared test					

TABLE IV.

Comparative incidence of symptoms by location of sinusitis

	Location						p-value†
	bilateral		left		right		
	N	%	N	%	N	%	
Nasal obstruction	21	100.0%	10	100.0%	16	100.0%	-
Purulent rhinorrhea	20	95.2%	9	90.0%	13	81.3%	0.392
Headaches	5	23.8%	2	20.0%	6	37.5%	0.543
Feeling of facial pressure	3	14.3%			5	31.3%	0.108
Facial pain	1	4.8%			3	18.8%	0.177
Hyposmia	3	14.3%	1	10.0%	1	6.3%	0.733
Cacosmia	2	9.5%					-
Anosmia			1	10.0%			-
Total	21	100.0%	10	100.0%	16	100.0%	
†Pearson Chi-squared test							

TABLE V.

Incidence of symptoms compared by patients' gender

	Gender				p-value†
	male		female		
	N	%	N	%	
Nasal obstruction	26	100.0%	21	100.0%	-
Purulent rhinorrhea	21	80.8%	21	100.0%	0.056
Headaches	8	30.8%	5	23.8%	0.596
Feeling of facial pressure	5	19.2%	3	14.3%	0.715
Facial pain	3	11.5%	1	4.8%	0.617
Hyposmia	1	3.8%	4	19.0%	0.158
Cacosmia			2	9.5%	-
Anosmia			1	4.8%	-
Total	26	100.0%	21	100.0%	
†Pearson Chi-squared test					

TABLE VI.

Incidence of symptoms compared by age group of patients

	Age group						p-value†
	<= 30 years		31-60 years		> 60 years		
	N	%	N	%	N	%	
Nasal obstruction	10	100.0%	33	100.0%	4	100.0%	
Purulent rhinorrhea	8	80.0%	30	90.9%	4	100.0%	0.477
Headaches	3	30.0%	9	27.3%	1	25.0%	0.978
Feeling of facial pressure	1	10.0%	6	18.2%	1	25.0%	0.755
Facial pain	1	10.0%	3	9.1%			0.813
Hyposmia	1	10.0%	3	9.1%	1	25.0%	0.620
Cacosmia			2	6.1%			-
Anosmia			1	3.0%			-
Total	10	100.0%	33	100.0%	4	100.0%	
†Pearson Chi-squared test							

The average incidence of the number of days of hospitalization of selected patients is around 2 or 3 days – 37 patients from 47 (78.72%). The longest hospitalization was of 9 days in case of one patient.

The average number of days of hospitalization was 3.13 ± 1,345, being statistically significantly higher in women than in men (with 3.57 ± 1,660 days compared to 2.77 ± 0.908 days) and in older patients compared to the others (tab. VII). Thus, for patients over 60 years of age the average length of hospitalization is 5.00 ± 2.944 days, while for those under 30 years the average length of hospitalization is practically halved (2.40 ± 0.516 days). Adult patients fall between the two extremes, with an average length of hospitalization of 3.12 ± 1.053 days.

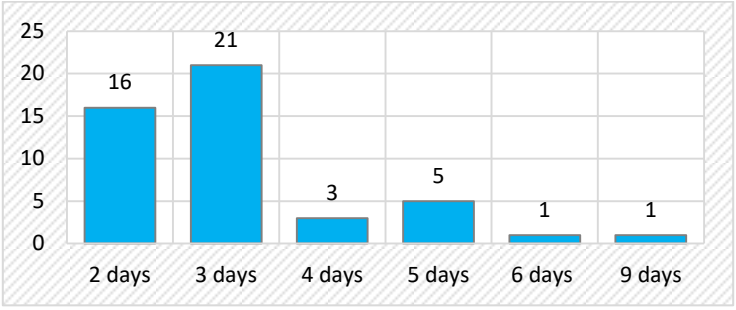

Fig. 6. Incidence by number of hospital days

Also, the mean number of days of hospitalization is statistically significantly higher in patients with bilateral sinusitis (with a mean of 3.90 ± 1,640 days) compared to other patients, for whom the mean hospitalization is two and a half days. The mean number of days of hospitalization is slightly lower in the 7 patients with odontogenic sinusitis compared to the others (with a mean of 2.57 ± 0.535 days versus 3.22 ± 1.423 days), but the difference is not pronounced enough to be statistically significant.

TABLE VII.

Days of hospitalization

Hospitalization days		N	Mean	Std. Deviation	Min	Max	Median	p-value
Gender	male	26	2.77	0.908	2	5	3.00	p = 0.044†*
	female	21	3.57	1.660	2	9	3.00	
Age group	<= 30 years	10	2.40	0.516	2	3	2.00	p = 0.031‡*
	31-60 years	33	3.12	1.053	2	6	3.00	
	> 60 years	4	5.00	2.944	2	9	4.50	
Sinusitis location	bilateral	21	3.90	1.640	2	9	3.00	p<0.001**
	left	10	2.40	0.516	2	3	2.00	
	right	16	2.56	0.512	2	3	3.00	
Sinusitis type	Rhinogenic	40	3.22	1.423	2	9	3.00	p = 0.310†
	Odontogenic	7	2.57	0.535	2	3	3.00	
Total		47	3.13	1.345	2	9	3.00	

†Mann-Whitney test; ‡Kruskal-Wallis test; p<0.05* statistically significant; p<0.01** statistically highly significant

We also comparatively investigated the number of days of hospitalization according to the presence/absence of observed symptoms (tab. VIII). No statistically significant differences were found, although in general the number of days of hospitalization tends to be slightly higher in patients presenting with symptoms compared to others.

TABLE VIII.

Comparative study of the number of days of hospitalization according to the presence/absence of characteristic symptoms

Hospitalization days		N	Mean	Std. Dev.	Min	Max	Median	p-value
Purulent rhinorrhea	present	42	3.14	1.372	2	9	3.00	0.854
	absent	5	3.00	1.225	2	5	3.00	
Headaches	present	13	3.15	0.899	2	5	3.00	0.386
	absent	34	3.12	1.493	2	9	3.00	
Feeling of facial pressure	present	8	2.88	0.991	2	5	3.00	0.667
	absent	39	3.18	1.412	2	9	3.00	
Facial pain	present	4	2.50	0.577	2	3	2.50	0.307
	absent	43	3.19	1.385	2	9	3.00	
Hyposmia	present	5	3.00	1.732	2	6	2.00	0.426
	absent	42	3.14	1.317	2	9	3.00	
Cacosmia	present	2	4.00	1.414	3	5	4.00	0.214
	absent	45	3.09	1.345	2	9	3.00	
Anosmia	present	1	3.00	.	3	3	3.00	0.813
	absent	46	3.13	1.360	2	9	3.00	
Total		47	3.13	1.345	2	9	3.00	

†Mann-Whitney test; $p<0.05$* statistically significant; $p<0.01$** statistically highly significant

DISCUSSION

Treatment of odontogenic sinusitis involves two stages: drug therapy and surgery. In the case of the patients included in the study, some of them first received antibiotic medication (VANCOMICIN combined with MEROPENEM) but the results were ineffective. Pre-operative medical treatment includes: identification of the causative factor of dental nature and its suppression, topical nasal steroids, antibiotic therapy (after antibiogram from nasal swab), nasal irrigation, antiseptics, anti-allergic.

Dental maxillary sinusitis is a well-recognized condition in both the dental and ENT communities. It is estimated that more than 30% of cases of maxillary sinusitis may have underlying dental pathology (11). Failure to identify a dental cause can frequently lead to progression to chronic sinusitis, which is often associated with serious complications (12). The close anatomical proximity of the root apices of teeth, particularly the second molar, to the maxillary sinuses gives dental conditions considerable potential to be a source of dissemination of infection into the maxillary sinuses. Untreated dental infections, such as periapical abscesses or deep dental caries, may allow bacteria to enter the maxillary

sinuses through natural communication channels or pathological perforations of the sinus walls (13).

The incidence of odontogenic sinusitis is low, accounting for 10-12% of all forms of sinusitis. However, despite the significant prevalence of dental infections, in recent years these conditions appear to be experiencing a significant increase in incidence, recent studies indicate that dental conditions associated with odontogenic sinusitis are increasing, with an estimated incidence of approximately 40% (1, 26).

In our study the incidence was almost 15%, possibly due to late presentation to dental treatment (or none), deficitary access to medical facilities in rural isolated areas, etc.

It is often refractory to initial antibiotic therapy due to its polymicrobial, predominantly anaerobic nature.

The condition should always be considered by otolaryngologists and dentists when a patient presents with unilateral nasal symptoms that do not respond to medical treatment, especially recent dental treatment in the past. Thus, the diagnosis of odontogenic sinusitis requires multidisciplinary team, systematic dental imaging examination and computed tomography, including three-dimensional techniques such as cone beam computed tomography (14, 15) in some cases.

A common consensus on the treatment plan for odontogenic sinusitis is not yet established; and the ideal therapeutic management algorithm is not yet standardized. However, there are studies that have reported promising results with different approaches, including endoscopic sinus surgery and dental surgery.

Endoscopic sinus surgery is especially recommended when the osteomeatal complex is blocked and the sinus mucosa is significantly thickened. In this context, elimination of the dental source of infection, such as endodontic treatment of the causative tooth or elimination of periapical or periodontal infection, is essential for prevention of sinusitis recurrence (16). Recent studies have highlighted the benefits of concurrent management of endoscopic sinus surgery and dental surgery, which can ensure complete resolution of infection. In addition, antibiotic treatment is often used as first-line treatment to control infection and reduce inflammation. It is also important to point out that common procedures in dental practice, such as insertion of dental implants or sinus lift procedures, may affect the maxillary sinus (17). Therefore, these procedures should always be preceded by an otolaryngologic examination to assess the status of the osteo meatal complex and to minimize the risk of complications. The success of dental implants can be significantly influenced by the health of the maxillary sinus and the integrity of its mucosa.

There are various opinions on the methods of treatment of dental sinusitis, which may include medical management, dental treatment or surgery (18). Medical management may involve administration of antibiotics to control infection and nasal irrigation with saline to reduce inflammation and remove sinus secretions (19, 20). Dental treatment may include endodontic therapy to treat root infections or tooth extraction in severe or irreparable cases. Endoscopic sinus surgery may be necessary in cases where there are complications or obstructions of the maxillary sinuses (21).

Despite the diversity of therapeutic ap-

proaches, it is important to emphasize that the therapeutic protocol of odontogenic sinusitis is not yet standardized (22, 23). However, it is clear that this complex condition requires a mandatory multidisciplinary approach, involving close collaboration between dentists, ENT and, in some cases, other relevant specialists. Only a comprehensive assessment and a personalized treatment plan, tailored to the specific needs of each patient, can ensure effective and successful management of odontogenic sinusitis.

Although odontogenic sinusitis is a relatively common condition, especially in certain age groups, it is underdiagnosed because of its non-specific symptoms. In addition, it can frequently be confused with rhinogenic sinusitis and thus not treated properly. A multidisciplinary team composed of an ENT specialist, a specialist in dento-alveolar or maxillo-facial surgery and a dentist is required for correct diagnosis and optimal therapeutic management.

Treatment of odontogenic sinusitis can involve a complex approach, usually divided into two distinct stages: non-surgical and surgical. However, it is crucial to emphasize that the therapeutic success of any intervention is often conditional on the resolution of the primary etiological factor, which is dental in nature. In the absence of effective management of this fundamental issue, treatment outcomes may be limited or even suboptimal (24).

Odontogenic sinusitis usually presents unilaterally affecting the maxillary sinus adjacent to the affected tooth or the inflamed dento-alveolar region. However, in the absence of adequate treatment it can be complicated by pan- or polysinusitis, orbital complications, general complications sometimes life-threatening. In its management, tooth extraction and curettage of osteolytic areas are considered crucial steps in the favorable post-operative and remote course. In cases where medical treatment is ineffective or insufficient, surgery is required.

Surgical intervention is in some cases delayed until the patient's hemodynamic balance is achieved. Some patients have severe co-morbidities such as liver disease with altered clotting factors, sepsis or organ failure, diabetes mellitus, cardiovascular disease, which may delay surgery. However, in emergency cases, especially those with orbital abscess consecutive with visual impairment, and those with generalized septic status, immediate surgical intervention is appropriate, requiring careful assessment of surgical treatment and intraoperative vital risk (25, 26). The treatment of choice is usually surgical; performing functional endoscopic rhinosinusional surgery (FESS) with favorable postoperative outcome in most cases.

Apical periodontitis, periodontal disease and iatrogenic foreign body extrusion into the sinus are the main causes of OS. Although the exact prevalence of dental sinus pathology is still controversial, ENT physicians are increasingly aware of its importance. In cases of treatment-resistant or unsatisfactory sinusitis, a dental origin should be considered and properly investigated. This requires close collaboration between dentists and ENT specialists for a comprehensive assessment and appropriate management of the condition.

CONCLUSIONS

Odontogenic sinus pathology is a growing but frequently under-diagnosed and

under-recognized condition, whose dimensions and impact on patients' health require a multidisciplinary approach.

By recognizing and properly managing dental sinus pathology, the quality of life of patients can be significantly improved and severe complications associated with the condition can be prevented.

It is essential that physicians from both specialties are aware of this link and work together effectively to ensure optimal care for affected patients.

FESS is a good option for treating odontogenic sinusitis, with good results if it is conducted correctly and performed after or concomitant with dental cause correction. It has replaced more invasive procedures used commonly for odontogenic sinusitis treatment, such as anterior sinus wall trephination, with advantages like shorter hospitalization, less pain and faster recovery.

CONFLICT OF INTEREST AND FUNDING

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PERCEPTION OF MEDICAL PROFESSIONALS ON RADIOLOGICAL SAFETY PRACTICES IN THE WORKPLACE

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PERCEPTION OF MEDICAL PROFESSIONALS ON RADIOLOGICAL SAFETY PRACTICES IN THE WORKPLACE (Abstract): Radiation protection aims to reduce unnecessary exposure to radiation to minimize the harmful effects of ionizing radiation, this goal is achieved by using different means and methods of radiation protection. The **aim** of this study was to analyze the knowledge, perceptions, and attitudes of medical personnel in the N-E Region of Romania regarding radiation protection and the effects of ionizing radiation. **Materials and methods:** The study was carried out based on the use of a structured questionnaire for data collection, a questionnaire that was distributed to the medical staff (doctors, nurses, radiologists) from hospitals and medical centers in the N-E Region of Romania. **Results:** The results show that most respondents know about the stochastic and deterministic biological effects of ionizing radiation, but a significant group has incomplete, average, or uncertain knowledge about this topic. **Conclusions**: These aspects suggest a need for additional education in the field of radiation protection in the workplace. **Keywords:** RADIATION PROTECTION, IONIZING RADIATION, BIOLOGICAL EFFECTS.

INTRODUCTION

In the medical field, ionizing radiation has become an inevitable tool used for diagnosing and treating a variety of medical conditions. Ionizing radiation is radiation (electromagnetic or corpuscular) that has enough energy to ionize the atoms (molecules) of the substance with which it interacts.

As their use has evolved, so have the cumulative doses of lifetime radiation that both patients and healthcare providers (healthcare professionals) receive. This requires a thorough understanding of the risks of radiation exposure and radiation dose reduction techniques in order to prevent biological effects. It was observed that although there were extraordinary benefits, there were associated dangers, equally great, not only for patients who underwent X-ray investigations, but also for the personnel who operated the facilities using X-rays. In addition to wearing radiation protection equipment (lead aprons, thyroid protection collars, lead salt glasses, gloves with lead insert), there were recommendations on limiting the exposure time (exposures as short as possible), along with X-ray beam filtering and the use of collimation (1-8).

Radiation exposure produces important biological effects, and prolonged exposure can lead to cell death. Biological effects are divided into stochastic effects and deterministic effects. The stochastic effects are due to cellular changes (DNA) and proliferation towards malignant diseases. The severity of the effects is independent of the dose but may increase with increasing dose. There is no threshold dose, the effects can occur even at very low doses, but the probability of the effect increases proportionally with the dose. The deterministic effects are due to cell death and have a threshold dose – a few Grays. They are specific depending on the affected tissue, and the severity of the effect is dose-dependent, the threshold being different from individual to individual (3, 14**).**

In the case of the use of CBCT (Cone Beam Computed Tomography), it has been observed that there are genotoxicity and cytotoxicity effects at the level of exfoliated oral epithelial cells. Cytotoxicity can lead to nuclear changes and usually causes lethal injury or necrosis (15).

Radiation protection is the totality of methods and means of reducing the harmful effects of ionizing radiation. Radiation protection aims to minimize the harmful effects of ionizing radiation by reducing unnecessary exposure to radiation. Ionizing radiation is used in the medical field to diagnose and treat a variety of medical conditions. The more they are used, the higher the cumulative lifetime radiation doses received by both professionally exposed personnel and patients (1).

After the discovery by W. C. Roentgen of X-rays in November 1895 and the increasingly accelerated use in the twenty-first century, the role of radiation protection was highlighted as an important tool for the management of health protection measures against the risks (for people and the environment) arising as a result of the use of ionizing radiation (5, 8).

A particular concern has been observed with long-term irradiation, at low, cumulative doses, which can lead to an increase in malignant diseases and genetic mutations, effects that can propagate to future generations. Thus, it is important to analyze the risk-benefit factor when using irradiation techniques (14). In order to reduce the radiation dose, the aim was to implement strategies to improve dose optimization, to draw up standard protocols, as well as to implement the concept of good practices (7, 12).

The radiation protection system aims to prevent the occurrence of deterministic effects and to reduce the occurrence of stochastic effects. Medical personnel working both in interventional radiology and in specialties that use interventional radiology techniques (cardiology, neurosurgery, orthopedics, urology, pulmonology, vascular surgery) are exposed to ionizing radiation coming from artificial sources (radiological installations), their protection is ensured by the use of lead screens (lead aprons, lead screens) (2, 16).

In the case of interventional radiology, the leaded apron with or without a collar is the standard clothing used against ionizing radiation. It is designed sleeveless, so the armhole can be an unprotected area, and the radiation scattered from the patient can contribute to an increase in doses to different organs of the operator and thus increase the effective dose (6). Since the equipment mentioned above is quite heavy and has a drawback, the absence of sleeves, we tried to introduce a much lighter protective equipment, in order to observe its effectiveness against ionizing radiation. Thus, it was found that the proposed new equip-

ment reduces the dose by approximately 98.1% and 90.1%, respectively. With the addition of radiation protection shields, a dose reduction of 99.0% and 98.2% was observed (17).

Another method of dose reduction is the "As Low as Reasonably Achievable" (ALARA) principle. This principle was first introduced in the nuclear energy sector and was later adopted in the field of radiology and medical imaging, with the aim of warning professionally exposed personnel that medical imaging should be used with justification (13).

Studies conducted so far indicate a low level of knowledge of radiation exposure in medical imaging among both healthcare professionals and patients (4). Studies from Greece and Turkey have highlighted significant deficiencies in radiation protection, influenced by educational level and professional experience, recommending continuous training and review of medical training to correct misperceptions and improve the quality of services (9, 10, 11).

In Romania, radiological safety in medicine is regulated and studied within the norms imposed by CNCAN (National Commission for the Control of Nuclear Activities). Research and regulations focus on diagnostic, interventional, and nuclear medicine radiology, with an emphasis on staff training, risk assessment, and implementation of radiation protection programs. For example, mandatory radiation protection courses for healthcare professionals and occupational exposure monitoring programs to ionizing radiation are required, tailored to the specific risks of each medical practice (18-20). What is important to emphasize is the lack of studies carried out at the national level, studies that assess the level of knowledge and attitudes regarding radiation protection. Thus, the purpose of this study was to analyze the knowledge, perceptions, and attitudes of medical personnel in the North-East Region of Romania regarding radiation protection and the effects of ionizing radiation.

MATERIALS AND METHODS

The observational study carried out in the North-Eastern region of Romania, between May 20 and June 15, 2024, aimed to analyze the knowledge, perceptions, and attitudes of medical personnel (doctors, nurses, radiologists) regarding radiation protection and the effects of ionizing radiation. The data were collected through a structured questionnaire, developed based on the literature and the research objectives, after obtaining the opinion of the Ethics Committee of "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

The sample, consisting of 100 participants selected by a convenience technique, ensured adequate representativeness for statistical analyses.

The questionnaire, applied online between May 20th and June 15th, 2024, through the Google Forms platform, included questions about the biological effects of radiation, known methods of radiation protection, optimal exposure intervals, and workplace safety measures. Before data collection, a pilot phase was carried out to validate the instrument. Only fully completed questionnaires were considered, and the process included coding and statistical analysis of data, providing a detailed insight into the level of training and awareness of medical staff.

Statistical analysis was descriptive, presented in the form of frequencies, percentages, and mean values for the variables of interest, and the correlation analysis where Pearson or Spearman correlation coefficients were used to examine the rela-

tionships between the variables. The threshold of statistical significance was set at p=0.05.

RESULTS

The analysis was conducted on a representative sample of 95 respondents. Of the total participants, 75.8% have bachelor's or post-secondary studies (in the case of nurses), 10.5% have master's degrees, and 13.7% have completed postgraduate studies. 45.3% of respondents have 10 years or less of seniority in the specialty, which suggests that almost half of the respondents are at the beginning or middle of their career (fig. 1).

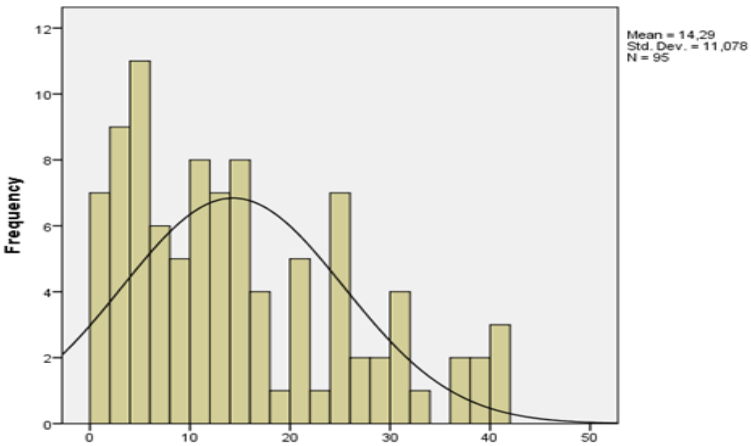

Fig. 1. Distribution of years of seniority of medical professionals

The perception of the safety status was achieved through the analysis of indicators, such as knowledge of the stochastic biological effects of ionizing radiation, fear of radiation effects, known radiation protection methods, optimal exposure range, and safety measures at work. After analyzing the results, it was found that most of the respondents (74.7%) have knowledge about the stochastic and deterministic biological effects of ionizing radiation, which shows a high level of information among the staff. A significant group (22.1%) has incomplete or average knowledge, suggesting a possible need for further education in this area. Very few respondents (2.1%) have no knowledge or are uncertain about this topic, which is positive, but still indicates the need for continuous awareness (tab. I).

TABLE I.

Do you know the stochastic/deterministic biological effects of ionizing radiation?

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Yes	71	74.7	74.7	74.7
	Not	1	1.1	1.1	75.8
	Partially	21	22.1	22.1	97.9
	Ns/Nr	2	2.1	2.1	100.0
	Total	95	100.0	100.0	

From the analyses performed, a moderate positive correlation can be observed Pearson = 0.234, (Sig. 2-tailed) = 0.022 between the knowledge of these effects and the level of study. This suggests that as the level of education increases, knowledge of the biological effects of radiation tends to increase as well. Moreover, there is a significant but weak negative correlation between fear of ionizing radiation and level of education, suggesting that people with higher education tend to have less fear of radiation (-0.263, p = 0.010) (tab. II).

TABLE II.
The correlation between the level of education and knowledge/fear in relation to ionizing radiation

		Studies
Studies	Pearson Correlation	1
	Mr. (2-tailed)	
	N	95
Do you know the stochastic /? deterministic biological effects of ionizing radiation?	Pearson Correlation	,234*
	Mr. (2-tailed)	,022
	N	95
Are you afraid of ionizing radiation?	Pearson Correlation	-,263*
	Mr. (2-tailed)	,010
	N	95

The most harmful of all the diseases listed are leukemia and hematological diseases. The damage to the digestive system has an average of 2.83, which indicates a lower perception of harmfulness compared to other effects, and with a moderate perception (mean = 3.64) we have effects on the immune system. All perceptions of harmful effects (cancer, genetic mutations, leukemia, and thyroid disease) are strongly correlated with each other, with correlation coefficients ranging from 0.656 to 0.817. This indicates that those who consider one effect to be harmful tend to attach similar importance to the other effects (tab. III).

We found a negative correlation between the knowledge of these biological effects and the fear of radiation (-0.213). which suggests that there is a relatively weak association between the two investigated variables. which is nevertheless statistically significant (0.038). Thus. as the level of knowledge about the biological effects of ionizing radiation increases. the fear of ionizing radiation tends to decrease. and vice versa (tab. IV).

From the analysis of the data. we find that 62.1% of people say that they do not have a certain fear of ionizing radiation. but there is a considerable percentage that says that there are certain aspects that can lead to fears in the activity they do.

Concerns about ionizing radiation and the assessment of its biological impact highlight significant correlations between fear of radiation and the perception of serious conditions associated with exposure to it. For cancer. the correlation coefficient is 0.390. indicating a moderate and statistically significant positive correlation (p = 0.000) between fear of radiation and the belief that it is a serious effect of radiation exposure. This suggests that people with a

greater fear of radiation tend to consider cancer as one of the most severe consequences of such exposure. For genetic mutations. the correlation coefficient is 0.220. statistically significant at the level of 0.05 (p = 0.032). which reflects a weak positive correlation between fear of radiation and its perception as harmful effects. Respondents with an increased fear of radiation are more likely to perceive genetic mutations as dangerous. although this association is less strong compared to that for cancer (tab. V).

TABLE III.

Perception of the biological effects of radiation

What biological effects do you consider to be more harmful? Scale 1-5	A	B	C	D	E	F	G	H	I	J
MEAN	**4.25**	**4.15**	**4.31**	**4.01**	**3.01**	**3.05**	**3.81**	**3.64**	**2.83**	**3.64**
A	1									
B	.758**	1								
C	.817**	.760**	1							
D	.671**	.656**	.713**	1						
E	.328**	.307**	.296**	.301**	1					
F	.357**	.286**	.363**	.269**	.790**	1				
G	.448**	.390**	.533**	.454**	.414**	.354**	1			
H	.464**	.436**	.410**	.421**	.435**	.390**	.646**	1		
I	.420**	.368**	.389**	.331**	.623**	.567**	.517**	.627**	1	
J	.590**	.502**	.645**	.491**	.310**	.344**	.507**	.534**	.578**	1
Mr. (2-tailed)	**.000**	**.000**	**.000**	**.000**	**.002**	**.001**	**.000**	**.000**	**.000**	**.000**
N	**95**	**95**	**95**	**95**	**95**	**95**	**95**	**95**	**95**	**95**

A - [Cancer]. B- [Genetic mutations]. C- [Leukemia and hematological disorders]. D- [Thyroid disorders]. E- [Radiological burns]. F- [Acute radiation syndrome]. G- [Reproductive system damage]. H- [Eye system damage] I- [Damage to the digestive system]. J- [Suppression of the immune system]

TABLE IV.

Analysis of correlations between knowledge of biological effects and fear of ionizing radiation

		Do you know the stochastic/ deterministic biological effects of ionizing radiation?	Are you afraid of ionizing radiation?
Do you know the stochastic / deterministic biological effects of ionizing radiation?	Pearson Correlation	1	-.213*
	Mr. (2-tailed)		.038
	N	95	95
Are you afraid of ionizing radiation?	Pearson Correlation	-.213*	1
	Mr. (2-tailed)	.038	
	N	95	95
*. Correlation is significant at the 0.05 level (2-tailed).			

TABLE V.

Correlations between fear of radiation and the perception of serious conditions associated with exposure to it

Correlations		Are you afraid of ionizing radiation?	What biological effects do you consider to be more harmful?			
			Cancer	Genetic mutations	Leukaemia and hematological disorders	Thyroid disorders
Are you afraid of ionizing radiation?	Pearson Correlation	1	.390**	.220*	.323**	.266**
	Mr. (2-tailed)		.000	.032	.001	.009
	N	95	95	95	95	95
**. Correlation is significant at the 0.01 level (2-tailed) / *. Correlation is significant at the 0.05 level (2-tailed).						

In the case of leukemia and hematological disorders. the correlation coefficient is 0.323. significant at the level of 0.01 (p = 0.001). indicating a moderate positive correlation. People who show a greater fear of radiation are more inclined to consider leukemia and hematological disorders as harmful effects of radiation. For thyroid disorders. the correlation coefficient is 0.266. significantly at the level of 0.01 (p = 0.009). which indicates a moderate positive correlation. but relatively weaker than that observed for leukemia or cancer. There is a standard deviation of responses above 1 within the variables "Reproductive system impairment" and "Immune system suppression." which suggests a wide dispersion of opinions. indicating that participants have different perceptions of the severity of these effects (tab. 3).

In the study on the recognition of radiation protection methodologies. several techniques were evaluated to determine the level of familiarity and perception of their effectiveness among participants. Lead barrier screens. with a score of 90.6%. stand out as a widely accepted and considered effective method. Personal dosimetry. which obtained an average score of 60.4%. is not the most recognized method of radiation protection but indicates a high appreciation of its importance in safety measures (fig. 2).

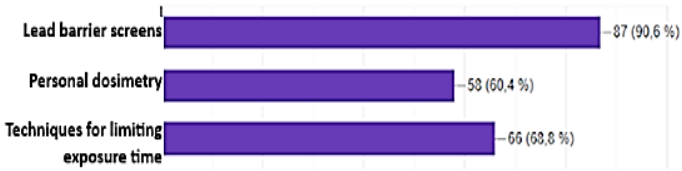

Fig. 2. Known radiation protection methods

Concerning the category of various protective methods (classified under "Other.."). the most frequently mentioned methods of radiation protection are the collimation of the radiation beam and the use of personal protective equipment. such as the protective apron with Pb insert and the collar with Pb insert. to protect the thyroid.

each of which is mentioned several times. Also mentioned were the collimation. the use of filters. the walls plated with lead or barite. the leaded glass. and the performance of radiological investigations as rarely as possible.

When asked about the optimal interval between exposures for optimization (referring to professionally exposed personnel - doctors. nurses. medical bioengineers. physicists). 24.2% of the participants opted for an interval of one hour. 11.6% for a day. 10.5% for a week and 8.4% for a month. while 45.3% gave other varied answers (tab. VI).

TABLE VI.

Best exposure interval for optimization

		Frequency	Percent
Valid	1 hour	23	24.2
	1 day	11	11.6
	1 week	10	10.5
	1 month	8	8.4
	Another answer	43	45.3
	Total	95	100.0

In terms of protection measures. respondents were questioned about the following variables: confidence in safety measures. regular updating of these measures. consultation with experts on radiation safety. and collaboration with radiation protection experts (tab. 7).

Regarding trust in security measures. the media of 7.94 indicates a relatively positive assessment of trust in existing security measures. although it is not perfect. The median of 9.00 suggests that most respondents rate this confidence close to the top of the scale. The standard deviation of 2.633 indicates moderate variability in responses. Positive kurtosis (0.553) suggests a slightly flat distribution. indicating that there are more very high ratings than in the case of a normal distribution (tab. 7).

TABLE VII.

Degree of confidence in radiation safety

How do you rate on a scale from 1 (not at all) to 10 (very much) the following statements?		[I am confident in the safety measures in place in the workplace]	[Security measures are periodically updated according to new technologies and research in the field]	[The occurrence of incidents related to workplace safety is low]	[We are regularly consulted on radiation safety]	[We call on radiation protection experts in case of incidents that occur in the workplace]
N	Valid	95	95	95	95	95
	Missing	0	0	0	0	0
Mean		7.94	7.81	6.75	7.29	8.23
Median		9.00	9.00	9.00	9.00	10.00
Std. Deviation		2.633	2.818	3.501	3.118	2.804
Kurtosis		.553	-.047	-1.445	-.888	.370
Std. Error of Kurtosis		.490	.490	.490	.490	.490

The results highlight that those who say they are confident in the safety measures are fully aware of the biological effects considered more harmful. There is a very strong and statistically significant relationship between the updating of security measures regularly and the level of confidence of employees in the security measures implemented in the workplace. The Pearson coefficient of 0.889 indicates a very strong and positive correlation. This means that as the clarity and availability of emergency procedures are present. employee confidence in radiation safety measures also increases. The meaning of 0.000 confirms that this relationship is extremely robust.

Moreover. in this examination. the associations between knowledge of radiation protection methods and confidence in safety measures were analyzed. The Pearson coefficient of 0.261 indicates a weak positive association between understanding the use of lead screens and confidence in safety protocols. suggesting that a deeper awareness of the protective role of these barriers contributes to increased confidence in workplace safety. although the influence is not particularly pronounced.

In terms of the perception regarding the periodic updating of security measures. the general perception is positive. but the variation in responses indicates that some employees believe that this aspect can be improved (mean: 7.81 - standard deviation: 2.818). Regarding the degree of occurrence of incidents related to safety at work. the average of responses is 6.75. showing a greater concern among employees regarding security incidents. However. the perception is quite varied. with a high dispersion of the answers provided (tab. VIII).

Regarding the periodic consultation of staff in relation to radiation safety. the variation in responses is relatively large. suggesting that some employees feel that they are not consulted often enough on issues related to radiation safety.

TABLE VIII.

Correlations between trust in security measures and different indicators

		Do you know the stochastic / deterministic biological effects of ionizing radiation?	What biological effects do you consider to be more harmful? [Cancer]	Known Radiation Protection Methods (Barrier Lead Screens)	How efficient is the equipment today?	How do you assess how the introduction and adoption of new technologies is managed?	[Security measures are periodically updated according to new technologies and research in the field.
I am confident in the security measures in place at work.	Pearson Correlation	-.299**	.212*	.214*	.390**	.261*	.889**
	Mr. (2-tailed)	.003	.039	.037	.000	.010	.000
	N	95	95	95	95	95	95

DISCUSSION

The present study was carried out starting from the following variables. namely. the knowledge of the stochastic biological effects of ionizing radiation. the fear of the effects of radiation. the known radiation protection methods. the optimal exposure interval. and the safety measures at the workplace.

In terms of knowledge of the stochastic

biological effects of ionizing radiation. the analysis showed that some of the respondents have adequate knowledge about the effects of radiation. but we have an important number of those who show that they have incomplete knowledge. indicating the need for additional education. The moderate positive correlation between knowledge of the effects and level of education suggests that more advanced education is associated with a better understanding of the effects of radiation. These conclusions are also supported by the cross-sectional study carried out in Greece involving 132 members of the medical staff. including nurses. radiographers. and doctors. with a response rate of 97% (10). A study in Nigeria found that 92% of medical personnel demonstrated a solid knowledge of radiation safety. but only 81% complied with protective measures (20). In Sri Lanka. nurses showed satisfactory awareness of radiation protection. but gaps in understanding the effects of radiation persisted. indicating a need for further training (21).

In terms of fears about the effects of radiation. the perception of risks varies significantly. with healthcare professionals and patients often having limited knowledge and fears related to exposure. The fear of radiation decreases with the increase in the level of education. having a significant negative correlation coefficient. The negative correlation between knowledge about the effects of radiation and fear of it suggests that a better understanding of the effects of radiation helps reduce fears. although fear is greater in the case of cancer. leukemia. and genetic mutations. Numerous studies reiterate the need for a high level of knowledge about radiation protection (22). There is a fairly unified perception regarding the harmfulness of biological effects. Analyzing the average of the responses on the knowledge of these effects. the following can be found: for carcinogenic effects. there is an average of 4.25 responses. which indicates that most consider cancer as one of the most harmful effects of ionizing radiation. In terms of genetic mutations. the average of 4.15 suggests that they are considered to be very harmful.

In terms of radiation protection methods. various radiation protection methods have been implemented to reduce the risks associated with radiation exposure. These include the use of protective equipment (lead aprons. collars for thyroid protection). limiting exposure time. filtering the X-ray beam. and collimation. By applying these measures. significant reductions in the effective dose have been achieved (8. 16. 17). Our research supports these conclusions. from applied questionnaires showing that lead barrier screens are the most known and appreciated (90.6%). and personal dosimetry has a moderate recognition (60.4%) (fig. 2). However. awareness of radiation protection methods has a weak correlation with trust in security measures. indicating that a better understanding of protection does not significantly influence confidence in security measures. Correlated with our results. a survey in Karachi revealed that although radiology workers had a basic understanding of radiation protection. their confidence in patient protection measures was insufficient (23).

Other research. however. has shown a significant correlation between understanding radiation and protective practices. but this has not extended to trust in safety measures (“An empirical analysis of the relationship between understanding. attitudes. and practices toward radiation – Focusing on the population eligible for South Korean National Health Insurance Service Examination”) (24).

Regarding the optimal exposure range**.** the ALARA principle ("As Low as Reasonably Achievable") remains an essential benchmark. which aims to minimize radiation exposure by justifying each exposure. This approach focuses on minimizing the radiation dose received. both for patients and occupationally exposed staff (13). From our results. the optimal interval between exposures varies significantly. with most respondents indicating short intervals (for 1 hour). and a considerable proportion giving varied answers. Overall. the implementation of these optimization strategies is essential to protect healthcare professionals while ensuring high-quality imaging (25).

The study also looked at trust in workplace safety measures. This is relatively high. but variable. The very strong correlation between the regular updating of security measures and the level of trust suggests the importance of keeping the measures up to date in order to ensure a high level of confidence in these measures. Especially in interventional radiology and other specialties that use radiological techniques. safety measures are essential. These include wearing protective equipment and using lead screens. Studies have shown that new protective equipment can significantly reduce the dose of radiation. but there are still unprotected areas. such as the arms. that require additional solutions to minimize exposure (6. 17).

The main limitation in the present study was represented by access to medical professionals and the presence of non-probability sampling.

CONCLUSIONS

The analysis shows that the level of education of medical professionals significantly influences the knowledge about the effects of ionizing radiation. Most respondents have a good understanding of these effects. although there is a need for continuous training. An inverse correlation was observed between the level of education and fear of radiation. with those with advanced education being less fearful. The fear of radiation is directly proportional to the perceived severity of these conditions. The variability of perceptions of the optimal exposure range suggests the need for clearer guidelines.

Confidence in radiation safety measures is high. but constant updates and consultations with experts are essential to maintain it. A well-protected and educated work environment reduces perceived radiation-related risks.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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26.

TOXOPLASMOSIS IN NORTH-EAST ROMANIA. DESCRIPTIVE STUDY COUNTING CASES BETWEEN 2010 AND 2016

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TOXOPLASMOSIS IN NORTHEAST ROMANIA. DESCRIPTIVE STUDY COUNTING CASES BETWEEN 2010 AND 2016 (Abstract): Infection with the parasite protozoa *Toxoplasma gondii* has a worldwide distribution. The mandatory intracellular parasite can infect humans, practically all warm-blooded animals, including mammals and birds. The study aim was to highlight descriptive epidemiology data on toxoplasmosis in patients admitted to a regional infectious disease hospital in North East Romania. **Material and methods**. We conducted a descriptive longitudinal study on a group of 160 patients admitted to the Iasi Clinical Hospital of Infectious Diseases with the diagnosis of toxoplasmosis, during 2010 and 2016. **Results**. Annual distribution of patients with toxoplasmosis had a maximum of frequency in 2010 (34 cases – 21.25%) and a minimum in 2016 (6 cases – 3.75%), with an increasing trend for the whole interval of study. Most of the patients were females (111 cases – 69.37%; M/F ratio = 0.44), from urban area (59.0%; U/R ratio = 1.43). The most affected age group was that of 15-24 years (38 cases – 23.75%), followed by 25-34 years (36 cases – 22.50%). Children aged between 1-4 years were less affected (3 cases – 1.87%). Either infants aged 0-1 year, or elderly persons over 74 did not admitted with toxoplasmosis. **Conclusions**. The frequency and severity of the disease are used to assess its medical relevance, and data on this can be collected in specific studies or surveillance systems. Targets for the future could be the implementation of a surveillance system of toxoplasmosis of any kind (acquired or congenital), a vaccine and, over time, a zero-incidence level of the disease.
Keywords: TOXOPLASMOSIS, EPIDEMIOLOGY, WATERBORNE DISEASE, MARINE, WILD FELID.

The world, whatever it is, the land, the navy or the wings, is full of toxoplasmosis. Infection with the parasite protozoa *Toxoplasma gondii* has a worldwide distribution. The mandatory intracellular parasite can infect humans, practically all warm-blooded animals, including mammals and birds. The first description was made by Nicolle and Manceaux in 1908 in rodent populations in North Africa, but its entire lifecycle was only definitively understood in the late 1960's, with the discovery of the cat's central role as

the definitive host for the sexually parasitic cycle and the spread of oocysts through faeces. His medical significance remained unknown until 1939, when *T. gondii* was identified in the tissues of a congenital infected child, and the veterinary importance became known in 1957 when it was found to have caused abortion in sheep. The discovery of the Sabin-Feldman test in 1948 led to the recognition that *T. gondii* is a common parasite of the warm-blooded host with a worldwide distribution. The real significance of the clinical implications of toxoplasmosis in humans was unknown until the first reports of cases of congenital toxoplasmosis, subsequently the role of *T. gondii* infection in immunocompromised patients was recognized in the mid-1970's and the concept of reactivation of the infection was subsequently investigated. In the last decade, the development of new genotyping tools has led to discoveries in understanding the global evolution of *T. gondii* phylogenetics and the recent advances in studies related to the degree of virulence of the protozoan associated with some genotypes (1, 2, 3, 4, 5).

Approximately 25-30% of the world's human population is infected with *Toxoplasma*. In fact, prevalence varies greatly between countries (from 10 to 80%) and often in a particular country or between different communities in the same region. The decrease in seroprevalence (10-30%) was observed in North America, Southeast Asia, North Europe, and the Sahel region of Africa. Moderate prevalence (30-50%) was found in Central and South European countries, and in Latin America and African tropical countries there were high prevalence rates (1, 2).

Screening of the large diversity of *Toxoplasma* host species has no specific test. The sensitivity and specificity of the techniques depend on animal species and the cutoff values are difficult to establish because the reference sera in experimentally infected animals are missing. Even when these sera are available for a species, they do not reflect natural conditions because experimental animals are often infected with high doses and sometimes by unnatural pathways that can cause excessive antibody titers. The modified agglutination test (MAT) seems to be most appropriate for a large number of species, but specific tests for enzyme immunoabsorption (ELISA) have been developed for certain species of domestic animals (1, 2, 3).

The incidence of toxoplasmosis in the general population in Romania and the incidence of congenital malformations were decreased according to the data reported to the Center for Calculation and Sanitary Statistics during 2000 and 2011. The mean incidence was 1.45‰ with the highest value (1.97‰) in 2003 and the lowest (1.15‰) in 2000. The highest average incidence was in the environment urban (1.96‰) and the age group under 1 year (2.24‰) (7).

In Romania, toxoplasmosis is a disease with mandatory reporting. Cases of *T. gondii* have been reported since 2007 as a "single case report of the communicable disease case" within 5 days of the detection of the suspect or confirmed case (8).

The study aim was to highlight descriptive epidemiology data on toxoplasmosis in patients admitted to a regional infectious disease hospital in North-East Romania.

MATERIAL AND METODS

We conducted a descriptive longitudinal study on a group of 160 patients admitted to the Iasi Clinical Hospital of Infectious Diseases with the diagnosis of toxoplasmosis, during 2010 and 2016. The data were collected from patient records and reported

files, respecting the confidentiality of the information. Inclusion criteria were limited to diagnosis of toxoplasmosis, and exclusion criteria did not refer to gender, residence area, or any age group. Secondary diagnoses and co-morbidities did not take into consideration, also.

The data was processed with the MS Excel 2010 software.

RESULTS

Annual distribution of patients with toxoplasmosis had a maximum of frequency in 2010 (34 cases – 21.25%) and a minimum in 2016 (6 cases – 3.75%), with an increasing trend for the whole interval of study (fig. 1).

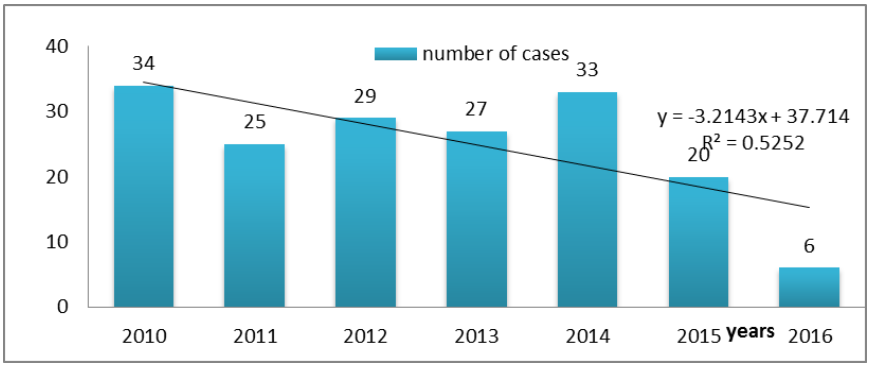
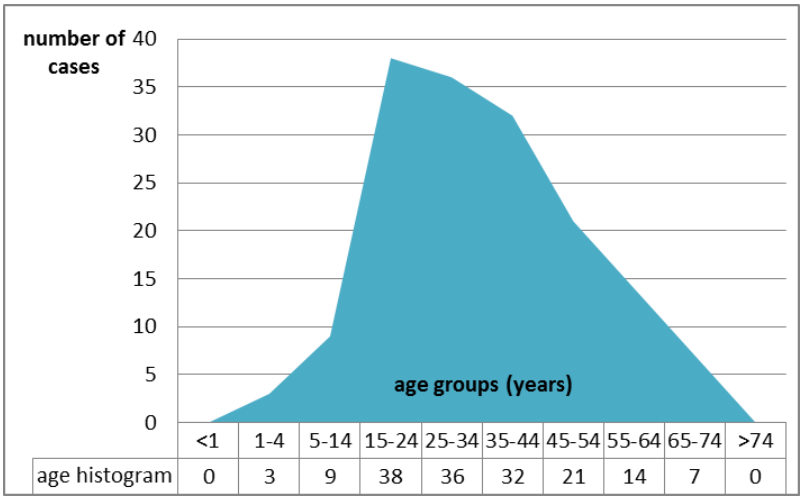


Fig. 1. Distribution of cases with toxoplasmosis by year of study

Most of the patients were females (111 cases - 69.37%; M/F ratio = 0.44), from urban area (59,0% ; U/R ratio = 1.43). The most affected age group was that of 15-24 years (38 cases – 23.75%), followed by 25-34 years (36 cases – 22.50%). Children aged between 1-4 years were less affected (3 cases – 1.87%). Either infants aged 0-1 year, or elderly persons over 74 did not admitted with toxoplasmosis (fig. 2).



	<1	1-4	5-14	15-24	25-34	35-44	45-54	55-64	65-74	>74
age histogram	0	3	9	38	36	32	21	14	7	0

Fig. 2. Age histogram of patients with toxoplasmosis

Most of patients had a diagnosis of toxoplasmosis without any specification of site or organ involvement (149 cases – 93.12%, M/F = 0.43). There are several cases suffered from toxoplasmosis with ocular involvement (2 cases, both males – 1.25%), as well as other organs (9 cases – M/F = 2/7) (fig. 3).

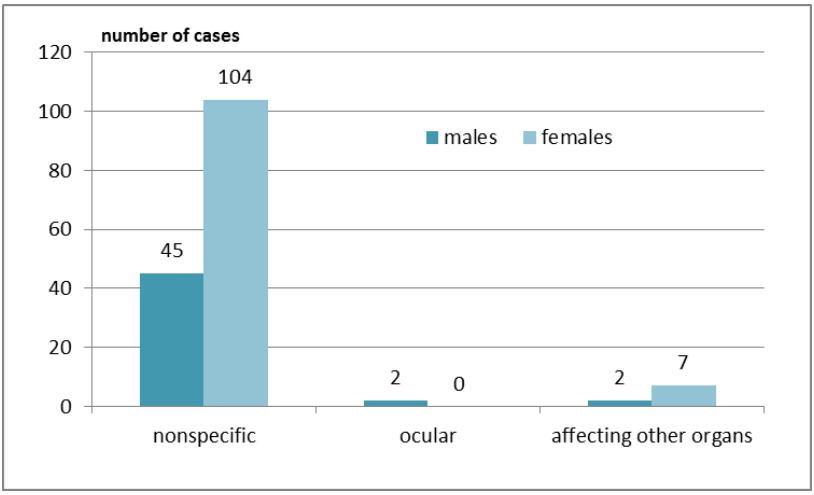


Fig. 3. Toxoplasmosis cases related to gender and specific sites

Distribution by month of admission showed a cluster of cases diagnosed during April - May and another one during September, with a maximum in May (21 cases – 13.12%) and a minimum in December (5 cases – 3.12%) (fig. 4).

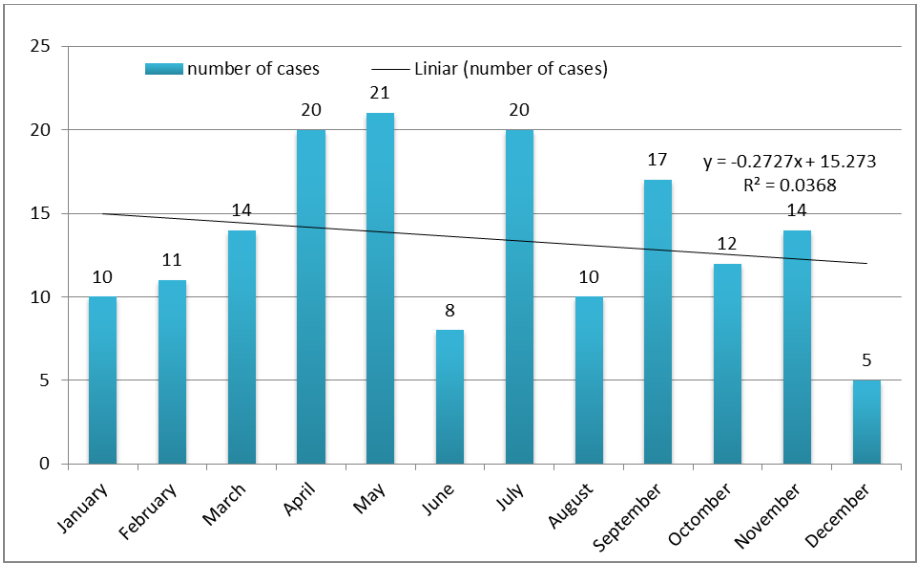


Fig. 4. Distribution of cases by month of admission

Seasonal distribution highlighted a predominance of cases admission during Spring (55 cases – 34.37%), then in Autumn (43 cases – 26.87%), Summer (38 cases – 23.75%), and Winter (26 cases – 15.01%) (fig. 5).

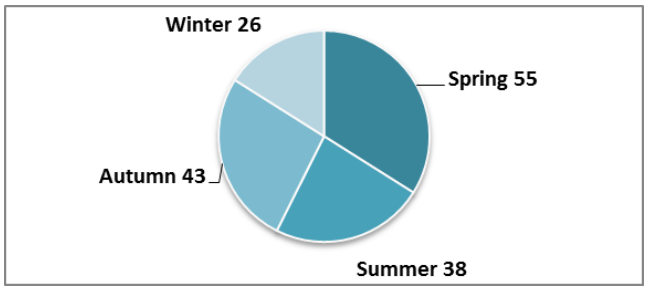


Fig. 5. Seasonal distribution of cases

As compared with hospitalization days due to all diagnoses were of 540,721 for all 72064 cases admitted with infectious diseases, during 2010 and 2016, hospitalization days for 160 patients with toxoplasmosis were 939, from which nonspecific – 895 days, ocular – 8 days, and toxoplasmosis affecting other sites – 36 days (tab. I)..

TABLE I.
Average length of stay

Diagnosis	Number of cases	Hospitalization days
All	72064	540721
Toxoplasmosis - nonspecific	149	895
Toxoplasmosis - ocular	2	8
Toxoplasmosis - other organs	9	36

DISCUSSION

Toxoplasmosis has been identified in mammals and birds which more than 350 host species, mammals and birds, most of them in the wild.

Peridomestic felid species habiting near farms or wild species can contaminate the environment by eliminating oocysts. Intermediate wildlife will subsequently become contaminated when the felids are present in the environment. Of the 39 feline species in the world, serological and parasitologic studies have shown that 31 species have *T. gondii* infection. In wild felids, the seroprevalence is high and can reach 100%. The processes leading to the infestation of wildlife populations are complex, involving physical, biological, ecological, and zoological factors and characteristics. The dry and warm climate is considered to be unfavorable to the survival of the oocysts. Countries with such climate report lower values of the prevalence of toxoplasmosis in wild animals, but countries in wet tropical regions report increased levels of toxoplasmosis prevalence. The susceptibility of the host species to *T. gondii* infection is a condition for the occurrence of diseases. Studies have shown that some species are resistant or heal spontaneously. Other studies have highlighted that the lifespan of animal species (dependent on their size and weight) influences the risk of infestation and explains the low incidence rates (1-5%) of toxoplasmosis in small rodents (9, 10).

Food behavior is another risk factor, the prevalence of infection being often lower

in ruminants than in omnivores and carnivores, due to the cumulative efficacy of the predator cycle of the parasite. Among the wild mammals in the Amazon forest (French Guyana), terrestrial mammals were significantly more exposed to *T. gondii* than arboreal mammals, due to ground-based behavior in carnivores. In the northern hemisphere, a high prevalence was found in carnivores (black bears and red foxes) or in omnivorous species such as wild boars that are exposed to infections by ingestion of both oocysts and tissue cysts. An ecological approach to studying the parasite movement in wildlife includes factors such as bird migration, fragmentation of the landscape (through rivers, roads, cultivated areas, localities, etc.), oocyst dispersion, or predatory behavior of different species of felids in various wild environments. Marine mammals such as sea otters, dolphins, seals and mollusks have recently been studied, with prevalence ranging between 47 and 100%. These marine mammals serve as sentinels for environmental contamination by oocytes through the drainage of freshwater into the marine ecosystem (1, 12, 13).

There are many risk factors which can influence the seroprevalence values of toxoplasmosis in humans. Higher prevalence values were observed for tropical countries with wet and warm climates and the lowest in countries with arid or cooler climate. Anthropogenic factors explain much of the variation in human seroprevalence, including eating habits, hand washing, eating meat and cooking, cleaning vegetables, etc. Other economic, social or cultural factors, water quality and sanitation are worthy of consideration. Seroprevalence increases with age, but age-related infection rates vary by country and socio-economic level. The assessment of maximum seroprevalence can be achieved in childhood in populations living in poor hygienic conditions, probably related to contamination of water, food, or by the ingestion of oocysts (1, 14).

Contaminated water is an important way of transmitting infection to humans in areas where non-filtered surface water is used for consumption and probably also in areas where fresh water is in contact, for example for recreation. For example, in a city located in the northern state of Rio de Janeiro (Brazil), adjusted age-adjusted seroprevalence was 84% for the lower socio-economic group compared to 62% for seroprevalence and 23% for higher socio-economic groups. Most people (up to 84%) of the lower socio-economic population were infected by the age of 15, while the infection was mainly obtained after the age of 20 in the higher socio-economic population (from about 20% for 20-29 age groups to 70% for the 40-49 age groups). In a multivariate analysis of the risk factors, this was attributed to water supply differences, with the poorest populations living in areas with unfiltered water. These different models of *Toxoplasma* infection depending on socio-economic levels may be more relevant in under-developed tropical countries, but in the United States, toxoplasmosis has also been considered a poverty-related infection. Increased socio-economic levels, along with improved hygiene conditions, changes in farm systems, frozen meat consumption, and sterilized food cats have led to a steady decline in seroprevalence in most industrialized countries over the last decades. In the United States, a national study found a decrease in age-adjusted *T. gondii* prevalence in 12-49 age groups, from 14.1% in 1988 - 1994 to

9% in 1999 – 2004. In France, seroprevalence in pregnant women was approximately 80% in the early 1960's, around 66% in the 1980's, 54% in 1995 and 44% in 2003, while the average age of pregnant women increased. This decrease in seroprevalence was observed in all areas where it was studied in Europe. For example, in the Netherlands, seroprevalence decreased from 35.2% in 1995 - 1996 to 18.5% in 2006 - 2007 in women at reproductive age (1, 2, 5, 15, 16, 17).

CONCLUSIONS

Our study highlighted that it is important to correctly report cases of toxoplasmosis in the general population, as well as to pregnant women, fetuses, neonates and children as this contributes to the motivation of the different screening programs currently performed worldwide. The frequency and severity of the disease are used to assess its medical relevance, and data on this can be collected in specific studies or surveillance systems. Targets for the future could be the implementation of a surveillance system of toxoplasmosis of any kind (acquired or congenital), a vaccine and, over time, a zero-incidence level of the disease.

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NEWS

TREATMENT OF ERYTHEMATO-TELANGIECTATIC ROSACEA WITH BRIMONIDINE

In a study by Micali et al, the outcome of a group of patients with erythematotelangiectatic rosacea treated with brimonidine alone or combined with a vascular laser was evaluated. The patients were affected by persistent erythema and varying degrees of telangiectasias. The patients were evaluated using erythema-directed digital photography by VISIA-CR™ system and X10 dermoscopy. A single application of brimonidine 0.33% gel was used for patients showing marked background erythema and minimal telangiectasias. A session of Nd: YAG laser was used for patients in the second group showing marked erythema and telangiectasias, followed by a reevaluation 1 month later after a single application of brimonidine. At the end of the therapy, an Investigator Global Assessment of outcome was done. A marked reduction of the background erythema was observed in all patients 6 h following the application of brimonidine. In the second group, there was a marked reduction of the erythema in all cases 6 h after brimonidine application, but not of telangiectasias. Complete clearing of facial erythema was observed following the treatment with brimonidine 1 month after the Nd: YAG laser session. The results of the study showed that the preliminary evaluation of the vascular component by erythema-directed digital photography and dermoscopy is helpful for selection of the appropriate treatment in erythematotelangiectatic rosacea (Micali G, Dall'Oglio F, Verzì AE, Luppino I, Bhatt K, Lacarrubba F. Treatment of erythematotelangiectatic rosacea with brimonidine alone or combined with vascular laser based on preliminary instrumental evaluation of the vascular component. *Lasers Med Sci* 2017 Sep 9. doi: 10.1007/s10103-017-2318-3).

Teodora Vremera

MULTIDRUG RESISTANT URINARY TRACT INFECTION – IS *KLEBSIELLA SPP* THE FIRST ENEMY TO FIGHT WITH?

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MULTIDRUG RESISTANT URINARY TRACT INFECTION - IS *KLEBSIELLA SPP*. THE FIRST ENEMY TO FIGHT WITH? (Abstract): *Klebsiella spp* is the second causative bacteria of urinary tract infection. **Aim:** To identify the profile of urological patients with *Klebsiella spp.* urinary tract infection. **Material and methods:** Review of the medical records of all patients with multidrug resistant urinary tract infection (MDR-UTI) admitted to the Iasi Urology Clinic between 2013 and 2016. **Results:** A total number of 753 patients with *Klebsiella spp.*-positive urine culture aged 18 to 95 years, mean age of 66.3 years were included in the study. The elderly was the age group most affected by *Klebsiella spp.* UTI. One quarter (25.89%) of patients had a neoplasm history and one third (31.07%) poor hygiene conditions. Almost one quarter of patients (24.70%), had a history of antibiotic use in the last three months and 520 patients had different urinary stents, most frequently bladder catheter (n=385). *Klebsiella spp.* strains resistance to penicillin was 40-99%, to cephalosporins 38-88%, fluoroquinolones 85-94%, gentamicin 80%, carbapenemes 21-33%, colistin 66%, Fosfomycin 21%, TMP-SMX 90% and to Piperacilin 75%. **Conclusions:** MDR-UTI due to *Klebsiella spp.* remains a challenge for clinicians both in terms of treatment and prevention. **Keywords**: KLEBSIELLA, ANTIBIOTIC RESISTANCE, UTI.

Urinary tract infections (UTIs) are one of the most common microbial diseases encountered in medical practice affecting people of all ages are. Worldwide, the prevalence of UTIs was estimated to be around 150 million persons per year (1). It is well known that UTI is the single most common hospital-acquired infection. While traditionally the most commonly encountered bacteria in UTI is *E. coli, Klebsiella spp*. is well-known as a causative agent of both community and hospital acquired pneumonia, bacteremia, and UTIs. In large epidemiological studies *Klebsiella spp* is the second most common causative bacteria of UTI (1, 2). Besides its high incidence we need to better understand this pathogen because of the increase in the number of vulnerable patients and in antibiotic resistance. The aim of our study was to identify the profile of urological patients with *Klebsiella spp.* UTI.

MATERIAL AND METHODS

In this prospective study, the medical records of all patients with multidrug re-

sistant urinary tract infection (MDR-UTI) admitted and treated to the Iasi Urology Clinic between January 1st, 2013 and January 1st, 2016 were reviewed. We considered MDR-UTI those urine cultures with bacteria resistant to more than 3 antibiotics of different classes or all antibiotics of a single class, regardless of the urological and other comorbidities (3). Data on age, sex, prior antibiotic treatment in the last three months, the presence of a urinary catheter, history of recurrent cystitis, menopause, habitual constipation and diabetes mellitus were collected. We have also evaluated the possibility of proper hygiene through the availability of running water and indoor toilet facilities at home.

Statistical analysis was made using Student t-Test for 2 Independent Means and Chi-Square test, calculated online on http://www.socscistatistics.com/tests/Default.aspx , statistical significance was defined as p value ≤ 0.05.

RESULTS

Between 2012-2016, 1742 patients, 1171 (67.22%) men and 571 (32.77%) women aged 18-95 years, mean age 65.05 years (SD=14.69) were diagnosed in our clinic with UTI. Although we expected that *E. coli*, to be most frequently identified bacteria, *Klebsiella spp.* - 43.22% (n=753) was the most commonly isolated bacteria, followed by *E. coli* - 20.78%, *Pseudomonas* - 14.17%, and other bacteria – 21.83%. A total number of 753 patients with *Klebsiella spp.*-positive urine cultures were included in the study, 506 (67.19%) males and 247 (32.80%) females. The age of patients with *Klebsiella spp.* UTI ranged from 18 to 95 years, with a mean age of 66.3 years. The mean age of female patients with UTI was 59.76 years, significantly lower than that of the male patients, 68.59 years (p=0.0001, t= 7.64973). The age-group distribution is shown in the Table I.

TABLE I
Age-group distribution of UTI patients

Age	18-30	31-40	41-50	51-60	61-70	>71
n= 753	18	19	46	108	195	367
%	2.3	2.5	6.1	14.3	25.8	48.7

The elderly was the age group most affected by *Klebsiella spp*. UTI with a frequency of 74.62%. A total of 195 (25.89%) patients had a history of cancer. Bladder cancer (43.07%, n=84) was the most common, followed by prostate cancer (21.02%, n=41) and cervical cancer (19.48%, n=38). The other cancers are shown in the chart below (fig.1).

Almost one quarter of patients (24.70%, n=186) had a history of antibiotic use within the last three months. The most frequently used antibiotic was ciprofloxacin (20.43%, n=38), followed by ceftriaxone (17.74%, n=33). Other antibiotics used were amoxicillin + clavulanic acid (8.06%, n=15), Tazocin (8.60%, n=16), cefixime (6.98%, n=13), Cefoperazone + Sulbactam (6.45%, n=12), gentamicin (4.30%, n=8), carbapenems (5.37%, n=10), ceftazidime (3.76%, n=7), colistin (1.61%, n=3), TMP-SMX (2.68%, n=5) and 26 (13.97%) patients could not specify.

Other identified predisposing factors were recurrent cystitis (18.06%, n=136), menopause (15.40%, n=116), habitual constipation (9.42%, n=71) and diabetes melli-

tus (8.36%, n=63). We were surprised to find that almost one third of patients (31.07% (n=234) had no access to running water and 28.41% (n=214) did not have indoor toilets at home.

The presence of a urinary stent was identified in 520 patients. The most frequently encountered was the bladder catheter (74.03%, n=385). Elderly patients have a significantly higher rate of a catheter dwelling (p=0.00001, χ^2=32.1311), as shown in Table II.

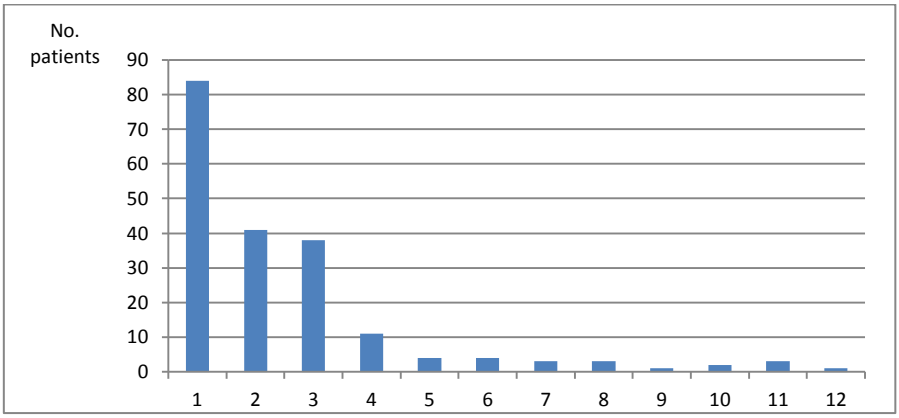

Fig. 1 Cancers in the history of the study patients.
(Legend: 1-bladder tumor, 2- prostate cancer, 3-cervical cancer, 4- colon cancer, 5- upper urothelial tumor, 6- penile cancer, 7- lymphoma, 8- gastric cancer, 9- lung cancer, 10- breast cancer, 11- ovarian cancer, 12-renal cancer)

TABLE II.

Distribution of urinary catheters

	JJ Stent	Bladder catheter	Cystostomy catheter	Nephrostomy
Patients <60 years	22	44	1	15
Patients >60 years	76	341	4	17
Total	98	385	5	32

TABLE III.

In vitro* antibiotic resistance rate of *Klebsiella spp.

Antibiotic	Resistant strain/total urine cultures	Percent of resistant stains
Ampicillin	742/743	99.86
Ampicillin +Clavulanic acid	525/570	92.10
Amoxicillin+ Clavulanic acid	299/743	40.24
Ceftriaxone	442/497	88.93
Cefuroxime	684/697	98.13
Cefixime	59/167	35.32
Ciprofloxacin	405/473	85.62
Norfloxacin	330/386	85.49
Levofloxacin	32/34	94.11
Piperacillin	393/520	75.57

Antibiotic	Resistant strain/total urine cultures	Percent of resistant stains
Gentamicin	581/726	80.02
Imipenem	236/707	33.38
Meropenem	361/730	49.45
Ertapenem	35/166	21.08
Colistin	55/83	66.26
Fosfomycin	7/33	21.21
TMP-SMX	67/74	90.54
Vancomycin	19/20	95
Teicoplanin	25/71	35.21

DISCUSSION

Usually, *Klebsiella spp.* is a bacterium in humans and other mammals, colonizing the gastrointestinal tract, skin, and nasopharynx. According to Tzouvelekis et al. (4), in the early 1970s both the epidemiology and spectrum of infections caused by *Klebsiella spp.* changed dramatically when this bacterium was established in the hospital environment and became a (still) leading cause of nosocomial infections. Although it produces only moderate amounts of chromosomal penicillinases, *Klebsiella spp*. is known as a notorious "collector" of MDR plasmids. In the first decade of the new millennium, a global crisis of unprecedented dimensions was noticed due to the rapid dissemination of MDR *Klebsiella spp*. strains producing "carbapenems" encoded by transmissible plasmids.

Ramanathan et al. (5) noticed that UTIs account for up to 40% of all healthcare-acquired infections and nearly 80% of all UTI occur in patients with short-term urinary catheters and are considered as catheter-associated UTI (CAUTI). In surgical patients, rates of UTI range from 1.8% to 4.1% based on surgery type, and development of UTI is correlated with increased duration of hospital stay and mortality.

Traditionally it is considered that women are significantly more likely to experience UTI than men. One third of women will have had at least 1 episode of UTI requiring antimicrobial therapy by the age of 24 years. Almost half of all women will experience one UTI during their lifetime (6). In our study, the men/female ratio was 2:1. This situation can be explained by the fact that our patients did not have community-acquired UTIs, being hospitalized for various urological diseases. This sex ratio is like that reported by Milan et al. (7) in a group of 589 patients, of which only 72 were diagnosed with infection on admission and were classified as CAUTI.

Although in our study patient age ranged from 18 to 95 years, we had significantly more old males (54.58%). This finding is not unusual because the incidence of UTI in men without indwelling catheters increases substantially after 60 years of age. According to Schaeffer (9), the incidence of all UTIs among older men is approximately half that among older women, but infection rates among men in the community who are older than 80 years of age approach those among women in the same age range. Older men often have structural and functional abnormalities of the urinary tract such as benign prostatic hyperplasia, which can cause UTI owing to obstruction and impaired urine flow. Moreover, these patients can have comorbidities associated with an increased susceptibility to infection, such as diabetes mellitus. However, in

our study group only 8.36% of the total number of patients had diabetes mellitus.

In our study, a quarter (25.89%) of patients had a cancer history. Most of them (83.58%, n=163) had a pelvic cancer. According to Rolston (9), the factors that increase the risk of infection in neoplastic patients include neutropenia, disruption of anatomic barriers, obstruction due to primary or metastatic tumor, diagnostic/therapeutic surgery, prosthetic devices or advanced age. Obstructive uropathy and complicated UTIs go hand in hand and are often recurrent and difficult to eradicate. In patients with retroperitoneal or pelvic cancers acute or chronic ureteral obstruction is a complication of the malignancy. Prompt decompression of the obstruction is required. For this procedure ureteral stents or, more frequently, percutaneous nephrostomy tubes are used. According to Bahu et al. (10) the use of stents and nephrostomy tubes is associated with a significant rate of pyelonephritis and eradication of such infections is difficult to accomplish.

Urinary catheter, especially bladder catheters are only used in neoplastic patients. Older men, as most patients in our study group, often need bladder catheters for urinary retention due to benign prostatic hyperplasia. In our study, of the 385 patients with bladder catheter indwelling, 88.57% were over 60 years of age. According to Ramanathan (5) the presence and duration of catheterization are the strongest risk factors for bacteriuria development. Approximately 10% to 25% patients with bacteriuria progress to symptomatic UTI and 1% to 4% develop urosepsis. In patients with indwelling urinary catheters or in patients with a recent history of indwelling urinary catheterization, the catheter serves as the most common route of access for microorganisms into the bladder. Bladder catheters facilitate bacteriuria by two ways: either through direct inoculation of the bladder during catheter insertion or through biofilm ascension along the catheter.

MDR-UTI has a very vague definition. According to Magiorakos et al (11) a bacterial isolate was considered non-susceptible to an antimicrobial agent when it tested resistant, intermediate or non-susceptible when using clinical breakpoints as interpretive criteria, and not epidemiological cut-offs provided by the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the Clinical and Laboratory Standards Institute (CLSI). In literal terms, MDR means ‘resistant to more than one antimicrobial agent', but no standardized definitions for MDR have been agreed upon yet by the medical community. Many definitions are being used to characterize patterns of multidrug resistance in Gram-positive and Gram-negative organisms. For these germs, the definition most frequently used is ‘resistant to three or more antimicrobial classes'. Another method used to characterize bacteria as MDR, is when they are ‘resistant to one key antimicrobial agent’, carbapenems for *Klebsiella spp.*

Prior antibiotic use can be associated with a high prevalence of resistance in patients with UTI. After analyzing more than 5,000 urine culture, Bidell et al (2) noticed that more than two prior antibiotic exposures were associated with slightly higher incidences of fluoroquinolone non-susceptibility, multidrug resistance, and extended-spectrum β-lactamase phenotype compared with no or one exposure. The authors suggest an increased risk for resistant Gram-negative pathogens among hospital patients with UTI occurring ≥3 days after admission. Micek et al (12) re-

vealed that exposure to antimicrobial agents in the previous 90 days is associated with significantly higher hospital mortality, longer hospital stays and higher hospital costs. The authors concluded that clinicians and hospital administrators should consider the potential impact of recent antibiotic exposure when formulating empiric treatment decisions for patients with severe infections attributed to Gram-negative bacteria. In our study, almost one quarter of patients (24.70%) had a history of antibiotic use within the last three months. Fluoroquinolones, especially ciprofloxacin were the antibiotics most frequently used prior to admission. This has leaded to a high incidence of fluoroquinolone nonsusceptibility in patients with *Klebsiella spp* UTI, ranging from 94.11% (levofloxacin) to almost 86% (ciprofloxacin and norfloxacin).

The European Association of Urology (13) recommends routine daily personal hygiene to maintain meatal hygiene, meaning soap and water is sufficient to achieve this goal. Special attention must be given to educating non-circumcised patients to clean underneath their foreskin daily to remove smegma, as this may increase their risk of developing a UTI. Surprisingly, almost one third of patients had no access to running water and had no indoor toilets. This situation makes them have a poor hand and genitalia hygiene. It becomes difficult for many of our patient which have urinary catheter, to follow Septimus et al. (14) recommendations for a good hand hygiene before and after catheter insertion or bag manipulation to prevent device-related healthcare-associated infections.

The rise in antibiotic resistance of *Klebsiella spp.* represents a growing threat to patients worldwide, especially when we should treat infections with carbapenem-resistant *Klebsiella spp.* (CRKP). The treatment of these infections is problematic, as few therapeutic options are available. In a review by Tansarli et al. (15) the antimicrobial susceptibility of *Klebsiella spp* isolates in CAUTI varied at the following rates: amoxicillin-clavulanic acid, 0 to 69%; cefotaxime, 43 to 100%; ceftazidime, 40 to 100%; imipenem, 100%; ciprofloxacin, 53 to 100%; nalidixic acid, 51 to 94%; gentamicin, 60 to 100%; amikacin, 100%; trimethoprim-sulfamethoxazole, 0 to 56%; nitrofurantoin, 40 to 78%; tetracycline, 43 to 69%; and fosfomycin, 100%(15). In one of the few *Klebsiella spp.* UTI in hospitalized patients by Ben Haj et. al. 16) the susceptibility of all *Klebsiella spp.* isolates to the tested antibiotics was the following: amoxicillin-clavulanic acid, 60%; cefotaxime, 66%; nalidixic acid, 48%; ofloxacin, 73%; ciprofloxacin, 74%; gentamicin, 69%; tobramycin, 70%; amikacin, 97%; trimethoprim-sulfamethoxazole, 57%; nitrofurantoin, 68%; and fosfomycin, 77%. In our patients *Klebsiella spp.* had a high rate of resistance to ampicillin +/- clavulanic acid (>90%), cephalosporins (88-98%) excepting cefixime (35%) or vancomycin (95%). We also encountered high rates of resistance to trimethoprim-sulfamethoxazole (90%) and fluoroquinolones (85-94%), often used as first line of treatment for uncomplicated UTI and gentamicin (80%). For these antibiotics, our results are very like those obtained by El Bouamria et al. (17) in a three-year study of 321 *Klebsiella spp.* positive urine cultures.

The trend of *Klebsiella spp.* incidence in our geographical area seems to be constant. In a report by Pricop et al. (18) between January 2013 and July 2014 the incidence of *Klebsiella spp.* UTI was of 46.15%. It had the same spectrum of re-

sistance throughout the study period: resistant to ampicillin, amoxicillin + clavulanic acid, cephalosporins (cefuroxime, cefotaxime, ceftazidime, cefepime), gentamicin, fluoroquinolones (norfloxacin, ciprofloxacin). *Klebsiella spp* MDR-UTI was associated with the insertion and replacement of "JJ" stents (33.33%), endourological interventions (transurethral resection of prostate - TUR-P 14.10%, transurethral resection of a bladder tumor - TUR-BT 23.08%), diabetes mellitus (19.23%), bladder tumors (17.95%) or prostatic neoplasm (14.10%), status of urethrovesical catheter carrier (46.15%) and with the insertion of probes for percutaneous nephrostomy (15.38%)(18).

The inappropriate antibiotic use in Romania is a major problem. Medicine consumption in Romania, especially antibiotics, is one of the highest across the European Union. In a survey conducted by the European Commission in 2015 Romanians consumes 70% more antibiotics than the average Europeans (19,20). Every day 3.3% of Romania's population takes antibiotics. Moreover, 70% of Romanians believe antibiotics kill viruses, as compared to 49% of the Europeans in a similar case; also, 55% of Romanians believe antibiotics are efficient in flu and cold, as compared to 41% of Europeans. A similar problem is encountered in Romanians hospitals. Tarcea et al. (21) evaluated the use of restricted antibiotics in an academic hospital in Romania. The authors found that the most commonly prescribed antibiotics were vancomycin, imipenem and meropenem, but they were used inappropriately in 49.71, 46.55 and 44.06 % of the cases.

CONCLUSIONS

Our study revealed that in our geographical area the hospitalized patient with *Klebsiella spp.* MDR-UTI is often an elderly man with a history of pelvic cancer or urinary retention due to BPH which required the use of a urinary stent, frequently a bladder catheter, sometimes with poor hygiene condition for which the usually used antibiotics are not effective. Our study stresses the need for frequent re-evaluation of the prevalence of pathogens and antibiotic resistance and the adjustment of the empirical first-line treatment.

Our study revealed that in our geographical area the hospitalized patient with *Klebsiella spp.* MDR-UTI is often an elderly man with a history of pelvic cancer or urinary retention due to BPH which required the use of a urinary stent, frequently a bladder catheter, sometimes with poor hygiene condition for which the usually used antibiotics are not effective. Our study stresses the need for frequent re-evaluation of the prevalence of pathogens and antibiotic resistance and the adjustment of the empirical first-line treatment.

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HEPATOCELLULAR CARCINOMA WITH CENTRAL SEVERE ARTERIOPORTAL SHUNT-ENDOVASCULAR APPROACH

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HEPATOCELLULAR CARCINOMA WITH CENTRAL SEVERE ARTERIOPORTAL SHUNT-ENDOVASCULAR APPROACH (Abstract): The prognosis of hepatocellular carcinoma is related to the extension of the lesion, as well as to the liver function at the time of diagnosis and associated arterioportal shunts. **Case report**: We report a 70-year-old woman with C virus - related cirrhosis, hepatocellular carcinoma and central severe arterioportal shunt, presenting with upper digestive tract hemorrhage and refractory ascites, treated by embolization of the shunt and trans catheter arterial embolization of the tumor. **Results:** After treatment, both ascites and esophageal varices improved, with good general status for three months. Afterwards, a massive esophageal bleeding uncontrolled by emergent endoscopic hemostatic techniques caused rapid deterioration and death. **Conclusions:** Hepatocellular carcinoma with central severe arterioportal shunt is a challenging clinical situation, whose prognosis is influenced mainly by portal hypertension. Embolization of the shunt is the first option to reduce the pressure in the portal system and to ensure the endovascular treatment of the tumor. **Keywords:** HEPATOCELLULAR CARCINOMA, ARTERIOPORTAL SHUNT, EMBOLIZATION

Liver cancer is the ninth most common cancer in women, the second most common cause of death from cancer worldwide, and accounts for 7% of all cancers (1). Hepatocellular carcinoma (HCC) represents more than 90% of primary liver cancers and its prognosis is related to the extension of the lesion, as well as to the liver function at the time of diagnosis (1). Furthermore, HCC frequently involves portal vein (PV) and creates arterioportal shunts (APS), worsening portal hypertension (PHT) and increasing the risk of gastrointestinal bleeding and hepatic encephalopathy (2).

The present report describes a patient with HCC and central severe APS treated by embolization of the shunt followed by trans catheter arterial embolization of the tumor.

CASE REPORT

A 70-year-old woman, who has been diagnosed with C virus - related cirrhosis for 8

years, was referred to our hospital for rapid deterioration of liver function with refractory ascites and upper digestive tract bleeding. The patient's laboratory data at admission are presented in table I. Child's classification status was B (Child-Pugh score 8).

TABLE I

Laboratory data at the admission, after initial embolization and at 1 month follow-up

Laboratory data	Admission	2 days after TAE1	9 days after TAE 1	3 days after TAE. 2
Total bilirubin level mg/dL (N: 0.2-1.2)	1.50	1.08	1.02	0.96
AST IU/L (N: 5-31)	73	43	35	27
ALT IU/L (N: 5-32)	112	72	52	46
GGTP IU/L (N: 8～59)		119	203	
WBC count /μL,	4010	5910		
RBC count × $10^{4/}$ μL	362	346	377	325
HGB level g/dL	11.3	10.7	11.7	11
hematocrit %	33.1	31.3	33.4	33
platelet count × 0^3/μL	90	82	132	85

AST - aspartate aminotransferase level; ALT - alanine aminotransferase level; GGTP - γ-glutamyl trans peptidase; WBC - white blood cell; HGB - hemoglobin; RBC - red blood cell;

Upper gastrointestinal endoscopy revealed severe esophageal varices and emergent sclerotherapy was performed to control bleeding. Abdominal ultrasound (US) identified a hyper vascular lesion in the 4th segment of the liver, and hepatofugal high velocity flow in PV trunk and branches (up to 190 cm/s) with arterial waveform (fig.1). No thrombus was found in PV. Massive ascites was noticed in all peritoneal spaces. Computed tomography (CT) of the abdomen, with tri-phase acquisition after injection of contrast medium, identified a low-density lesion in the 4th segment only on non-enhanced and late scan. An APS located in *porta hepatis* with early and strong enhancement of enlarged PV trunk at early hepatic arterial phase was seen. An arterial variant was also disclosed, with replaced common hepatic artery (HA) originating from superior mesenteric artery (SMA).

The final diagnosis was HCC with central severe APS and refractory PHT. The patient was then referred to the interventional radiology unit. A common HA arteriography showed a major APS centrally located (between right HA and PV trunk with multiple feeders), but a tumor stain was not visible because of the shunt (fig. 2a). A super selective catheterization of the right HA was performed with microcatheter (3F, Renegade) followed by angiography. Due to the numerous feeders and enlarged right HA and PV, we decided to perform coils trans catheter arterial embolization (TAE) for the major APS. Three coils (Azur Pure, 4 mm in diameter, length 4 and 6 mm) were introduced into the right HA with partial occlusion of the APS (approximately 80%) (fig. 2b).

The follow-up Doppler US performed 1 week after revealed lower velocity in PV but with persistent APS. Clinical status slowly improved with diminishing rate of ascites recurrence.

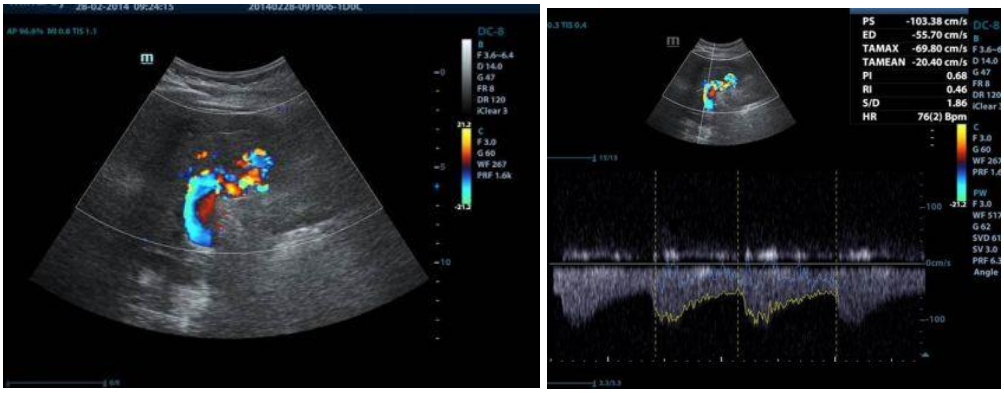

Fig. 1. Liver Doppler ultrasound: a) mixed arterial and venous flow in PV; b) hepatofugal PV flow with high velocity arterial waveform in the portal trunk and branches.

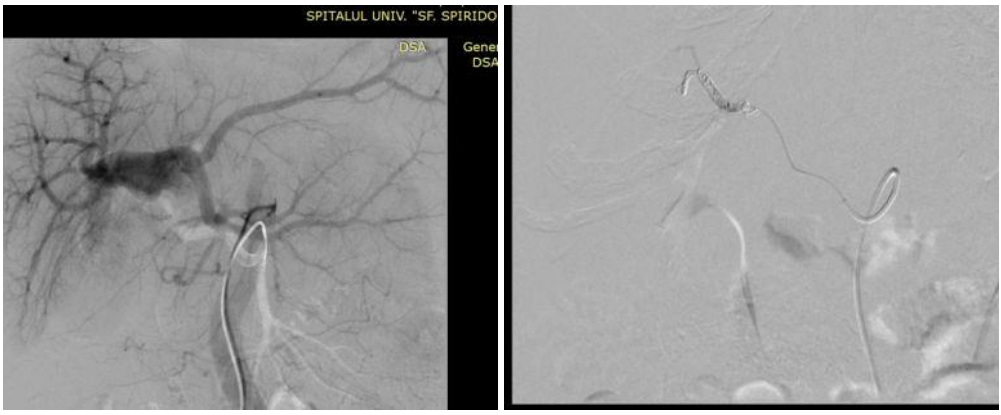

Fig. 2. a) Digital subtraction hepatic arteriography: major APS centrally located (between right HA and PV trunk with multiple feeders); b) TAE: coils into the right HA.

Consequently, we have performed a second TAE within 3 weeks, completing the embolization with Gelfoam and occluding the shunt (fig. 3). The left HA arteriography showed HCC feeders, which were super selective embolized with Embosphere microspheres 300-500 µm. The celiac trunk (splenic, left gastric and left phrenic arteries) was catheterized, but no tumor feeders were depicted.

After treatment, ascites and esophageal varices, as well as laboratory findings improved (tab. I). Doppler US revealed reduced sized PV (14 mm), without thrombosis (fig. 4). The patient was discharged one week after with a small amount of ascites.

Three months later the patient presented a massive esophageal varices bleeding. Emergent CT showed revascularization of the HCC, with arterial supply from left HA, right internal mammary and right gastroepiploic arteries, and an APS in the lower part of the lesion. The right HA was still embolized. Unfortunately, the massive esophageal bleeding was uncontrolled by emergent endoscopic hemostatic techniques, with rapid deterioration and death.

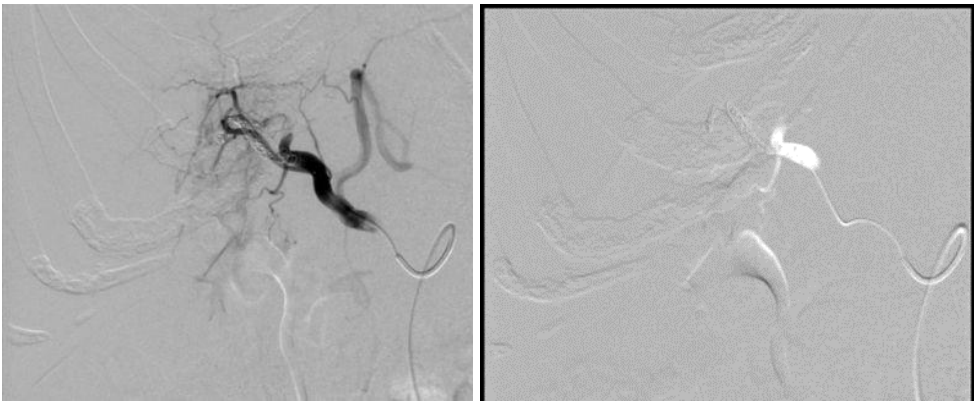

Fig. 3. a) Hepatic arteriography after 3 weeks: persistence of APS;
b) completion of the embolization with occlusion of the shunt.

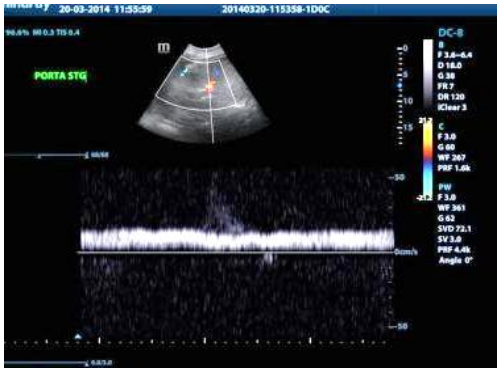

Fig. 4. Liver Doppler US - follow-up after embolization: reduced sized PV (14 mm), without thrombosis and normal hepatopetal flow in the left portal branch

DISCUSSION

Several clinical and imaging classifications of APS are available (3). A central APS is in *porta hepatis* and a severe APS consists in opacification of PV trunk and/or the first-order branches with enhancement at the early hepatic arterial phase, without or with early enhancement of HCC foci (3).

APS occurs in 60% of patients with HCC, marked APS of the main, right or left PV accounting for half of these patients (2, 4). Lesions associated with severe APS shows no enhancement at the arterial phase in dynamic CT due to reduced arterial flow to the tumor, as presented in our case (3).

TAE is the standard treatment for intermediate stage HCC, but there is no consensus about optimal treatment of HCC associated with central severe APS (1). EASL-EORTC Clinical Practice Guidelines have no reference related to the treatment of this complication of HCC, even if microscopic vascular invasion involves 20% of tumors of 2 cm in diameter and APS occurs in more than half of patients with HCC (1).

Moreover, HCC associated with central severe APS is a challenge for interventional radiologist since embolic material can pass through the shunt reducing the effectiveness of the treatment and producing damage of PV branches with higher risk of liver ischemia. Therefore, temporary or permanent occlusion of the supplying arteries of the APS is required before endovascular treatment of the lesion. For severe APS, the procedure is also mandatory to ameliorate PHT (5-11).

TAE of severe APS can be achieved by several embolic materials used alone or combined: gelatin sponge, ethanol, PVA

particles, N-butyl cyanoacrylate, coils (5-11). The choice of embolic agent is based on shunt size, velocity, location, angio-architecture and specific actions of each embolic agent. Coils are generally recommended only for simple APS owing to the difficulty to reach each feeder in complex shunts, but we choose them because of severity of the APS and multitude of feeders which render the selective embolization impossible.

The prognosis of HCC patients with severe APS is related to severe PHT and subsequently, to variceal bleeding, refractory ascites and portal encephalopathy (1). Surgical treatment is reported only for some severe resistant or recanalized APS with recurrent uncontrolled esophageal variceal hemorrhage (12). For centrally located APS the procedure of choice should be a major hepatectomy, unsafe in patients with poor liver function.

CONCLUSIONS

Our case outlines the challenging situation of HCC with central severe APS. Embolization of the shunt is the first option to reduce the pressure in the portal system, as well to render the endovascular treatment of the tumor more efficient and to favor patient survival.

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PREVALENCE OF MYOPIA AMONG MEDICAL STUDENTS FROM "GRIGORE T. POPA" UNIVERSITY OF MEDICINE AND PHARMACY

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PREVALENCE OF MYOPIA AMONG MEDICAL STUDENTS FROM "GRIGORE T. POPA" UNIVERSITY OF MEDICINE AND PHARMACY (Abstract): **Introduction**: There are scientific evidences that the etiology of juvenile-onset myopia involves both genetic and environmental factors. Excessive near work represents the most frequently cited environmental factor associated with the development of this disease. **Objectives:** The aim of our study was to assess some epidemiological aspects of myopia in today`s medical students from "Grigore T. Popa" University of Medicine and Pharmacy, including the prevalence of this disorder, and the differences between generations. **Materials and methods:** A questionnaire was shared via the internet on the social groups of the University`s Medical Students. Five hundred and seventy-six (576) students filled the survey online: from the 1st year - 105 students; 2nd year - 86; 3rd year - 121; 4th year - 80; 5th year – 86, and from the 6th year - 98 students. **Results:** 73.8% of the students included in the study suffer from myopia. At a first glance, the analysis of myopic students' distribution by the university year showed no significant difference: 1st year – 73.3%; 2nd year – 74.4%; 3rd year – 71.9%; 4th year - 77.5%; 5th year - 68.6%, and 6th year – 77.6% ($p<0.005$). The percentage of the 6th year students that entered the university with myopia was 43.8%. Furthermore, we observed that the number of students that already had myopia before entering the university has progressively increased. To emphasize how important the difference is, this year 64% of the 1st year students already were diagnosed with myopia. **Conclusions:** These results open a debate regarding the causes of the increasing number of students suffering from myopia, including the difficult academic requirements, the behavioral changes, the genetic factors or the poor quality of nutrition. **Keywords**: PREVALENCE, MYOPIA, STUDENTS, UNIVERSITY OF MEDICINE AND PHARMACY.

Myopia, also known as near-sightedness, is a common refractive error developed when a distant object`s image is formed anterior to the retina instead of on the retina. This occurs when the eyeball becomes too long and prevents incoming light from focusing directly on the retina (1). The consequence is blurred vision and requires refractive correction at any diopter for clear focus. It may also be caused by an abnormal shape of the cornea or lens.

For more than 100 years the etiology of

myopia has been discussed and searched, raising a debatable question, whether it is an environmental or a genetic disorder. The studies conducted state that an interaction is involved. However, the exact nature and contribution remains elusive (2). In 2010, myopia was found to be the second most common cause for blindness globally, affecting 108 million persons. Myopia can further cause cataract, glaucoma, retinal detachment and myopic macular degeneration, consequently leading to irreversible vision loss. The American Academy of Ophthalmology's latest meta-analysis predicts that by 2050 there will be one billion people worldwide with high myopia, 7.5 more times than in 2000, bringing both serious economic implications and better eye care facilities throughout the Globe (3).

The aim of our study was to assess some epidemiological aspects of myopia in today`s medical students from "Grigore T. Popa" University of Medicine and Pharmacy, including the prevalence of this disorder, the differences between generations, and the factors that can influence the occurrence of the disease, to analyze if the results found are enclosed in those of other studies regarding the relationship between the development of myopia and the high academic requirements. Most of the factors are modifiable, so the contribution of primary prevention is very important for these subjects.

MATERIAL AND METHODS

A cross-sectional study was carried out among students from "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, Romania. This study was based on a questionnaire shared via the internet on the social groups of the University`s Medical Students to obtain data regarding age, sex, year of study, urban or rural background, eye-sight problems and characteristics, family history, behavioral aspects such as the time spent studying, the type of lighting used and eating habits and the frequency of eye check-ups. The questionnaire was filled in online, anonymously, in two weeks (during May 2016) by five hundred and seventy-six (576) students from each university year (I - VI).

RESULTS

The 576 students' distribution was, as it follows: 1st year - 105 students; 2nd year - 86; 3rd year - 121; 4th year - 80; 5th year – 86, and from the 6th year - 98 students (tab. I). From the 576 participants, there were 487 (84.5%) females and 89 (15. 5%) males. From these, 74.5% (363 out of 487) were women with myopia and 25.5% (62 out of 89) were men with myopic impairment (tab. II).

TABLE I

The distribution of students by year of study

Year of study	Number	Percentage (%)
I	105	18.2
II	86	14.9
III	121	21.0
IV	80	13.9
V	86	14.9
VI	98	17.1
Total	**576**	**100**

73.8% of the students included in the study had myopia (425 students) (tab. II). Analyzing based on the question of diopters, we classified the student`s myopia into four categories: less than -1D, between -1D and -3D, between -4D and -7D and lastly, more than -7D. Most students range from less than -1D (37%) and -1D to -3D (49%), indicating a mild myopia that is not predisposed to further complications (fig. 1).

TABLE II

The distribution of students by gender and health status

Gender	Myopia students			Healthy students			Other categories			Total	
	No.	%		No.	%		No.	%		No.	%
		from total students	from total women		from total students	from total women		from total students	from total women		
Female	363	85.4	74.5	92	80	19	32	88.9	6.5	**487**	**84.5 (from total students)**
Male	62	14.6	69.7	23	20	25.8	4	11.1	4.5	**89**	**15.5 (from total students)**
Total	**425**	**73.8 (from total students)**		**115**	**20 (from total students)**		**36**	**6.2 (from total students)**		**576**	**100**

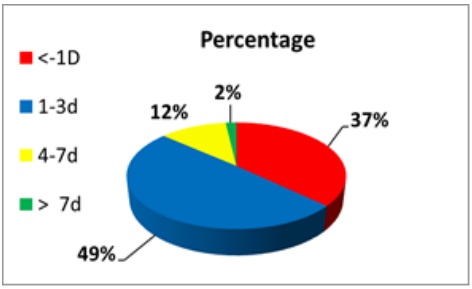

Fig. 1. The distribution of students with different degree of myopia

At a first glance, the analysis of myopic student's distribution by the university year showed no significant difference (p>0.05): 1st year – 73.3%; 2nd year – 74.4%; 3rd year – 71.9%; 4th year – 77.5%; 5th year - 68.6%, and 6th year – 77.5% (tab. III).

TABLE III

The distribution of students by health status and by study year

Health status	Study year												Total	
	I		II		III		IV		V		VI			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Presently with myopia	77	73.3	64	74.4	87	71.9	62	77.5	59	68.6	76	77.6	**425**	**73.8**
Healthy	22	21	20	23.2	26	21.5	12	15	20	23.2	15	15.3	**115**	**20**
Students with other sight problems	6	5.7	2	2.4	8	6.6	6	7.5	7	8.2	7	7.1	**36**	**6.2**
Total	**105**	18.2	**86**	14.9	**121**	21.0	**80**	13.9	**86**	14.9	**98**	17.1	**576**	**100**

The number of students that already had myopia before entering the university has progressively increased, especially in 1st, 3rd (57.4%), and 4th year (61.3%), as opposed to those who entered the university several years before (5th – 50.8% and 6th years – 53.9%) (tab. IV). To emphasize how important the difference is, we observed that 67.5% of the 1st year students already were diagnosed with myopia.

TABLE IV

The distribution of students already diagnosed with myopia by study year

Health status	Study year												Total	
	I		II		III		IV		V		VI			
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Already diagnosed with myopia	52	67.5	33	51.6	50	57.4	38	61.3	30	50.8	41	53.9	**244**	**42.4**

More students from urban areas developed myopia – 77.4% as opposed to those from the rural areas 63% (tab. V). Furthermore, the students coming from cities have a higher frequency of developing high myopia, almost twice as the students from rural areas, due to food quality, time spent indoors versus outdoors and economic factors (tab. V).

TABLE V

The distribution of students by health status, degree of myopia and origin

Characteristics	Rural area		Urban area	
	No	%	No	%
Health status	**146**	**100**	**430**	**100**
Myopia	92	63	333	77.4
Healthy	44	30.1	71	16.5
Others	10	6.8	26	6
Degree of myopia	**92**	**100**	**333**	**100**
< 1D	35	38.0	123	36.9
1-3D	49	53.3	160	48
4-7D	7	7.6	43	12.9
> 7D	1	1.1	7	2.1

Our study showed that there are differences between food diet used before admission in the University and during the University years (fig. 2, 3). Before admission, for students with myopia, there was a predominance of food diet bought from supermarket (54.5%) compared with food bought from the producer (45.5%). Also, there are differences between the percentage of food bought from the producer among students with myopia and healthy students (45.4% *versus* 50.4%) (fig. 2).

After entering the University, the students with myopia, had the same diet behavior as before, but with more important differences between the percentages of students which bought the food from supermarket *versus* producer (84% *versus* 16%). The healthy students changed their diet behavior, with a predominance of food

bought from the supermarket (70.4%) compared with that bought from the producer (29.6%) (fig. 3).

Regarding the genetic factors involved in the development of myopia, there are no significant differences between those who have a positive family history and those who show no family history ($p > 0.05$) (tab. VI). The 12 students who developed high myopia of more than -7D have 100% family history.

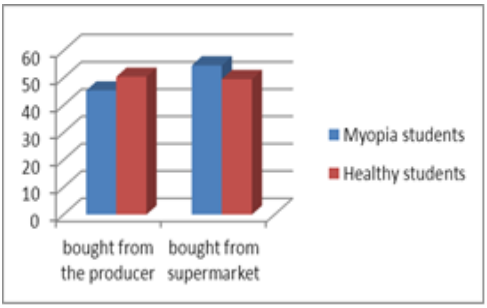

Fig. 1. Nutrition distribution before entering the University

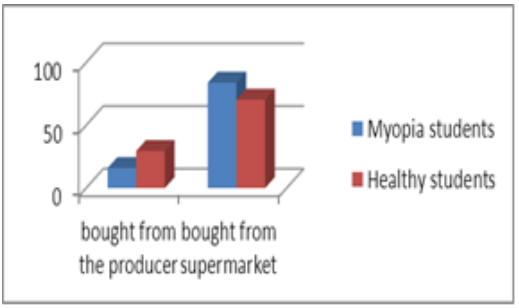

Fig. 3. Nutrition distribution before entering the University

TABLE VI

The familial distribution of myopia

Degree of myopia	Family cases		No family cases		Total	
	No	%	No	%	No	%
< 1D	82	19.4	76	17.7	158	37.1
1-3D	127	29.8	82	19.4	209	49.2
4-7D	25	5.9	25	5.9	50	11.8
> 7D	8	1.9	0	0	8	1.9
Total	**242**	**57**	**183**	**43**	**425**	**100**

45% of the medical students questioned had yearly eye exam and 49% had eye exam once every two years or less. Just a small number of students had the eye examination twice per year (6% = 22 students) (fig. 4).

We observed that more than half of students with myopia spent over 4h per day studying during the university year (51.5%). Also, more than 50% of healthy students spent less than 4h per day studying during the university year (52.2%) (fig. 5).

There were no significant differences ($p > 0.05$) between students with myopia (> 6h = 83.1%) compared with healthy students (> 6h = 82.6%) regarding the number of hours spent studying during the exam sessions (tab. VII).

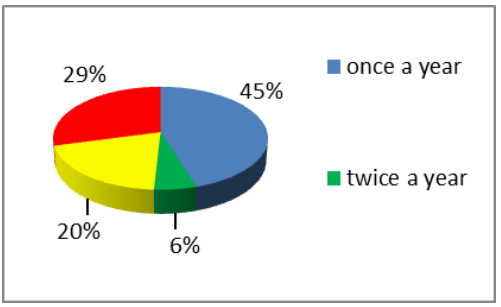

Fig. 4. Eye examination frequency

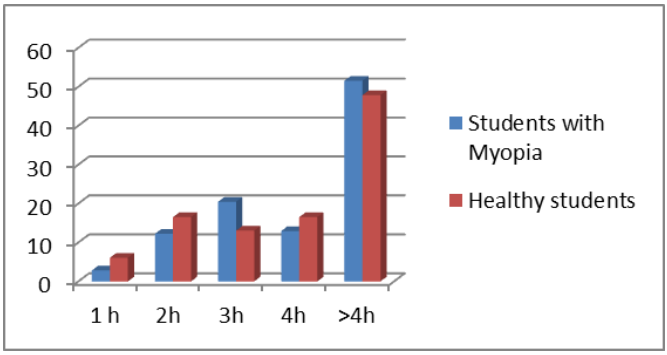

Fig. 5. Hours spent for study during the university year

TABLE VII

Hours spent studying during exam sessions

Number of hours spent studying during exam sessions	Students with myopia		Healthy students	
	No	%	No	%
1h	1	0.2	0	0
2h	1	0.2	0	0
3h	2	0.5	1	0.9
4h	11	2.6	4	3.5
5h	18	4.2	5	4.3
6h	39	9.2	10	8.7
>6h	353	83.1	95	82.6
Total	**425**	**100**	**115**	**100**

DISCUSSION

In our study the frequency of myopia among medical students was higher than the value found by Chaudhry for Pakistan (73.8% versus 57.6%) (4), and was superior to the values for Norway and Denmark (50%), India (45%), Turkey (33%), and Poland (32%) (5). The prevalence of myopia among medical students from "Grigore T. Popa" University of Medicine and Pharmacy – Iasi was close to that found in China (71%) and lower than the values from Singapore (82%) or Taiwan (93%) (5). However, there are differences between the methodology of these studies and the comparison could have some limitations.

The number of students that already had myopia before entering the university has progressively increased, especially in 1st and 2nd year, as opposed to those who entered the university several years before. To emphasize how important the difference is, this year 64% of the 1st year students already were diagnosed with myopia. This aspect was found in similar studies, throughout Europe, Asia and the United States which were conducted in regions with both large populations and high rate of myopia, raising awareness about the con-

stantly increasing educational standards (4, 5, 6, 7).

Our findings regarding the presence of myopia before the admission in medical University were like those founded by other researchers: most students developed myopia before admission (4).

Studies conducted in the early 20th century on isolated communities with no or little schooling revealed a 0.4% incidence of myopia. The reason is simple: these communities lived a hunter-gatherer subsistence, eating what they produced. 200 years ago, a dietary shift happened, refined goods being slowly introduced, with high a high glycemic load, as opposed to the minimally processed food our ancestors consumed (8). Nowadays, the western dietary patters lead to hyperglycemia, hyperinsulinemia, and type 2 diabetes. These metabolic conditions can induce and affect myopia (9).

Before the Revolution in December 1989, Communist Romania had little access to processed western goods, filled with carbohydrates and sugar. After this event, the democratic regime meant free economic changes. The changes first arrived in the cities and slowly entered the villages, therefore affecting all generations of medical students. Our results that illustrate the nutrition differences before entering the university showed that most students bought their goods directly from the producer, with no preservatives and additives, as opposed to the situation they find themselves in now, agreeing with other studies (9).

As shown in other studies those people with high myopia (-7D) have genetic mutations located on chromosome 7q36 which is inherited autosomal dominant (9). Our results were in accordance with studies from the scientific literature which showed an important family history among subjects with high myopia (4, 9).

Both excessive near work and high educational demands over several years are risk factors for the development and progression of myopia among medical students, especially if they have an initial high prevalence (12). Our study`s findings agreed with other studies, since an average student spends more than 6 hours a day studying during exam sessions, and more than 4 hours a day in a normal university year, which can consequently affect his vision, especially if already diagnosed with myopia (4, 5, 12).

Regardless of their myopia problems, since they undergo a high amount of stress, perform a high amount of near work and are prone to high academic demands it is advisable that they have an eye examination every year, especially if they are already diagnosed with myopia.

Our study has the following limitations: it was not conducted in collaboration with the Ophthalmology department in order to verify the student`s myopia compared to the diopters claimed; more females responded to the questionnaire than males; it is possible to have response bias, with more subjects with myopia choosing to participate than students without myopia impairment; it is prone to subjectivity since the questionnaire was online submitted and the responses may or not be accurate; it is difficult to distinguish the limit between youth-onset myopia (15-20 years) and adult-onset myopia (>20 years) since studies shown a different origin (12).

CONCLUSIONS

In our study, the prevalence of myopia was high among students from “Grigore T. Popa” University of Medicine and Pharmacy – Iasi, Romania and was the most important

among younger students (study years I-III). These results open a debate regarding the causes of the increasing number of students suffering from myopia, including the difficult academic requirements, the behavioral changes, the genetic factors or the poor quality of nutrition. Also, our findings raise the awareness regarding myopia among medical students and the need for primary and secondary prevention.

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WHAT CAN WE FIND BEHIND A PARIETAL ABSCESS? CASE REPORT

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WHAT CAN WE FIND BEHIND A PARIETAL ABSCESS: CASE REPORT (Abstract): We report a rare case of a 71-year-old female who presented to the First Clinic of Surgery from "Sf. Spiridon" Hospital Iasi, Romania with a mass in the left iliac fossa that started to discharge a feculent secretion five days prior to presentation. The patient had a history of constipation, several episodes of hematochezia associated with significant weight loss over the past 6 months. Following evaluation, a diagnosis of enter cutaneous fistula secondary to a locally advanced sigmoid cancer was made. The patient underwent semi-elective resection of the sigmoid tumor (measuring 20 cm) with 10 cm safe margins, and of the involved lymph nodes and abdominal wall. A termino-terminal colo-rectal anastomosis was performed. The patient was discharged the 10th postoperative day after an uneventful evolution for oncological evaluation. The authors presented this case in order to illustrate the challenges associated with diagnostic and therapeutic management of advanced colorectal cancer that has been discovered through a parietal abscess. **Keywords**: LOCALLY ADVANCED COLON CANCER, ENTERO-CUTANEOUS FISTULA, ABDOMINAL WALL ABSCESS, FECULENT SECRETION.

Colorectal cancer is currently the third most common cancer in the world according to the World Cancer Research Fund International, with 1.4 million cases diagnosed in 2012, and the second malignity as cause of death (1). *Enter cutaneous fistulas* (ECF) in colorectal cancer are rare as they usually occur in case of inflammatory bowel disease, diverticulitis, pancreatitis, radiotherapy, and trauma (external or iatrogenic) (2-4). The authors currently describe the case of a 71-year-old woman who presented with a parietal abscess in the left iliac fossa due to a locally advanced sigmoid colon cancer.

CASE REPORT

A 71-year-old female patient originating from a rural area presented to the Emergency Room, with lower abdominal pain for almost a week, a parietal mass in the left iliac fossa that appeared five days prior to the current presentation and discharge of a feculent secretion that started the previous day.

The patient reported severe weight loss

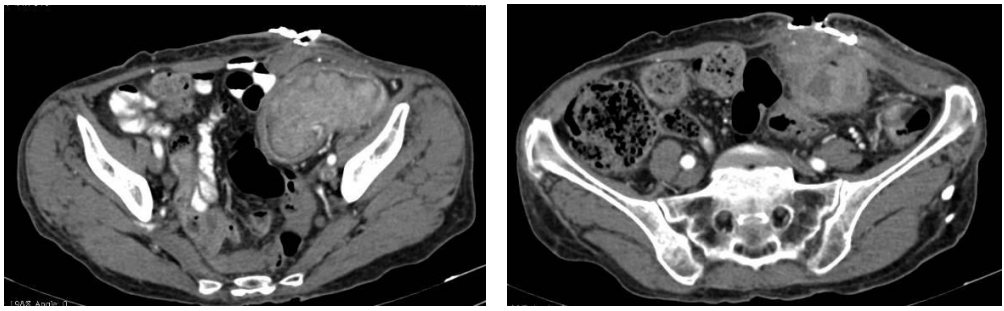
(15 kg) in the past 6 months, a long history of constipation (more than 10 years) and several episodes of hematochezia in the past 3 months. Concerning the surgical history, the patient presented hysterectomy for uterine fibroma 24 years prior to the current presentation complicated by incisional hernia during the same year.

Physical examination revealed stable vital signs, no fever, pale teguments, and a body mass index of 17 kg/m^2, no palpable hepatomegaly, no evidence of ascites, no cardiovascular, respiratory or urinary tract abnormalities. The clinical examination revealed a lump in the left iliac fossa a lump of approximately 15 cm was covered by teguments, with inflammatory signs (pain, red and warm teguments), and a feculent smelling secretion discharged during the manipulation. A diagnosis of parietal abscess probably secondary to an ECF was suspected in the actual clinical context.

Laboratory test results revealed mild leukocytosis (10.16 x 10^3 cells/μL), mild anemia (hemoglobin 11.6 g/dL with a normal hematocrit), thrombocytosis (621 x 10^3 cells/μL), and an increased C-reactive protein (15.03 mg/dL).

An ultrasound examination was also performed and revealed a mass of approximately 17 cm centered on the sigmoid colon, adjacent fat with inflammatory signs and a superficial collection that could not be separated from the sigmoid mass. An ECF secondary to a locally advanced sigmoid cancer was suspected based on these findings, and a computed tomography examination was indicated for preoperative staging.

The Computerized tomography examination revealed a soft tissue mass of 17 cm occupying the entire lumen of the sigmoid colon, with a significant enhancement after contrast administration (40 Hounsfield Units), in contact with a parietal breach in the left iliac fossa, compressing the adjacent small bowel loops without evident signs of invasion, and surrounded by fat, characterized by an inflammatory aspect. Lymph nodes of 12 and 14 mm were noticed in the mezosigmoid. The liver enhanced homogenously and presented no suspect lesions. There was no evidence of intraperitoneal free fluid (fig.1 a, b).

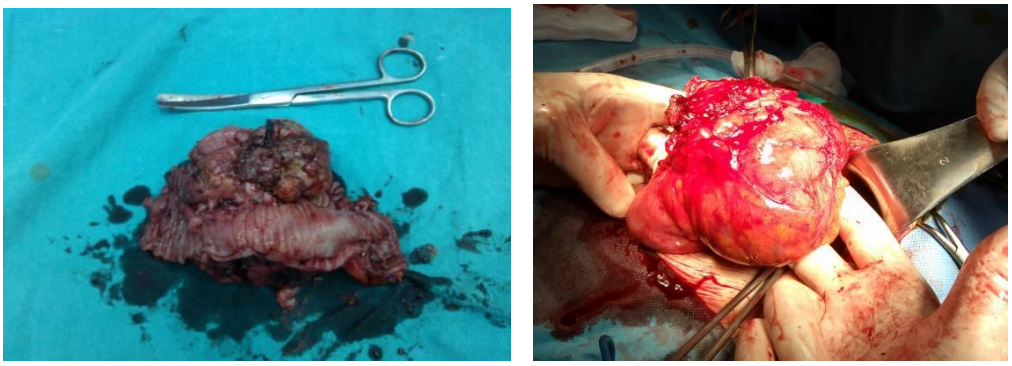


a. **b.**

Fig. 1. a, b. CT aspects of the sigmoid mass after contrast administration:
a. enhancing tumor occupying the entire sigmoid lumen;
b. intimate contact of the tumor with the parietal breach.

After the computerized tomography examination, the patient was prepared for surgery and she was operated. *A navel-to-pubis laparotomy* was performed while the

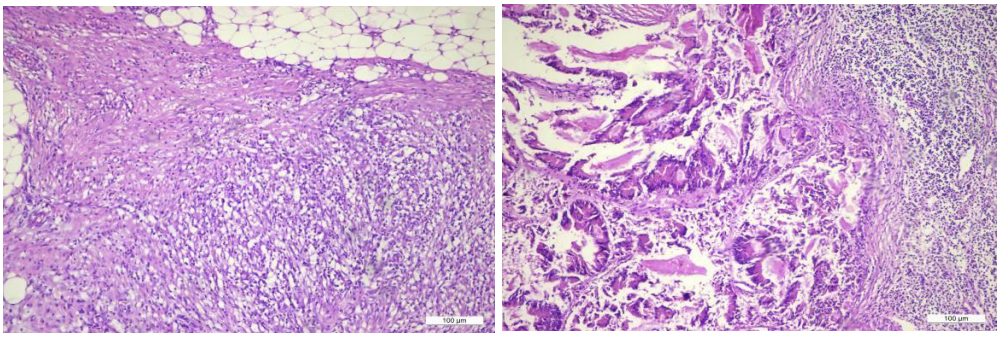
inspection of the peritoneal cavity showed a tumor of approximately 20 x 20 cm with a malignant aspect that invaded the abdominal wall by traversing the rectus sheath, 2/3 of the tumor being located outside the abdominal cavity. No peritoneal or liver metastases were noted during surgical inspection. The tumor was resected together with 10 cm of sigmoid colon on each side (fig. 2 a, b). Adjacent lymph nodes were removed with the tumor section of the sigmoid colon. A colo-rectal termino-terminal anastomosis was performed and the parietal breach was excised as well.



a. **b.**

Fig. 2. a, b. Tumor aspect: a. intraluminal tumor aspect; b. exterior tumor aspect.

Histopathological examination concluded moderately differentiated adenocarcinoma of colic origin, ulcerated, with infiltration and serosal effraction, and extensive areas of hemorrhage and necrosis. The parietal fragment consisted in muscular, adipose and conjunctive tissue with adenocarcinoma infiltration, and extensive areas of necrosis and hemorrhage (fig.3 a, b).



a. **b.**

Fig. 3. a, b. Histopathological aspects of the excised tumor and lymph nodes:
a. Sub serosa necrosis and desmoplastic reaction;
b. Lymph node metastasis of adenocarcinoma (hematoxylin and eosin stain)

The post-operative diagnosis established a loco regional advanced sigmoid cancer (T4bN1aMx (G2)).

The patient was discharged the 10th post-

operative day after an uneventful evolution and was referred for oncological evaluation and treatment to the Oncological Clinic. In addition to surgeons, specialists in nutrition, intensive-care medicine, nursing, wound care, made part of the treatment team.

After six months from the surgical intervention the patient came to a postoperative check after chemotherapy treatment and according to the clinical examination and computed tomography evaluation there were not found secondary neoplasia lesions.

DISCUSSION

Colorectal cancer is among the rarest causes of ECF and exceptionally manifests in this way. ECF usually occurs at any time after surgical interventions, spontaneous fistulas being uncommon (15-25%), usually occurring in patients that received radiotherapy (weeks or years after as mucosal ulcerations persist), have an inflammatory process (bowel disease, pancreatitis), bowel obstruction, or suffered an abdominal trauma (5). Bowel surgery and anastomosis after previous irradiation increases the risk of fistulation (6). According to the Food and Drug Administration website, out of 13.554 patients with colon cancer, only 8 (0.06%) presented with ECF. White et al. performed a study in 1973 concerning abdominal wall abscess as the presenting sign in colon cancer, analyzed 16.000 patients treated at the Massachusetts General Hospital and concluded that only 9 patients (0.05%) with colon cancer presented with abdominal wall abscess secondary to a colocutaneous fistula (3).

A throughout examination is the key point of a rapid and accurate clinical diagnosis.

In case of ECF due to malignant diseases, the only options are surgical resection (*en bloc* if possible) completed by enteric bypass or diversion (7). The presence of a malignant disease is a total contraindication to surgical closure of an ECF. In our particular case, the patient ignored a long period of time lasting constipation and hematochezia. The presentation in our emergency room occurred only when she was alarmed by the discharge of a feculent secretion from a cutaneous lesion.

In the present case, ECF has been already suspected in the emergency room at the moment of the first physical examination. Computed tomography examination is the preferred method of investigation as, compared to barium enema, provides information on the part of the bowel involved, type and extension of bowel disease, fistulous tract, presence of an abscess, other organs involved in the process (8, 9, 10).

Chapman et al. described in an article published in 1964 the key principles of ECF care, principles that apply no matter the etiology: correction of intravascular volume deficit, drainage of abdominal abscess, control of fistula effluent, and skin protection (11). Additional surgical management is required in patients with ECF due to cancer, and the surgical intervention can be performed in an elective or semi-elective manner, like was the case of our patient that underwent preoperative imaging studies for cancer staging. Depending on the size of the cutaneous breach and degree of involvement of adjacent tissues the closure of the abdomen may be performed primary or with a biological mesh that acts like a fascial bridge (12).

Untreated, ECF are associated to a high morbidity and mortality due to sepsis, malnutrition, electrolyte imbalance, and possible massive bleeding in case of vascular erosion.

ECF that complicate an advanced cancer are often associated with a poor overall survival and quality of life as they delay or restrain the administration of an adjuvant therapy.

CONCLUSIONS

Our case is a particular example of an unusual presentation of colorectal cancer as an ECF with abscess formation. The authors presented this case in order to illustrate the challenges associated with diagnostic and therapeutic management of advanced colorectal cancer that has to undergo semi-elective surgery with limited adjuvant therapy.

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THE RELEVANCE OF CYTOLOGICAL DIAGNOSTIC IN THE MAMMARY GLAND CANCER

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THE RELEVANCE OF CYTOLOGICAL DIAGNOSTIC IN THE MAMMARY GLAND CANCER (Abstract): Breast cancer is the second leading cause of cancer death in women, while in Eastern Europe the most common form of diagnosed cancer. Out of the multiple possibilities of early detection of mammary neoplasia that have been elaborated, only mammography has proved to be a simple, efficient method and of a high sensitivity, almost 90% However, the cytological confirmation of diagnosis allows us to perform the preoperative radiotherapy treatment or poly chemotherapy. **Material and methods**: we analyzed the informative value of these diagnosis methods in stage I mammary gland cancer (MGC). In this way, in the present paper we demonstrated that collecting samples through fine-needle aspiration biopsy allows the cytological confirmation of the diagnosis of stage I MGC in 30.7% cases. **Results and discussion**: In stage I MGC young patients, under 35 years, the cytological confirmation rate is 22.2% and is lower as compared to the cytological confirmation rate in patients older than 35 years which is 37.9% Also, for a tumor diameter < 0.5 cm, the prevalence of cytological confirmation was only 10.3%, while for the diameter of 0.6 – 1.0 cm the cytological confirmation was around 40.0%. Therefore, in order to improve the cytological diagnosis confirmation rate the tumor biopsy through the USG of the mammary glands is required. Moreover, the cytological investigation of the smear obtained by the first and second puncture was instrumental in confirming the diagnosis in 41.3% and 17.4% cases; the subsequent repetition of the punctures was not useful as it helped to confirmation of the diagnosis only in 9.3% cases. The frequency of diagnosis cytological confirmation depends on the tumor histopathological form and type of growth. **Conclusions:** Thus, the lowest prevalence was in the mixed forms - 12.5% cases, lobular cancer - 24.4% cases, while regarding the type of growth, for the rare forms the cytological confirmation rate was 7.7% and 31.5% cases for the schiros growth type. **Keywords**: MAMMARY GLAND CANCER, CYTOLOGY.

Breast cancer is the most common oncologic disease in women and is one of the major public health issues. Worldwide, breast cancer is the second leading cause of cancer death in women, while in Eastern Europe the most common form of diagnosed cancer. Among the most frequent involved factors in the outset of breast

cancer are included: the hereditary factor - genetically breast cancer develops in 3-5 cases; the genetic factors BRCA 1 and BRCA 2, should be more carefully studied, which up to now have been confirmed as having the greatest impact in breast cancer development risk, age over 35 years, hormonal contraception, incorrect nutrition, therapy with hormonal preparations, ionizing radiation, psychosocial factors and mental stress (1).

The questionable risk factors that are presented in various studies are very different and numerous, in some authors being citing over 300 in number, but none established with certainty. Immune factors along neuroendocrine factors are regarded as responsible for the clinical development of breast cancer (2).

Cancer research is a priority in all the laboratories of the world, in terms of uncovering the appearance causes of the malignant process, understanding the mechanisms of development, but most of all, the discovery of early diagnostic methods and effective treatment. Ignorance, fear of diagnosis, lack of health education and of efficient programs for prevention and screening, from our country cause diagnosis of the disease to be detected in the majority of cases in advanced stages, when treatment remains only palliative and very costly, in this cases the patient's suffering being immense (3).

Out of the multiple possibilities of early detection of mammary neoplasia that have been elaborated, only mammography has proved to be a simple and efficient method of a high sensitivity (almost 90%). In the same time, if the clinical examination is performed by an experienced physician, mammography can play an important role in screening. Moreover, the clinical diagnosis of palpable tumors usually does not present any difficulties because the clinical symptoms that indicate suspicion or clinical diagnosis of MGC are well-known (4).

Morphological factors of prognosis include the following: presence of tumoral emboli of the lymphatic and blood vessels of both tumor and adjacent mammary glandular tissue. The presence of tumoral emboli in the blood and lymph vessels was noticed in 14-29.5% MGC patients, who progressed after surgical treatment (5).

According to MoHceenico B. M., the most important criteria to assess the prognosis of MGC are: 1. The diameter of the tumor. 2. The degree of differentiation of the tumor after Bloom-Richardson. 3. The status of the regional lymph nodes. 4. The degree of expression of the steroid hormones receptors (RE, RP). 5. RNM synthesis activity markers: marking indices, the S-phase fraction, thymidine kinase activity, - Ki-67. 6. Growth factors receptors, including the oncogenes: epidermal growth factor receptor (EGF-R), HER2/neu, insulin-like growth factor receptors (IGF-R), somatostatin receptors. 7. Tumor suppressor genes - p53, -Nm 23 (6).

Also, due to the successes of molecular biology in the arsenal of researchers and clinicians there is a number of accurate indices which contributing to the assessment of predicting the efficacy of the MGC treatment and prognosis and selecting adjuvant and neoadjuvant therapy tactics in combined and complex MGC treatment. The problem concerning the researches in this field is to determine the set of informative indices, which would complement one another, which would enable at a minimum possible examination price to ensure the selection of the most efficient method in the treatment of each patient. The research spectrum in each particular case can depend on the progress stage, the age and

concomitant pathology of the patient, planned therapy and material technical basis of the institution.

In this way, it is generally accepted that the actual methods of MGC treatment which include the surgical component, radiotherapy, chemotherapy (CT) and hormone therapy (HT) could ensure a 90 - 95% rate of over 5 years' survival in stage I of MGC (7).

Still, the cytological confirmation of diagnosis allows us to perform the preoperative radiotherapy treatment or poly chemotherapy; which is why we analyzed the informative value of these diagnosis methods in stage I MGC.

Therefore, even with the mammographic or ultrasonographic clinical conclusion or assessment on the malignant character of the pathological process in the mammary gland, the neoadjuvant treatment becomes feasible only when the diagnosis is confirmed by one of the morpho-pathological methods, such as the cytological or histopathological method. According to the data in the international literature the cytological confirmation in the tumors with dimensions of less than 2.0 cm is problematic and does not exceed 20-25% cases as opposed to the extended malignant processes (8).

MATERIALS AND METHODS

The material of this study includes clinical and laboratory data analysis, the informational value of various investigation and treatment methods on 680 patients, selected between 1996 and 2004.

The patients included in the study were divided into three groups: the retrospective group, the prospective group and the control group.

In this way, the retrospective group included 496 stages I MGC patients, diagnosed and treated during 1996-2004. The retrospective group was divided into 2 groups according to the type of clinical development of tumoral process after treatment: the 1st group including 419 patients without progress for 5 years after the treatment, and the 2nd group including 77 patients with the progressing tumoral process after treatment.

On the other side, the prospective group included 66 stages I MGC patients, diagnosed and treated between 2007 and 2008. For the patients in the prospective group there were assessed the molecular expressions of the HER2/neu molecular markers, CEA tumoral markers, CA-15.3, hormonal receptors RE and RP, hormonal homeostasis and immunological indices.

Finally, the control group comprised of 118 patients, from which 56 patients with Ha, Ilb, or lila stage MGC, and 62 patients with benign diseases.

In the control group the immunological and homeostasis hormonal parameters were studied for comparison with the stage I MGC.

A special inquiry was developed for this study, which included 150 parameters regarding special body or tumor features, the informational of various methods of investigation and the results of different treatment variants applied.

Data analysis

The decisive prognostic factors determination was performed by discriminate analysis. By applying discriminate analysis - within the groups of the retrospective lot the decisive prognostic factors of the clinical evolution variance were determined in the stage I MGC patients.

RESULTS

In this way, the cytological investigation conducted on 475 patients confirmed the diagnosis of cancer of the mammary gland

only in 146 patients, which represented 30.7% cases. The cytological confirmation correlates with the age of the stage I mammary gland cancer patients (tab. I). As a matter a fact, as shown in the table, the diagnosis of MGC was cytological confirmed in 22.2% cases for the patients up to 35 years; in 24.1% cases for the 35-49 years' patients - and in 37.9% cases for the 50-year-old and older patients (tab. I).

TABLE I

The accuracy of cytological confirmation of the malignant process in stage I breast cancer patients by age.

No	Patients Age	No. of recorded patients	Cytological confirmed diagnosis	
			No. abs.	%
1	< 35 years	27	6	22.2
2	35-49 years	216	52	24.1
3	> 50 years	232	88	37.9
4	Total:	475	146	30.7

$X^2 = 11.06$, $p < 0.05$

Also, the cytological conclusion variants were: cancer - 30.7%, the cancer suspicion conclusion -18.9% cases, proliferative process with atypical - in 19.4% cases.

For the other cases other variants of cytological diagnosis, without suspicion of cancer, have been established.

Of these the cytological conclusion for 1 in each 3 patients, which represents 17.5% - consisted in normal glandular epithelial cells without proliferation or atypical signs (tab. II).

TABLE II

Cytological conclusion variants in stage I MGC versions by the number of conducted aspiration biopsies

No.	Cytological analyses conclusion	Quantity of conducted aspiration biopsies							
		1		2		3-4		Total:	
		283 patients		138 patients		54 patients		475 patients	
		No.	%	No.	%	No.	%	No.	%
1	Cancer	117	41.3	24	17.4	5	9.3	146	30.7
2	Suspicion of cancer	34	12.0	35	25.4	21	38.9	90	18.9
3	Proliferation with	22	7.8	50	36.2	20	37.0	92	19.4
4	Glandular epithelial cells	46	16.3	29	21.0	8	14.8	83	17.5
5	Lipids, erythrocytes,	64	22.6	0	0	0	0	64	13.5

$\chi^2 = 38.37$, $p < 0.01$

In addition, as it can be seen in table II, the results for the cytological confirmations by number of conducted aspiration biopsies were: the first biopsy was cytological confirmed in 41.3% cases, for the second biopsy the cytological investigation of the smear from the samples allowed the diagnosis cytological confirmation in 17.4% cases, while the aspiration biopsies repeated 3-4 times cytological confirmed the diagnosis only in 9.3% cases.

Also, we have to mention that the correct cytological conclusion prevalence analysis depending on the tumor diameter revealed that for a tumor diameter < 0.5 cm the correct conclusion of MGC was at-

tained only in 10.3% cases, for 0.6 – l.0 cm in 40% and for l.l – 2.0 cm – in 34.3% cases (tab. III).

Moreover, the diagnosis cytological confirmation frequency depends on the histopathological form and type of tumor growth. 31.6% cases were cytological confirmed for the ductal form, for the lobular form 24.4% cases were cytological confirmed, for the mixed forms (ductal and lobular) 12.5% cases, and for the rare forms that unite a string of highly differentiated forms of MGC (tubular, papillary, medullar, mucinous and other.) - in 41.7% cases (tab. IV).

In addition, the prevalence of the cytological confirmation is depending on the morphological type of the tumor: for the solid growth MGC type, 66.1% of the cases were cytological confirmed; for the schiros growth type 37.5% were cytological confirmed, while the lowest frequency rate of cytological confirmation was in the mixed forms - 7.7% cases (tab. V).

TABLE III

The frequency of the cytological confirmation for the malignant process in patients with stage I MGC by size of the tumor

No.	Size of tumor	No. of marked patients	Cytological confirmed diagnosis	
			No. abs.	%
1	< 0.5cm	39	4	10.3
2	0.6-l.0cm	160	64	40.0
3	l.l-2.0cm	213	73	34.3
4	Total:	412	141	34.2

$\chi^2 = 35.0, p > 0.05$

TABLE IV

Frequency of cytological confirmation in patients with stage I MGC by tumor morphological variant

No.	Morph pathological variants	No of patients	Cytological confirmation	
			No. abs.	%
1	**Ductal**	345	109	31.6
2	**Lobular**	86	21	24.4
3	**Ductal and lobular**	8	1	12.5
4	**Rare forms**	36	15	41.7
5	**Total**	475	146	30.7

$\chi^2 = 65.0, p > 0.05$

TABLE V

Frequency of cytological confirmation in patients with stage I MGC by tumor morphological type.

No.	Morphological type	No. of Patients	Cytological confirmation	
			No abs.	%
1	**Solid**	59	39	66.1
2	**Schiros**	56	21	37.5
4	**Rare forms**	260	20	7.7
5	**Unknown**	100	-	-
6	**Total**	475	146	30.7

$\chi^2 = 5.0\ p > 0.05$

It must be also mentioned that although the cytological diagnosis was confirmed only in 146 patients who represented 30.7% of cases, for the other 329 patients, 69.3%, the morph pathological diagnosis was determined by intraoperative extemporaneous histological investigations.

DISCUSSION

In this way, as previously mentioned, it is generally accepted that the actual methods of MGC treatment which include the surgical component, radiotherapy, chemotherapy (CT) and hormone therapy (HT) could ensure a 90 - 95% rate of over 5 years' survival in stage I of MGC (7). Still, the cytological confirmation of diagnosis is allowing us to perform the preoperative radiotherapy treatment or poly chemotherapy; which is why we analyzed the informative value of these diagnosis methods in stage I MGC.

Therefore, although with the mammographic or ultrasonographic clinical assessment on the malignant character of the pathological process in the mammary gland being available, the neoadjuvant treatment becomes feasible only when the diagnosis is confirmed by one of the morphopathological methods, such as the cytological or histopathological method. According to the data in the international literature, the cytological confirmation in the tumors with dimensions of less than 2.0 cm is problematic and does not exceed a rate of 20-25% cases as opposed to the extended malignant processes (8).

Moreover, serum tumoral markers are used to assess mammary neoplasia evolution: the carcinoembryonic antigen (CEA) and the CA 15.3 (cancer antigen 15.3) (9). In this way, the CA 15.3 concentration occurs in women with the diagnosis of breast cancer, and its usefulness lies in evaluating the evolution of the illness, response to treatment and relapse assessment. The CA 15.3 tumor marker is a mucin-like antigen, with high molecular weight, issued primarily by cells of mammary glandular carcinoma. CA 15.3 is defined by its reactions with two monoclonal antibodies: one of them (115D8) binds to an antigen from human milk; the other (DF3) bounds to a membrane fraction of the carcinomatous cells.

The CA 15.3 blood titer increased in patients with advanced process, drops after chemotherapy treatment and rises again in the case of relapses or metastasis (in particular bone metastasis). Although the sensitivity of this marker in detecting early stages is reduced, lately there is a increasing tendency towards the idea of introducing it as a method of screening in the female population in the age group 45-54 years old (pre-and postmenopausal), considered to be the age group most prone to breast cancer. Also, determining molecular markers is an important tool for the diagnosis, treatment, assessment of therapy response. A slight physiological increase of the CA 15.3 antigen is registered in 4-7% of the nursing women and in about 8% of the pregnant women (9).

Moreover, carcinoembryonic antigen (CEA) is a specific marker for the digestive tract cancers (colorectal, stomach, pancreas, liver, biliary vesicle); subsequently, clinical studies have shown an increase in other malignant diseases (lung, mammary gland, ovary, cervix, prostate).

Historically speaking, the carcinoembryonic antigen was first described in 1965 by Gold and Freedman in a patient

with colorectal carcinoma. The carcino-embryonic antigen is a glycoprotein with the molecular weight of 220 kDa containing approximately 40% proteins and 60% carbohydrates. In this way, it is one of the onco fetal products during embryonic life and fetal development. Its production is suppressed after birth, reaching very low levels into adulthood, less than 3 ng/ml in non-smokers, less than 5 ng/ml in smokers. Immunohistological, the carcino embryonic antigen can be determined in elevated concentrations in the fetal gastrointestinal tract and pancreas, as well as in colorectal adenocarcinoma, and in lower concentrations in the normal intestinal tissue, intestinal mucosa, exocrine pancreas, and in the liver. The function of antigen carcino embrionar is not fully known; it is assumed that the CEA bound to the cellular membrane serves as mechanism for intracellular recognition functioning as a receptor. In addition, CEA is also assigned an immunosuppressive effect through the induction of macrophages and suppressor T lymphocytes (10).

Also historically speaking, we could also mention the unique research carried out in 1962 by H. Bloom, W. Richardson, E. Harries. The authors were able to trace the fate of 250 untreated patients mainly in stage III-IV MGC between the years 1805 and 1933: the median survival was 2.7 years, and the last patient died in the 19th year of surveillance without any treatment (2).

Of particular relevance in this context is also the fact that the prediction factors mirror the relations of reciprocity between tumor biology and treatment (for example, RE, RP and hormonotherapy; HER2/neu and herceptin therapy) (11). In fact, the prediction factors may also have prognosis significance. In this way, MGC is considered to be a heterogeneous pathology, with clinical evolution ranging in various patients from severe aggressiveness to the relatively benign.

CONCLUSIONS

MGC represents a polymorphic and pathogenic disease and it cannot be admitted that all subgroups of patients will obtain identical results from one tactic of treatment determined for all the patients with MGC. Thus, the concept of MGC both clinical and patho morfological, combines different cell clones depending on its microstructure and biology. As a result, the evolution of the disease, the prognosis and the effectiveness of the treatment may vary in different patients at the same stage, depending on the degree of malignancy of the tumor, its histopathological structure, the degree of expression of molecular markers identification and also immunoresistance.

Also, some numbers-related conclusions could be represented by the fact that: collecting samples through fine-needle aspiration biopsy allows the cytological confirmation of the diagnosis of stage I MGC in 30.7% cases. In stage I MGC young patients, under 35 years, the cytological confirmation rate is 22.2% and is lower as compared to the cytological confirmation rate in patients older than 35 years which is 37.9.%. For a tumor diameter < 0.5 cm, the prevalence of cytological confirmation was only 10.3%, while for the diameter of 0.6 – 1.0 cm the cytological confirmation was around 40.0%.

Therefore, in order to improve the cytological diagnosis confirmation, rate the tumor biopsy through the USG of the mammary glands is required.

The cytological investigation of the smear obtained by the first and second

puncture was instrumental in confirming the diagnosis in 41.3% and 17.4% cases; the subsequent repetition of the punctures was not useful as it helped to confirmation of the diagnosis only in 9.3% cases. Moreover, the frequency of diagnosis cytological confirmation depends on the tumor histopathological form and type of growth. Thus, the lowest prevalence was in the mixed forms - 12.5% cases, lobular cancer - 24.4% cases, while regarding the type of growth, for the rare forms the cytological confirmation rate was 7.7% and 31.5% cases for the schiros growth type.

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GIANT INTRALIGAMENTARY UTERINE LEIOMYOMA AND ITS COMPLICATIONS

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GIANT INTRALIGAMENTARY UTERINE LEIOMYOMA AND ITS COMPLICATIONS (Abstract): Uterine leiomyomas are benign tumors arising from uterine smooth muscle. Although their pathogenesis remains unclear, they are the most common tumor of the female reproductive tract, occurring in as many as half of women older than 35 years. Uterine leiomyomas represent the most common benign tumors of the female reproductive tract. Giant uterine leiomyomas are very rare and represents a great diagnosis and therapeutic challenge. Uterine leiomyoma is one of the most frequent types of tumours and it is diagnosed in 20-40% of the women of reproductive age. Until the age of 50, approximately 70% of the white women and less than 80% of the black women will have had at least one leiomyoma. The frequency of the emergence of uterine leiomyoma in black women is significantly higher than in white women.
Keywords: UTERINE GIANT FIBROID, SURGERY, HYSTERECTOMY.

Around 200,000 hysterectomies, 30,000 myomectomies and thousands of selective uterine artery embolizations are performed annually in the United States to reduce the size of uterine leiomyomas (4, 5).

These lesions disturb the uterus functions and generate excessive bleeding, anemia, defective implantation of embryos, recurrent miscarriages, premature labour, pelvic discomfort and urinary incontinence; they can also imitate or mask malignant tumours.

The size of the leiomyoma and its location generate characteristic manifestations. Thus, in the case of submucuous leiomyomas, there are excessive or irregular bleedings, infertility, as well as recurrent miscarriage. Subserous leiomyomas developing in the peritoneal cavity can exercise pressure, which is perceived by the patient as pelvic discomfort, and intramural leiomyomas located in the myometrial wall are an intermediary grouping (7, 12).

CASE REPORT

Patient M.B., 44 years old, coming from the rural environment, working as a salesperson, is admitted in 2nd Obstetrics and Gynecology Clinic, at "Cuza Voda" Maternity Hospital of Iaşi for: moderate pain in the hypogastrium and left iliac fossa, pelvic load sensation, repeated metrorrhagias (excessive genital bleeding at irregular intervals, of prolonged duration of the flow > 6 days), enlarged abdomen, pollakiuria, slow bowel movement, pain in the left lower limb.

The personal pathologic history reveals that, 14 years before, the patient underwent surgery for left adnexectomy due to left

ovarian cyst, and her family history is insignificant. At the time of her admission, the patient's general state was good, and the examination of her apparatuses and systems reveals chronic constipation (slow bowel movement), and pollakiuria.

Upon bimanual abdominal palpation, in the left iliac fossa and hypogastrium an abdominal-pelvic tumour is found, which is hard, irregular, of firm consistency, painful upon palpation, of reduced mobility, and with the transversal diameter over 8 cm. The digital vaginal examination reveals a cartilaginous cervix with the internal orifice closed, the uterine body enlarged on the whole, deviated to the left, of polycyclic outline, impalpable annexes.

The abdominal ultrasonography reveals a tumour formation that is hyperechogenic, non-homogeneous, well limited, of 24.4/31 cm, non-visualized annexes, no free fluids in the peritoneal cavity; in the left kidney, there is a 1st degree left hydronephrosis, due to the compression of the tumour. Laboratory tests reveal a mild anemia upon admission (fig. 1).

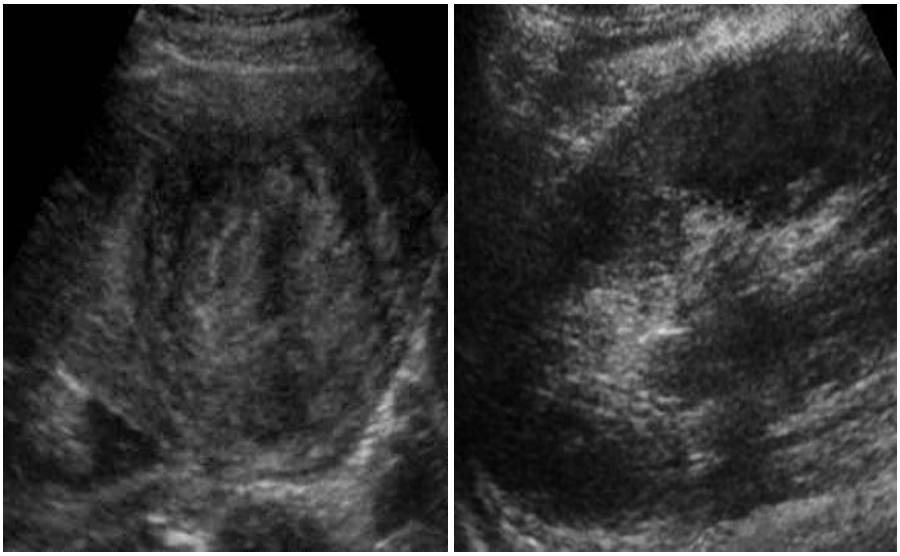

Fig. 1. Transabdominal ultrasound aspects of the uterine leiomyoma

Based on the clinical and paraclinical data, the set diagnosis is uterine polyfibromatosis with an intraligamentary nodule of 25/30 cm, for which surgical therapy is chosen. Surgery is performed and the following is found during surgery: enlarged uterus of approximately 11/9/5 cm, with multiple subserous fibromatous nodules with diameters between 3 and 5 cm, an adherent intraligamentary fibromatous nodule of approximately 25/30 cm, and a posterior isthmic subserous nodule of approximately 7 cm, left annex absent by previous surgical intervention, right annex of normal aspect. Total hysterectomy and right adnexectomy are performed. Difficult hemostasis due to exuberant vascularization of the leiomyoma, but eventually efficient. Two intraabdominal meshes with hemostatic role are left inside as well as a drain in the Douglas space. Postoperative pieces were submitted to an anatomopathologic examination (fig. 2, 3, 4).

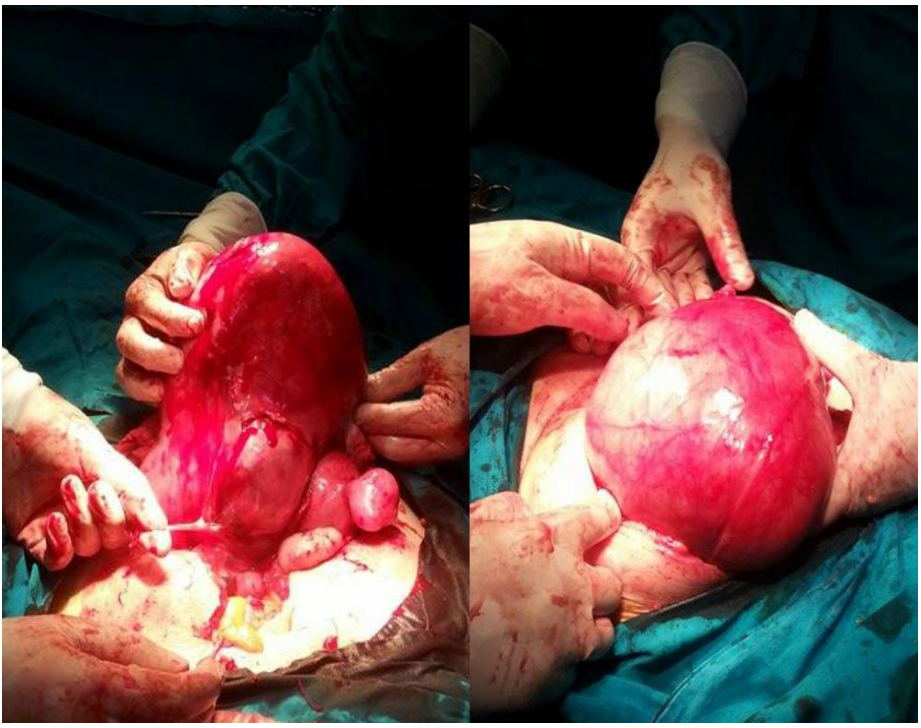

Fig. 2. Intraoperative image (uterine leiomyoma)

Fig. 3. Intraoperative aspects of the pelvic tumour

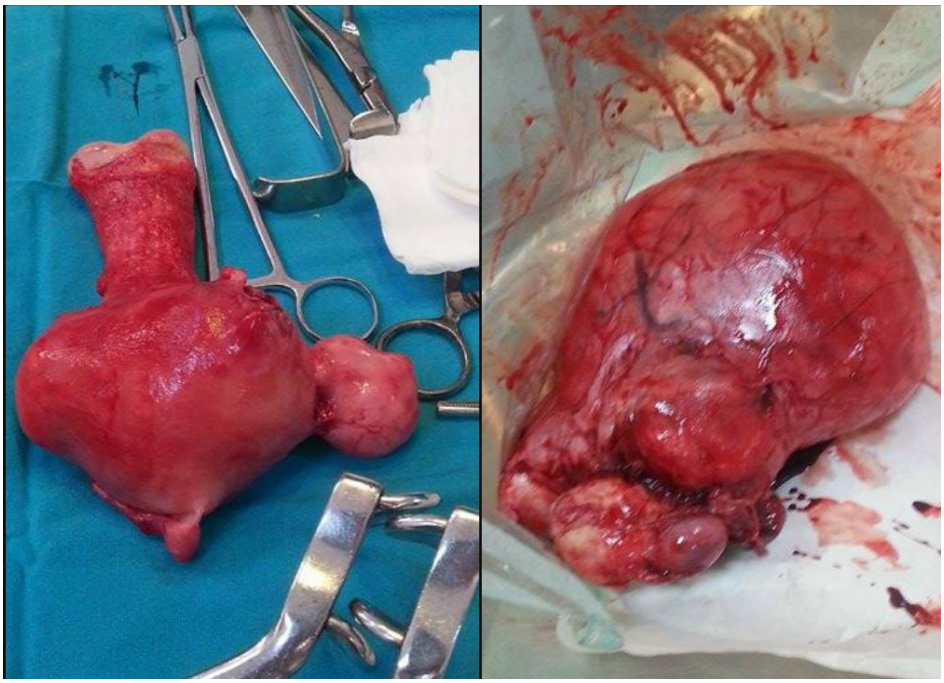

Fig. 4. Operative pieces (uterine body + cervix; intraligamentary uterine leiomyoma of 25/30 cm)

After surgery, following laboratory tests, the patient was transfused with two units of iso-group iso-Rh blood. Her evolution is favourable, she resumed feeding and bowel movement, the abdominal meshes and drain are supressed the second day after surgery. A sample was collected from the wound, which was positive for Staphylococcus OR, sensitive to Ciprofloxacin.

In the anatomopathologic examination, the following is found in the examined sections: subserous nodular leiomyoma (max diameter 33 cm) with densely vascularized areas and hyaline degeneration with no mitotic activity, necrosis or haemorrhage, intramural nodular leiomyoma (diameter = 3cm), endometrium with simple hyperplasia and exacerbated chronic inflammation, non-specific chronic cervicitis, right ovary: follicular cysts, simple serous cyst (2cm), right exacerbated chronic salpingitis.

DISCUSSIONS

Depending on the size and location, the uterine leiomyoma can put pressure on the uterine organs, generating a series of "borrowed" symptoms, of variable intensity, some forms being well tolerated, but others generating important complications.

Generally, leiomyoma is not painful, but in case pain emerges, then we can speak of a trophic complication of the tumour, compressive phenomena exercised on other organs and sometimes even an association to another pathology. Large tumours generate a load sensation in the pelvis, the submucous polypoid ones – spasmodic or colical pain, and in the case of enclosure of a leiomyoma in the true

pelvis, most frequently with isthmic location, there is pelvic pain radiating in the lower limbs due to the compression on the roots of the sciatic nerve. The latter phenomena appears in less than 1% of the cases and is favoured by pregnancy.

The leiomyoma location is important also in detecting other functional disorders, therefore in case of compression on the urinary ducts, there are urination disorders such as pollakiuria, which is generated due to the hindrance of the bladder distension by the leiomyoma. But the most important is the renal-urethral impact, which affects silently the upper urinary ducts, and in the case under discussion the leiomyoma – being intraligamentary – generates hydronephrosis, which comes to compromise the renal function. The posterior development of the leiomyoma can be accompanied by strangury or chronic constipation.

In the case of a leiomyoma with pelvi-abdominal development, the surgical intervention is more laborious, of high difficulty. Most times, the median incision is extended to the left paraumbilical area for increased visibility. The surgical option is determined most often by the tumour size, the organs need to be accurately recognized, avoiding the opening of the urinary bladder in the lower angle of the abdominal approach path. In order to avoid haemorrhage, the contact with the tumour surface should be as tight as possible.

In 1952, Forgue spoke about the precise notions of the detachable areas of the pelvic peritoneum, about the virtual subserous spaces where these tumours develop, and about the lesion risks involved by visceral displacements, which the surgeon should consider.

The surgical difficulty as regards a pelvic tumour of large size is not related to the total size of the uterus, but rather to the presence of included leiomyomas, those tumours developed inside the areas of detachment of the visceral peritoneum.

The intraoperative problems are represented by the probable existence of adhesions between the uterus and neighbouring organs, the presence of intense vascularization feeding growing tumours with haemorrhagic rick. Several planes must be crossed in order to reach the true cleavage surface, these planes can include the ureters; the vessels feeding the tumour can be numerous and large, in some cases as big as the inferior vena cava. Thus, they need to be ligatured before acting in this area (1, 9).

Due to its location, the leiomyoma can displace the uterus, can elongate the vagina or even elevate the urinary bladder. This way, the relations to the organs around are changed, the main risk of the surgical intervention being injuries cause to the uterus. The advantage that the gynaecological surgeon has in case the tumour is benign is the fact that the uterus is displaced laterally by the tumour and not enclosed in it, as is the case of endometrial adenocarcinoma or cervical cancer, which invade one or both parameters from the very first stages. Therefore, we consider that procedures such as catheterizing the uterus before surgery or other imaging methods of evaluation of the exact location of the ureteral route are not absolutely necessary if the paraclinical evaluations prior to surgery support the diagnosis of benign uterine tumour. We consider that the attentive opening of the parameter with the evaluation "at sight" of the parameter elements and avoiding injuring them are the optimum procedure in such cases. The entire pelvic anatomy can be completely changed.

Hence, the surgical technique is not the

standardized one. The surgical intervention begins from the best exposed uterine side. At first, either adnexectomy is performed on that side, or the interadnexal hysterectomy technique is applied ,followed by adnexectomy. Hysterectomy is performed concomitantly on both uterine sides, with the purpose of mobilizing the uterine body and exteriorizing it as much as possible. Another technique that can be performed consists in the initial sectioning of the uterosacral ligaments, which increases the uterus mobility. Total hysterectomy is also possible, but it involves higher blood loss and surgery takes longer. It would be optimum for the hysterectomy duration to be as short as possible and the blood loss to be as low as possible. This way, the surgical technique can be simplified by practicing first subtotal hysterectomy or polymyomectomy followed by the totalization of the intervention and potentially adnexectomy. The totalization of the intervention by extracting the cervix immediately after the subtotal hysterectomy is not subject to the same technical difficulties as totalization of a hysterectomy performed years before. In such cases, the visceral peritonization of the cervical stump determines the elevation of the urinary bladder, which is injured during surgery, while uncovering the cervix. There is no such risk in our intervention (11, 12).

CONCLUSIONS

In our opinion, the therapy for the uterine leiomyoma must start from the particularities of this tumour: it is a benign tumour with negligible risk of turning into a malignant one, and the symptoms that the patient describes are given by afferent complications and not the leiomyoma itself, and it is when the uterine bleeding becomes abundant leading to anemia that it overcomes the stage of sign and it becomes a complication.

The entire pelvic anatomy can be completely changed. Hence, the surgical technique is not the standardized one. It would be optimum for the hysterectomy duration to be as short as possible and the blood loss to be as low as possible. This way, the surgical technique can be simplified by practicing first subtotal hysterectomy or polymyomectomy followed by the totalization of the intervention and potentially adnexectomy.

Also, the section of utero-sacral ligaments at the beginning of the histerectomy is an efficient technique of making the uterus mobile.

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NEWS

THE IMPORTANCE OF MOLECULAR BIOLOGY TESTS IN THE DIAGNOSIS OF *CLOSTRIDIUM DIFFICILE* INFECTION

C. difficile is an anaerobic bacterium that causes pseudomembranous colitis after antibiotic therapy. Between 3% and 7% of healthy people are colonized with *C. difficile*, but the percentage increases among people hospitalized. *C. difficile* infection diagnosis is based on clinical manifestation and is confirmed by the immunoassay test for *C. difficile* toxins and PCR for bacterial DNA. A recent study showed that the exclusive use of PCR in the diagnosis of *C. difficile* infection without immunological tests for toxins may conduct to over diagnosis and overtreatment. The duration of diarrhea during treatment, rate of complications and mortality rate for patients Tox−/PCR+ were comparable to patients without *C. difficile* (Tox−/PCR−) and lower than Tox+/PCR+ patients. These findings suggest that the treatment is not necessary for patients who were positive by PCR testing but negative by toxin immunoassay (Polage CR, Gyorke CE, Kennedy MA, Leslie JL, Chin DL, Wang S, Nguyen HH, Huang B, Tang YW, Lee LW, Kim K, Taylor S, Romano PS, Panacek EA, Goodell PB, Solnick JV, Cohen SH. Overdiagnosis of *Clostridium difficile* infection in the molecular test era. *JAMA Intern Med*. 2015. doi:10.1001/jamainternmed.2015.4114).

Cătălina Luncă

BURNOUT SYNDROM AMONG PUBLIC AMBULANCE STAFF

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BURNOUT OUT SYNDROM AMONG PUBLIC AMBULANCE STAFF (Abstract): Healthcare professionals are frequently confronted with urgent situations and a high-risk human intervention. They are usually exposed to what is called burnout syndrome. **Aim:** To identify the effects of burnout syndrome on the professional conduct and attitudes of doctors and nurses who work in the Romanian public ambulance service. Secondary, the causal relationships between burnout and various socio-demographic variables were analyzed. **Material and methods:** The 20-item Toronto Alexithymia Scale (TAS- 20), Maslach Burnout Inventory and Job Satisfaction Questionnaire were administered to 122 ambulance doctors, nurses and drivers (62 females and 60 males). **Results**: The degree of job satisfaction is the most important indicator of burnout syndrome. Significant differences were found between low and high alexithymic subjects. Women are more susceptible to experience higher levels of burnout than men. The level of burnout is influenced by the combined effect of job satisfaction and alexithymia. **Conclusions**: Burnout syndrome is a common problem among people working in the emergency medical system. The causes of job-related burnout have to be identified in order to apply an appropriate level of burnout intervention program and to increase the efficiency of coping strategies. **Keywords**: ALEXITHYMIA, BURNOUT SYNDROME, MEDICAL STUFF, AMBULANCE SERVICE

The term "burnout" was first used in the USA in the '70s among employees of social care services. Typical symptoms of burnout are classified as: physical - fatigue, frequent headaches, gastrointestinal disorders, insomnia, changes in eating habits, lowered immunity and frequent infectious conditions, cardiovascular disorders, muscle disorders; psychological - feelings of guilt, negativism, low motivation, mood swings, irritability, low empathy and self-confidence, mnemonic disorders, and behavioral - frequently absent from or late for work, refusal of dialogue, a tendency to avoid telephone contacts and postpone meetings, low performance, conflicting relationships with family and friends (1, 2, 3). Studies in the field showed that work satisfaction and alexithymia correlate with the burnout syndrome obtaining important results for the understanding, as well as prevention of this phenomenon (4).

Despite the fact that initially burnout was not looked upon as a serious scientific topic, research on it started to flourish from the beginning of the 1980s. From 1975 to

1980, the number of articles published on burnout increased from 5 per year to over 200. Since the beginning of this century, over 6000 articles on burnout have been published (1). Significant aspects of burnout have been studied at length in countries with a tradition in business development, especially from the perspective of preventing and treating such symptoms. Three quarters of employees believe that occupational stress is more significant nowadays than it used to be for the previous generation. Workplace problems are more strongly associated with health complaints than with any other problem, for example, financial or even family problems (5, 6).

Researches were focused on specific groups, the representative ones targeting workers in healthcare (doctors and nurses), social care services, education and students (7,8,9,10).

Comparative studies between risk groups in the same professional field were conducted in various countries, such as Canada and the Netherlands, and the results indicated that the levels of exhaustion and depersonalization of Canadian teachers were higher than those of their Dutch colleagues (11).

In Switzerland, for instance, there are specialized clinics for the recovery of individuals affected by burnout. People take action in order to address this issue without being or feeling stigmatized. In countries like USA, the Netherlands or Spain, burnout is acknowledged as a temporarily disabling condition and people receive specialized help in order to overcome this situation and regain socio-professional functioning (2). Burnout is a transient state, but one that cannot be overcome without taking certain measures.

In Romania, a study conducted in 2007 by Competent Consulting, European Commission agency, on a sample of 2000 subjects showed that 40% of them were affected by occupational stress and 39% by burnout at emotional level (12). In our country the key issue at stake is correct identification of burnout, as it is commonly mistaken for depression or chronic stress, seeing as there is no "tradition" of treating this problem. The burnout syndrome has no immediate manifestations, but appears as a gradual reaction to emotional exhaustion and prolonged exposure to stressors, which, in their turn, lead to a higher degree of dehumanization and professional dissatisfaction (13).

The rising incidence of the burnout phenomenon in Romania caused an increase in specialist interest in this field. Most research work is focused on healthcare professionals: one third of anesthesiologists (mostly women) included in a study conducted in 2012 showed that high levels of exhaustion, daily concerns and workload were the major predictors of depersonalization (14,15).

MATERIAL AND METHODS

The aim of this research was to determine the relationship between work satisfaction, alexithymia, length of service, gender and the occurrence of the burnout syndrome among public ambulance staff.

The research hypotheses were: 1. burnout syndrome in public ambulance staff varies in relation with the level of professional satisfaction and alexithymia, length of service and gender; 2. There are interaction effects between the independent variables level of professional satisfaction, level of alexithymia, length of service and gender and the dependent variable burnout.

The research variables of the study

were: the burnout syndrome as dependent variable and independent variables:

1. Job satisfaction, with two levels: low and high;

2. Alexithymia, with two levels: low and high;

3. Length of service, with two levels: 1-15 years and 16-35 years;

4. Gender: male and female;

5. Professional categories - auxiliary medical staff (nurses) and ambulance drivers.

A number of 122 ambulance doctors, nurses and drivers (62 females and 60 males) from two public ambulance services on North-Eastern Romania voluntarily accepted to participate in this study.

To measure alexithymia we used the 20-item Toronto Alexithymia Scale (TAS 20), which assesses alexithymia as a multifaceted construct, consisting of (1) difficulties in identifying emotions (DIF), (2) difficulties in describing emotions (DDF) and (3) externally oriented thinking style (EOT). TAS 20 consists of 20 self-descriptive statements, and has a good internal consistency (Cronbach alpha coefficient 0.85).

To measure the burnout phenomenon we used the MBI with 3 subscales: emotional exhaustion (the feeling of being emotionally exhausted and depleted by one's work); depersonalization (emotional coldness and impersonal reactions towards the recipients one's service), personal accomplishment (feelings of competence and accomplishment in working with other people).

To assess professional satisfaction we used the Work Satisfaction survey. This includes four factors: Remuneration and promotion (Cronbach alpha =0.820); Leadership and interpersonal relationships (Cronbach alpha =0.760); Organization and communication (Cronbach alpha =0.738). The Cronbach alpha coefficient for the entire survey was 0.872.

RESULTS

Important interaction effects were found between the level of professional satisfaction, level of alexithymia, length of service and gender and the results of the dependent variable burnout:

a. there is a combined interaction effect between the level of professional satisfaction and the level of alexithymia on the burnout variable ($F(3.118)=7.25$; $p= 0.008$), subjects with low levels of professional satisfaction and high alexithymia having the highest burnout values;

b. there is a combined effect of gender and alexithymia on burnout level ($F(3.118)=4.34$, $p=0.03$), female subjects with high alexithymia having the highest burnout values;

c. there is a combined effect of professional satisfaction and length of service on burnout level ($F(3.118)=11.79$, $p=0.001$), subjects with lengths of employment of over 16 years and low professional satisfaction having the highest burnout values;

The second hypothesis emphasizes the relationship between the burnout syndrome in healthcare professionals working in emergency care services and the level of professional satisfaction, level of alexithymia, length of service and gender.

a. relationship between burnout and the level of professional satisfaction. Results obtained after using t tests on independent samples emphasize the fact that there are significant differences between subjects with high levels of job satisfaction ($M=22.13$) and subjects with low levels of job satisfaction, in what concerns the perceived degree of burnout ($t (120) = 6.56$,

p=0.000), in that subjects with high levels of job satisfaction have lower levels of burnout syndrome (M=22.13), compared to subjects with low levels of job satisfaction (M=42.81);

b. relationship between burnout and the level of alexithymia. The *t test* for independent samples reveals significant differences (t (120) = -2.65, p=0.000) in the level of burnout depending on the level of alexithymia, in that those with high levels of alexithymia have higher burnout levels (M=38.23) than those with low levels of alexithymia (M=28.79);

c. As to the influence of the variable length of service on the perceived level of burnout, the research hypothesis was not confirmed;

d. The last hypothesis of the study, which claims that there is a significant female-male difference in burnout, was confirmed. The *t test* for independent samples (t (120) = -6.37, p=0.000) shows that female subjects (M=43.39) have higher levels of burnout than male subjects (M=23.25);

e. there are no significant differences between the perceived burnout and subjects' professional categories (nurses and ambulance drivers).

CONCLUSIONS

Our study emphasizes the fact that the level of professional satisfaction is an important indicator of the emergence of burnout syndrome; the higher the level of job satisfaction, the lower the possibility of becoming the victim of an "internal combustion" (burnout). We may talk about a causal relationship between professional satisfaction and burnout. Much like professional satisfaction, alexithymia also plays a major role; a high level of alexithymia is associated with a high level of burnout, because the inability to identify and express emotions often leads to estrangement from others and from oneself. Alexithymia causes many problems which hinder man's normal functioning in society. Women are more likely to exhibit higher levels of burnout than men. This fact may be explained in terms of gender differences, which make women a lot more susceptible; they tend to be more emotionally involved in their work, to show more soft spots for their patients, and to be more subjective.

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NEWS

ATRIAL FIBRILLATION FROM THE PATHOLOGIST'S PERSPECTIVE

Atrial fibrillation (AF) is the most common sustained arrhythmia encountered in clinical practice. Electrophysiologically, it is characterized by a high rate of asynchronous atrial cell depolarization causing a loss of atrial contractile function and irregular ventricular rates. For a long time, AF was considered as a pure functional disorder without any structural background. Only in recent years, new mapping and imaging techniques have identified atrial sites which are predominantly involved in AF initiation and maintenance, i.e., the pulmonary veins (PVs) and the surrounding left atrial posterior wall of the myocardium. Morphological analysis of these myocardial sites has demonstrated significant structural remodeling. This architectural myocardial disarrangement is induced by the arrhythmia itself and the associated cardiovascular disorders. At the same time, the structural remodeling is also capable of sustaining AF, thereby creating a sort of pathogenetic vicious circle. This review focuses on current understanding about the structural and genetic bases of AF with reference to their classification, pathogenesis, clinical implications and cure. Ablation procedures performed in these specific left atrial sites has very often proved efficient in restoring sinus rhythm in patients suffering from AF. In conclusion, AF itself and the associated cardiovascular disorders are capable of inducing in the atrial myocardium various degrees of remodeling at the histologic and ultrastructural level. The extent of structural remodeling goes hand in hand with the clinical history of AF. As the disease evolves from paroxysmal to chronic, this remodeling spreads to larger portions of the atrial wall often making both classic antiarrhythmic drugs and ablation of the sole PV outlets ineffective. On this basis, the most urgent strategy is preventing the structural remodeling—with special regard to interstitial fibrosis, by early treatment of the arrhythmia as well as its underlying cardiovascular diseases (Corradi D. Atrial fibrillation from the pathologist's perspective. Cardiovascular Pathology 2014, 23: 71–84).

Doina Butcovan

ASSESSMENT OF THROMBOPHILIC STATUS IN TYPE 2 DIABETIC PATIENTS: THE LINK BETWEEN PAI-1 POLYMORPHISM AND THE ONSET OF COMPLICATIONS

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ASSESSMENT OFTHROMBOPHILIC STATUS IN TYPE 2 DIABETIC PATIENTS: THE LINK BETWEEN PAI-1 POLYMORPHISM AND THE ONSET OF COMPLICATIONS (Abstract): Diabetes is a very common disease characterized by a prothrombotic status involving both the chronic activation of the clotting system and a decrease in the endogenous fibrinolytic capacity. The existing data regarding the hemostatic alterations in diabetic patients are insufficient and contradictory due to the heterogeneity of the studied population, the presence of cardiovascular complications, glycemic status, other associated risk factors, and the type of treatment. The metabolic changes found in diabetic patients cause disturbances in platelet activity, hemostasis, endogenous fibrinolysis and rheology favoring a prothrombotic status. These systemic changes may lead to an extensive atherosclerotic process derived from the thrombophilic status, thus partially explaining the frequent coronary atherosclerosis in type 2 diabetic patients. Moreover, the hemostatic alterations seen in diabetic patients seem to be responsible for the microvascular localization of coronary atherosclerosis. Recent studies indicate that increased PAI-1 levels are correlated with an increase in the incidence of diabetes mellitus and its complications, such as diabetic neuropathy, diabetic retinopathy and ischemic cardiomyopathy. PAI-1 is synthesized both by endothelial cells and hepatocytes. Endothelial cell activation causes an increase in tissue thromboplastin secretion, a decrease in thrombomodulin expression, a diminished tissue plasminogen activator synthesis and an increase in PAI-1 production. Despite all this, the data regarding the association between PAI-1 levels and the incidence of diabetes mellitus remain unclear. **Keywords:** TYPE 2 DIABETES MELLITUS, THROMBOPHILIC STATUS, PAI-1 POLYMORPHISM.

Diabetes is a very common disease that is characterized by a prothrombotic status involving both the chronic activation of the coagulation system and a decrease in endogenous fibrinolytic capacity. The studies comparing the prevalence of coronary diseases in diabetic and nondiabetic patients showed a threefold higher incidence of atherosclerosis, with an earlier clinical onset and a twofold higher cardiovascular risk in diabetic patients.

Hyperlipoproteinemia, platelet activation and changes in fibrinogen levels seem to play an important role in the onset and progression of diabetic complications. The existing data regarding the hemostatic

changes in diabetic patients are insufficient and inconclusive due to the heterogeneity of the studied population, presence or absence of cardiovascular complications, glycemic status, other associated risk factors, and the type of administered treatment received (1).

It is well known that the vascular diseases in diabetic patients have a multifactorial etiology, being influenced by the changes in thrombosis and thrombolysis balance, consisting mostly in the activation of the thrombotic process, thus leading to ischemic complications.

The association of increased LDL-cholesterol levels, abnormal trigliceride levels, small and dense LDL-cholesterol particles and diminished HDL-cholesterol levels leads to the atherogen lipoproteic phenotype, which is correlated with an early onset of coronary disease and extensive atherosclerosis process in type 2 diabetic patients. Moreover, small and dense LDL particles seem to be a marker for a series of anomalies including the decrease of HDL-cholesterol levels, the increase in apoB concentrations, diminished insulin sensitivity and procoagulant anomalies (increased PAI-1 levels) (2). The association between the atherogenic lipoproteic phenotype and other proatherogenic anomalies is suggestive of a genetic predisposition. Therefore, in 1990 it was proven that the genetic locus responsible for small and dense LDL particles is also associated with increased trigliceryde levels, apoB, VLDL and IDL levels and decreased HDL-cholesterol and apoA1 levels. Even more, genetic linkage studies showed that LDL particle size and HDL and LDL cholesterol levels are correlated with an allele of hepatic lipase gene. The genetic mutations affecting the *Cholesteryl Ester Transfer Protein* (CETP) locus situated on the 16 th chromosome can influence, through its role in the reverse transport of cholesterol, the LDL size and trigliceryde levels (3).Furthermore, there is a constant relation between the atherogenic lipoproteins (oxidized LDL and LP(a)) and the vascular endothelium, thus leading to a diminished fibrinolytic process. The effect is an imbalance between coagulation and fibrinolysis that favors thrombosis which eventually causes the occurrence of thrombotic complications (4,5).Hypercoagulability in type 2 diabetes mellitus seems to result especially from the synthesis of factors that activate the coagulation system and factors that inhibit fibrinolysis (such as factor VII or PAI-1). The hemostatic anomalies in type 2 diabetic patients may also reside from the synthesis in the adipose tissue of inflamatory citokines that can also mediate insulin resistance, such as IL-6 and TNF-alpha (6).

ASSESSING THE PROTHROMBOTIC STATUS IN TYPE 2 DIABETES MELLITUS PATIENTS

Recent studies showed that both endogenous fibrinolytic parametres and platelet activity are changed in type 2 diabetic patients at increased cardiovascular risk. The metabolic alterations that cause a change in rheology, platelet activity, hemostasis and endogenous fibrinolysisfound in diabetic patients favor the occurrence of a thrombophilic status. These changes can cause an extensive atherosclerotic process derived from the thrombophilic status, thus partially explaining the frequent coronary atherosclerosis in type 2 diabetic patients. Even more, the hemostatic alterations seen in diabetic patients seem to be responsible for the microvascular localization of coronary atherosclerosis (7, 8).

The plasminogen activator inhibitor-1 (PAI-1) belongs to the serine protease inhibitor superfamily, and plays a key role in the regulation of extracellular matrix degradation. Studies have indicated that the increase in PAI-1 level was related to the incidence of diabetes mellitus (DM) and its complications, such as an increased risk of diabetic nephropathy (DN), diabetic retinopathy (DR) and diabetic coronary artery disease (CAD). PAI-1 is synthesized by the endothelial cells and hepatocytes. Endothelial cell activation determines an increase in tissue thromboplastinsecretion, a decrease in thrombomodulin expression and an increase in PAI-1 synthesis. Some studies indicated the presence of elevated PAI-1 serum levels in diabetic patients (9).Structurally modified lipoproteins such as VLDL or LDL have the capacity of releasing PAI-1, thus causing a decrease in the fibrinolytic plasmatic capacity, determined by a diminished conversion of plasminogen to plasmin. There are 5 identified polymorphisms of PAI-1 gene: -675 4G/5G, -844 A>G, Ala15Thr, Val17Ile and Asn195Ile; the presence of these polymorphisms may increase the cardiovascular risk in diabetic patients (10). The PAI-1 gene is situated on the 7 th chromosome and contains 8 introns and 9 exons. The 4G/5G polymorphism (the deletion/insertion of guanosine in the 675 position of the PAI-1 gene promoter) is a major genetic determinant of PAI-1 levels. Some authors suggested that the association of the 4G allele with other thrombophillic defects (such as factor V Leiden, prothrombin gene mutation, hyperhomocysteinemia, diminished protein C and protein S activity) increases the risk of thrombosis. In diabetic patients with coronary ischemic disease the presence of 4G/4G genotype was associated with an increased risk of sudden death.

LESSONS FROM CLINICAL TRIALS

Some of the oldest studies that evaluated the association between PAI-1 polymorphism and the risk of type 2 diabetes mellitus showed a positive correlation between the 4G allele of type 1 plasminogen activator and the risk of diabetes mellitus in Pima Indians, in this population the incidence of noninsulin-dependent diabetes being very high. On the contrary, some subsequent studies analyzing the Caucasian population did not manage to show an association between PAI-1 polymorphism and diabetes mellitus. A case-control study on 856 Tunisian patients with type 2 diabetes mellitus showed an increased incidence of 4G/4G genotype, thus suggesting that this polymorphism may be a prothrombotic risk factor associated with type 2 diabetes mellitus. Many studies analyzed the incidence of PAI-1 polymorphism in diabetic Asian patients, but did not succeed to prove a significant statistic correlation between 4G/5G allele and the risk of diabetes (6).

A meta-analysis published in 2013 analyzed the results of 9 studies including 1219 patients, from which 5 studies referred to Caucasian population, 3 studies included Asian patients and 1 study was conducted on Pima Indians. This meta-analysis demonstrated a slight significant statistic correlation between 4G/4G polymorphism and the risk of diabetes complicated with neuropathy. In the subgroup analysis by ethnicity, significantly increased risks were observed among the Caucasian population (for 4G/4G versus 5G/5G, for 4G/4G versus 5G/5G + 4G/5G). In the stratified analysis by average diabetes duration, PAI-1 variation was found associated with a high risk of diabetes retinopathy in patients with duration of diabe-

tes longer than 10 years (for 4G/4G versus 5G/5G).

Another meta-analysis conducted in 2013, consisting of 20 studies, tried to assess the relationship between PAI-1 polymorphism and the risk of developing diabetes mellitus and its complications. Therefore, 6 studies including 1333 patients analyzed the association between PAI-1-675 4G/5G polymorphism and the risk of diabetes, 7 studies including 1060 patients assessed the correlation between this polymorphism and diabetic nephropathy, 10 studies with 1327 patients evaluated the correlation with diabetic retinopathy and 4 studies with 610 patients measured the risk of coronary artery disease in type 2 diabetic patients. The results of this meta-analysis suggested that there is no conclusive association between PAI-1 4G allele and the risk of diabetes mellitus or of developing complications, no matter the type of the evaluated population or diabetes duration.

CONCLUSIONS

The existing data are rather contradictory, some studies suggesting that high PAI-1 levels are constantly present in patients with type 2 diabetes mellitus or with insulin resistance, other studies denying this hypothesis. PAI-1 675 4G/5G polymorphism determines the deletion/insertion of a single guanosine, thus causing the expression of an additional linkage DNA *situs* for a protein with a repressing role in the transcription process, which will have an increased role in PAI-1 plasma levels. Therefore, this polymorphism may become a genetic risk factor for diabetes mellitus. The elevated PAI-1 levels may be correlated with a high incidence of such diabetic complications as diabetic nephropathy, diabetic retinopathy and coronary artery disease, but the results of the existing trials remain unclear and inconclusive. In these conditions unrevealing new genes that contribute to the thrombophillic status present in diabetic patients remains an important fundamental research theme.

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THE PROGNOSTIC SIGNIFICANCE OF P53, BCL2 AND MIB1 EXPRESSIONS RELATED WITH OTHER CLINICOPATHOLOGICAL VARIABLES IN SEROUS OVARIAN CARCINOMAS. A CLINICOPATHOLOGICAL STUDY IN PERITONEAL FLUIDS

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THE PROGNOSTIC SIGNIFICANCE OF P53, BCL2 AND MIB1 EXPRESSIONS RELATED WITH OTHER CLINICOPATHOLOGICAL VARIABLES IN SEROUS OVARIAN CARCINOMAS. A CLINICOPATHOLOGICAL STUDY IN PERITONEAL FLUIDS (Abstract): **Objective:** The first cytological study examining the expression of P53, BCL2 and MIB 1 expressions in correlation with other clinicopathological parameters in ascitic fluids of patients with serous ovarian carcinomas. **Materials and Methods**: Fifty women 35-75 years old were diagnosed cytologically and confirmed histologically after operation in the University Hospital of Crete. All carcinomas were serous type and eight(8) of grade I, eighteen(18) of grade II and twenty two (22) of grade III. All carcinomas were staged according to the Figo criteria. Fifteen (15) were of Figo stage III and thirty five (35) were of Figo stage IV. For p53 and bcl-2, staining was evaluated on a semiquantitative scale depending on the number of cells showing positivity. For MIB1, the percentage of positive nuclei was calculated. Main outcome measure(s): The expression of P53, BCL2 and MIB 1 (Ki 67) correlated with tumor grade and Figo stages were estimated by chi-square (x^2). **Results:** The expression of P53 and MIB1 were found to be statistically significant ($p<0,005$) correlated with Figo stage and tumor grade. A statistical significant correlation was also found between BCL2 expression and tumor Grade ($p<0,005$) but not between BCL2 expression and Figo Stage. The study found a high expression of P53 (64%) and MIB1 (72%) and an expression of BCL2 (48%) in ascitic fluid of patients with ovarian carcinoma. A statistically significant correlation between P53 and MIB1 expression correlated with tumor grade and Figo stage ($p<0,005$) and a statistically significant correlation between BCL2 expression and tumor grade but no with the Figo stage was found ($p<0.005$).There was a positive correlation between P53 and MIB1 .No significant association was found between P53 and BCL2 expression or MIB1 labeling index. **Conclusion(s)**: Our data show significant differences in the expression of these markers in ovarian tumors and suggest a possible role for these tumor-associated genes as supplemental tools in prognosis and further definition of the biologic potential of these tumors. **Keywords**: OVARIAN CANCER, P53, BCL2, MIB1, APOPTOSIS.

Cancer of the ovary is one of the most common causes of cancer deaths in women and continues to present a challenge despite advances in our knowledge of the disease

during the past 20 years. The etiologic factors involved in the pathogenesis of ovarian cancer are poorly understood (1).

Recent studies have emphasized the importance of programmed cell death or apoptosis in the maintenance of tissue homeostasis and pathogenesis of tumors (2).Tissue homeostasis is maintained by the balance between cell proliferation and cell loss. There is evidence that much of the cell loss observed is due to programmed cell death or apoptosis (3). Apoptosis is a distinct form of cell death that can result from the activation of a genetically regulated cell suicide program or from cell injury induced by various stimuli. Types of cell damage that initiate apoptosis include: 1) exposure of cells to ionizing radiation 2) hyperthermia 3) toxic exposures 4) viral infections 5) cytokines 6) chemotherapeutic agents.

Numerous genetic factors have been identified as important contributors to the regulation of both cell proliferation and apoptosis. Aberrant regulation of both these processes, which stems from the malfunctioning of such genes and their products, plays an important role in tumor growth and development. The P53 protein produced in response to DNA damage restrains cellular proliferation by binding to specific regions of DNA and regulates the expression of other genes responsible for the cell cycle arrest, DNA repair, and initiation of apoptosis. The P53 gene also regulates expression of the anti-apoptotic BCL2 gene (4). Mutations of the P53 gene constitute the most common molecular genetic change associated with many cancers, including ovarian cancers (5, 6). The BCL2 gene is located on chromosome 18, and encodes the BCL2 protein (7). BCL2 is unique among the proto-oncogenes, as it is located on the mitochondrial membranes and interferes with programmed cell death independently of its ability to promote cell division(8). BCL2 belongs to a still growing family, the members of which are able to form homo- or heterodimers with one another. The most important regulators of apoptosis are found among these dimmers (9, 10)

Proliferating cell nuclear antigen (PCNA) or Ki-67(MIB 1) is an axillary protein of DNA polymerase,an enzyme involved in the catalysis of DNA synthesis and repair. It has been shown that the total proliferative compartment of the tumor can be determined by the expression of this protein (11).

The present study examined the extent of apoptosis, apoptosis regulatory proteins P53 and BCL2 as well as the extent of tumor cell proliferation reflected by MIB1 expression in serous ovarian tumors.

MATERIAL AND METHODS

We studied, retrospectively, 50 women 35-75 years old (mean age 55 years) with serous ovarian carcinoma. All patients were referred and treated surgically at the University hospital of Crete in the Department of Gynecology and Obstetrics, diagnosed cytologically and confirmed histologically in the Department of Pathology-Cytology.

The cytological; histological determination was done by cytologist and pathologist and histological type and grade assigned according to the World Health Organisation Classification (12).All carcinomas were serous type and eight(8) of grade I, eighteen(18) of grade II and twenty two (22) of grade III, as determined by histopathology. All carcinomas were staged according to the Figo criteria. Fifteen (15) were of Figo stage III and thirty five (35) were of Figo stage IV.

For the immunocytochemical staining the following primary monoclonal antibodies were used. Monoclonal mouse anti – human P53 protein (DAKO) that labels wild type and mutant type P53 protein, monoclonal mouse anti-human BCL2 oncoprotein, clone 124 (DAKO) and Ki-67(MIB1), rabbit monoclonal expressed in G1-S-, M-, and G-2 phases of the cell-cycle(Thermo Scientific), at a dilution of 1/50, 1/50 and 1/200, respectively. A standard alkaline -phosphatase method (APAAP) was used with 1-h incubation in a microwave oven at 750 watts, 3 times. A very light hematoxylin counterstaining was performed before cell counting. Tissues from the respective primary serous tumors were used as positive controls. Peritoneal fluids with endometriosis from Yale series (University-based endometriosis referral center) were used as negative controls too. In each specimen (at least 500 cells) in ten high power fields (x 400) were examined (light microscopy).For the statistical analysis chi-square (x^2) test was used.

RESULTS

P53 accumulation was documented in 32 (62%) of the 50 cases showed a nuclear accumulation in more than 10% of neoplastic cells. Cytoplasm immunoreactivity was never encountered. P53 accumulation was positively correlated with grade (grade I 2/8, grade II 10/18, and grade III 20/24) ($p<0,005$) and Figo stage (stage III 6/15 and stage IV 26/35) ($p<0,005$) (tab. I).

BCL2 immunoreactivity

BCL2 immunoreactivity was observed in 24 (48%) of the 50 cases: it was restricted to the cell cytoplasm in more than 10 % of neoplastic cells.An inverse correlation, with grade was found (grade I 8/8 , grade II 9/18 and grade III 7/24) $p<0,005$ but no any correlation with Figo stage (stage III 7/15 and stage IV 17/35) (tab. II).

TABLE I

P53 expression and relationship with other clinical and pathological variables in ovarian carcinomas

Tumor Grade		
I	2/8(25%)	p<0.005
II	10/18(55,6%)	
III	20/24(83,4%)	
FIGO Stage		
III	6/15(40%)	p<0,005
IV	26/35(74,3%)	

TABLE II

BCL2 expression and relationship with other clinical and pathological variables in ovarian carcinomas

Tumor Grade		
I	8/8(100%)	p<0,005
II	9/18(50%)	
III	7/24(29%)	
FIGO Stage		
III	7/15(46,6%)	
IV	17/35(48,5%)	

Proliferative fraction

MIB 1 labeling index (L.I.) in 36 (72%) of the 50 cases. The expression was nuclear in more than 30 % of the neoplastic cells. The MIB 1 L.I. was positively correlated with grade (grade I 2/8, grade II 12/18 and grade III 22/24) and Figo stage (stage III 5/15 and stage IV 21/35) ($p<0,005$) (tab. III).

Correlations among P53, BCL2 and MIB 1 immunostaining

There was a positive correlation between P53 and MIB1 expressions whereas no significant correlation was found between P53 and BCL 2 or BCL 2 and MIB1 (tab. IV).

TABLE 3

MIB 1 expression and relationship with other clinical and pathological variables in ovarian carcinomas

Tumor Grade		
I	2/8 (25%)	p<0.005
II	12/18 (66.6%)	
III	22/24 (90%)	
FIGO Stage		
III	5/15 (33.3%)	p<0.005
IV	21/35 (60%)	

TABLE 4

Relationship between P53, BCL2 , and MIB 1 expressions

Variable	Reference value	RR	p-value
p53 accumulation	<10% stained cells	2.769	0.0005
BCL2	<10%	1	0,676
MIB 1	<30%	2,499	0,004

DISCUSSION

The prognosis of advanced-stage ovarian cancer patients has remained largely unchanged over the past 20 years (13).The available clinical and pathological prognostic factors have thus far proven insufficient to clearly define prognostic subgroups of patients.

Several studies have been devoted to identifying novel prognostic indicators for ovarian carcinomas , but the actual prognostic and /or predictive implications of a variety of biological parameters (P53 gene aberration and protein accumulation, BCL2, tumor growth fraction and ploidy) in these malignancies are still highly controversial (14). It s exceedingly difficult to ascertain from the currently available data whether and/or the extent to which evaluation of the above parameters contributes to a better definition of the biology and clinical behavior of ovarian carcinomas, in addition to the information already provided by the conventional clinical (age, performance status) and pathological (histological type, grade and stage of the neoplasms, presence of ascites) prognostic indicators. The aim of this study was to access the prognostic and predictive implications of P53 accumulation, BCL2 expression as regulator of apoptosis and tumor proliferative fraction evaluated by the MIB 1 monoclonal antibody against the Ki-67 antigen in a series of 50 ovarian

carcinoma patients correlated with the grade and stage (15).

Our results in accord with previous reports (14,16,17,18,19,20,21) nuclear P53 accumulation was ascertained in more than 50 % of ovarian carcinomas and was found to be statistical correlated with the tumor grade and Figo stage. The possible prognostic value of P53 aberrations in ovarian carcinomas has been addressed in several studies, many of which documented an adverse prognostic effect of P53 protein accumulation and that nuclear accumulation of P53 protein in more than 10 % of neoplastic cells was significantly correlated with worse disease-free and overall survival (16).

In our study BCL 2 expression was found in 48% of our cases and in more than 10% of neoplastic cells was significantly correlated with the grade of the tumors but not with the Figo stage. In other studies BCL 2 immunoreactivity was not correlated with grade or stage of ovarian tumors (14, 1) but was related to histopathologic subtype, and positive staining was seen most frequently in serous (77%) and endometrioid (56%) carcinomas, but was less common in mucinous carcinomas (20%) (1).

This study clearly showed that MIB 1 was positively correlated with grade and stage of the tumors expressed in 72% of our cases and in more than 30% of the neoplastic cells. A positive correlation between MIB 1 labeling index and P53 accumulation was ascertained, whereas no significant association was found between BCL2 immunoreactivity and P53 accumulation or MIB 1 labeling index in accord with another previous study. (14)

Both P53 accumulation and MIB 1 immunoreactivity correlated significantly with a worse prognosis and a reduced overall survival but BCL2 expression has no independent effect on the survival of patients.

The molecular characterization of ovarian cancer in different subtypes is the cornerstone to build a personalized approach in the era of personalized medicine (22, 23, 24, 25, 26, 27, 28, 29, 30, 31).

CONCLUSIONS

Our results indicate that the simultaneous evaluation of p53 accumulation and MIB 1 labeling index (L. I.) has independent prognostic indications in serous ovarian carcinomas. Our data show significant differences in the expression of P53, BCL 2, and MIB1 in ovarian tumors and suggest a possible role for these tumor-associated genes as supplemental tools in prognosis and further definition of the biologic potential of these tumors.

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MECKEL'S DIVERTICULUM - A RARE CAUSE OF INTESTINAL OBSTRUCTION IN ADULTS

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MECKEL'S DIVERTICULUM - A RARE CAUSE OF INTESTINAL OBSTRUCTION IN ADULTS (Abstract): Although many people have Meckel's diverticulum, only some experience any symptoms, most under the age of 10. In adults it is usually asymptomatic but approximately 4% develop complications. Meckel's diverticulum is usually diagnosed in the first years of life and after that the risk of the complications decreases with increasing age, with no predictive factors for the development of complications. We describe the case of a 34-year-old man admitted in the emergency department with diffuse abdominal pain, nausea, flatulence and lack of transit for feces and gas. The patient had been previously operated for peritonitis due to a perforated ulcer. Clinical examination and paraclinical investigations (abdominal radiography and ultrasound) suggested the diagnosis of intestinal obstruction, probably produced by adhesions due to previous abdominal intervention. The diverticulum was resected using a linear stapler and the patient recovered without any complications. Small bowel obstruction due to Meckel's diverticulitis may be caused by entangled loop of small bowel around a fibrous cord, intussusception, volvulus, or incarceration within a hernia sac. The discovery of a Meckel's diverticulum complication in a mid thirties patient represented an intra-operatory surprise and is the peculiarity of the case. **Keywords:** MECKEL'S DIVERTICULUM, INTESTINAL OBSTRUCTION, COMPLICATION.

Meckel's diverticulum (MD) is an outpouching of the distal ileum, due to persistence of the congenital vitello-intestinal duct, located at the anti-mesenteric border, usually within 45-60 cm of the ileocecal valve (1).

Considered the most common congenital malformation of the gastrointestinal tract (present in 2-4% of population), it was first described in a paper published in 1809 by the German anatomist, Johann Friedrich Meckel (1781-1833), who characterized it as a remnant of the omphalo-mesenteric duct. In the fetal life, it connects the yolk sac to the intestinal tract and usually it obliterates in the 5th to 7th week of life. If this process fails, the congenital anomalies develop, leading to the residual fibrous cords, umbilical sinus, omphalo-mesenteric fistula, entero-cyst and most commonly, Meckel's diverticulum (2).

Meckel's diverticulum is lined mainly by the typical ileal mucosa as in the adjacent small bowel. However, other ectopic

tissue (most common gastric, duodenal, colonic, pancreatic, endometrial mucosa) may be found, usually near the tip (3, 4).

Although many people have Meckel's diverticulum, only some experience any symptoms, most under the age of 10. In adults it is usually asymptomatic but approximately 4% develop complications such as obstruction, due to the intussusception or adhesive band (14-53%); ulceration (less than 4%); diverticulitis; and perforation (2, 5, 6).

Tumoral transformation (carcinoid tumor, stromal tumor, carcinoma, adenocarcinoma), intra-ductal papillary mucinous adenoma of pancreatic tissue and vesico-diverticular fistulae are rare complications.

CASE PRESENTATION

We describe the case of a 34-year-old man admitted in the emergency department with diffuse abdominal pain, nausea, flatulence and lack of transit for feces and gas. The patient had been previously operated for peritonitis due to a perforated ulcer. Clinical examination and paraclinical investigations (abdominal radiography and ultrasound) suggested the diagnosis of intestinal obstruction, probably produced by adhesions due to previous abdominal interventions.

Exploratory laparotomy was decided and performed under general anesthesia and moderate dilation of the small bowel was discovered. During arduous adhesiolysis, an intestinal volvulus caused by the Meckel diverticulum adhesions was unveiled. It had an unusual length of over 20 cm and was torsioned in pelvis through massive viscero-visceral and viscero-parietal adhesions (fig.1).

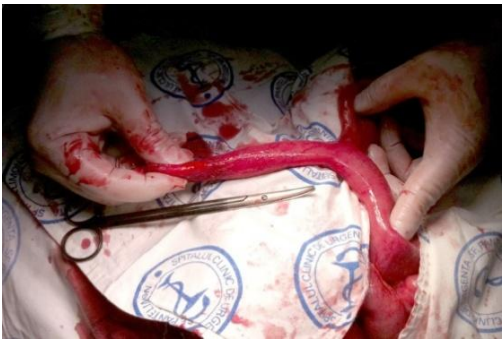

Fig. 1. Meckel's diverticulum after dissection and isolation

After cutting the adhesions and devolvulation, the diverticulum was resected using a linear stapler, fired at its base of implantation (fig. 2, fig. 3).

Fig. 2. Diverticulum resection using a linear stapler

Fig. 3. Final aspect of mechanical suture line

A peritoneal lavage was performed with dextran solution and mesenteric novocaine injection. The viability check confirmed that the intestinal loop regained its color and peristalsis. The patient recovered without any complications.

DISCUSSION

Meckel's diverticulum is usually diagnosed in the first years of life and after that the risk of the complications decreases with increasing age, with no predictive factors for the development of complications. Although the incidence of MD is equal in both sexes, complications are more frequent in men than in women, with a male-female ratio of about 3:1 (7, 8, 10).

Mackey (9) published a large retrospective study with a total of 402 patients with a diagnosed MD over a period of fifty years. Of all complications, 70.1% occurred in patients younger than 40 years of age. According to Ludtke (8), most diverticular complications were seen in children younger than 2 years of age. Leijonmarck (11) found a lifetime risk of complications from MD of 3.7% at 16 years, 2% at 30 years and almost zero when older. The more recently published Mayo clinic experience with 1,476 patients of Park (10) found significantly more symptomatic MD in patients younger than 50 years of age.

Adults develop obstruction or, less frequently, symptoms of inflammation. Hemorrhage is less common and is usually the result of heterotopic gastric or pancreatic mucosa causing ulceration. Small bowel obstruction due to Meckel's diverticulitis may be caused by entangled loop of small bowel around a fibrous cord, intussusception, volvulus, or incarceration within a hernia sac. Obstruction is usually demonstrable on plain films of the abdomen and the cause of the small bowel obstruction might be shown by CT. (5)

Due to the rarity of cases in adults, Meckel's diverticulum is still misdiagnosed preoperatively. The surgeon should always consider this if a patient has abdominal pain and/or vomiting and physical examination and ultrasonography findings are not suggestive of appendicitis or air-fluid levels are found on abdominal X-ray. If no surgical pathology is found during laparotomy, it is advisable to carefully inspect the small intestine, particularly the last segment, where Meckel's diverticulum is most commonly discovered.

CONCLUSIONS

The proper management of asymptomatic Meckel's diverticulum is still controversial. It is difficult to predict which fortuitous diagnosed patients can become symptomatic. Morbidity rates after resection of incidentally found MD are much lower than those after resection of symptomatic MD. The risk for future complications of a non-resected MD must be weighed against the risk of complications after a resection in order to justify a prophylactic approach.

The discovery of a Meckel's diverticulum complication in a mid thirties patient represented an intra-operatory surprise and is the peculiarity of the case.

ACKNOWLEDGEMENTS

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NEWS

NANOFIBERS: AN IMPORTANT ALTERNATIVE IN THE TREATMENT OF WOUNDS CAUSED BY BURNS

Nanofibers and incorporation of antimicrobial compounds in these scaffolds are an important alternative way to control skin infections. The most common technique to prepare nanofibers is electrospinning. Nanofibers are produced from different polymers, treated in a specific manner to form threads with diameters from a few micrometers to nanometers. Also these structures can be used as drug delivery systems. The possibility to incorporate antimicrobial compounds to nanofibers, open a new perspective in biomedical engineering. Release of antimicrobial compounds, or other compounds that can accelerate the healing process, can be controlled by selecting polymers of specific composition. A localized delivery system is required to control the level and the rate of antimicrobial compounds delivered to the wounds. An example of drug delivery system is the incorporation of tanic acid on the surface of chitosan nanofibers, generated by electrospinning process. The antibacterial activity of chitosan is based on the damaging interaction of this polycation with the negatively charged surfaces of bacteria. In the same time, tanic acid inhibits bacterial growth for *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. With the involvement of scientific community, electrospinning became one of the most powerful tools for fabricating nanomaterials with a wide range of applications. (Xua F, Wenga B, Gilkersonb R et al. Development of tannic acid/chitosan/pullulan composite nanofibers from aqueous solution for potential applications as wound dressing. *Carbohydr. Polym*, 2015; 115: 16-24).

Oana Dragostin

THE MEDICO-PSYCHO-SOCIAL IMPLICATIONS OF PREGNANCY DURING ADOLESCENCE

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THE MEDICO-PSYCHO-SOCIAL IMPLICATIONS OF PREGNANCY DURING ADOLESCENCE (Abstract): The issue of maternity in adolescence is an important one due to the increasing number of adolescent mothers in our country, as well as in other countries, because of the risks involved by a pregnancy at this age, especially from a medical perspective, but also from a social one. **Material and methods**: The current study was conducted between 2012 and 2017 on a group of 103 adolescent mothers aged between 12 and 19 years of age, with a total of 109 children admitted to the Pediatric Recovery Department of Iasi "Sf. Maria" Emergency Hospital for Children. **Results**: The distribution of adolescent mothers by age was: 12%-12 years old, 10%-13 years old, 11% -14 years old, 12%-15 years old, 13%-16 years old, 14%-17 years old, 15%-18 years old, 15%-19 years old. Consideration was given to the existence of acute illnesses during the pregnancies for the mothers included in the study, the background, the level of education, the jobs and the marital status of the young parents, the birth weight of children, the Apgar score, the complications of the newborns, the Rh incompatibility between mother and child, etc. **Conclusions**: These data raise the need for an over-specialization – Infantile Obstetrics – that should deal with the issue of pregnancy in adolescents. In order to have a lower level of unwanted consequences on mother and fetus the close collaboration between the pediatric and the obstetrics sectors is very important for the pregnancy at this age group. **Keywords**: ADOLESCENT MOTHERS, PREGNANCY IN UNDERAGE WOMEN, ADOLESCENCE AND PREGNANCY.

Adolescent pregnancy is one of the most important public health issues today. It is a global phenomenon with both social and medical implications. It is defined as the occurrence of pregnancy in females under the age of 19 years. Annually, 16 million adolescents give birth to children and about 2 million of them are under the age of 15 years. In Romania, approximately 13 million newborns from mothers under the age of 19 were recorded in 2013. According to the National Institute of Statistics, around 90,000 girls aged 12-18 became mothers in 2009-2012 (1). UNICEF

data show that Romania is one of the top countries in Europe in terms of number of teenage births (2).

Pregnancy during adolescence involves several medical complications along the pregnancy as well as in the postnatal period, due to the immaturity of the organism at this age and the lack of medical education caused by the low level of schooling or poverty, which prevents the access to specialized medical consultations – most of the expectant mothers go to a doctor when they are in labor or they simply do not know they are pregnant until the second trimester (3, 4). What is even worse is that adolescents sometimes become multiparous mothers, which means even more medical complications. In addition, Romania is among the countries with the highest number of teenage pregnancies. The phenomenon is not so widely known, and the importance given to this issue is not as big as it should be (1, 3).

MATERIAL AND METHODS

The current study was conducted between 2012 and 2017 on a group of 103 adolescent mothers aged between 12 and 19 years of age, with a total of 109 children admitted to the Pediatric Recovery Department of Iasi "Sf. Maria" Emergency Hospital for Children. The data was processed using *Microsoft Excel* 2010.

Several variables were considered: the sex of the newborn, the background, the mother's age, the marital status, the level of education, the occupation, whether the pregnancy was medically monitored, the caesarean indication, the number of pregnancies, the number of deliveries, acute illnesses, chronic illnesses discovered during the pregnancy, the presence of risk factors, the place of birth, the mode of delivery, the presentation, the APGAR score at 1 minute and need to repeat it, the gestational age of the newborn, its weight and length at birth, the compatibility between mother and fetus, the presence of jaundice and its therapy, the type of diet, the treatments administered in the maternity hospital, the anthropometric data on admission to the Pediatric Recovery Department, the type of diet, the mode of administration, the weight gain obtained in the Pediatric Recovery Department, the number of days of hospitalization, pathogen carriage on admission to the Pediatric Recovery Department, treatment, the antibiogram conducted.

RESULTS

Most of these young mothers were secondary school students (58%), 21% were high-school or vocational school students, 21% graduated only 4-6 grades or are illiterate. Most pregnancies were not monitored from the health perspective (79%), some of the pregnant teenagers did not see a doctor before the moment of the delivery, while the rest of them, 21%, attended prenatal counseling, but rarely and very rarely. We also considered mothers' capacity to provide financial support to their newborns. Thus, 85% of mothers became homestay mothers after their children were born, dropping out of school; 15% continued their studies, but none of them had a job. Sometimes, during pregnancy or labor, there may be conditions that require the obstetrician to perform the caesarean section-thus, 76% had no indication for a caesarean section, while the rest of 24% required a caesarean section. 61% of mothers had another pregnancy in the past, 28% had 2 previous pregnancies, 9% had 3 previous pregnancies and 2% had 4 previous pregnancies (fig. 1).

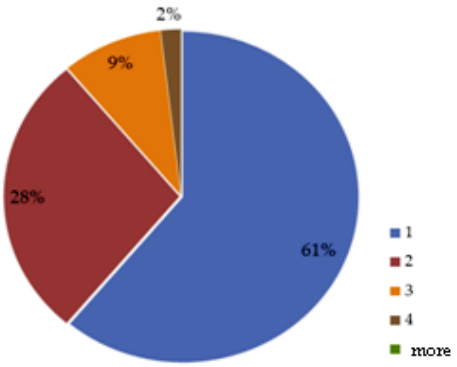

Fig. 1. Number of pregnancies among adolescent mothers included in the study

The number of deliveries for these mothers was also monitored. 71% of them had a previous delivery, 23%-had 2 previous deliveries and 6% had 3 previous deliveries. Consideration was given to the existence of acute illnesses during the pregnancies of the mothers included in the study: 91 of them had no acute illness, 3 of them had urinary tract infection, one of them had ureterohydronephrosis, one of them had pregnancy-induced hypertension, 3 of them had vulvovaginitis and 3 of them had anemia. Since a pre-existing pathology can influence the current pregnancy, the chronic diseases of these mothers were noted, as follows: 97 of them had no history of pathologies, 3 had mental retardation, one had neoplasm, one had lues and one had chronic anemia. 88% did not show risk factors, 2% consumed ethnobotanical substances, 5% smoked, 2% took medicines without medical indication and 3% drank alcohol. Regarding the place of delivery, 66% of deliveries took place at the "Cuza-Voda" Obstetrics and Gynecology Clinical Hospital in Iasi, 10% took place at the "Elena-Doamna" Obstetrics and Gynecology Clinical Hospital, 19% took place in other maternity hospitals in the Moldavian area and 5% were medically assisted in the ambulance. In terms of the type of delivery, 76% of the adolescent mothers delivered their babies naturally, the remaining 24% delivered their babies by caesarean section. There were various fetal presentations – 82% of the babies had a cephalic presentation, 13% had a pelvic presentation and 5% had a transverse presentation. APGAR scores were monitored one minute after birth: 7%-APGAR of 9-10, 54%-APGAR of 7-8, 16%-APGAR of 5-6, 12%-APGAR of less than 4; in 7% of the newborns, doctors were unable to identify the APGAR score. APGAR scores five minutes after birth: 13%-APGAR of 9-10, 61%-APGAR of 7-8, 14%-APGAR of 5-6, 3%-less than 4; in 9% of the newborns, doctors were unable to identify the APGAR score. The newborns of adolescent mothers were classified according to the gestational age and weight criteria: LBW-61%, VLBW-20%, ELBW-7%, ILBW-12%. For the length at birth, the statistical results are: 21%-between 32 and 40 cm, 50%-between 40 and 45 cm, 28%-between 45 and 50 cm and only 1% over 50 cm. The statistical data also included the presence / absence of maternal and fetal compatibility, with the following findings: compatibility in 41% of cases, incompatibility in 17% of cases and unknown in 42% of cases. In 69% of newborns, jaundice was present, while in 31% it was absent. 71% of the cases needed phototherapy, while in 29% of the cases it was not necessary. The newborn's diet in the maternity hospital was also considered to be of interest and was included in the statistical data: natural feeding in 1% of cases, mixed feeding in 18% of cases and artificial feeding in 81% of cases. At the time of admission to the Pediatric Recovery Department, the following investigations were per-

formed: 2%-nasal exudates, 1%-pharyngeal exudates, 1%-eye discharge, 14%-gastric aspiration; in 82% of the children, no investigations for pathogen carriage were performed. The germs identified in the investigations were: *Klebsiella spp.*-9%, E. coli-9%, *Pseudomonas aeruginosa*-6%, *Enterobacter spp.*-3%, *S. aureus*-5% and unidentified in 68 % of cases. In 30% of cases, antibiotic therapy and symptomatic treatment were performed. Weight on admission in the Pediatric Recovery Department: 27%-between 1,700 and 2,000 grams, 67%-between 2,000-2,500 grams and 6%-between 2,500-2,700 grams. In the Pediatric Recovery Department, the feeding mode was: 50%-gavage and bottle feeding and 31% of the cases –bottle feeding exclusively. The type of infant nutrition in the Pediatric Recovery Department was predominantly artificial (fig. 2).

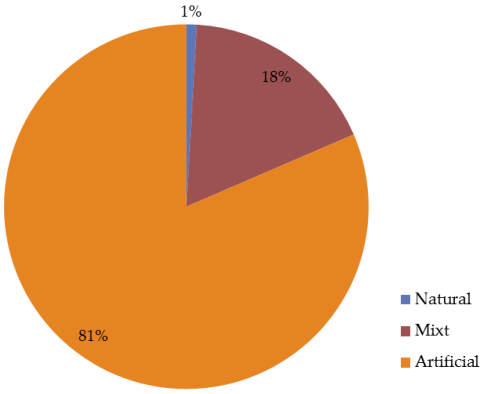

Fig. 2 The type of feeding received by the new born in the Maternity Hospital

The weight on discharge from the Pediatric Recovery Department was also monitored: 20%-between 2,000 and 3,000 grams, 58%-between 3,000 and 4,000 grams, 21%-between 4,000 and 5,000 grams and 1%-over 5,000 grams. The weight gain was also statistically determined in the Pediatric Recovery Department: 29%-between 100 and 1,000 grams, 53%-between 1,000 and 2,000 grams, 17%-between 2,000 and 3,000 grams and 1%-over 3,000 grams. The number of days of hospitalization within the department: 25%-between 9-30 days, 49%-30-60 days, 23%-60-90 days and 3%-more than 90 days. The sex distribution of neonates from teenage mothers includes 46% males and 54% females. In terms of place of origin, most of the teenage mothers come from rural areas: 75%. The distribution of teenage mothers by age groups: 35%-between 12 and 14 years, 39%-between 15 and 17 years, 26%-between 18 and 19 years.

DISCUSSION

A study conducted in 2011 in the US showed that pregnancy among adolescents and especially among those who are unmarried has medical and socio-economic effects on mother and child (1, 5). In another study conducted in India, all adolescent mothers were married. A child was generally conceived about 4-8 months after marriage (6, 7). A study conducted in Turkey showed that young mothers also give birth to children following sexual abuse, and built-up stress increases the risk of premature birth. The biological age of mothers shows the predominance of the age group between 17 and 19 years of age (8, 9). A study conducted in Timisoara showed that the predominant age at which adolescents become mothers is 16-17 years old (1). According to a study conducted in New York, teenage girls who get pregnant do not complete their middle school or high school studies, while those who continue their high school studies also show the desire to do post-secondary studies (1, 2).

Another study conducted in Romania shows that most adolescent mothers have completed their middle school education, but they do not continue with high school education (8). According to a study conducted in Finland and published in 2013, most adolescent mothers included in their database frequently participated in antenatal checks and tests (10). Another study conducted in Timisoara showed that the newborns generally came from pregnancies that were not medically monitored and that most of them were premature (6). A study conducted in Korea showed that pregnant teenagers saw an obstetrician very seldom compared to pregnant women who were not underage. 15% of the teenage mothers never sought any prenatal care service throughout their pregnancy. Regarding the teenage mother's occupation, most of them (85%) were homestay mothers, while the remaining 15% were students. Teenage mothers whose husbands did not work were more prone to be underweight compared to those whose husbands worked. Adolescent pregnancy is associated with socio-economic decline-most pregnancies occur in developing countries and in poor families (4, 7). A 8-year study conducted in the North Eastern part of Moldavia, published in 2014, showed that the number of cesareans was higher among adolescent mothers (natural birth: caesarean delivery ratio was 1:3). The cesarean distribution by age groups among adolescents shows that these girls are not physically prepared to become mothers (8, 10).

CONCLUSIONS

From our research we concluded that a pregnancy during adolescence is based on unstable ground, both from a psychosocial point of view (poor social and economic conditions, most of the young women belong to ethnic groups with early marriage, elderly partners, abuses, alcohol and drug consumption, the influence of the entourage and the media) and from a medical point of view – an immature body that supports a pregnancy (adolescence represents the period of sexual development paralleled by incomplete maturation, with the development of the gonads and the appearance of secondary sexual characters). The impact of pregnancy during this period is reflected both by the social impact on the mother (resulting in school dropout, repercussions on the mental state, depression, anger, stigmatization by others) and by several medical consequences both for the mother and for the fetus: improper conditions for development in intrauterine life – restricted intrauterine growth, abortion in intrauterine life – birth defects, low birth weight, prematurity, anemia, increased risk for malformations, predisposition to infection, impaired psychosomatic development, growth / speech difficulties, behavioral disorders and emotional dysfunctions. To have a lower level of unwanted consequences on mother and fetus, a close collaboration between the pediatric and the obstetrics sectors is very important for the pregnancy in adolescents.

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NEWS

SURGICAL MANAGEMENT OF PERI-IMPLANT FEMORAL FRACTURES

Peri-implant fractures represent an important problem for the health system due to their increased incidence, the high average age of the affected population and the exorbitant costs that the treatment implies. As the number of implants used in orthopedics increases, it is inevitable that associated fractures become even more common. A significant increase is the number of trochanteric fracture nailing procedures accompanied by peri-implant femur fracture complications. A group from Czech Republic study and analyzed 849 patients with per trochanteric fracture, which had surgically managed with short nail and then analyzed those patients who suffered postoperative peri-implant fractures. Peri-implant fractures were divided into 3 groups according to the established classification, which served as a therapeutic guide. Following her study, they identified 21 postoperative femoral fractures (2.5%). They found 4 fractures(2 with locked nails and 2 with unlocked nails) in the distal portion of the femur, and 17 peri-implant fracture that occurred in the area of the nail and just below the nail tip. Of the 17 postoperative femoral fractures only one patient had distal dynamic locking. Based on this the risk of peri-implant fracture after unlocked distal nailing is higher than after locked nailing. In conclusion peri-implant fractures are a distinct topic in fractures. Trochanteric fractures with internal fixation and distally unlocked short nails does not prevent peri-implant fractures of the femur.We would draw attention to the potential risks associated with the omission of distal locking (Jiří Skála-Rosenbaum, Valér Džupa, Radek Bartoška, Pavel Douša, Petr Waldauf, Martin Krbec. Distal locking in short hip nails: Cause or prevention of peri-implant fractures. *Injury* 2016; 47(4): 887-892).

Manuela-Gabi Boboc (Poroh)

IMPROVED EXPERIMENTAL BURN MODEL ON RABBITS

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IMPROVED EXPERIMENTAL BURN MODEL ON RABBITS (Abstract): **Objective**: The goal of our study was to find an easy and suitable skin burn model for rabbits, to identify the temperature and contact time to reach a deep 2nd degree burn, and to find the best wound dressing fixation method. **Material and Methods**: We used New Zealand male rabbits as experimental models. On a pilot study, with a copper device heated to temperatures between 43-48 Celsius degrees, the burn was inflicted using contact times of 3, 4, 5, and 7 seconds. The burn depth was evaluated histological. Twenty-four rabbits were used to evaluate four dressing fixation methods: regular dressing with retention bandages; skin stapler; collars and body resin cast. **Results**: A 2nd degree deep burn was obtained at 43 degrees for 4 seconds. The retention elastic bandages were removed within hours and the skin staplers were removed with teeth leaving wounds on the skin. The collars were more successful but produced superficial neck lesions. The body resin casts were the most successful. **Conclusions:** The best contention method was the body resin cast. **Keywords**: SKIN BURN MODEL, RABBIT, DRESSING CONTENTION METHOD, BODY CAST

We aimed at finding the ideal experimental burn model to identify an animal with skin properties similar to humans and possibility of good wound dressing contention. On *Pubmed*, most experimental models are rats (1-5), Guinea pigs (6), rabbits (7-12) and pigs (13). In the last 15 years of searching on *Pubmed*, we have found few articles dealing with rabbit skin burn models. Finding a suitable burn model in animals is not an easy task. A review of the literature revealed several studies that attempted to describe a model of skin burns in rodents, most commonly using Wistar rats. Most did not explain how the dressings were kept in place without hurting the animal. Our goal was to find the proper temperature to produce a deep 2nd degree burn and to identify the best dressing contention method for rabbits.

MATERIAL AND METHODS

The study was performed on New Zealand male rabbits. The experiment was approved by the Ethics Committee of our institution and by the University of Agri-

cultural Sciences and Veterinary Medicine Ethics Committee and is part of a research national grant project.

Study design

A) We investigated the ideal temperature to reach a 2nd degree deep skin burn

Five male rabbits with an average weight of 2.5 kg were used to identify the ideal temperature to reach a deep 2nd degree skin burn. For the entire period, the animals were fed with rabbit food and water *ad libitum*. All procedures were performed under general anesthesia with intramuscular Ketamine 50 mg/kg and Xylasine 5 mg/kg.

Each rabbit had hair removed on each flank. A very fine and short hair of 0.20mm remained on the skin. For removing this hair, we used a commercial depilatory cream. The contact time with the cream was 25 minutes, according to the manufacturer's instructions. The cream was then removed and the skin was washed with water and then dried (fig. 1). For the next 24 hours no procedures were performed, allowing time for the skin irritation to disappear.

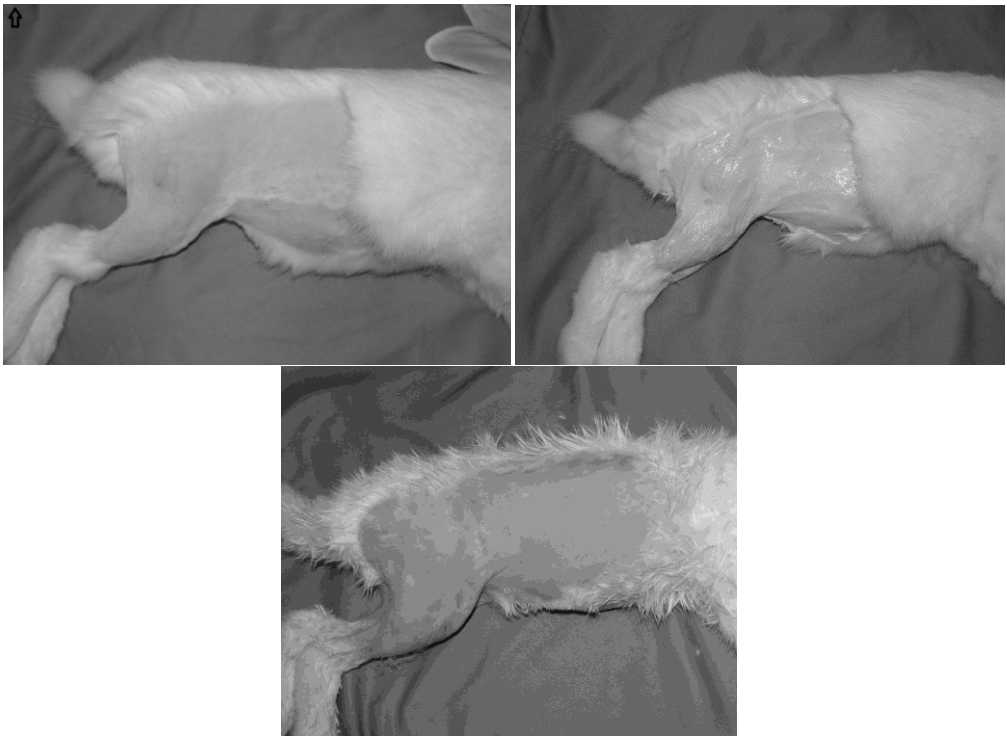

Fig. 1. Preparing the skin for the burn to be inflicted
A. The hair is removed with an electric shaver; B. The depilatory cream is in place; C. The skin appearance immediately after washing. Note some skin irritation.

The area to be burned was marked with a permanent skin-marker. Each animal had four burns, two on each flank (fig. 2). A 350 g copper device was used to inflict the burns (fig. 3). The device was introduced in boiling water and kept for 30 seconds. The temperature was measured using an infrared thermometer (Fluke 62 mini infrared

thermometer, accuracy: ±1ºC). When the device reached the desired temperature, it was applied. Because the copper has a better thermal conductivity compared with other materials, the temperature decreased slowly with an average of 1ºC per three seconds at room temperature.

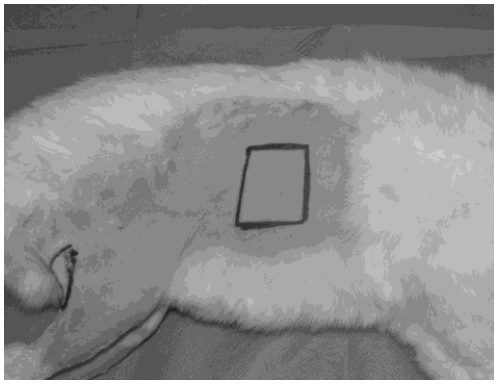

Fig. 2. The skin area to be burned is marked

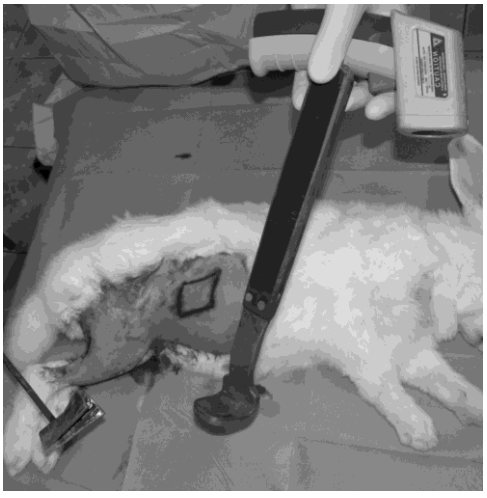

Fig. 3. The device used for the burn. The temperature was infrared measured.

All the procedures were performed under aseptic conditions, after cleaning the skin with diluted Povidone Iodine solution. No additional pressure was applied manually, except for the device's own weight. The device's copper volume was 19.28 cm^3 and the contact surface was 14.4 cm^2. Using contact times of 3, 4, 5 and 7 seconds and the device's temperatures of 43°C, 44°C, 45°C, 47°C, and 48°C (for each timing), burns were inflicted on the marked areas (two on each flank). The time between measuring the device's temperature to skin application was less than one second on a digital stopwatch.

The wounds were covered with dry gauze to keep them clean. On the third day, biopsies were taken from the burned areas to evaluate the depth. From each burned area one biopsy was taken.

B) Evaluation of the best dressing contention method

We compared four dressing contention methods on 24 New Zealand white male rabbits with an average weight of 2.5 kg, separated into 4 groups. Each rabbit's flank was inflicted with a burn.

Group I: We used the simplest method: dressing and retention bandages circumferentially (Peha Haft®, Hartman) (fig. 4). Each dressing was planned to be changed every 2nd day.

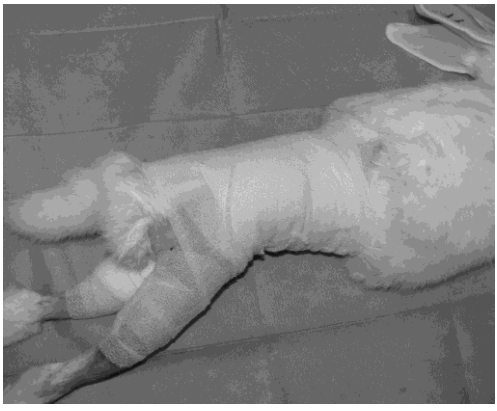

Fig. 4. Group I: Retention bandages placed circumferentially

Group II: The dressing was fixed with skin staplers (fig. 5) and retention bandages circumferentially (Peha Haft®, Hartman) applied on top. Each dressing was planned to be changed every 2nd day.

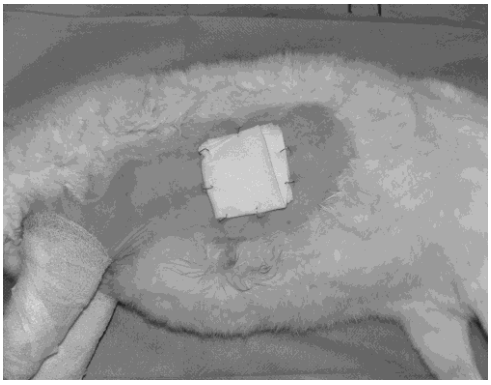

Fig. 5. Group II: The dressing fixed with skin staplers

Group III: The same as described in group II plus collars (fig. 6). Each dressing was planned to be changed every 2nd day.

Fig. 6. Group III: The collar in place

Group IV: The same as in group II, with fewer skin staplers and a body resin cast on top. The cast was placed circumferentially with narrower openings compared to the middle part. After the resin hardened, the cast was cut longitudinally and the sides connected with Omnifix® (Hartman) (partial elastic adhesive dressing) to allow better thoracic movement during respiration (fig. 7). The dressings were changed every 2nd day under general anesthesia for a period of 2 weeks.

Fig. 7. Group IV: Body resin cast placed circumferentially

RESULTS

A) The ideal temperature to reach a deep 2nd degree skin burn

The skin biopsies collected were histological evaluated and it was found that a deep 2nd degree burn can be obtained at 43° C for 4 seconds (fig. 8). Since the burn surface is not uniform, we considered a deep 2nd degree burn if more than 75% of the area was involved.

B) Evaluation of the best dressing contention method

Group I: The dressing fell down within hours after the rabbit woke up. The dressings were set again, but under a bit more tension. It was hard to find the proper tension in the retention bandages. This way, the dressings were in place, but four of the rabbits died 24 hours later. The autopsy revealed hemorrhagic ulcers in the stomach and bowel, most probably due to post-traumatic stress and administration of NSAIDs. Pleural effusion and pulmonary edema was associated. The treatment with

ketoprophen was stopped to the other groups and Phytomenadione and Omeprazole was initiated for the next days.

Group II: The dressings were in place for a longer time (hours up to one day). In the end all rabbits were able to remove them with the teeth.

Group III: The collars were useful and stopped the rabbits from removing the dressing for the next 48 hours. All the rabbits were very agitated and had superficial neck wounds at the site of collar. Their movements were highly restricted.

Group IV: This was the best contention method. After the first 48 hours, the cast was shortened in four rabbits, in order to prevent pressure sores at the hip joint.

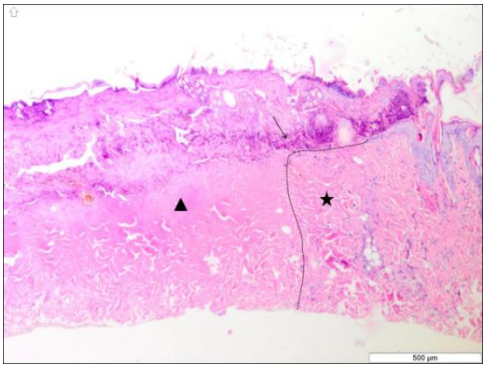

Fig. 8. Histopathology of full thickness skin damage, with ulceration and fibrinoleukocytic exudate. Severe, interstitial inflammatory infiltrate in the papillary dermis dominated by neutrophils, lymphocytes, and macrophages, delimiting the lesion (arrow). In the upper dermis there is linear fibroblastic and collagen proliferation along with newly formed blood vessels. Loss of normal architecture with homogenization of the collagen bundles in the reticular dermis (triangle). Perilesional normal skin (star) is delimited by dotted line. Hematoxylin and eosin stain, Bar=500µm.

After the dressings fell down twice in groups I-III, the contention method was changed to body resin casts and all animals (groups I-IV) were involved in a further study on topical creams for burn wounds.

DISCUSSION

Several types of burn models are described in the literature, most focusing on thermal injuries. Such injuries could be obtained after direct contact with hot water or with a hot surface. The contact temperature and the time are the main factors influencing the burn depth. There is a wide variety of reported contact temperatures and time to reach a 2nd degree burn.

Knabl et al (7) reported partial skin thickness burn on 9 New Zealand rabbits using an aluminum stamp of 85g heated up to 80°C and 14 seconds contact time. No details about the methods of dressing fixation were described. They recommend using rabbits of the same strain and weight in the anagen hair growth phase for better evaluation. Von Bullow et al (8) described a skin burn model on 10 New Zealand rabbits. Full thickness burns were achieved by the application of heated brass probes at 100°C for more than 30 seconds on the backs of the rabbits. No details about the weight and volume of the probe are described. According to the same author (8), superficial partial thickness burns can be produced with a contact time of 7 seconds. There are no data about the type of dressing. In their study on 15 rabbits, Aksoy et al (9) obtained 3rd degree skin burn injuries using a brass plate weighting 500 g, with a surface diameter of 3.5 cm. Before applying it to the skin, the instrument was left in boiling water at 100°C for 15 minutes. A contact time of 15 seconds was used to obtain a full thickness burn injury. It was mentioned that no topical antimicrobial treatment was applied. In this

study a delayed wound healing model was obtained by removing the *panniculus carnosus* layer, and three weeks later a 3rd degree burn was inflicted on the skin flaps.

Wang et al (10) obtained a full thickness burn area after 10 seconds of contact with 90-93°C water. The dressings on the burned areas were fixed with the tie-over method. No details about follow-up of wound dressing were reported. In another study on 16 New Zealand male rabbits where no burns were inflicted but full thickness wounds were performed to check different topical agents, the wounds were covered with a self-adhering wrap-around bandages. The rabbits were checked twice a day for the bandages to be in place and the dressings were changed daily without anesthesia.(11). The same type of dressing fixation was used by Jurjus et al in their study on deep 2nd degree skin burns obtained using an aluminum stamp heated to 80° and a contact time with the skin of 25 seconds (12). Unlike the study mentioned before, these dressings were changed after sedating the animals.

A possible explanation for this great variety of temperature and time can be explained by using different instruments for obtaining the burn, each with a different capacity for releasing the heat. Heated brass (1, 8, 9, 13), heated copper instruments (3), aluminum instruments (6, 7, 12), or even contact with hot water (10) were all tested to obtain an experimental burn model. It is important to know the time that elapsed from measuring the device's temperature until the inflicted burn. We chose to use a copper device because it has better thermal conductivity compared to other nonferrous metals or iron.

We decided to use rabbits for this experimental research, because they are larger than other rodents, easy to handle, and allow good follow-up. When working on small animals for burn models, some results can be negatively affected by the burn size. The contraction can be significant enough to alter the wound evaluation. In small animals the burn size can be a limiting factor for the experiment. Researchers dealing with different models of burns know that maintaining the dressings in place for a longer period of time (days) is a difficult task.

CONCLUSIONS

According to our results, this burn model seems to be better than those existing on rabbits and offer details about obtaining a deep 2nd degree burn and describes a good contention method. The best contention method was the body resin cast. We hope this information will be useful for those researchers working on rabbits.

DECLARATION OF INTEREST

Lucian Fodor is currently receiving a grant (PN-II-RU-TE-2011-3-002) from CNCS-UEFISCDI. Raluca Sobec, Codrin Dobreanu, Marius Fodor, Cristian Magdas are members of the same grant team. For the remaining authors none were declared.

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NEWS

CYTOLOGICAL ANALYSIS OF THE PERIODONTAL POCKET IN PATIENTS WITH AGGRESSIVE PERIODONTITIS AND CHRONIC PERIODONTITIS

Oral exfoliative cytology includes the study and interpretation of the features cells exfoliated from the oral mucosa. The aim of a study realized by a group of argentinian researchers was to analyze cytological changes in the periodontal pocket of patients with different clinical stages of aggressive periodontitis and chronic periodontitis. Patients, aged between 24-54 years, of whom 41 were diagnosed with aggressive periodontitis, 40 with chronic periodontitis, sub-classified as mild, moderate and severe periodontitis, and 40 healthy individuals who were the control group. For the cytological study were taken samples of the epithelium of the periodontal pocket. Superficial and intermediate cell values were significantly greater in patients with aggressive periodontitis than in patients with chronic periodontitis or the control group. Histiocyte number was higher in patients with chronic periodontitis than in those with aggressive periodontitis, and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with chronic periodontitis, and there were differences between patients with periodontitis and the control group. The cytological study demonstrated that patients with aggressive periodontitis had greater tissue damage, shown by the increase in intermediate and superficial cells of the epithelium of the periodontal pocket compared to the group of healthy subjects and to a lesser extent, to patients with chronic periodontitis. Only superficial cells made it possible to differentiate the sub-stages of the disease (Cecilia EC, Myriam AK, María EL. Cytological analysis of the periodontal pocket in patients with aggressive periodontitis and chronic periodontitis. *Contemp Clin Dent*, 2014; 5(4): 495-500).

Irina Grădinaru

THE PSYCHO-EMOTIONAL PROFILE OF THE HIV-POSITIVE NAÏVE PATIENT

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THE PSYCHO-EMOTIONAL PROFILE OF THE HIV-POSITIVE NAÏVE PATIENT (Abstract) **Aim:** The paper aims to outline the naïve HIV-positive patient's profile, in terms of feelings and emotions post-diagnosis. **Material and methods**: The evaluation took place from January 2011 to December 2013 in the Psycho-social Assistance Office of the "Sfânta Parascheva" Infectious Diseases Hospital in Iaşi. We evaluated 146 patients newly diagnosed with HIV infection, both in terms of immunological and virusological and from a psychological perspective, using the Hamilton Anxiety Scale (HAMA), Beck Depression Inventory II (BDI) and the clinical interview. **Results**: In a period of 3 years (January 2011 - December 2013) we registered in the HIV/AIDS Regional Center in Iaşi, 146 new cases of HIV infection in adults, with distribution by years as follows: 46 in 2011, 45 in 2012, 55 in 2013; 39 cases were in Iaşi, 39 in Suceava, 26 in Botoşani, 18 in Neamț 18, 16 in Bacău and 6 in Vaslui. Of these, 51 were women and 95 men, aged between 24 and 46 years. From the immunological point of view, 11% of patients had values of CD4 > 500 (16 patients), 47 % between 500 and 100 (69 patients) and 42 % < 100 (61 patients). From a psychological perspective, the clinical interview revealed a state of fear in 68.5 % of cases (fear of death, fear of complications, fear of other people's reaction to the diagnosis), confusion in 62 % of cases (in terms of diagnosis, the mode of infection, the future), anger in 27 % of cases (against the source of infection, against themselves, against God or divinity), guilt and self-blame in 7 % of cases. Beck Depression Inventory (BDI) revealed moderate depression in 14% of patients; mild depression in 27% of patients and 58 % of patients presented no symptoms of depression. Regarding the anxiety scale HAMA, in 54 % of cases it showed mild anxiety, average anxiety in 28 % of cases, 14% severe anxiety and 3% (4 cases) very severe anxiety. **Conclusions**: Patients newly diagnosed with HIV are a vulnerable population with a specific psycho-emotional profile. Multidisciplinary knowledge - in medical, psycho –emotional and social terms – of the patient's characteristics and needs helps to support adherence to the antiretroviral therapy and improve the quality of life. **Keywords:** HIV/AIDS, NAIVE PATIENT, PSYCHOLOGICAL PROFILE, ANXIETY, DEPRESSION

The newly diagnosed HIV-positive patient (also known as naive) represents a challenge for both infectious diseases doctor and psychologist, in terms of proper management. In order to best treat the patient, it is important to establish a relation-

ship based on the knowledge and the understanding of his needs, expectations, and his distinct psycho-emotional profile (1).

On receiving the HIV-positive diagnosis, the patient experiences a series of specific emotions: first, denial of the disease; then anger towards himself and others; this is followed by bargaining or "negotiation" of different aspects of the disease; after that comes the depression, the sense of "losing everything"; the last stage is acceptance, when the patient fully understands the HIV diagnosis and accepts it in his life (2). In most countries being HIV-positive is associated with stigma and discrimination, and that is why many patients feel that having contracted this disease is shameful and that it leads to social rejection – losing friends, social status, and ultimately their value as human beings (1, 2).

This is why the moment after the first HIV-positive diagnosis is critical in a patient's evolution. It is very important that the infectious diseases doctor work with a specially trained psychologist that can offer support and guidance to the naive patient. The psychologist can help the patient fully understand the impact that HIV/AIDS will have on his social and personal life and can also offer assistance in coping with the antiretroviral therapy (ART). This is one of the most difficult aspects of living with HIV/AIDS – having to take ART for the rest of one's life, and also coping with side-effects (3, 4).

In order to assist the newly diagnosed HIV/AIDS patient and to create the doctor-patient and psychologist-patient relationship, it is essential to understand the psychological and emotional profile of the patient. This paper aims to outline the naïve HIV-positive patient's profile, in terms of feelings and emotions post-diagnosis

MATERIAL AND METHODS

The evaluation took place from January 2011 to December 2013 in the Psychosocial Assistance Office of the "Sfânta Parascheva" Infectious Diseases Hospital in Iași.

Newly diagnosed patients, also known as naive patients, were evaluated in terms of immunological and virusological status and were also interviewed by the psychologist. After the clinical interview, they were also evaluated using the Hamilton Anxiety Scale (HAMA) and the Beck Depression Inventory II (BDI).

The Hamilton Anxiety Scale is of the first rating scales developed to measure the severity of anxiety symptoms. It consists of 14 items, each defined by a series of symptoms. It is designed to measure both psychological anxiety (mental agitation and psychological distress) and somatic anxiety (physical complaints related to anxiety) (5, 6).

The Beck Depression Inventory is one of the most widely used instruments for measuring the severity of depression. It is a self-report inventory and it consists of 21 questions with multiple-choice (7, 8).

In a period of 3 years (January 2011 - December 2013) we registered in the HIV/AIDS Regional Center in Iași 146 new cases of HIV infection in adults, with distribution by years as follows: 32% in 2011, 31% in 2012, 37% in 2013 (fig.1).

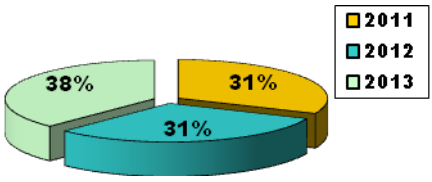

Fig. 1 Patient repartition in 3 years

Patients were admitted from all of the 5 counties in Moldova: 26.7% cases were in Iaşi, 26.7% in Suceava, 17.8% in Botoşani, 12.3% in Neamț, 10.9% in Bacău and 4.1% in Vaslui (fig. 2).

There was a higher percent of male patients – 65% men and 35% women (fig. 3), aged between 24 and 46 years.

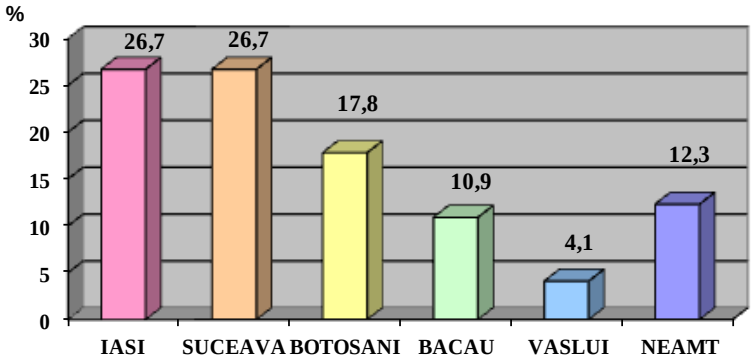

Fig. 2 Repartition in the Moldova area

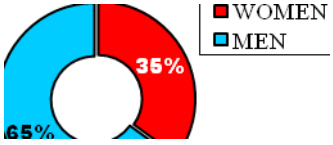

Fig. 3 Gender distribution

RESULTS

From the immunological point of view, 11% of patients had values of CD4 > 500 (16 patients), 47 % between 500 and 100 (69 patients) and 42 % < 100 (61 patients) (fig. 4).

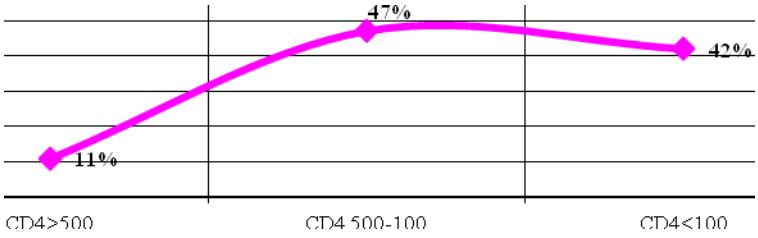

Fig. 4. CD4 cell count distribution

From a psychological perspective, the clinical interview revealed a state of fear in 68.5 % of cases (fear of death, fear of complications, fear of other people's reaction to the diagnosis), confusion in 62 % of cases (in terms of diagnosis, the mode of infec-

tion, the future), anger in 27 % of cases (against the source of infection, against themselves, against God or divinity), guilt and self-blame in 7 % of cases.

Beck Depression Inventory (BDI) revealed moderate depression in 14% of patients, mild depression in 27% of patients and 58 % of patients presented no symptoms of depression (fig. 5).

Regarding the anxiety scale HAMA, in 54 % of cases it showed mild anxiety, average anxiety in 28 % of cases, 14% severe anxiety and 3% (4 cases) very severe anxiety (fig. 5).

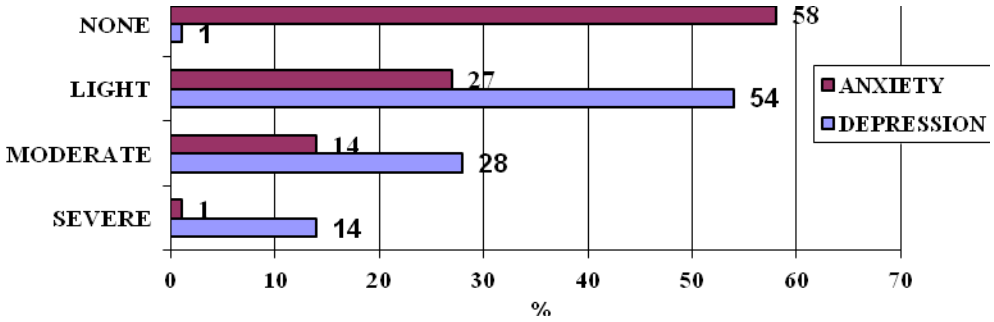

Fig. 5 Anxiety and depression evaluation

DISCUSSION

The number of new HIV/AIDS cases is continually increasing, with transmission mainly through unprotected sexual contact (homo- and heterosexual, prostitution) and use of unsterilized needles (drug users, tattoo and piercing) (9, 10).

In the North-Eastern part of Romania, the number of new HIV cases was large, with a rate of 35% per year, and many of the patients were in an advanced stage of the disease. The patients came from different backgrounds: multiple sexual partners, slow-progressers form the "pediatric cohort", homosexual men.

From a psychological point of view, they all had common emotional patterns: fear or lack of perspective for the future, lack of respect for one's self, sadness up to depression (11). Anxiety was high, and we believe this to be caused by both fear of dying and fear of social rejection.

A common emotion found in many of the patients in our study was guilt or shame. This is caused by a social phenomenon that associates HIV/AIDS with stigma, and leads to discriminatory behavior. In Romania, in spite of all the current existing information, AIDS is still, subconsciously, considered a shameful disease, contracted by marginalized populations that exhibit undesired behavior. With this in mind, the naive patient struggles to accept the positive diagnosis and not to consider him/herself as part of that discriminated group.

When establishing the best antiretroviral treatment, the infectious diseases doctor is obliged to offer the patient the best ART regimen catered to his or her needs. As specific as this treatment is, it only helps the patient at a molecular level. It is the psychologist's task to support the moral and emotional process of accepting the HIV diagnosis and beginning ART (12). The psychologist's expertise allows him to

guide the patient through all the five stages of acceptance of the diagnosis. This will have long term impact on the patient's adherence to therapy.

CONCLUSIONS

In our study, we highlighted the fact that patients newly diagnosed with HIV are a vulnerable population with a specific psycho-emotional profile.

Multidisciplinary knowledge - in medical, psycho –emotional and social terms – of the patient's characteristics and needs helps to support adherence to the antiretroviral therapy and improve the quality of life.

The psychologist's role is as important as the role of the infectious diseases doctor in managing the HIV-positive patient, due to his support and guidance in the continuous process of living with AIDS.

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POLYCYTHEMIA VERA SYNDROME-AS A PROTROMBOTHIC STATE

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POLYCYTHEMIA VERA SYNDROME-AS A PROTROMBOTHIC STATE (Abstract): **The aim** of our study was to investigate whether the disorder of coagulation and fibrinolysis factors are mechanisms that contribute to the prothrombotic state in patients with polycythemia vera (PV) syndrome with or without cardiovascular disease (CVD), in order to identify the patients having high risk for thrombotic events. **Material and methods:** The study comprises 20 patients divided in 2 groups: 10 patients with PV syndrome (PV) and 10 pacients with PV and cardiovascular diseases associated (PV+CVD). The patients were tested by determining three factors of coagulation profile: protein S, free fraction (PS), antithrombin III (AT III) and Protein C (PC). **Results:** The level of the three parameters were found significantly modified in the both groups ($p<0.05$); comparing the results between the two groups of patients, in the second group (PV+CVD) the level of the parameters were significantly lower than in the first group (PV). **Conclusions:** In PV syndrome the risk for thrombosis is also due to the changes in coagulation factors. Patients with associated cardiovascular disease, present a more severe risk for trombothic events, so regarding the disorder of coagulation factors, this represent a major mechanism implicated in the etiology of thrombosis in these categories of patients. **Keywords:** THROMBOSIS, POLYCYTHEMIA VERA, PROTEIN S

Polycythemia vera (PV) is a clonal disorder arising in a multipotent hematopoietic progenitor cell that causes the accumulation of morphologically normal red cells, white cells, platelets, and their progenitors in the absence of a definable stimulus and to the exclusion of nonclonal hematopoiesis (1. 2). Described for the first time in 1892, polycythemia vera is not a new disease and while uncommon, with an incidence of at least 2 per 100,000 (3). Multiple factors are likely to contribute to the pathogenesis of thrombosis, including increased cell mass, possibly platelet number, activation of platelets and leukocytes and their interaction to form platelet leucocyte aggregates, in addition to prothrombotic circulating and endothelial factors. Endothelial activation may also have been due to elevated levels of vascular endothelial growth factor, which has also been reported to be elevated in PV (4. 5. 6). Activated leukocytes (neutrophils and monocytes) promote coagulation by release of granule contents, formation of aggregates with platelets. Subsequent effects include plate-

let leukocyte aggregates playing a pathogenic role in triggering monocyte tissue factor expression as well as superoxide anion and inflammatory cytokine release causing endothelial activation and damage. Besides the procoagulants mechanisms, the thrombotic risk is also amplified by the association of the other risk factors for thrombosis represented by advanced age, history of thrombotic vascular diseases, repeated phlebotomies, hypercholesterolemia and smoking (7, 8).

MATERIAL AND METHODS

The study comprises 20 adult patients, diagnosed according to Polycytemia Vera Study Group criteria, divided in 2 groups: 10 patients with PV syndrome (PV) and 10 pacients with PV with CVD associated (PV+CVD). Patients' age ranged from 61 to 80 years, significantly higher in the PV+CVD group ($p<0.05$). The old of the disease ranged from 1 to 4 years, significantly higher in the PV+CVD group ($p<0.01$).The patients were tested by determining three factors of coagulation profile: Protein S (PS), Antithrombin III (AT III) and Protein C.

RESULTS AND DISCUSSIONS

Protein S, Ag (PS): In the studied groups, the mean values of PS not significantly differ between genders, but in males from PV group, the mean values of PS are significantly lower than those observed in the PV+CVD group ($p<0.05$). In both studied group, it was observed a direct correlation between PS values and the patients' age; low values of PS were recorded in 66% of elderly patients from PV group and in 85% of elderly patients from PV+CVD group.

We have been observed a direct strong correlation between PS values and the age of the disease, both in the PV group ($r=0.76$) and in the PV+CVD group ($r=0.88$).

On the studied groups, comparing PS values, we have been observed the following characteristics:

- PS had the most homogeneous individual values in the PV+CVD group (12.15 CV%);
- in PV+CVD group, all of the patients had the PS values below normal limit (65%), significantly more cases compared with PV group (just 14 cases over limt) ($\chi^2=4.60$; GL=1; $p=0.027$);
- the mean values of PS are statistically significantly lower in PV+CVD group compared with PV group ($t=5.54$; GL=38; $p<0.001$). (fig. 1, 2, 3).

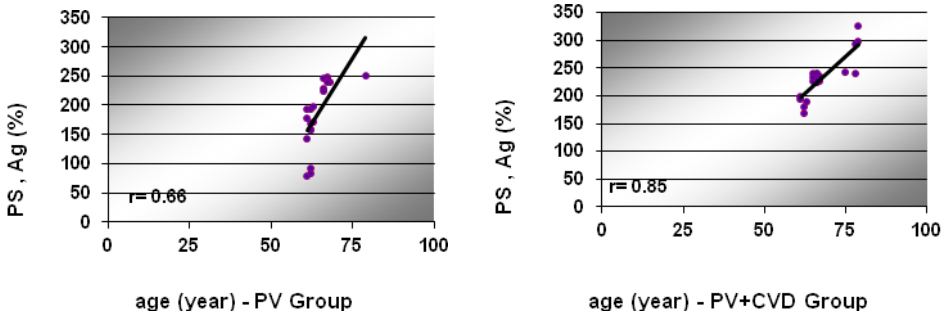


Fig. 1. Correlations between PS, Ag values and patients' age

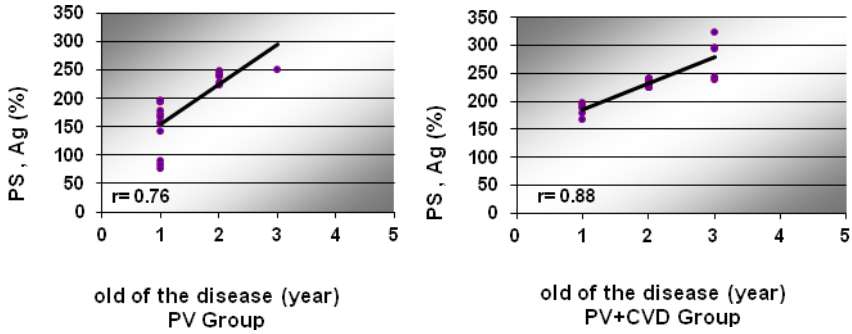


Fig. 2. Correlations between PS, Ag values and the old of the disease

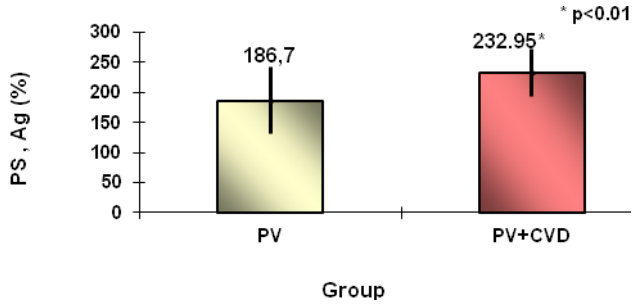


Fig. 3. The mean values of PS, Ag in the studied groups

Antithrombin III, Ag (AT III): On the studied groups, the mean values of AT III did not significantly differ between genders. On both studied groups, it was observed a direct correlation between AT III values and the patients' age; low values of AT III were recorded in 85% of elderly patients from PV group and in 89% of elderly patients from PV+CVD group.

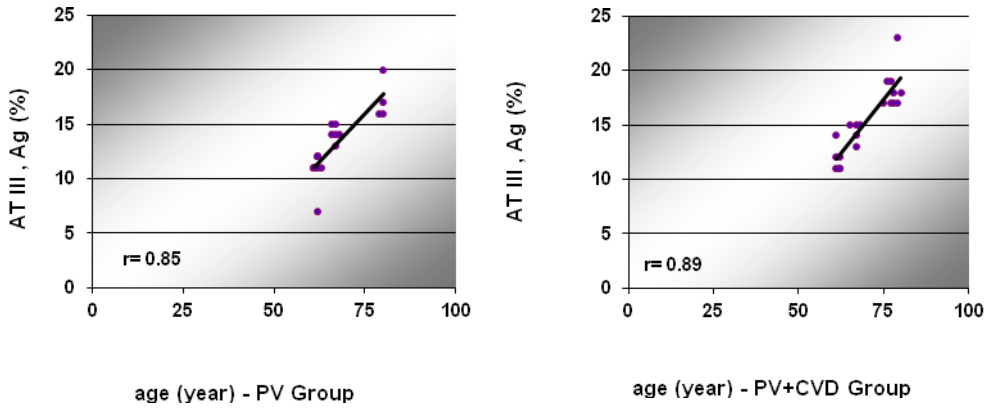


Fig. 4. Correlations between AT III, Ag values and patients' age

It was observed a direct strong correlation between AT III values and the age of the disease, both in the PV group (r=0.92) and in the PV+CVD group (r=0.89).

On the studied groups, comparing AT III values, we have been observed the following characteristics:

- the AT III individual values have a large dispersion, but the most homogeneous individual values was found in the PV+CVD group (12.66 CV%);
- we found the AT III levels below normal limits (65%) in all of the 20 patients from PV+CVD group and in 19 patients from PV group, without registering a significant statistically differences between these groups (χ^2=1.03; GL=1; p=0.311);
- the mean values of AT III are significantly lower in PV+CVD group compared with PV group (t=2.78; GL=38; p<0.05) (fig. 4, 5, 6).

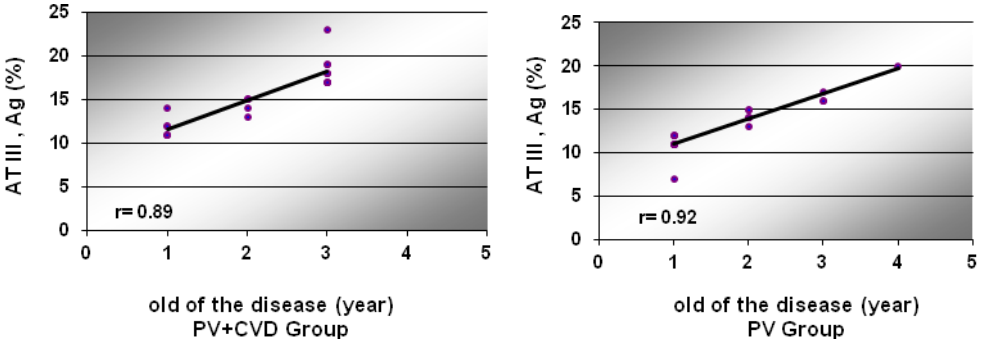


Fig. 5. Correlations between AT III, Ag values and the old of the disease

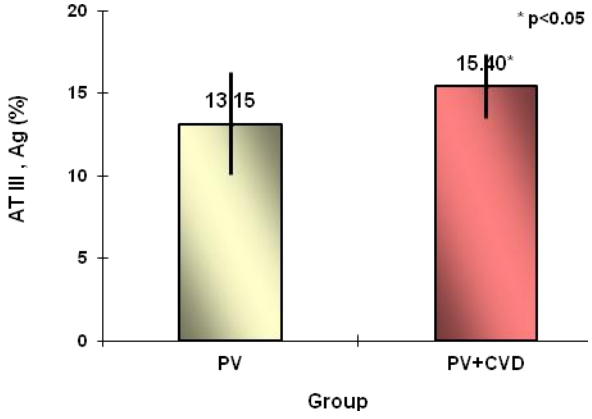


Fig. 6. The mean values of AT III, Ag in the studied groups

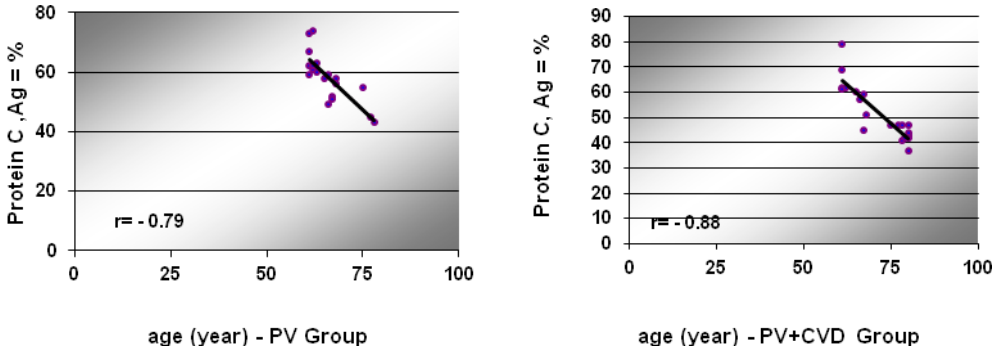
Protein C, Ag (PC): In the PV group, the mean values of protein C, Ag differ significantly between genders (p<0.05); in the PV+CVD group, in women, the values of this parameter are significantly lower compared with the values observed in women, from PV group (p<0.05). In both studied groups, it was observed an indirect

correlation between protein C values and the patients' age; low values of protein C were recorded in 79% of elderly patients from PV group and in 88% of elderly patients from PV+CVD group.

We also have been observed a strong indirect correlation between protein C values and the old of the disease, in both, in the PV group (r=0.84) and in the PV+CVD group (r=0.89).

On the studied groups, comparing protein C values, we have been observed the following characteristics:

- The variation of individual protein C, Ag values are large, but the most homogeneous series of values were found in PV+CVD group (14.49 CV%);
- We found the protein C, Ag level below normal limit (65%), in 18 patients from PV+CVD group and in 17 patients from PV group, without registering significant statistically differences between these groups (χ^2=0.23; GL=1; p=0.633);
- The mean values of protein C are significantly lower on PV+CVD group compared with PV group (t=2.42; GL=38; p<0.05) (fig. 7, 8, 9).



F

ig.7. Correlations between Protein C, Ag values and pacients' age

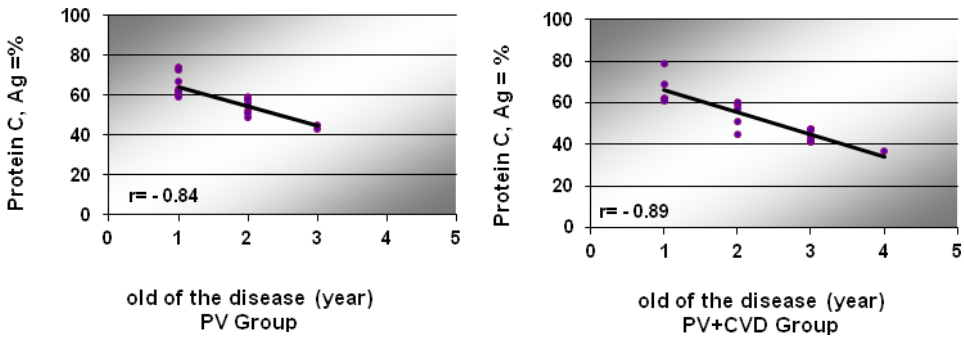


Fig. 8. Correlations between Protein C, Ag values and the old of the disease

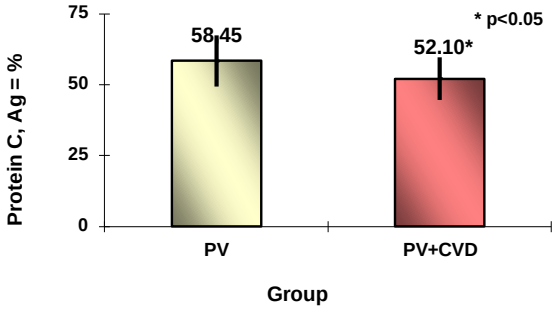


Fig. 9. The mean values of Protein C, Ag in the studied groups

DISCUSSION

Thrombosis and bleedings are the major causes of morbidity and mortality in PV. Large vessel arterial thrombosis in PV involves the cerebral, coronary and peripheral arterial circulations, while microcirculatory disturbances may cause erythromelalgia and digital ischemia. This hypercoagulable state in the group of patients with PV, could in part be explained by the known hypercoagulable condition associated with malignancy, by increases in white blood cells (WBC) count, but polymorphonuclear (PMN) enzyme involvement is possible. Activated PMN release reactive oxygen species and intracellular proteases, which can act on the endothelial cells and platelets and may modify the hemostatic balance toward a prothrombotic state. Endothelial activation may also have been due to elevated levels of vascular endothelial growth factor, which has also been reported to be elevated in PV (9). Leukocyte elastase and cathepsin G can induce detachment or lysis of endothelial cells and can modify endothelial cell functions involved in thromboregulation-that prevent thrombin-induced prostacyclin production, induce plasminogen activator inhibitor release and proteolyze endothelial surface components such as thrombo-modulin (10). Furthermore, the potential thrombogenic effects of PMN-derived proteases include the direct potent platelet activation elicited by cathepsin G. Elastase can directly proteolyze and inactivate natural inhibitors of blood coagulation, including protein C, protein S, tissue factor pathway inhibitor, antithrombin, and heparin cofactor II. This inactivation may contribute to local progression of coagulation reactions at the inflammation sites (11).

CONCLUSIONS

In PV syndrome the risk for thrombosis is also due to the changes in coagulation factors. Patients with associated cardiovascular disease, present a more severe risk for trombothic events, so regarding the disorder of coagulation factors, these represent a major mechanism implicated in the etiology of thrombosis in these category of patients. We may hypothesize that hypercoagulable state associated with malignancy, increases in WBC count, endothelial dysfunction, and reduced levels of physiologic anticoagulants (antithrombin III, proteins C and S) and decreased fibrinolytic activity that in part may be secondary to increased plasma levels of plasminogen activator inhibitor could explain, the pathogenesis of the thrombo-philic state in PV.

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NEWS

SERUM REACTIVITY TO *DEMODEX*-ASSOCIATED *BACILLUS OLERONIUS* PROTEINS IN ROSACEA PATIENTS

There is increasing evidence that suggests the role of *Bacillus oleronius*, isolated from within *Demodex folliculorum* mite in the induction of rosacea. A group of researchers tried to determine the correlation between the level of sebum and the density of *D. folliculorum* and the serum reactivity to the 62 and 83 kDa proteins of *B. oleronius* in patients with erythematotelangiectatic rosacea. Serum reactivity to *B. oleronius* proteins was detected in 82.6% of the rosacea patients compared to 26.9% of controls. In this group of patients, the level of sebum was lower and the density of *Demodex* populations on their faces was statistically higher than in controls, suggesting a potential role for this bacterium in the aetiology of rosacea (Jarmuda S, McMahon F, Zaba R *et al*. Correlation between serum reactivity to Demodex-associated Bacillus oleronius proteins, and altered sebum levels and Demodex populations in erythematotelangiectatic rosacea patients. *J Med Microbiol*. 2014; 63(Pt 2):258-62. doi: 10.1099/jmm.0.065136-0).

Teodora Vremeră

SELF-MEDICATION WITH ANTIMICROBIAL DRUGS AMONG UNIVERSITY STUDENTS IN A NORTHEAST REGION OF ROMANIA

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SELF-MEDICATION WITH ANTIMICROBIAL DRUGS AMONG UNIVERSITY STUDENTS IN A NORTHEAST REGION OF ROMANIA (Abstract): Self-medication with antimicrobial drugs is an important problem in the world and may lead to serious consequences for healthcare systems. **Objective:** To evaluate the prevalence and patterns of self-medication with antimicrobial drugs among university students in a Northeast region of Romania. **Material and methods:** A cross-sectional questionnaire-based study was conducted to collect data from medical and non-medical students who lived in residence halls. Data were analyzed using descriptive statistics and the chi-square test, when applicable. **Results:** Out of the 320 questionnaires distributed, a total of 281 students completed and returned the questionnaires. Among these, 115 (41%) respondents admitted to have used at least one antibiotic in the six months prior to the survey, 44% of whom did not seek medical advice (irrational self-medication). The most common antimicrobial drugs used for self-medication were amoxicillin (37%), amoxicillin-clavulanate (33%), ciprofloxacin and penicillin (14%) and the most frequently reported reasons for self-medication were respiratory and oral infections (31%), common cold (25%), and genitourinary infections (20%). Some students mentioned the use of more than one antimicrobial drug, for more than one disease. **Conclusions:** self-medication with antimicrobial drugs is a relatively common practice among students in Romania. This suggests the need for interventions to prevent the irrational use of antimicrobial drugs, such as the implementation of national programs for public education regarding the risks and consequences for the health of irrational use of antimicrobials. **Keywords:** SELF-MEDICATION, ANTIMICROBIAL DRUGS, STUDENTS.

Self-medication is defined as the use of any type of drug to treat self-diagnosed disorders or symptoms, without seeking the advice of a physician, either for diagnosis, prescription or surveillance of treatment. The World Health Organization (WHO) introduced the term "rational" self-medication, which limits the use of medications without prescription only to over-the-counter (OTC) drugs, thus helping prevent and treat minor illnesses that do not require medical consultation and save medical resources for more severe cases (1). Health authorities for each country have to ensure that only safe drugs are made available OTC and that patients have all the information regarding the drug usage, contraindications and side-effects (2-4).

Self-medication with antimicrobial drugs, a form of irrational use of medi-

cines, is a common phenomenon in many countries, with major implications for the public health: emergence of resistant microorganisms, particularly in developing countries, increased risk of drug interactions and side effects, treatment failures and prolonged hospital stays, wastage of medical resources and increased morbimortality. Self-medication is more common among the youth and it is associated with several factors related to modern society, such as less time for seeking medical advice, increased costs of medical consultations, poor compliance to treatment and aggressive pharmaceutical publicity (5-11).

Self-medication practices vary widely in different populations and previous studies have shown that age, gender, economic status, education level and medical knowledge are important predictors of self-medication (12-15). Students are a population of great interest regarding self-medication practices since they represent a privileged segment of the population, with access to information. Moreover, the attitude of medical students regarding self-medication may reflect in their future medical practice.

To the best of our knowledge, in Romania there are no studies regarding the self-medication practices with antimicrobial drugs among university students. Therefore, a cross-sectional study was conducted to evaluate the prevalence and patterns of self-medication with antimicrobial drugs among medical and non-medical university students. We aimed to describe the most common antimicrobial drugs used for self-medication and the ailments students treat with antimicrobial drugs.

MATERIAL AND METHODS

This cross-sectional questionnaire-based study was conducted on a sample of students from residence halls of two universities ("Grigore T. Popa" University of Medicine and Pharmacy and "Alexandru Ioan Cuza" University) from Iași, in the Northeast of Romania, from October to December 2011. The students were selected by convenient sampling. The study was approved by the Ethics Committee of the "Grigore T. Popa" University and written consent was obtained from each participant after a briefing on the objectives of the study.

The including criteria were: medical or non-medical students enrolled in the above-mentioned universities, aged between 18 and 31 years old, both genders, healthy. The non-medical students group included students in the first 2 years of medical studies, who did not study pharmacology and therapeutics. Information regarding demographic characteristics (age, gender, type and year of university studies, social environment) and antimicrobial drugs use in the six months prior to the study were collected through anonymous, self-administered questionnaires with open- and close-ended questions. The questionnaires, written in Romanian language, were pre-tested and validated on a sample of 100 students to determine the application time and to clarify possible questions from the students. If antimicrobial drugs use was confirmed in the previous six months, supplementary information was asked: the name antimicrobial drug, duration of the treatment, the person who recommended the treatment and the disease treated with antimicrobial drugs. The collected data were registered in a database and analyzed using SigmaPlot 11, descriptive statistics and the chi-square test in order to test the difference between proportions. A p value of less than 0.05 was considered significant. Some of the questions had multiple

answers; therefore the sum total of percentages is not always 100%.

RESULTS

Of the 320 questionnaires distributed, 281 students completed and returned the questionnaires, with a response rate of 87.81%. The demographic data of medical students and non-medical students are presented in tab. I.

TABLE I

Demographic characteristics of the respondents

Variable		Medical (n=141)	Non-Medical (n=140)
Age (mean±SD, years)		23 ± 1	21 ± 1
Sex	Male	38	18
	Female	103	122
Social environment	Rural	27	52
	Urban	114	88

Out of the 281 respondents, 115 (41%) reported that they had used at least one antibiotic in the six months previous to the survey. Antimicrobial drugs were used without medical advice (irrational self-medication) by 51 students (44%), while 64 students (56%) followed medical advice. Irrational antimicrobial drugs use was reported by 27 students (53%) as a self-recommended treatment, by 16 students (31%) according to pharmacist advice and by 17 students (33%) according to a friend or relative advice. Some students mentioned the use of more than one antimicrobial drug. There was no significant difference between medical and non-medical students (p=0.81) regarding the use of antimicrobial drugs in the six months before the survey. However, in relation to the source that influenced irrational self-medication, self-prescription was mentioned by 38% of medical students compared to only 9% non-medical students (p=0.003). These findings show that medi-

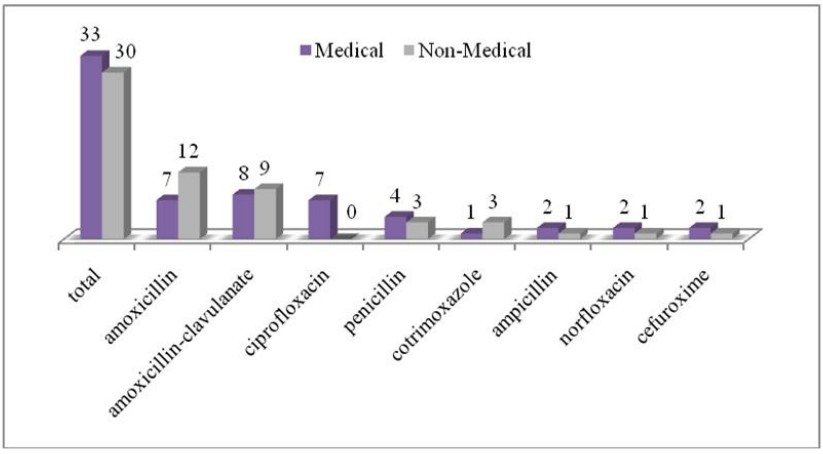

Fig. 1. The most commonly used antimicrobial drugs for self-medication (more than one antimicrobial drug were used by a person).

cal students are more confident in their knowledge regarding antimicrobial drugs use. Non-medical students mentioned pharmacists (19%) and friends or relatives (23%) for advice on antimicrobial drugs use compared to medical students, who mentioned taking the advice of the pharmacist and friends or relatives in 9%, respectively 7% of cases. There was a significantly higher prevalence of self-medication with antimicrobial drugs among students from urban areas (p=0.002) (fig. 1).

The majority of students reported self-medication with antimicrobial drugs for respiratory infections and oral infections (31%), common cold (25%), genitourinary infections (20%), while fewer students (8%) self-medicated for pain, fever, conjunctivitis, eczema or enterocolitis. Some persons mentioned more than one disease.

DISCUSSION

Despite the national regulations that restrict the dispensation of antimicrobial drugs without prescription, our study indicates a relatively high prevalence of irrational self-medication with antimicrobial drugs in the Northeast of Romania. Irrational use of antimicrobial drugs was reported in 44% of our students' population. This rate is lower than countries such as Ghana (70%) and similar to countries such as Turkey (44.1%) and China (47.8%) (10, 11, 14). Other studies on self-medication with antimicrobial drugs have focused on the general population and reported variable prevalence rates between 7.3% in Indonesia (5) and 77.9% in Greece (15).

Compared to the results obtained by Olayemi et al. (16), showing a very high (80%) prevalence of self-medication with antimicrobial drugs among students from Medical and Pharmaceutical sciences compared to 42.11% of students in Social Sciences and 27.78% of Engineering students, we found that medical students had a higher frequency (38%) of self-prescription of antimicrobial drugs compared to non-medical students (9%), who more frequently had followed the advice of the pharmacist or friends and relatives. Medical students from higher years of studies, attending courses of pharmacology and therapeutics, are generally more confident in their own knowledge regarding antimicrobial drugs use and, hence, mention self-recommended antimicrobial drugs more frequently. Of particular concern is the high percent of 31% of students advised by a pharmacist regarding the antibiotic use for self-medication. Though national regulations restrict the issuing of antimicrobial drugs without a prescription, it seems that regulatory authorities often lack resources to enforce them.

The main limitation of this study consisted in the self-administered nature of the questionnaires. Therefore, the data was based on information provided by students' recollection only. Since our study included only university students, who represent a well-informed group of individuals, our population sample may not be representative for the general population. Other limitations include the small sample group and its sampling frame based on convenience, which could be a source of possible bias.

CONCLUSIONS

The results of this study confirm that self-medication with antimicrobial drugs is a relatively common practice among medical and non-medical students in Northeastern Romania. Self-medication with antimicrobial drugs is associated with serious risks such as emergence of microorganism resistance, drug interactions and toxicity, wastage of medical resources and increase

in morbi-mortality. There is need for national education programs regarding the risks of irrational self-medication with antimicrobial drugs, particularly among students, who represent the young, well-informed population. Furthermore, we consider that there is need for a stronger enforcement of the regulations regarding the sale of antimicrobial drugs and the pharmaceutical publicity.

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DEVELOPMENT AND VALIDATION OF A SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF NEW PRESERVATIVES FROM PHARMACEUTICAL FORMS

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DEVELOPMENT AND VALIDATION OF A SPECTROPHOTOMETRIC METHOD FOR QUANTITATIVE DETERMINATION OF NEW PRESERVATIVES FROM PHARMACEUTICAL FORMS (Abstract): **Aim**: A spectrophotometric UV-VIS fast, sensitive and simple method was developed for quantitative determination of two new antimicrobial preservatives (oxi-acetyl-mandelic acid and oxi-propionyl mandelic acid) and validated according to validation quides. **Material and methods**: The absorption spectra were recorded at 200–400 nm, and the absorbances were measured at 256 nm for oxi-acetyl mandelic acid and 258 nm for oxi-propionyl mandelic acid. Optimum working conditions were established and the method was validated. The developed and validated method was applied to determine these two preservatives from ointments, in which the preservatives were incorporated. **Results:** The method has a good linearity in the concentration range 0.1–1.5 mg/ml (for oxi-acetyl mandelic acid) and 0.1–1 mg/ml (for oxi-propionyl mandelic acid). The RSD for the precision of the method was 0.96328 for oxi-acetyl mandelic acid and 0.797612 for oxi-propionyl mandelic acid. The relative standard deviation was 0.726367% for oxi-acetyl mandelic acid and 0.726234% for oxi-propionyl mandelic acid. In the ointment samples, the concentration of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid were within the limits specified by pharmacopoeia for ointments. **Conclusions:** The developed and validated spectrophotometric method, for the determination of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid is simple, easy to perform, fast and cost effective. **Keywords:** VALIDATION, OINTMENTS, PRESERVATIVES, SPECTROPHOTOMETRIC METHOD, MANDELIC ACID

Mandelic acid is known as an antiseptic, being active against some pathogens (*Staphylococcus aureus*, *E. coli*, *Proteus* sp., *Aerobacter* sp. etc.). It is used in urinary infections (oral use), or dermic infections (inflammatory acne), for external use. Starting from this acid, we synthesized some mandelic acid derivatives, two of them (oxi-acetyl mandelic acid and oxi-propionyl mandelic acid) presenting high antimicrobial activity compared to mandelic acid.

For these two derivatives, oxi-acetyl mandelic acid and oxi-propionyl mandelic acid, were carry out some studies: there were confirmed their chemical structures (by elemental analysis, UV-VIS, IR, RMN ^{1}H spectral analysis), there were estab-

lished the minimum inhibitory concentrations (MCI=125 μg/ml for oxi-acetyl mandelic acid and MCI=500 μg/ml for oxi-propionyl mandelic acid) and lethal dose 50%, after oral administration (LD_{50}=800 mg/kg for oxi-acetyl mandelic acid and LD_{50}=3000 mg/kg for oxi-propionyl mandelic acid) (1, 2).

This paper presents the development of a fast and sensitive spectrophotometric UV-VIS method for oxi-acetyl mandelic acid and oxi-propionyl mandelic acid determination. The spectrophotometric method was validated according to validation quides and applied to oxi-acetyl mandelic acid and oxi-propionyl mandelic acid determination from pharmaceutical forms (ointments) (3, 4, 5).

MATERIAL AND METHODS

Apparatus:

- Jasco V530 spectrophotometer, quartz cells (1=1 cm);
- IKA-Werke basic ultrasonic bath.

Materials and reagents:

- oxi-acetyl mandelic acid, new synthesized preservative, obtained in faculty lab, for which we have validated the structure and established the MCI, the results have been previously published in journals;
- oxi-propionyl mandelic acid, new synthesized preservative, obtained in faculty lab, for which we have validated the structure and established the MCI, the results have been previously published in journals;
- absolute ethanol (p.a.) (Merck KGaA);
- cetyl alcohol, lanolin, vaseline, sunflower seed oil were produced by Redox, Bucureşti, România.

There were prepared oxi-acetyl mandelic acid and oxi-propionyl mandelic acid stock solutions and then were obtained suitable dilutions in absolute ethanol. Taking into account that those two preservatives are active at low concentrations, the determination was performed at concentrations close to the active ones. The absorption spectras were recorded between 200–400 nm, and the absorbances were measured at 256 nm for oxi-acetyl mandelic acid and 258 nm for oxi-propionyl mandelic acid in a 1 cm cell versus a blank of absolute ethanol.

The developed method was validated by studying the following parameters: linearity, detection limit, quantification limit, precision, in accordance with the bioanalytical method validation guidelines (3, 4, 5).

The developed and validated method was applied to determine these two preservatives from pharmaceutical forms (ointments). According to MCI`s, the preservatives were embedded in ointments in 0.0125 g% concentration for oxi-acetyl mandelic acid and in 0.05 g% concentration for oxi-propionyl mandelic acid. The ointments were prepared by the formula presented below:

Rp/	
Cetyl alcohol	5 g
Sunflower seed oil	20 g
Lanolin	5 g
Vaseline	20 g
Oxi-acetyl mandelic acid	0.0125 g
Distilled water q.s. ad	100 g

Rp/	
Cetyl alcohol	5 g
Sunflower seed oil	20 g
Lanolin	5 g
Vaseline	20 g
Oxi-propionyl mandelic acid	0.05 g
Distilled water q.s. ad	100 g

These two preservatives were extracted from ointments with gently heated absolute ethanol and, after filtration; the samples were spectrophotometric analyzed at 256 nm for oxi-acetyl mandelic acid and at 258 nm for oxi-propionyl mandelic acid, respectively.

RESULTS AND DISCUSSION

The method was validated by studying the following parameters: linearity, detection limit, and quantification limit, precision.

Linearity. There were prepared stock solutions of oxi-acetyl mandelic acid, respectively oxi-propionyl mandelic acid, from which were obtained the working solutions in a concentration range of 0.1–1.5 mg/ml (for oxi-acetyl mandelic acid) and 0.1–1 mg/ml (for oxi-propionyl mandelic acid). For each concentration, three determinations were performed and the average value of absorbance was calculated for each range (tab. I).

TABLE I

Absorbance values for establish the linearity of the method

No.	Oxi-acetyl mandelic acid concentration mg/ml	Absorbance (λ=256 nm)			Average
		I	II	III	
1	0.1	0.0375	0.0322	0.0437	0.0378
2	0.3	0.1675	0.1632	0.1598	0.1635
3	0.5	0.3331	0.3129	0.3290	0.3235
4	0.7	0.4589	0.4600	0.4656	0.4615
5	0.9	0.5999	0.6231	0.6202	0.6144
6	1.1	0.7325	0.7833	0.8179	0.7779
7	1.3	0.9128	0.9323	0.9311	0.9254
8	1.5	1.1109	0.9834	1.1991	1.0978
No.	**Oxi-propionyl mandelic acid concentration mg/ml**	**Absorbance (λ =258 nm)**			**Average**
		I	**II**	**III**	
1	0.1	0.1222	0.0982	0.1101	0.1102
2	0.3	0.2835	0.301	0.2989	0.2944
3	0.4	0.3899	0.3943	0.4101	0.3981
4	0.5	0.4952	0.4888	0.4903	0.4914
5	0.6	0.5921	0.5899	0.5932	0.5917
6	0.7	0.6854	0.7128	0.7001	0.6994
7	0.8	0.8201	0.7967	0.8024	0.8064
8	1	1.0098	1.0156	1.0167	1.0140

The average values of absorbance are graphically represented according to concentrations (fig. 1 and fig. 2).

Statistical analysis of experimental data leads to regression line equations, presented in table II. There were calculated the standard error of regression curve (SE), the Person correlation coefficient (r^2), the detection limit (LOD) and the quantification limit (LOQ). In tab. III are prezented the statistical data.

In conformity with the obtained data, the developed method was linear in the range of the studied concentration.

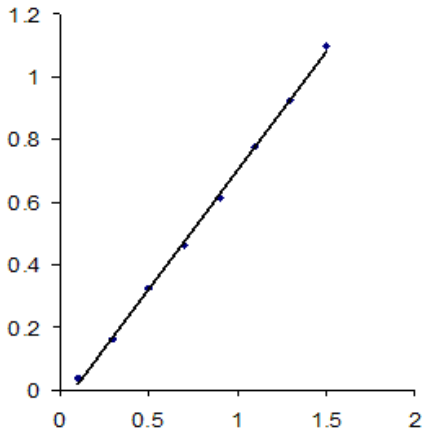

Fig. 1. Regression line for oxi-acetyl mandelic acid determination

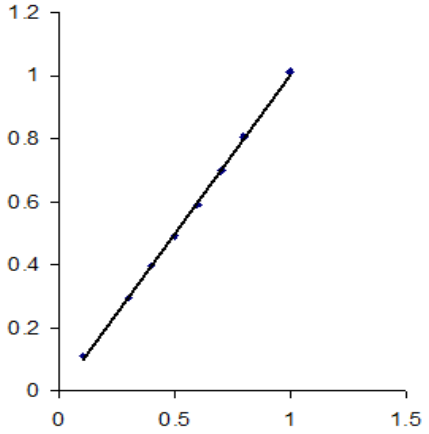

Fig. 2. Regression line for oxi-propionyl mandelic acid determination

TABLE II

Regression line equations for the two preservatives determination

Substance	Regression line equations
Oxi-acetyl mandelic acid	Absorbance = 0.75867x - 0.05671
Oxi-propionyl mandelic acid	Absorbance = 1.00851x - 0.00396

TABLE III

Statistical data regarding the two preservatives determination

	Oxi-acetyl mandelic acid	**Oxi-propionyl mandelic acid**
Person coefficient (r^2)	0.99897	0.99920
Standard error	0.012876	0.008863
Intercept	- 0.05671	- 0.00396
Slope	0.75867	1.00851
Detection limit (LOD) mg/ml	0.05	0.03
Quantification limit (LOQ) mg/ml	0.17	0.08

Precision of the system and method. Precision of the system was studied for the 1.3 mg/ml concentration of oxi-acetyl mandelic acid and 0.6 mg/ml concentration of oxi-propionyl mandelic acid, for 10 assays performed in the same conditions. The results and the statistical analysis for standard deviation (SD) and relative standard deviation (RSD %) are presented in table IV.

Processing data lead to a relative standard deviation RSD=0.96328% for oxi-acetyl mandelic acid and RSD=0.797612% for oxi-propionyl mandelic acid, compared to the maximum of 2% proposed by the European standards, so the systems are considered to be precise.

TABLE IV
System precision

	Oxi-acetyl mandelic acid	**Oxi-propionyl mandelic acid**
Det. no.	**Absorbance**	**Absorbance**
1	0.9234	0.5917
2	0.9231	0.5998
3	0.9111	0.6001
4	0.9323	0.6013
5	0.9101	0.5928
6	0.9321	0.6032
7	0.9278	0.5925
8	0.9109	0.5922
9	0.9215	0.5912
10	0.9311	0.5922
Average	**0.92234**	**0.5957**
Standard Deviation (SD)	**0.008885**	**0.004751**
Relative standard deviation (RSD) %	**0.96328**	**0.797612**

The precision of the method was investigated in a range of 20% compared to interest concentration, grouped on a number of 3 assays in 3 concentrations levels. Using the regression line equation the concentrations and recovery were calculated (tab. V).

From statistical analysis we obtained a relative standard deviation RSD= 0.726367% for oxi-acetyl mandelic acid and RSD=0.726234% for oxi-propionyl mandelic acid, compared to the maximum of 5% proposed by the european standards, so the proposed method is precise.

The analysis of the two preservatives from ointments. The presence of the oxi-acetyl mandelic acid and oxi-propionyl mandelic acid was carried out by comparing the spectras obtained after extraction with the standards ones. The obtained data, after the two preservatives quantification from the ointments were compared with the limits recommended by pharmacopoeia (tab. VI) (6, 7).

The experimental data shows us that the concentrations of oxi-acetyl mandelic acid and oxi-propionyl mandelic acid from the studied ointments are in the recommended ranges for ointments in pharmacopoeia (6, 7).

TABLE V

The calculated concentrations and recovery for the two preservatives

Oxi-acetyl mandelic acid				**Oxi-propionyl mandelic acid**			
Theoretic conc. mg/ml	**Absorbance**	**Calculated conc.**	**Recovery (%)**	**Theoretic conc. mg/ml**	**Absorbance**	**Calculated conc.**	**Recovery (%)**
1.1	0.7729	1.09	99.09	0.4	0.3998	0.4003	100.07
	0.7901	1.11	100.90		0.4049	0.4054	101.35
	0.7821	1.10	100,00		0.4001	0.4006	100.15
1.3	0.9312	1,30	100.00	0.6	0.5965	0.5953	99.23
	0.9265	1.29	99.23		0.6015	0.6003	100.05
	0.9335	1.31	100.76		0.5996	0.5984	99.74
1.5	1.0801	1.49	99.33	0.8	0.8024	0.7995	99.94
	1.0930	1.51	100.66		0.7998	0.7969	99.62
	1.0850	1.50	100.00		0.8112	0.8082	101.35
Statistical data		**Average**	**99.99**	**Statistical data**		**Average**	**100.16**
		SD	**0.72634**			**SD**	**0.727444**
		RSD %	**0.726367**			**RSD %**	**0.726234**

TABLE VI

The calculated concentrations and recovery for the two preservatives determination from ointments

Det. no.		**Theoretical conc. ± admissible deviation FRX**	**Calculated average conc. ± standard deviation**	**Recovery %**
1.	Oxi-acetyl mandelic acid	0.0125 ± 7.5%	0.01251 ± 0.0001	100.08
2.			0.01249 ± 0.0001	99.92
3.			0.01232 ± 0.0001	98.56
1.	Oxi-propionyl mandelic acid	0.05 ± 7.5%	0.0508 ± 0.0501	101.60
2.			0.0497 ± 0.0501	99.40
3.			0.0498 ± 0.0501	99.60

CONCLUSIONS

A simple, fast and sensitive UV-VIS spectrophotometric method was developed and validated for the assay of two new preservatives (oxi-acetyl mandelic acid and oxi-propionyl mandelic acid). The valida-

tion was performed according to validation guides. The developed and validated spectrophotometric method was applied to determine these two preservatives from pharmaceutical forms (ointments). The mean recovery in the ointments of the two preservatives was in the range of 98.56–101.60%.

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NEWS

RAMAN SPECTROSCOPY FOR DIFFERENTIATION OF CAUSATIVE AGENTS OF ONYCHOMYCOSIS

The discriminative power of Raman spectroscopy for differentiation of important dermatophytic and non-dermatophytic agents of onychomycosis in human nail was analyzed in a study by Smijs *et al.* An ex vivo nail model was used to determine the capacity of Raman spectroscopy to distinguish between *Trichophyton rubrum*, *T. mentagrophytes*, *T. tonsuran*s, *Scopulariopsis brevicaulis* and *Candida albicans*. For this purpose, Raman spectra (200/sample) were taken from 50-µm slices of infected nail using a 2500 High-Performance Raman Module and 785-nm diode laser. Analysis of spectra showed differences between the dermatophytes (with *T. rubrum* being most different) and between dermatophytes the non-dermatophytes *S. brevicaulis* and *C. albicans.* In conclusion, Raman spectroscopy allows for specific and non-invasive differentiation of causative organisms of onychomycosis (Smijs TG, Jachtenberg JW, Pavel S, Bakker-Schut TC et al. Detection and differentiation of causative organisms of onychomycosis in an ex vivo nail model by means of Raman spectroscopy. *J Eur Acad Dermatol Venereol*. 2013. doi: 10.1111/jdv.12324).

Teodora Vremera

SOME OBSERVATIONS ON THE OPTIMIZATION OF PRE-HOSPITAL EMERGENCY MEDICAL SERVICES FROM THE CITY OF IASI, ROMANIA

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SOME OBSERVATIONS ON OPTIMIZATION OF PRE-HOSPITAL EMERGENCY MEDICAL SERVICES FROM THE CITY OF IASI, ROMANIA (Abstract): In the Iasi City, with a population of 825,100 inhabitants, the number of requests for ambulance service has increased steadily for the past 17 years. One of the determining factors could be the development of the primary health care prevention. **Material and methods**: We take into study some factors which consider influencing the reorganization of emergency medical systems in the Iasi City territory. **Results**: According to the spatial analyzes, distribution of health units in Iasi City is concentrated type, developing in the downtown area, where there is also a high accessibility to health care services. There are some areas with a high population density, have low accessibility to hospital units, due to the presence of spatial network railroad as a spatial barrier, limiting interaction with other areas of the city. **Conclusions**: The analysis of optimization concept of Medical Services in a city like Iasi is about the challenge to understand the impact of the population health towards the development and organizing the territory, and, interrelated, how we can improve the population health by the best possible organization. **Keywords**: AMBULANCE SERVICE, HEALTCARE, EMMERGENCY CARE UNIT, CENTRAL PLACES THEORY

The evolution of the health status of the population is a phenomenon that could not be anticipated, but factors that influence it positively or negatively could be identified. Thus, situation is contradictory: although we live in a time when innovations in healthcare are increasingly diverse, living standards higher than that of previous periods, we are witnessing a growing demand for emergency medical services.

The Ambulance Service of Iaşi (A.S.I.) is a strategic medical unit, with legal personality and its specific work on standby 24 hours from 24, 365 days per year. A.S.I. is subordinated to the Department of Public Health Iasi. A.S.I. provides pre-hospital emergency care, both at application and during transport of patients (sick, injured, pregnant women) to the hospital. In addition, A.S.I. provides ambulatory care at the request, but also non-medical transportation. A.S.I. answers telephone calls coming from people and various medical units, with an average of 150 requests in 24 hours to about 4,500 requests a month, with a distribution of structurally relatively equal.

There is a higher share of major emergencies (disaster emergencies, collective and individual emergencies), followed by those of second degree and medical transport.

MATERIAL AND METHODS

Pre-hospital emergency medical services and medical transportation are provided of County Ambulance Service of Iasi (C.A.S.I.), for a population of 825,100 inhabitants (in 2008), of which 393,389 urban and 431,711 rural population. To better resolve requests, C.A.S.I. has organized ambulance substations in 9 towns in the county (Paşcani, Hîrlău, Tg. Frumos, Bivolari, Răducăneni, Podu Iloaie, Mircești, Vlădeni, Țibănaşi).

We take into study some factors which consider influencing the reorganization of emergency medical systems in the Iasi City territory.

RESULTS AND DISCUSSION

The number of requests for ambulance service has increased steadily for the past 17 years. One of the determining factors could be the development of the primary health care prevention.

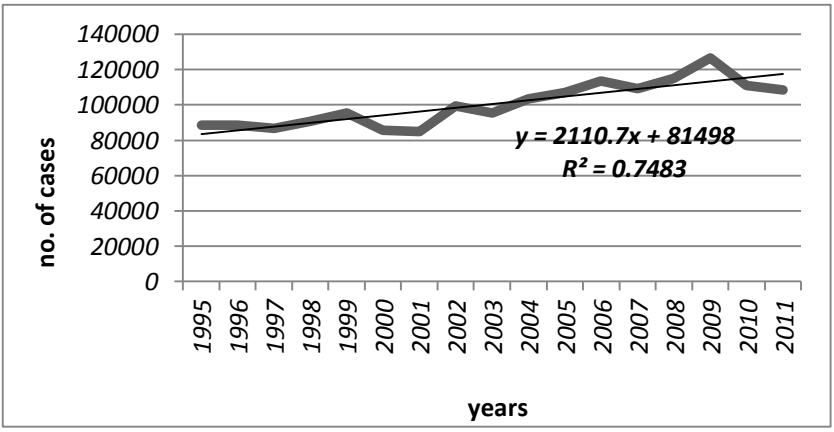

Fig. 1. Evolution of the number of requests in Iaşi (1995-2011) (source: C.A.S.I.)

TABLE I

The evolution of the ambulances number during the period 2006-2012 (source: C.A.S.I.)

Year	2006	2007	2008	2009	2010	2011	2012
C1/C2	2	2	2	3	3	3	4
B1/B2	14	16	19	19	17	17	18
A1/A2	10	11	9	9	8	8	8
Unclassified	16	16	10	10	8	8	8

The situation of fleet of ambulances is not good, according to European standards, if we consider the optimal number of ambulances reported to the population served (an ambulance with stretcher to 5000 inhabitants). Thus, at the end of May 2012, the situation was as follows: 38 ambulances, of which 4 are Resuscitation and Intensive Care Ambulances (C1/C2) and 18 are Emergency Ambulances and Resuscitation (B1/B2), 8 unassisted ambulance transport (A1/A2) and 8 unclassified (T1/T2) with crew formed by healthcare specific activity of 1, 2 or 3 people, including: physicians, nurses and ambulance drivers. These ambulances are sent to the area to resolve the 150 requests on average in 24 hours, a number that increases by 30-40% in case of outbreak (tab. I).

Force medical personnel consisted of 12 physicians, 121 nurses and 91 ambulance drivers (drivers trained to be the third person who fills specialized emergency response crew of the ambulance), at the end of May 2012. Program staff is working four shifts (day and night, 12-24, 12-48). Of the 15 hospitals of Iasi County, nine of them take major and second degree emergencies of the entire county, such as: "St. Spiridon" University Emergency Hospital, "St. Mary" Emergency Hospital for Children, "Elena Doamna" and "Cuza Voda" Obstetrics and Gynecology Hospitals, Clinic Hospital of Neurosurgery, "C.I. Parhon" Hospitay, TB Hospital, "St. Paraskeva" Infectious Diseases Hospital, "Socola" Psychiatric Hospital".

According to the spatial analyzes, distribution of health units in Iasi City is concentrated type, developing in the downtown area, where there is also a high accessibility to health care services (fig. 2, 3).

At first glance, the two maps do not differ too much. The focus of accessibility around of medical services is observed. This fact respects *Central Places Theory* conducted by Christaller and Lösch (central places are the result of conscious choices of places optimal location). Even if in these cases central places are not chosen by default, the hospitals focus only in a specific area of the city offers a high degree of vulnerability of most outlying addresses or buildings. Thus, in case of emergency, a large proportion of Iasi remains in areas which could consider not accessible or accessible to a longer time interval, comparatively with emergency medical facilities. In addition, the costs to serve that population are increased.

We measured ease of movement or reaching the place from the population addresses - barycenters buildings, respectively - to the nearest hospital. The situation would be different if we take into account that every hospital has one highly specialized profile. We have achieved this mapping by using the Network Analyst function. Neighborhoods in the northwest, west and southeast, such as Pacurari, Canta, Dacia, Nicolina, CUG, Bucium, with a high population density, have low accessibility to hospital units, due to the presence of spatial network railroad as a spatial barrier, limiting interaction with other areas of the city.

The problems of Pre-hospital Emergency Medical System (EMS). Despite the well-established structure of the Romanian health system (primary care, local clinics, hospitals, emergency care unit ECU, and pre-hospital medical units), in the emergency medical services gaps appear different from the education of the public, to the treatment and resolution of cases.

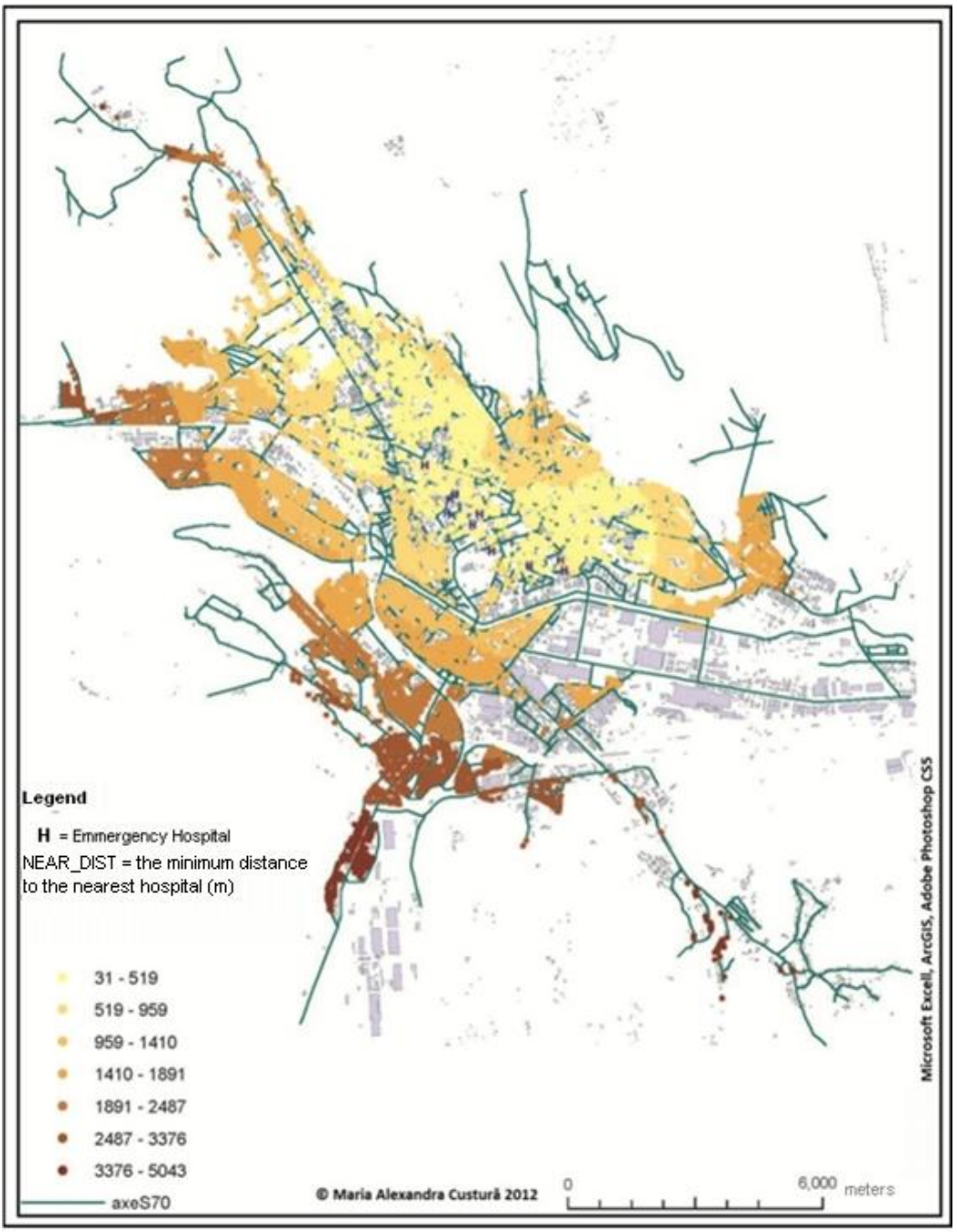

Fig. 2. Accessibility of public addresses to the nearest hospital (Iasi, Romania)

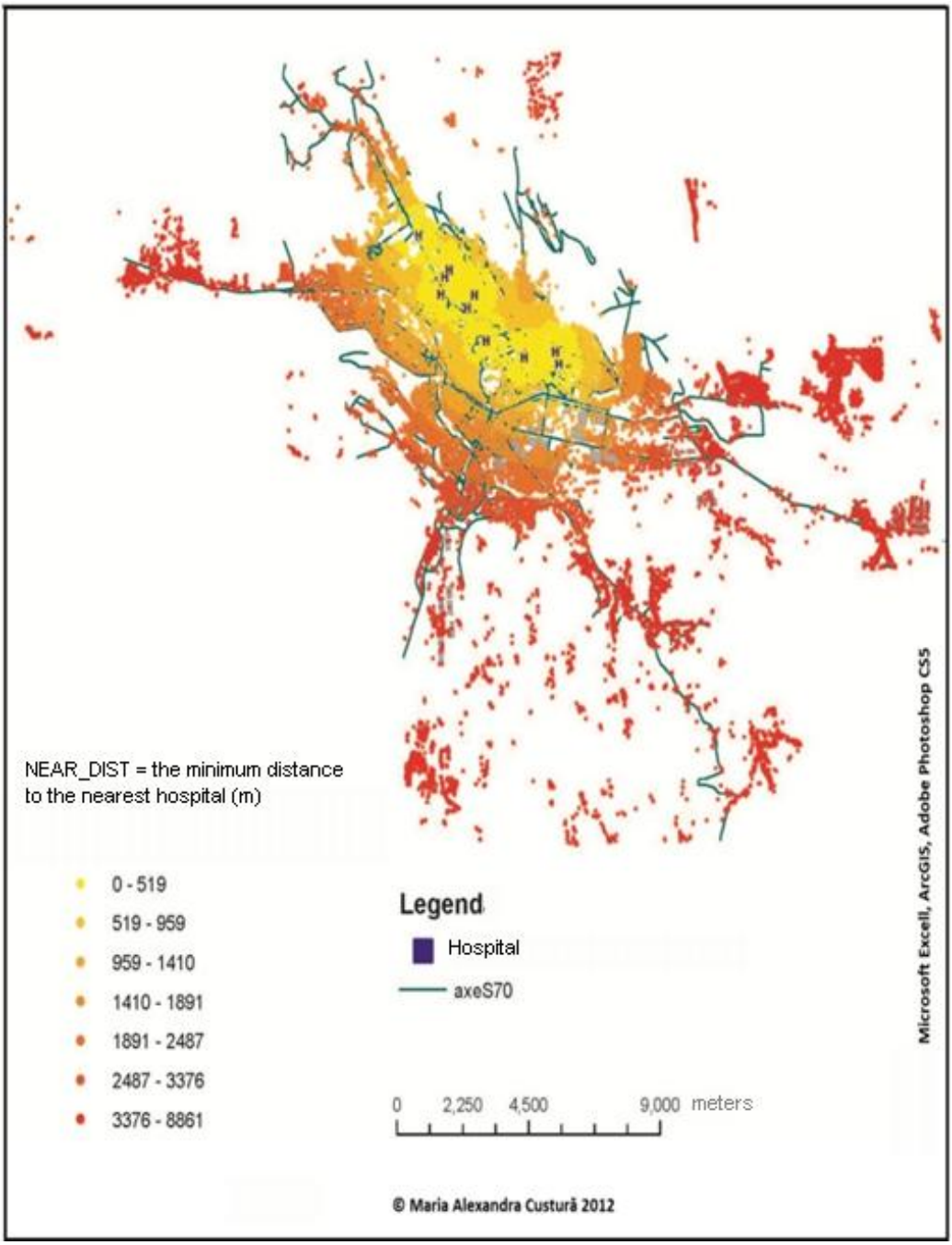

Fig. 3. Accessibility of central points of buildings (barycenters) to the nearest hospital (Iasi, Romania)

Education of the population is the way to prevent and treat some diseases, the attitude to the disease and how they require and expect to be given pre-hospital first aid. Western European countries have placed great emphasis on education of the population, leading to a better functioning of ECU and a growth of the population state of health (the prevention of major emergencies - secular people learning first aid medical).

In addition to public education, another problem is the lack of cases screening at the level of the primary health care, leading to overuse of pre-hospital emergency services for cases that can be resolved in family physician's office. The lack of a complex hospital unit where various cases could be admitted, fully equipped for diagnosis and treatment, leads to situations of patients' transfer, that endanger the state of it, and their resource consumption and wastage, both of those material and human.

Infrastructure is an impediment to the proper conduct of business by covering large distances, congestion because the permanent works to rehabilitate it. In addition to road infrastructure, that informational is insufficiently used. Another weakness of the Romanian medical system is the ratio between healthcare personnel and the deserved population, including the situation of the ECU also.

CONCLUSIONS

The concept of optimization underlies real need of improve Medical Services and, thus, the population welfare. Therefore, the analysis of this issue is about the challenge to understand the impact of the population health towards the development and organizing the territory, and, interrelated, how we can improve the population health by the best possible organization. This understanding requires not only the knowledge of the medical system problems or territory, but also the interdependencies that occur between the two.

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ANTIMICROBIAL RESISTANCE IN SEPTIC-PURULENT INFECTIONS

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ANTIMICROBIAL RESISTANCE IN SEPTIC-PURULENT INFECTIONS (Abstract): Evaluating the spread of antibiotic-resistant microorganisms from a multi-profile hospital. **Materials and methods:** There were analyzed 3940 strains of antibiotic-resistant microorganisms from patients with septic-purulent infections. **Results:** From the total, 51.44% samples of isolated strains showed resistance to antibiotics, including Gram negative (61.66%), and Gram positive ones (40.25%). A higher degree of resistance showed strains of *Acinetobacter spp*. (86.87%), *P. rettegeri* (83.40%) and *K. pneumoniae* (73.35%). Etiological structure of septic-purulent infections, as well as the degree of resistance, has proved to be varied, depending on the hospital profile unit. Isolated strains showed a higher resistance to penicillins and cephalosporins. **Conclusion:** Microorganisms isolated from patients with nosocomial septic-purulent infections have showed a higher resistance to antibiotics. **Keywords:** SEPTIC-PURULENT INFECTIONS, MICROORGANISMS, ANTIBIOTICS, RESISTANCE.

Nosocomial septic-purulent infections are characterized by poly etiological character, and highly resistant bacteria to antibiotics, due to uncontrolled use of antibiotics (1, 2, 3, 4, 5, 6).

Microorganisms with a high and broad spectrum of resistance to chemotherapeutics are involved in the etiological structure of nosocomial infections, thus requiring using reserve antibiotics or combinations of antibiotics, which are much more expensive. (4, 5, 7, 8). To develop the tactics of using antimicrobial drugs and the strategy of their purchase, our research aimed at accumulating information about the spread of antimicrobial resistance in some hospital settings.

MATERIAL AND METHODS

The paper refers to transversal epidemiological studies carried in 2010 based on the retrospective analysis of 3940 strains of microorganisms isolated from patients with septic-purulent infections (SPI) hospitalized in the National Center for Emergency Medicine. Study of antimicrobial resistance was performed by disk diffusion method and program WHONET – 5.6 was used for the epidemiological analysis.

RESULTS

The study revealed that the 3940 strains isolated from patients with SPI belonged to 51 species. This fact demonstrates the strong poly etiological character of this pathology, the Gram-positive microorganisms prevailing - 2237 (56.78%). From all strains, Gram-negative microorganisms represented 1624 (41.22%) and fungi constituted 79 (2.01%).

Our research showed the following predominance in the general etiological structure of SPI: *S. epidermidis* – 21.45%, *S. aureus* – 17.03%, *E. coli* – 11.14%, *E. faecalis* – 10.38%, *K. pneumoniae* – 5.36%, *P. aeruginosa* – 6.47%, *Acinetobacter sp*. – 3.07% and microorganisms from genus *Proteus* – 4.5%.

TABLE I

Etiological structure and antibiotic sensitivity / resistance of microorganisms isolated from patients with SPI

	Microorganisms (species)	Num. of strains	% from the group	% of total	Nr of antibiotic tests	Including the nr. of samples			
						sensitive		resistant	
						Nr.	%	Nr.	%
Gram-positive microoganisms	S. aureus	671	30.00	17.03	4132	2824	68.3	1308	31.6
	S. epidermidis	845	37.77	21.45	5192	2911	56.1	2281	43.9
	S. mutans	86	3.84	2.18	524	375	71.5	149	28.4
	S. pyogenes	121	5.41	3.07	732	581	79.4	151	20.6
	S. viridans	55	2.46	1.40	332	237	71.4	95	28.6
	E. faecalis	409	18.28	10.38	2241	922	41.1	1319	58.8
	E. faecium	14	0.63	0.36	80	58	72.5	22	27.5
	Other microorg.	36	1.61	0.91	234	139	59.4	95	40.6
	Total Gram pos.	**2237**	**100**	**56.78**	**13466**	**8046**	**59.7**	**5420**	**40.2**
Gram-negative microoganisms	Acinetobacter sp.	121	7.45	3.07	1295	170	13.1	1125	86.8
	E. coli	439	27.03	11.14	4131	2063	49.9	2068	50.1
	E. aerogenes	100	6.16	2.54	810	259	32.0	551	68.0
	E. cloacae	105	6.47	2.66	815	331	40.6	484	59.4
	K. oxytoca	30	1.85	0.76	269	109	40.5	160	59.5
	K. pneumoniae	211	12.99	5.36	1951	520	26.6	1431	73.3
	P. aeruginosa	255	15.70	6.47	1985	805	40.5	1180	59.4
	C. freundii	55	3.39	1.40	435	223	51.2	212	48.7
	C. diversus	10	0.62	0.25	96	51	53.1	45	46.8
	M. morganii	38	2.34	0.96	299	160	53.5	139	46.5
	P. mirabilis	89	5.48	2.26	855	302	35.3	553	64.6
	P. rettgeri	44	2.71	1.12	398	66	16.6	332	83.4
	P. vulgaris	44	2.71	1.12	385	195	50.6	190	49.3
	N. perflava	20	1.23	0.51	91	49	53.8	42	46.1
	P. alcalifaciens	12	0.74	0.30	116	8	6.9	108	93.1
	S. marcescens	34	2.09	0.86	257	108	42.0	149	58.0
	Other microorg.	17	1.05	0.43	98	59	60.2	39	39.8
	Total Gram neg.	**1624**	**100.0**	**41.22**	**14286**	**5478**	**38.3**	**8808**	**61.6**
Fungi	C. albicans	63	79.75	1.60	326	113	34.6	213	65.3
	C. krusei	12	15.19	0.30	65	31	47.7	34	52.3
	Other species	4	5.06	0.10	21	7	33.3	14	66.7
	Total fungi	**79**	**100.0**	**2.01**	**412**	**151**	**36.6**	**261**	**63.3**
	Total	**3940**	**100.0**	**100.0**	**28159**	**1367**	**48.5**	**1448**	**51.4**

Etiological structure of SPI in relation to the hospital profile of departments has proved to be varied. Gram-positive strains were predominant (52.38%, 72.69%, 94.57%, 69.33%) in trauma, maxillo-facial surgery, neurology and cerebrovascular diseases departments. In general surgery (septic and aseptic), anesthesiology, reanimation and intensive care unit, urology and neurosurgery departments Gram-negative strains were predominant (56.92%, 53.22%, 65.08%, 54.55%, 55.77%) (tab. II).

Analysis of antibiotic resistance of isolated strains (tab. I) revealed that from the overall testing (28159), it was recorded resistance to antibiotics in 14484 samples (51.44%). Gram negative microorganisms showed a higher degree of resistance (61.66%) compared to Gram positive ones (40.25%).

A higher degree of antibiotic resistance exhibited *E. faecalis* strains (58.86%) and *S. epidermidis* (43.90%). The majority of Gram-negative strains had an increased resistance to antibiotics, especially *Proteus, Enterobacter, Klebsiella and Pseudomonas*. *Acinetobacter sp*. have proved to be the most resistant strains (86.87%).

Analysis of antibiotic resistance of strains in relation to the group of antibiotics showed a higher resistance to penicillins (65.40%), cephalosporins (79.40%), including the first generation cephalosporins (74.76%), second generation (93.30%), third generation (73.80%) and fourth generation (85.50%). Sensitivity of microorganisms to these groups of antibiotics constituted: 34.60%, 20.60%, 25.24%, 6.70%, 26.20% and 14.50%, respectively.

TABLE II

Structure of isolated microorganisms from patients with septic-purulent infections in relation to the profile hospital unit

Micro organism species	Indices	*Asep. surg.	Sep. surg.	Asep. tr.	Sep. tr.	ICU	Urol.	MFS	CVD	Neur. surg.	Other departments
Tot. Gram+ microorg.	Nr.	55	494	110	527	122	86	174	52	22	35
	%	42.31	46.04	52.38	72.69	32.28	43.43	94.57	69.33	42.31	53.84
Tot. Gram- microorg.	Nr.	74	571	97	196	246	108	8	22	29	26
	%	56.92	53.22	46.19	27.03	65.08	54.55	4.35	29.33	55.77	40.00
Total fungi	Nr.	1	8	3	2	10	4	2	1	1	8
	%	0.77	0.75	1.43	0.28	2.65	2.02	1.09	1.33	1.92	12.31
Tot. microorg. strains	Nr.	130	1073	210	725	378	198	184	75	52	65
	%	100	100	100	100	100	100	100	100	100	100

*__Legend:__

Asep. surg – aseptic surgery; Sep. surg – septic surgery; Asep tr. –aseptic trauma; Sep. tr.– septic trauma; ICU – intensive care unit; Urol – urology; MFS – maxilo facial surgery; CVD – cerebrovascular diseases; Neur. surg. – neurosurgery.

Gram-negative microorganisms showed a higher resistance compared to Gram-positive ones (61.66%), especially to penicillins (79.78%), cephalosporins of I, II and IV generations - (89.60%, 93.60% and 85.40%) and were sensitive to penems (81.14%), aminoglycosides (51.60%) and quinolones (54.80%). Gram-positive microorganisms isolated from patients with SPI also showed a higher resistance to penicillins (57.47%), cephalosporins (65.85%), cephalosporins of I, II and IV generations (66.10%, 84.40%, 87.50%), and a higher sensitivity to aminoglycosides (87.65%), quinolones (77.94%), penems (61.54%) and oxazolidinones (93.10%) (tab. III, fig. 2).

Analysis of resistance in relation to species of the isolated strains (fig. 2 and 3). Of *S. aureus* strains, 64.70% were resistant to cephalosporins (64.70%), especially II (87.50%) and IV generation (80.00%). A higher sensitivity was recorded to aminoglycosides (90.36%), oxazolidinones (94.90%) and quinolones (85.87%).

Strains of *S. epidermidis* showed a higher resistance to penicillins (61.97%), second generation cephalosporins (82.60%), first generation cephalosporins (66.40%), IV generation cephalosporins (65.20%), also to macrolides (53.95%) and glycopeptides (60.0%), and an increased sensitivity to penems (88.24%), aminoglycosides (86.45%) and quinolones (71.92%).

Strains of *S. pyogenes* showed an obvious resistance to cephalosporins (85.70%) and a higher sensitivity to quinolones (96.70%), lincosamides (92.60%), macrolides (70.70%) and oxazolidinones (93.80%).

Strains of *E. faecalis* have revealed a higher resistance to penicillins (67.92%), lincosamides (76.70%), macrolides (68.90%) and glycopeptides (68.30%). These organisms werealso sensitive to beta-lactamase +inhibitors (78.80%), penems (87.50%), nitrofurans (80.00%) and oxazolidine (80.80%).

Acinetobacter sp. strains showed an increased resistance to quinolones (81.60%), aminoglycosides (72.80%), cephalosporins of all generations (98.30%), especially to III generation (97.85%) and II generation (97.80%) and to penicillins (95.30%). These strains showed sensitivity only to penems (47.50%).

E. coli strains have also proved to be highly resistant, especially to penicillins (73.90%), beta-lactam+inhibitor (66.70%), cephalosporins (73.00%), monobactams and tetracyclines (100.0%), and sensitive to penems (85.70%), aminoglycosides (62.40%) and quinolones (75.26%).

Strains of *E. aerogenes* have proved to be highly resistant to penicillins (88.30%), cephalosporins (87.50%), aminoglycosides (66.05%) and sensitive to penems (91.70%), nitrofurans (57.10%) and quinolones (47.80%).

E. cloacae strains showed resistance to penicillins (80.43%), cephalosporins II generation (90.0%), cephalosporins III generation (80.70%), aminoglycosides (51.15%) and sensitivity to penems (90.0%), quinolones (56.80%) and phenicols (61.10%).

Strains of *K. pneumoniae* have proved to be resistant especially to penicillins (97.10%), cephalosporins of all generations (92.95%), especially to cephalosporins I and II generations (96.10% and 97.70%), nitrofurans (83.30%), quinolones (59.80%) and sensitive to aminoglycosides (40.60%) and phenicols (51.80%).

From all strains of *P. aeruginosa*, resistant to penicillins have proved to be 77.90%, to cephalosporins (95.90%), to monobactams (97.00%), to nitrofurans (66.70%) and sensitive to penems (85.65%) and aminoglycosides (53.68%).

TABLE III

Evaluation of the sensitivity / resistance of microorganisms isolated from patients with SPI in relation to the group of antibiotics

Group of antibiotics	Microorganisms								Total			
	Gram positive				Gram negative							
	S		R		S		R		S		R*	
	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%	Nr.	%
Penicillins	894	42.53	1208	57.47	235	20.22	927	79.78	1129	34.60	2135	65.40
Beta-lactam+Inhib.	544	81.40	124	18.60	41	16.70	204	83.20	585	64.00	328	36.00
Cephalosporins I gen.	485	33.90	946	66.10	87	10.40	748	89.60	572	25.24	1694	74.76
Cephalosporins II gen.	7	15.60	38	84.40	90	6.40	1319	93.60	97	6.70	1056	93.30
Cephalosporins III gen.	78	41.50	110	58.50	469	24.70	1431	75.30	547	26.20	1541	73.80
Cephalosporins IV gen.	1	12.50	7	87.50	42	14.60	246	85.40	43	14.50	253	85.50
Total Cephalosporins	571	34.15	1101	65.85	688	15.52	3744	84.48	1259	20.60	4845	79.40
Monobactams	-	-	-	-	4	2.10	184	97.90	4	2.10	184	97.90
Penems	32	61.54	20	38.46	327	81.14	76	18.86	359	78.90	96	21.10
Aminoglycosides	1394	87.67	196	12.33	1704	51.60	1600	48.40	3098	63.30	1796	36.70
Quinolones	1544	77.94	437	22.06	2016	54.80	1662	45.20	3560	62.90	2099	37.10
Lincosamides	878	60.00	585	40.00	-	-	-	-	878	60.00	585	40.00
Macrolides	1779	54.45	1488	45.54	10	28.60	25	71.40	1789	54.20	1513	45.80
Nitrofurans	7	87.50	1	12.50	36	35.30	66	64.70	43	39.10	67	60.90
Oxazolidinones	122	93.10	9	6.90	-	-	-	-	122	93.10	9	6.90
Glycopeptides	257	51.40	243	48.60	-	-	-	-	257	51.40	243	48.60
Phenicols	19	79.20	5	20.80	417	57.80	305	42.20	436	58.40	310	41.50
Tetracyclines	5	62.50	3	37.50	-	-	10	100.0	5	27.80	13	72.20
Antifungals	-	-	-	-	-	-	-	-	151	36.65	261	63.35
Total	**8046**	**59.75**	**5420**	**40.25**	**5478**	**38.34**	**8808**	**61.66**	**13676**	**48.60**	**14484**	**51.40**

* S = sensitive; R = resistant

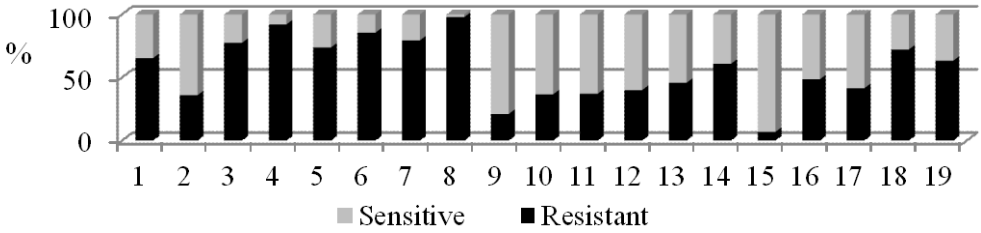

Fig. 1 Antimicrobial resistance / sensitivity of strains isolated from patients with SPI in relation to antibiotics group.

1) Penicillins; 2) Beta-lactam+Inhib.; 3) Cephalosporins I gen.; 4) Cephalosporins II gen..;5) Cephalosporins III gen.; 6) Cephalosporins IV gen.; 7) Total Cephalosporins; 8) Monobactams; 9 Penems; 10) Aminoglycosides; 11) Quinolones; 12) Lincosamides; 13) Macrolides; 14) Nitrofurans; 15) Oxazolidinones; 16) Glycopeptides; 17) Phenicols; 18) Tetracyclines; 19) Antifungals.

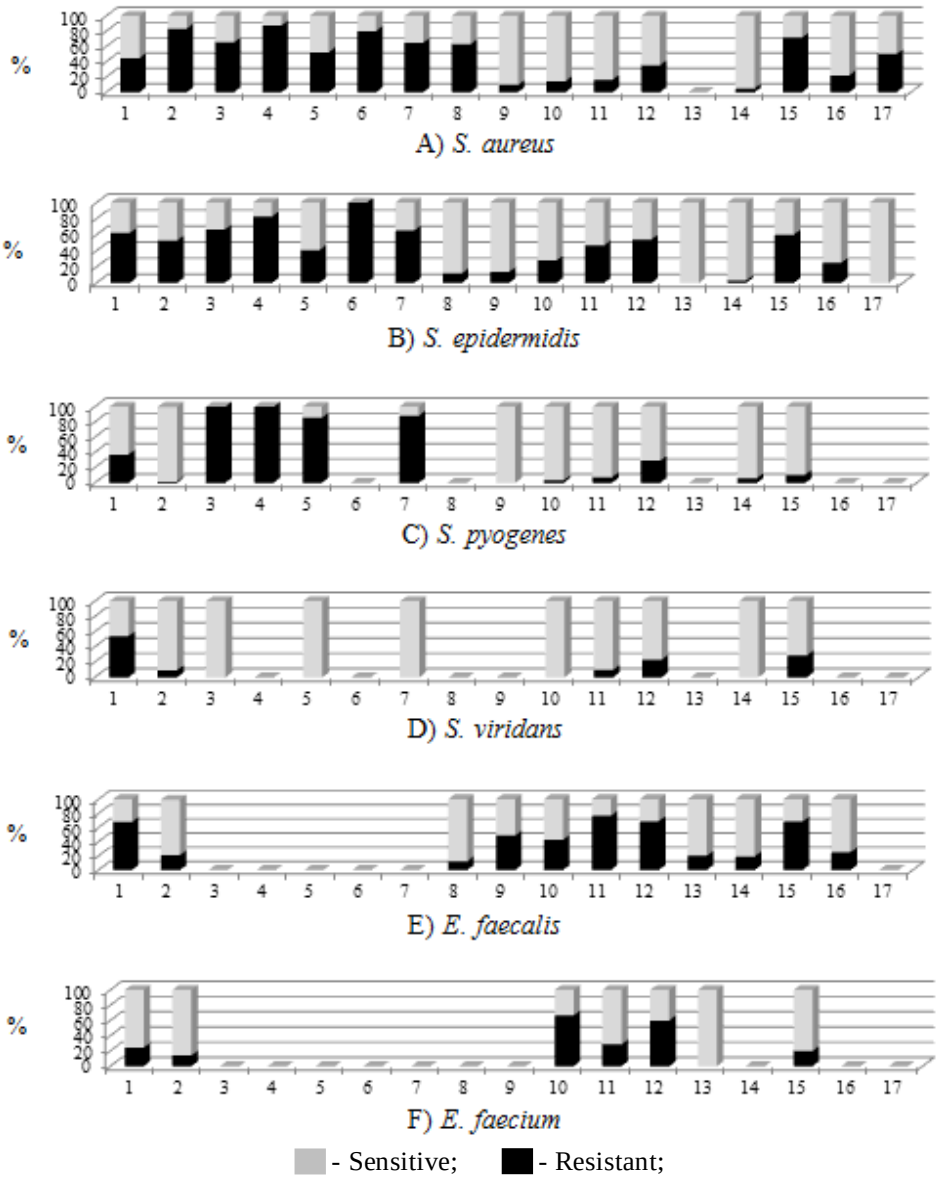

Fig. 2. Sensitivity / resistance of Gram positive microorganisms in relation to the type of microorganisms (A) *S. aureus*, B) *S. epidermidis*, C) *S. pyogenes*, D) *S. viridans*, E) E. faecalis, F) *E. faecium*) and group of antibiotics – 1) Penicilins, 2) Beta-lactam+inhibitor, 3) Cefalosporins I gen., 4) Cefalosporins II gen., 5) Cefalosporins III gen., 6) Cefalosporins IV gen., 7) Total Cefalosporins, 8) Penems, 9) Aminoglycosides, 10) Quinolones, 11) Lincosamides, 12) Macrolides, 13) Nitrofurans, 14) Oxazolidinones, 15) Glycopeptides,
16) Phenicols, 7) Tetracyclines.

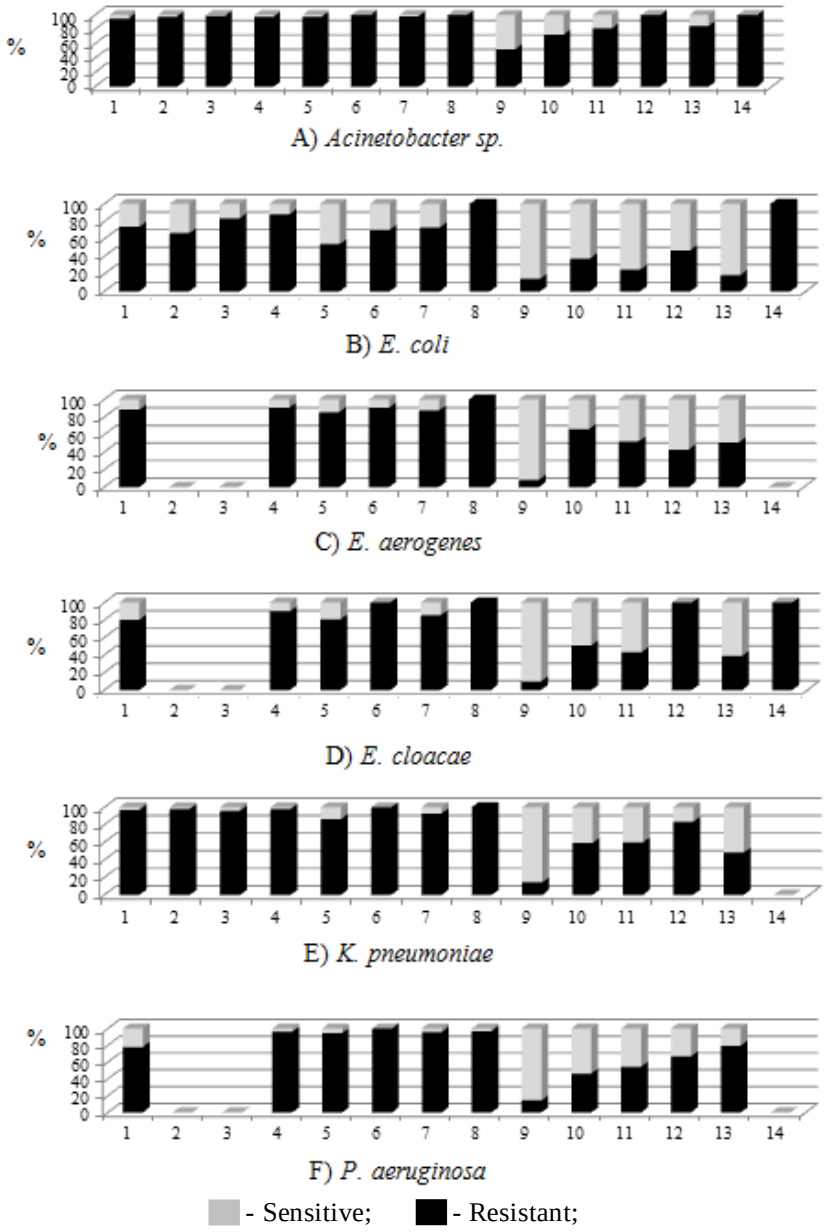

Fig. 3. Sensitivity / resistance of Gram negativemicroorganismsin relationto the type of microorganisms
(A) *Acinetobacter sp*., B) *E. coli*, C) *E. aerogenes*, D) *E. cloacae*, E) *K. pneumoniae*, F) *P. aeruginosa)*and group of antibiotics1)Penicilins, 2) Beta-lactam+inhibitor, 3) Cefalosporins I gen., 4) Cefalosporins II gen., 5) Cefalosporins III gen., 6) Cefalosporins IV gen., 7) Total Cefalosporins, 8) Monobactams, 9) Penems, 10) Aminoglycosides, 11) Quinolones, 12) Nitrofurans, 13) Phenicols, 14) Tetracyclines.

From the strains of the genus *Proteus*, a higher resistance was recorded at *P. rettegeri*, especially to penicillin (96.67%), quinolones (81.90%), cephalosporins (96.26%), aminoglycosides (71.90%), and sensitivity to penems (61.54%).

Strains of *S. marcescens* have shown a high resistance, especially to penicillins (80.00%), cephalosporins (86.60%), the second generation cephalosporins (95.65%), and had sensitivity to penems (50.00%), aminoglycosides (75.80%) and quinolones (65.30%).

CONCLUSION

Our research showed that septic-purulent infections were characterized by etiologic polymorphism and resistance to antibiotics, especially in Gram negative (61.66%) compared to the Gram-positive ones (40.26%). The most resistant to antibiotics have proved to be *Acinetobacter sp.* (86.87%), *P. rettegeri* (83.40%), *K. pneumoniae* (73.35%), *P. aeruginosa* (60.0%) and *S. marcescens* (58.0%). Strains isolated from patients with SPI showed high resistance to penicillins and cephalosporins, which are the antibiotics most commonly used in medical practice, which resulted in a permanent monitoring of the antibiotic resistance and administration of antibiotics in relation to the antibiogram results.

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MONOAMINE OXIDASE - A INHIBITION REVERSES ENDOTHELIAL DYSFUNCTION IN HYPERTENSIVE RAT AORTIC RINGS

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MONOAMINE OXIDASE-A INHIBITION REVERSES ENDOTHELIAL DYSFUNCTION IN HYPERTENSIVE RAT AORTIC RINGS (Abstract). **Aim.** Monoamine oxidases (MAOs) are mitochondrial enzymes, with 2 isoforms, A and B that convert biogenic amines to their corresponding aldehydes via a reaction that produces hydrogen peroxide. Since MAO-A is the predominant form at vascular level we hypothesized that MAO-A-dependent H_2O_2 production may contribute to the development of endothelial dysfunction and, MAOs inhibition could improve the vascular function, respectively. **Material and methods.**To this aim aortic rings were isolated from female adult spontaneously hypertensive rats (SHR) and their corresponding (Wistar-Kyoto) controls. The effect of MAO-A inhibitor, clorgyline (10µmol/l) on endothelium-dependent relaxation (EDR) in response to acetylcholine and endothelium-independent relaxation in response to sodium nitroprusside, was studied in isolated phenylephrine-preconstricted aortic segments in the presence of indometacine (10µmol/l). **Results.**In hypertensive group EDR was significantly decreased – maximal relaxation (% of KCl, mean±SD) being 37±3.5 in SHR vs. 3.7±1.8 in controls. If experiments were done in the presence of clorgyline, EDR in control segments was unaffected. However, the compound restored normal EDR in aortic segments from hypertensive rats (maximal relaxation - % of KCl, 13.7±2.3). **Conclusions**. Inhibition of the MAO-A isoform might be useful in restoring endothelium-dependent relaxation in this experimental model of hypertension in rat.
Keywords: ENDOTHELIAL DYSFUNCTION, EXPERIMENTAL HYPERTENSION, MONOAMINE OXIDASE.

Cardiovascular disease is a leading cause of morbidity worldwide and endothelial dysfunction (ED) represents both a widely investigated underlying mechanism and a therapeutic target. Accumulated lines of evidence indicate that increased production of reactive oxygen species (ROS) significantly contributes to ED pathogenesis. Four enzymatic systems have been systematically investigated as major sources of ROS generation by vascular cells: NAD(P)H oxidases, xanthine oxidase, uncoupled eNOS and mitochondrial respiratory chain (1). However, recent data have incriminated monoaminooxidases (MAO) with two functional isoforms, A and B, as another important source of deleterious hydrogen peroxide (H_2O_2) production (2). In particular, increased activity of myocardial MAO-A has been reported to contrib-

ute to ventricular dysfunction associated with postischemic reperfusion, and also to the maladaptative hypertrophy within the evolution of heart failure (3). Moreover, with respect to vasculature, MAO-A mediated ROS production has been shown to induce mitogenic signaling in smooth muscle cells by a process that may involve the activation of matrix metalloproteinase 2, which likely contributes to vascular wall remodeling (4). Enhanced MAO-A protein expression and consecutive generation of H_2O_2 in the cerebral arteries have been also demonstrated to be responsible for the exaggerated *in vitro* tension development induced by serotonin, in the isolated basilar arteries of SHR (5).

The unfavorable effects of MAO activation are antagonized by a couple of available selective MAO inhibitors (MAOI) that can be divided in: i) irreversible MAOI, such as clorgyline for MAO-A and selegiline for MAO-B and ii) reversible MAOI, such as moclobemide for MAO-A and lazabemide for MAO-B, respectively (6).

In the light of these new findings that consider MAO an important source of H_2O_2 not only in the brain, as widely recognized (7) but also in the heart, the aim of the present study was to determine whether inhibition of MAO-A, the predominant isoform in the cardiovascular system, could improve the vascular relaxation in hypertensive and normal rat vessels. We hypothesized that, similarly to ischemia/reperfusion injury and hypertrophy, the activity of this enzyme is increased in pathological conditions associated with ED, such as experimental hypertension, with the subsequent generation of a large amount of H_2O_2, that might cause a decrease in nitric oxide (NO); in this respect, MAO inhibition might correct the endothelial dysfunction by improving the NO-dependent vasodilatation.

MATERIAL AND METHODS

All experimental procedures used in this study were conducted in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The experimental protocol was approved by the Ethics Committee of the University for Medicine and Pharmacy of Timişoara, Romania.

Animals were fed *ad libitum* and housed under standard conditions (constant temperature and humidity of 22.5 ± 2° C and 55 + 5%, 12-h light/dark cycle). Twenty-four hours prior to the experiment solid food was withdrawn with no limitation in water supply.

Experiment protocol. Experiments were performed in vascular preparations (thoracic aortas) isolated from SHR and control female rats weighing 250-350 g (n = 6/group). The arterial segments were carefully cleared of connective tissue to not damage the intimal surface and cut into 2-3 mm wide rings. The rings were suspended between two parallel stainless steel hooks in two individual jacketed organ baths containing Krebs-Henseleit buffer (composition in mM: NaCl 118, KCl 4.7, CaCl2 2.5, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25, and glucose 11.1), gassed with a mixture gases of 95% O2 and 5% CO2 and maintained at 37°C. The upper hook was connected to a force transducer for isometric tension recording. The amplified analog signal from the force transducer was digitized using a data acquisition system (Digidata 1200B, Axoscope 10, and Molecular Devices Ltd).

The rings were equilibrated for 60 minutes at 37°C under 1.75 cN passive tension and the buffer was replaced every 15 min. To confirm the viability of vascular smooth muscle, vessel rings were contracted twice with KCl. A relaxation re-

sponse to acethylcholine, an endothelium-dependent vasodilator, of 15% or more from the stable tension induced by KCl was considered as functional endothelium. After washout and return to baseline the tissues were pre-contracted with 10-5 M phenylephrine (PE). Cumulative concentration-response curves (10^{-9} to 10^{-5} M) for: acetylcholine (ACh), as endothelial-dependent response, and sodium-nitroprusside (SNP), as endothelial-independent response, were recorded in the absence vs. the presence of MAO-A inhibitor (Clorgyline, 10^{-5} M). Indometacin (10^{-5} M), as inhibitor of cyclooxygenase, was present in the organ baths throughout the experiments in order to eliminate the influence of prostaglandine synthesis on vasodilator response.

Relaxation response of each vascular ring was assessed by measuring the reduction in vascular tone at cumulative doses of the vasodilator agent and expressed as percentage change from the stable tension produced by PE.

Statistical analysis. Acquired data were statistically processed using *GraphPad Prism 5* and *Microsoft Office Excel 2003* software (Microsoft Corporation). Central tendencies of the variables obtained from n different rings were expressed as mean (M) and the dispersion as the standard deviation (SD). Statistical comparison between two groups of averages was performed using t test.

In order to assess overall vascular reactivity, we chose to calculate the following variables in the equation for a Hill-type sigmoidal relationship: i) the estimated maximum response, ii) the concentration required to achieve 50% of maximum response (EC50 (-log [M]) and the iii) dose-response slope. In order to compare the dose - response curves, F test was used.

RESULTS

The rings isolated from SHR develop a pronounced attenuation of endothelium dependent-relaxation (EDR). In control segments, EDR was unaffected in the presence of the MAO-A inhibitor, clorgyline (10μmol/l). In contrast, the irreversible MAOI partially restored normal EDR in aortic rings harvested from SHR rats (fig. 1).

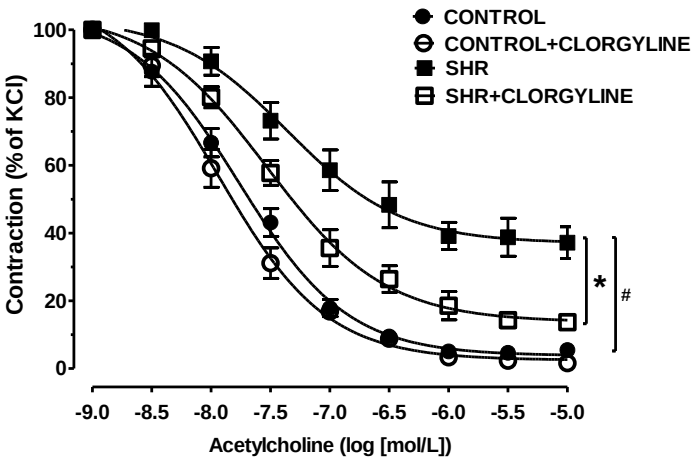

Fig.1. Acethycholine dose-response curve of aortic rings in SHR vs. CONTROL in the presence vs. the absence of the MAO A inhibitor, clorgyline (10 μmol/l) (*, # $p<0.0001$)

The relaxation induced by sodium nitroprusside was similar in both experimental groups, regardless the presence or the absence of clorgyline (fig. 2).

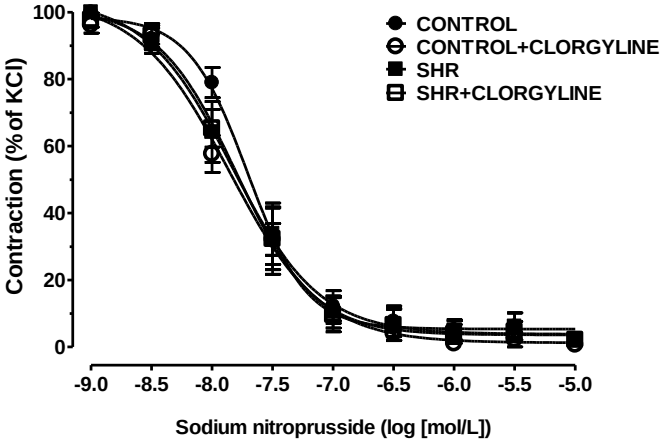

Fig. 2. Sodium nitroprusside dose-response curve of aortic rings in SHR *vs.* CONTROL, +/- clorgyline(10 μmol/l) in the presence of indometacin (10 μmol/l).

Preincubation with L-NAME, a potent eNOS inhibitor caused a significant reduction in relaxation response in SHR group as compared to control; also, relaxation in the rings from SHR group treated with clorgyline was improved as compared to the non-treated rings. This observation suggests that the mechanism underlying the improvement of relaxation could be NO-dependent (fig. 3).

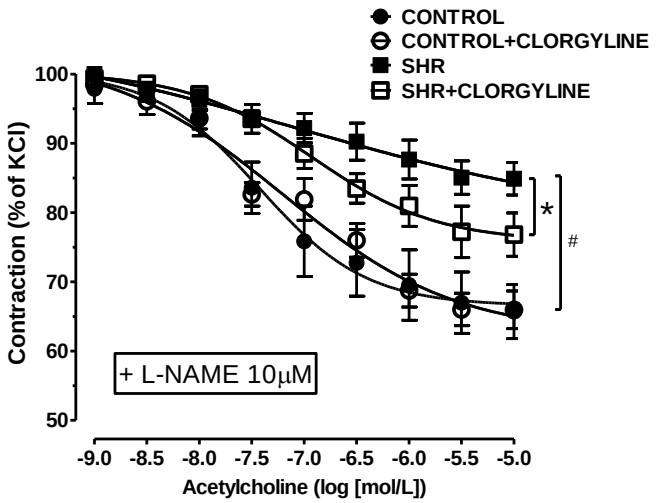

Fig. 3. Acetylcholine dose-response curve in aortic rings from SHR *vs.* CONTROL, +/- clorgyline (10 μmol/l) in the presence of L-NAME (10 μmol/l) and indometacin (10 μmol/l). (*, # $p<0.001$)

DISCUSSION

Increased production of radical species has been constantly associated to various pathologies, including hypertension, atherosclerosis, diabetes (8, 10). ROS-induced endothelial dysfunction is a well establishedpathomechanism but currently this phenomenon was attributed to the increased formation of the highly reactive superoxide anion (11), not to H_2O_2 with the former being considered the only responsible for scavenging NO (with the generation of peroxynitrite required for eNOS uncoupling). However, since hydrogen peroxide is freely diffusible through cellmembranes, it can further activate inflammation, induce apoptosis and promote endothelial dysfunction (12).

The roles of MAOs, in terminating the action of neurotransmitters in the central and peripheral nervous system and in the oxidation of dietary amines in extraneuronal tissues have been extensively studied (6,7). Thus, it has been shown that in vitro or in vivo application of L-deprenyl, an irreversible MAO-B inhibitor, produced vasodilatation via an increase in the amount of NO in brain tissue and cerebral blood vessels (13). NO inhibits mitochondrial monoamine oxidase activity and decreases outer mitochondria membrane fluidity, yet, the mechanism by which NO inhibits MAO remains unknown at present. However, it seems likely that the effect of NO on MAO activity is by a direct interaction of the compound or a metabolite with the protein (14, 15).

In the present study we focused on the role of MAO-A in the development of endothelial dysfunction in an experimental model of genetically induced hypertension. Ex vivo blockade of MAO A with clorgyline improved endothelium-dependent relaxation in vessels from hypertensive animals. Of note, this effect was not present in vessels isolated from the control animals. This observation strongly suggests that inhibition MAO activity in basal conditions do not interfere with vascular reactivity, most probably due to minimal generation of H_2O_2.

However, catabolism of monoamines results not only in H_2O_2, but also in production of ammonia, a much less investigated by-product (3). Generation of NH_3 could also explain the deleterious effects of MAO activation in vascular cells. NH_3 is known to be a mediator of cerebral vascular dysfunction in hepatic encephalopathy. Ammonia stimulated the formation of ROS by cerebral endothelial cells (16, 17) and reduction of ammonia levels in a liver failure model, improved endothelial function (18).

The present study has at least two limitations: first, MAO activity and H_2O_2 generation were not quantified and the experiments were restricted to the functional analysis of isolated vessels without addressing the effects of *in vivo* MAO inhibition. However, systemic MAO inhibition in this model is not suited to address the role of MAO in endothelial function. As MAOs are a main pathway of catecholamine degradation, the MAO inhibitor-induced accumulation of these vasoactive compounds may increase the already present hypertensive response and further promote vascular dysfunction (19). Also, it must be pointed out that information concerning the pharmacokinetics of MAO inhibitors is quite scarce. For instance, it is not clear whether the plasma concentrations achieved in patients treated for neurological disorders are in the range of the dosages tested in this study. Systematic pharmacokinetic studies

are undoubtedly necessary to establish the dose regimen necessary to achieve and maintain the concentrations of the circulating compounds in the micromolar range.

MAO inhibitors are currently used in psychiatry and neurology for the treatment of anxiety and depressive disorders, Parkinson's and Alzheimer's diseases, respectively (6). Therefore, no major problem should exist for their introduction in clinical studies concerning heart disease. Of note, MAO inhibition was already used in the late '60s in the treatment of angina pectoris (20, 21), although obviously the involvement of mitochondria and ROS generation was not even in its infancy. In a recent paper, Li et al demonstrated a therapeutic effect of an antidepressant medication (selective serotonin uptake inhibitor) in ameliorating experimental heart failure (22). If evidence will be provided for common novel pathways contributing to these co-morbid pathologies maybe, indeed, in the future antidepressants will be able to heal both minds and hearts (23). In this light, further studies addressing the potential role of MAO inhibitors in reversing an impaired vascular dysfunction are clearly warranted.

CONCLUSIONS

Inhibition of MAO-A improved the vasodilator response to Ach in hypertensive rat aortas whereas no effect was observed in control animals. Therefore, the MAO-A inhibitor, clorgyline, might be useful in restoring endothelium-dependent relaxation in situations of increased vascular oxidative stress and endothelial dysfunction.

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NEUROSURGICAL APPROACH IN ORBITAL PATHOLOGY - UNICENTRIC STUDY

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NEUROSURGICAL APPROACH IN ORBITAL PATHOLOGY - UNICENTRIC STUDY (Abstract): Orbital pathology represents a unique entity because is located in a border region that requires neurosurgery, ophthalmology and oncology collaboration. The modern surgery includes the orbital surgery and skull base surgery. **Material and Methods:** This study presents neurosurgical approaches in 17 cases of orbital pathology in a period of 4 years in the Neurosurgery Department of Emergency Hospital "Prof. Dr. N. Oblu" Iaşi. In most cases our team performed a fronto-temporal and pterional approach with orbital roof resection and tumor ablation. In our study there were 10 women (58.8%) and only 7 men (41.1%). 16 patients presented with exophthalmia, head each (8 patients), diplopia (3 patients), amblyopia (4 patients), ptosis (1 patients), rinoreea (1 patients) and nerveVII paresis (2 patients). **Results:** Postoperative there were remissions of exophthalmia and the other symptoms and there were not registered any complications**. Conclusions**: However the technique and cranial approach can be evaluated on case-by-case situation and depends on the location and extension of the lesion. The goals were patient safety, symptoms remission and tumor removal. **Keywords:** ORBIT, TRANSCRANIAL APPROACH, EXOPHTHALMOLOS.

Over a 4 years period there were admitted 17 patients with intra-orbital pathology and they went under surgery with microsurgery or macro surgery ablation of the lesions. In 15 cases our team performed an intracranial approach to the orbital region and in 2 cases an anterior approach to the lesion. The objectives were total removal of the tumor and symptoms remission.

MATERIAL AND METHODS

The study presented 17 patients with orbital tumors diagnosed on CT and MRI scan since 2007 until the end of 2010 with 10 women and 6 men with ages between 12-77 years old. All except one patient presented with exophthalmia. In most cases our team performed a pterional approach with frontal or orbital extension or both (7 cases) and a fronto-temporal approach with orbital extension (8 patients). For the other 2 cases an anterior approach was used. In one of these cases, a patient presenting with rinoreea, a median trans-facial ap-

proach with Le Fort osteotomy was made.

For the pterional approach the patients were positioned in supine position with the head tilted backwards by 20 degrees and turned 30-35 degrees to the lateral. Skin incision was made from zygomatic arch 1 cm in front of tragus, curving anteriorly, behind hairline (1, 11). We also need to perform orbital roof craniotomy for tumor macro or microsurgery ablation. Medially between the dorsal superior rectus and levator muscle and medial rectus muscle was located the optic nerve (7). When working near it, great care should be taken to avoid injury the central retina artery or ciliary nerve. It was not performed an orbital roof reconstruction after this (1, 7). One of the goals is total ablation of the tumor.

In 8 cases we use fronto-temporal approach with the head positioned rotated on 60^0 from vertical. Skin incision was made curved with anteriorly concavity from zygomatic arch to the medial line and 3 cm down on the medial line. The craniotomy was made in 4 holes. Performing orbital roof craniotomy was obtained visual access to the orbital tumor (1, 11). Our study comprised 10 females (58.8 %) and 7 males (41.1 %). 16 patients presented with exophthalmia, head each 8 patients, diplopia 3 patients, amblyopia 4 patients, 1 patients with ptosis, 1 with rinoreea and 2 patients with nerve VII paresis.

The predominated side of the tumors was left 58.8% (10 cases) and right 35.2 % (6 cases) and one case was bilateral. In 9 cases tumors were only in orbital region in 2 cases were orbital pathology with intracranial extension and in 6 cases were skull bases pathology with orbital extension. We encountered 3 cases of lachrymal gland adenoma, 3 cases of orbital non-Hodgkinl lymphoma, 1 cases of fibroma, 3 cases of skull base meningioma, 2 cases of carcinoma metastasis, 1 cases of orbital fibrosclerosis, 2 cases of hemangioma, 1 cases of orbital cavernoma and 1 cases of mucocele. For the most cases there was performed a transcranial approach with macroscopic resection of the tumor.

TABLE I
Pathology cases

PATHOLOGY	CASES
Lachrymal gland adenoma	3
Orbital non-Hodgkinl lymphoma	3
Fibroma	1
Skull base meningioma	3
Carcinoma metastasis	2
Fibrosclerosis	1
Hemangioma	2
Cavernoma	1
Mucocele	1

In 7 cases we used pterional approach with the total resection of the lesion in 71.4 % (5 cases) and subtotal in 28.5 % (2 cases). In 4 cases the lesions had orbital extension. In 4 cases our team used microsurgical technique with 3 cases of total removal of the tumor; in one cases with skull base meningioma with orbital extension the resection was subtotal. In 3 cases was performed a macroscopic technique with total removal of the tumors in all cases.

In 8 cases we use fronto-temporal approach and the total resection was in 75% (6 cases) and subtotal in 25% (2 cases). The extension of the lesion intracranial was in 2 cases and in 2 cases was skull base pathology with intra orbital extension and in 4 cases only orbital pathology. In all cases we used a macrosurgical technique.

In 2 cases the approach was anterior with macro- and microsurgical technique, the tumor resection was subtotal in both cases. The tumors had only orbital location without cranial extension.

Two patients with skull base meningiomas presented recurrence of the tumor and we performed a second intervention for complete tumor ablation.

Exophthalmia was resolved in all cases. N. VII paresis did not resolve after tumor resection. No patient died as a result of the surgical procedures (tab. II).

RESULTS

TABLE II

Clinical data of the patients with orbital pathology

No	Age	Sex	Side	Pathology	Surgical approach	Intervention	Intracranial extension	Intra-orbital extension	Degree of Resection
1	68	F	R	Pleomorphic adenoma of lacrimal gland	F-temporal	Macrosurgical	-	/	Total
2	77	F	R+L	Bilateral orbital Non-Hodgkin Lymphoma	F-temporal	Macrosurgical	-	/	Total
3	12	M	L	Fibroma	Median trans-facial with Lafort osteotomy	Microsurgical	-	/	Subtotal
4	60	F	R	Pleomorphic adenoma of lacrimal gland	F-temporal	Macrosurgical	-	/	Total
5	68	F	L	Carcinoma Metastasis	Left Pterional	Macro surgical	/	+	Total
6	67	F	L	Meningioma	Left Pterional	Microsurgical	/	+	Total
7	35	M	L	Hemangioma	Left Pterional	Microsurgical	/	+	Total
8	31	F	L	Meningioma	Left Pterional	Microsurgical	/	+	Subtotal
9	64	F	R	Non-Hodgkin Lymphoma	F-temporal	Macrosurgical	+	/	Total
10	68	M	R	Non-Hodgkin Lymphoma	Transpalpebral	Macrosurgical	-	/	Subtotal
11	55	M	L	Pleomorphic adenoma of lacrimal gland	Left Pterional	Macrosurgical	-	/	Total
12	37	F	R	Inflamatory fibrosclerosis	F-temporal	Macrosurgical	-	/	Subtotal
13	77	F	L	Carcinoma Metastasis	Left Frontal	Macrosurgical	/	+	Total
14	49	M	L	Cavernoma	F-pterional	Microsurgical	-	/	Total
15	40	F	R	Hemangioma	F-O-pterional	Macrosurgical	-	/	Total
16	56	M	L	Meningioma	F-temporal	Macrosurgical	/	+	Subtotal
17	56	M	L	Mucocele	F-temporal	Macrosurgical	+	/	Total

DISCUSSION

There are 2 types to approach orbital tumor pathology. One is anterior approach and the other one is trans-cranial approach. The anterior approach is used mainly by ophthalmologists and trans-cranial approach is used by neurosurgeons (3). The type of the approach aims the goals of the surgical treatment. However the tumors characteristics are important.

In 1913 Frazier (6) describes trans-cranial approach with orbital osteotomy, technique that suffered some modifications since then. In our study we used classical pterional approach with frontal extension in one case and fronto-temporal approach with classical craniotomy. Out team performed roof orbitotomy in 12 cases and roof and lateral wall orbitotomy in 2 cases. In 2 cases there was performed an anterior approach.

In trans-cranial approach the lesion resection was total in 80 % (12 cases) and subtotal in 20 % (3 cases). In anterior approach the lesion resection was subtotal (2 cases).

Although the approach is difficult, given the anatomical elements for a direct approach and tumor resection, we recommend a trans-cranial approach. In our study we found no significant differences between microsurgical technique and macro surgical technique.

Although surgical treatment remains the main treatment for most symptomatic orbital tumor pathology, lesions involving the skull base and the medial wall of the orbit are still difficult to treat. These lesions should be addressed by a multidisciplinary team. There is indication that good knowledge of the microanatomy of the orbit is necessary for a good outcome (7, 8).

CONCLUSIONS

For safety and better chance of total removal of orbital tumor pathology we recommend trans-cranial approach. However the technique and cranial approach can be evaluated on case-by-case situation and depends on the tumor location and extension. The goal is patient safety, symptoms remission and tumor removal.

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TUBERCULOSIS MENINGO-ENCEPHALITIS WITH POSITIVE CSF FOR KB AND SLOWLY FAVOURABLE EVOLUTION. CASE REPORT

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TUBERCULOSIS MENINGO-ENCEPHALITIS WITH POSITIVE CSF FOR KB AND SLOWLY FAVORABLE EVOLUTION. CASE REPORT (Abstract) **Background:** Meningo-encephalitis produce by Koch bacillus is a disease including various symptoms, with often atypical onset and evolution, and with an etiological diagnosis being rarely established. It therefore remains a disease with severe prognosis, despite adequate treatment. **Case report:** a 47 years old male from Neamt County, with chronic otitis in his recent medical history, comes to the emergency room for fever, severe headache, confusion, equilibrium disorders, influenced health status, and meningeal irritation signs. Given his otitis and antibiotic therapy received in recent medical history, the patient was initially diagnosed and treated for beheaded bacterial meningitis (348 elements/mm³, 89% lymphocytes, 5% granulocytes, proteinorrhachia 1.54 g/l). Clinical and paraclinical evolution was poor with persistent fever and headache, impaired mental state, and the spinal puncture showed increased pleocytosis (725 elements/mm³), lymphocytosis (78%), increased proteinorrhachia (2.12 g/l), and low levels of glycorrhachia and chlororrhachia. Tuberculosis meningoencephalytis was suspected (confirmed after 3 weeks through cultures on Lowenstein-Jensen medium: positive for *Mycobacterium tuberculosis*), and a tuberculostatic treatment associating four drugs and intravenous Ciprofloxacin was started. Although the treatment included substances against cerebral edema and neurotrophic agent, his evolution showed persistent headache syndrome. We noticed very slow decrease in CSF pleocytosis (after a month) of treatment the patient still had 240 elements/mm³, 85% lymphocytesand 15% granulocytes. Moreover, due to hepatic toxicity a reduction of the tuberculostatic doses was needed. **Conclusions:** The beginning of the tuberculosis infection symptoms is often surprising, with clinical signs regarding secondary determinations (meningoencephalitis), association with another infection (otitis – wrong diagnosis), as well as persistent yet slowly favorably evolving symptoms, both clinically and paraclinically (CSF parameters). The etiological agent is extremely rarely isolated, due to a very slow growth of the Koch bacillus on special culture mediums and also because such diagnosis is not often considered before starting the treatment. **Key words:** TUBERCULOSIS MENINGO-ENCEPHALITIS, PERSISTENT SLOWLY FAVORABLE CLINICAL AND BIOLOGICAL EVOLUTION.

Tuberculosis meningo-encephalitis is one of the most severe sites of tuberculosis infection evolution, being an extremely polymorphic disease, with frequently atypical onset and evolution and with an etiological diagnosis being rarely established,

with severe prognosis, despite adequate treatment. Tuberculosis still represents a very severe health problem, with high prevalence and mortality rates, frequently because of the lack of a fast and safe diagnostic algorithm (which often leads to late specific treatment), atypical onset and increasingly higher incidence of tuberculosis in the general population.

CASE REPORT

We present the case of a 47 years old male patient from Neamţ County who came to our clinic for fever, severe headache, confusion, equilibrium disorders, impaired health status, and positive signs of meningeal irritation.

His medical history included a chronic otitis during the previous months.

The onset of the disease was about 10 days before hospitalization and manifested itself through severe occipital headache, confusion status, equilibrium disorders, impaired general state. He went to Târgu Neamt Hospital where was evaluated by a neurologist, his native cranio-cerebral computer tomography being normal. He received treatment for cerebral edema, but the patient started to have fever and he was therefore transferred to the Infectious Disease Department of Târgu Neamţ . Chest X-ray showed a triangular heterogeneous opacity with opaque bands from center to periphery under the left hillum, for which antibiotic treatment was started (Amoxicillin- clavulanatum, then Ceftazidime + Gentamicin), with unfavorable evolution, persistent fever, headache and confused mental state, for which he was transferred to our clinic.

On admission: normal weight, subfebrility, influenced general state, confusion (partial temporo-spatial disorientation, hemodynamic and respiratory stable state, HR 88/min, BP 125/75 mmHg, diffuse headache, positive signs of meningeal irritation).

The paraclinical tests revealed normal white cells count (8720/mm³), neutrophilia (94.1%), HSR 25 mm/h, mild hepatic cytolysis (ASAT 67UI/L). The chest X-ray revealed a small zone of fibrosis of approximately 1.5 cm in diameter in the medial right pulmonary field. A lumbar puncture was performed, which revealed clear CSF, pleocytosis (348 elem/mm³, 89% lymphocytes, 5% granulocytes, high protein-rrhachia (1.54 g/l), low glycorrhachia (0.25g/l) and normal chlorurorrhachia (7.04g/l).

Because of the otitis in his recent medical history and previous antibiotic therapy administered at Târgu Neamţ Hospital, it was thought to be beheaded bacterial meningitis and treated with Ceftriaxon 4g per day + Ciprofloxacin 400 mg per day, treatment against cerebral edema (Dexametazon, Manitol), group B vitamins, drugs against fever and antalgic agents.

Seven days after admission the patient still had mild fever, non-severe confusion, influenced general state. The lumbar puncture was repeated and revealed increased pleocytosis (725 elem/mm³), 78% lymphocytes, 20% granulocytes, high proteinorrhachia (2.12 g/l), low glycorrhachia (0.29 g/l) and low clorurorrhachia (6.80 g/l). We suspected a tuberculosis meningoencephalitis (confirmed after 3 weeks by positive culture on Lowenstein-Jensen medium for *Mycobacterium tuberculosis*), so we started tuberculostatic treatment including four agents (Pyrazinamide 2g + Isoniazid 300 mg + Ethambutol 1,6 g + Rifampicin 750 mg) together with intravenous Ciprofloxacin.

The evolution was slowly favorable

with persistent headache, mild cerebral edema despite neurotrophic therapy, remission of fever and confusion, and a very slow CSF pleocytosis decrease (tab. I).

TABLE I
Evolution of CSF parameters from admission to discharge

	Admission	Day 7	Day 17	Day 33	Day 45
Elem/mm³	348	725	264	240	160
Sediment	Lf 89% Gr 5% Mf 6%	Lf 78% Gr. 20% Mf 2%	Lf 83% Gr.12% Mf 5%	Lf 85% Gr.15%	Lf 90% Gr. 10%
Ziehl Nielsen coloration	Negative				
Latex agglutination	Negative				
Bacterial cultures	negative				
Cultures on Lowenstein-Jensen medium	*M. tuberculosis*				
Proteinorrhachia	1.54	2.12	2.95	1.58	1.20
Glycorrhachia	0.25	0.29	0.30	0.40	0.60
Clorurorrhachia	7.04	6.80	7.04	6.93	7.00

The evolution was also marked by the appearance of hepatotoxic side effects, supported by the increased transaminase levels, which determined us to decrease the Rifampicin doses.

After one and a half month from admission to our clinic, the patient was discharged in good general state, without fever, good temporal and spatial orientation, but with a persisting mild headache, the native computer tomography performed being normal. We recommended the patient to continue his tuberculostatic treatment under monitoring from the TBC outpatient clinic in his town.

DISCUSSION

Tuberculosis meningitis is caused by tuberculosis infection dissemination in the meningeal structures, favored by bacteremia and *Mycobacterium tuberculosis* proliferation, in immunodepressed individuals. Tuberculosis meningitis remains a disease with severe prognosis, being the most life-threatening site of tuberculosis infection, with a high rate of mortality and important neurological sequelae (1, 2)

Despite global efforts to manage the tuberculosis infection, its prevalence has been increasing, with 9.4 millions of infected patients worldwide in 2008, and the highest incidence of 139:100000 inhabitants in Africa (3). When tuberculosis cases are quickly detected and treated, there is better control of this illness and the transmission risk decreases inversely proportional to time of treatment. Most likely, the dynamics of tuberculosis infection is strictly related to the fast detection of each case, thus reducing the transmission period (3).

There are some peculiar aspects in the modality of tuberculosis infection onset in this case, i.e. clinical symptoms in secondary sites of infection (meningo-encephalitis) or absence of personal epidemiological contact. Also, there was no pulmonary involvement in his medical history, as the X-rays performed, both at Târgu Neamţ and in our clinic, revealed no indication of any active pulmonary disease.

The presence of otitis in his recent medical history was misleading for diagnosis setting, as it made us suspect another bacterial cause of meningitis and hence delayed specific treatment delivery.

The etiological agent is extremely rarely isolated, due to the difficulties related to the growth of the Koch bacillus on special culture mediums and also due to the slow susceptibility index of this etiology before treatment. Since *Mycobaterium tuberculosis* takes a long time to be isolated on special mediums, and given the low number of positive reactions revealed by Ziehl Neelsen stain, it is often recommended to initiate the tuberculostatic therapy if such condition is suspected and not to wait for positive culture results.

Persistent headache and mild cerebral edema may be considered complications of tuberculosis meningitis despite a normal computer tomography.

The prognosis of the disease in this case may also be influenced by the secondary hepatic side effects which, when associated with digestive symptoms (nausea, vomiting, lack of appetite, abdominal discomfort), may influence the patient's adhesion to the treatment, especially if it is a long-term treatment. The large amount of pills to be taken every day is often another problem of therapy adherence and hence disease prognosis.

CONCLUSIONS

The tuberculosis infection onset was surprising, with clinical manifestations in secondary sites (meningo-encephalitis), the association of concomitant otitis (misleading), and the persistent yet slowly favorably evolving symptoms, from both the clinical and CSF parameters viewpoints. The isolation of the etiological agent is extremely rare, due to the difficult growth of the Koch bacillus on special mediums and also to the small susceptibility for this diagnosis, especially at the beginning of the disease, when the clinical and biological signs are atypical.

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PERFORMANȚA DIFERITELOR METODE DE SCREENING ÎN PRIMUL TRIMESTRU DE SARCINĂ PRIVIND ESTIMAREA RISCULUI PENTRU ANOMALIILE CROMOZOMIALE

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PERFORMANCE OF DIFFERENT METHODS OF ESTIMATING RISK SCREENING FOR CHROMOSOMAL ANOMALIES (Abstract): In the last 10 years, several studies have been carried out on additional ultrasound markers in the first trimester of pregnancy in order to improve detection rate of fetal numerical chromosome abnormalities (aneuploidy) and to reduce the rate of false-positive diagnosis. The purpose of this study was to evaluate the performance of various recommendations for which amniocentesis was performed followed by FISH testing in the diagnosis of aneuploidy. These evaluations were conducted in order to determine whether ultrasound aspects are associated with fetal aneuploidy and to estimate the risk level of individual markers using probability estimation analysis. **Material and methods**: The study has been carried out at the Clinical Hospital of Obstetrics and Gynecology "CuzaVodă" Iaşi, at the Laboratory of cytogenetic - prenatal diagnosis, during January 2004 – December 2011, on a target group of 1406 pregnant women. As part of this study, 1411 amniocentesis were performed. **Results**: increased efficiency of screening for fetal aneuploidy in the first trimester of pregnancy is obtained through combined method (maternal age over 35 years, increased nuchal translucency and the presence of double test risk) which has 100% detection rate and a rate false-positive result of 0%. The efficiency of this method is provided also by the relatively high risk (RR = 17.2) and its specificity (Sp = 100%). Making the assessment following the study false positive rate, it appears that a good method of risk assessment for aneuploidy is the combined evaluation of increased nuchal translucency (NT) with maternal age over 35 years (specificity 99.5%, a detection rate of 40% false positive rate of 0.45% and a relative risk of 7.09 for the presence of aneuploidy). **Conclusions**: The achievement of a correct prenatal diagnosis and the increase of the method efficiency, requires a correct selection of cases with aneuploidy risk assessment, based on the results of ultrasound and biochemical (double test risk) investigations correlated with advanced maternal age or previous presence of aneuploidy of children (Down syndrome, Edwards syndrome, Patau syndrome, Klinefelter syndrome and triplo X). **Key words**: FETAL NUMERICAL CHROMOSOMAL ABNORMALITIES, SCREENING METHOD, PRENATAL DIAGNOSIS

Orice sarcină porneşte de la posibilitatea existenței unei malformații cromozomiale. Pentru a calcula riscul individual, este necesară evidențierea riscului a priori ce depinde de vârsta maternă şi corelarea acestuia cu o serie de teste screening efectuate în puncte cheie ale sarcinii. Anii '70 au adus în prim plan corelarea riscului de trisomie 21 cu vârsta maternă avansată, dar costurile şi riscurile ridicate asociate amniocentezei au impus limitarea indicației acesteia doar pentru gravidele peste 40 de ani. Consecutiv, grupul de risc crescut a beneficiat de o reducere a vârstei materne la 35 de ani. Anii '90 s-au caracterizat prin combinarea vârstei materne şi a valorii translucenței nucale la 11-13+6 săptămâni de gestație ceea ce a permis identificarea a aproximativ 75% dintre fetuşii afectați cu o rată de screening pozitiv cotată la 5%. Asocierea translucenței nucale şi a biochimiei serice materne în primul trimestru de sarcină, reprezentată de dublu test (β hCG liber şi PAPP-A) determină identificarea a circa 85-90% din fetuşii afectați (1,2).

Echipa condusă de Kypros Nicolaides a stabilit liniile generale ale *screening*-ului prenatal în ultimii ani. Astfel, dacă inițial se considera că esențiale sunt morfologiile de trimestru I şi II, actualmente se promovează ideea că esența diagnosticului prenatal se află în ecografia de 11-13 săptămâni (3).

Scopul studiului a fost acela de a evalua performanța diferitelor metode de *screening* pentru care s-a practicat amniocenteză urmată de efectuarea testului FISH în vederea diagnosticării anomaliilor cromozomiale numerice fetale. Ca şi indicații au fost evaluate: vârsta maternă avansată, markeri ecografici fetali (translucența nucală crescută), markeri serici materni (dublu test cu risc crescut). Această evaluare a fost realizată pentru a determina dacă aspectele ecografice, biochimice şi vârsta maternă avansată sunt asociate cu aneuploidiile fetale, pentru a estima gradul de risc a markerilor individuali folosind analiza probabilității de estimare.

În ultimii 10 ani au fost realizate mai multe studii suplimentare privind markerii ecografici din primul trimestru de sarcină pentru a îmbunătăți rata de detecție a aneuploidiilor şi de a reduce rata de diagnostic fals-pozitiv (4).

MATERIAL ŞI METODĂ

Studiul s-a efectuat în Spitalul Clinic de Obstetrică şi Ginecologie „Cuza Vodă" Iaşi, în Laboratorul de citogenetică - diagnostic prenatal, în perioada ianuarie 2004 – decembrie 2011 pe un lot care a inclus un număr de 1406 de gravide. În cadrul studiului au fost practicate 1411 amniocenteze sub ghidaj ecografic în vederea efectuării testului FISH care permite detectarea anomaliilor numerice ale cromozomilor 21, 13, 18, X şi Y.

Amniocentezele au fost efectuate de medici cu experiență din cele trei Clinici ale Spitalului Clinic de Obstetrică şi Ginecologie „Cuza–Vodă" Iaşi, astfel încât să se obțină suficient lichid amniotic de la prima încercare, evitându-se astfel repetarea procedurii, scăzând riscul pentru complicații.

Studiul statistic a abordat două aspecte: statistica descriptivă şi statistica analitică. Statistica descriptivă prezintă clasificarea şi sintetizarea datelor de observație. Aceasta concentrează informația existentă în datele respective cu ajutorul anumitor indicatori statistici ce exprimă caracteristici sau tendințe ale fenomenului studiat. Analiza corelației statistice a permis identificarea unor dependențe specific statistice şi adaptate la formele complexe şi variate din medicină. În urma aplicării testelor statistice

s-au luat în discuție principalii parametrii de interes, iar în funcție de valorile acestora s-au stabilit concluziile.

REZULTATE ŞI DISCUȚII

Performanța diferitelor metode de screening în primul trimestru de sarcină privind aneuploidiile fetale

Vârsta maternă este un criteriu de screening sărac, deoarece un număr mare de copii cu aneuploidii (frecvent sindrom Down) sunt născuți de femei mai tinere de 35 de ani. Colegiul American de Obstetrică şi Ginecologie (The American College of Obstetricians and Gynecologists - ACOG) nu mai recomandă utilizarea vârstei materne de 35 de ani ca un punct critic pentru practicarea amniocentezei (5,6).

Screening-ul bazat pe **vârsta maternă** pentru un cut-off de 35 de ani permite definirea grupului cu risc crescut pentru aneuploidii fetale (anomaliile cromozomiale numerice). Vârsta maternă avansată (n= 628) a avut o rată de detecție a anomaliilor cromozomiale numerice de 5,10% (n=32). Analiza gradului de asociere a vârstei materne peste 35 de ani cu prezența aneuploidiilor a demonstrat prin rezultatul testului Yates faptul că nu există o asociere semnificativă, aspect ce demonstrează că vârsta maternă avansată nu este un factor de predicție semnificativ în apariția unei sarcini cu anomalie cromozomială numerică (χ^2=1,02, r=-0,129, p=0,2609; 95%CI).

Evaluarea raportului de şansă (OR= 0,77) şi a riscului relativ (RR=0,78) susține încă odată concluzia potrivit căreia vârsta maternă avansată nu este un factor de predicție semnificativ în estimarea unei sarcini cu aneuploidie, riscul relativ având valoare subunitară.

Utilizând rezultatul testului FISH ca metodă *gold standard*, s-au calculat sensibilitatea, specificitatea, valorile predictiv pozitive şi predictiv negative dar şi acuratețea privind utilizarea vârstei materne avansate ca metodă de evaluare a riscului pentru prezența aneuploidiilor. Valoarea predictiv pozitivă a vârstei materne a fost de 5,1%, iar valoarea predictiv negativă a fost de 93,49%. Sensibilitatea testului a fost de 38,55% şi este mai mică comparativ cu specificitatea vârstei materne (55,12%), aspect ce demonstrează încă odată că în cazul gravidei cu vârstă peste 35 ani putem să ne aşteptăm la rezultate fals pozitive însă acuratețea fiind mică acestea nu se corelează semnificativ cu vârsta maternă peste 35 de ani.

Translucența nucală crescută a fost evidențiată în 3,33% din cazuri (n=47).

Translucența nucală crescută a avut o rată de detecție a aneuploidiilor de 19,15% (n=9). Corelația dintre translucența nucală crescută şi prezența anomaliilor cromozomiale numerice a evidențiat (testul neparametric Pearson) faptul că există o asociere semnificativă, aspect ce demonstrează faptul că translucența nucală crescută este un factor de predicție semnificativ pentru prezența anomaliilor cromozomiale numerice (χ^2=15,45, r=0,61, p=0,00008, 95%CI). Raportul de şansă (OR=4,13) şi riscul relativ (RR=3,53) demonstrează prezența unui risc crescut pentru anomalie cromozomială numerică în cazul prezenței translucenței nucale crescute, în lotul analizat acesta fiind de 3,53 ori mai mare comparativ cu riscul prezent în cazul absenței translucenței nucale crescute. Valoarea mare a specificității (97,14%) indică faptul că probabilitatea de a avea o anomalie cromozomială numerică în contextul prezenței translucenței nucale crescute este mare. Rata rezultatelor fals pozitive în cazul translucenței nucale crescute a fost de 2,86%.

În lotul de studiu 12,33% din cazuri (n=174) au prezentat **dublu test** cu risc crescut, dintre acestea doar 5,75% au fost însoțite de aneuploidie cromozomială (n=10).

Rezultatele au demonstrat faptul că prezența dublului test cu risc crescut nu prezintă o asociere semnificativă cu prezența anomaliilor cromozomiale numerice, aspect ce demonstrează faptul că dublul test cu risc crescut nu este un factor de predicție semnificativ pentru prezența anomaliilor cromozomiale numerice (χ^2=0,0065, r=-0,01, p=0,935 95%CI). Raportul de şansă (OR=0,97 → 0,46-1,99; 95%CI) şi riscul relativ (RR=0,97 → 0,51-1,85; 95%CI) demonstrează prezența unui risc mic pentru anomalie cromozomială numerică în cazul dublului test cu risc crescut. Valoarea specificității (87,65%) indică faptul că probabilitatea de a avea o anomalie cromozomială numerică în contextul rezultatului de risc crescut dat de dublu test este moderată şi mai mică comparativ cu probabilitatea de estimare a prezenței translucenței nucale crescute (97,14%). Rata rezultatelor fals pozitive este de 12,35%, valoare de asemenea mai mare comparativ cu cea calculată în cazul translucenței nucale crescute.

Evaluarea metodelor combinate de screening în primul trimestru de sarcină.

Asocierea vârstei materne ≥ 35 ani şi translucenței nucale crescute ca metodă de predicție în aneuploidiile fetale

Vârsta maternă avansată şi prezența translucenței nucale crescute a fost identificată în 0,71% din cazuri (n=10). Rata de detecție a anomaliilor cromozomiale numerice în cadrul metodei de screening combinate (vârstă maternă ≥35 ani + translucență nucală crescută) a fost de 40% (n=4). Gradul de asociere a rezultatelor pozitive (risc crescut) a testului combinat (vârstă+TN) cu prezența anomaliilor cromozomiale numerice demonstrează faptul că această evaluare combinată este un factor de predicție semnificativ pentru prezența anomaliilor cromozomiale numerice (χ^2=15,423 r=0,83 p<<0,05 95%CI) (tab. I).

Evaluarea raportului de şansă (OR= 21,18 → 2,59-45,75; 95%CI) şi a riscului relativ (RR=7,09 → 3,22-15,61; 95%CI) demonstrează prezența unui risc foarte mare pentru anomalie cromozomială numerică în cazul asocierii vârstei materne peste 35 de ani şi translucenței nucale crescute (tab. III). Valoarea foarte mare a specificității (99,5%) indică faptul că probabilitatea de a avea anomalie cromozomială numerică în contextul evaluării combinate date de vârsta maternă avansată şi translucența nucală crescută este mare. Rata rezultatelor fals pozitive este de 0,45%, valoare de asemenea mică comparativ cu cele calculate în cazul analizei individuale a vârstei materne avansate şi translucenței nucale crescute (tab. II).

Asocierea vârstei materne ≥ 35 ani, translucenței nucale crescute şi dublu test cu risc crescut ca metodă de predicție în aneuploidiile fetale

Rata de detecție a anomaliilor cromozomiale numerice în cadrul metodei de screening combinate (vârstă maternă ≥35 ani + translucență nucală crescută + dublu test cu risc crescut) a fost de foarte mare, în lotul de studiu fiind întâlnit un singur caz, acesta fiind asociat cu aneuploidie cromozomială (trisomie 18). Corelarea rezultatelor pozitive (risc crescut) a testului combinat (vârstă+TN+dublu test) cu prezența anomaliilor cromozomiale numerice a evidențiat (testul neparametric Yates) faptul că există o asociere semnificativă, aspect ce demonstrează faptul că această evaluare

combinată este un factor foarte bun de predicție pentru prezența anomaliilor cromozomiale numerice (χ^2=3,51, r=0,87, p=0,00006, 95%CI) (tab. I). Datorită numărului foarte mic de cazuri nu a putut fi evaluat raportul de şansă. Evaluarea riscului relativ (RR=17,2 → 13,94-21,21; 95%CI) demonstrează prezența unui risc foarte mare pentru anomalie cromozomială numerică în cazul asocierii vârstei materne peste 35 de ani, translucenței nucale crescute şi dublu test cu risc crescut (tab. III).

În evaluarea performanței metodei de screening privind evaluarea combinată - vârstă maternă avansată, translucență nucală crescută şi dublu test cu risc crescut, numărul mic de cazuri din lotul de studiu a avut o influență semnificativă asupra rezultatelor. Astfel, prezența unui caz unic a determinat o specificitate mare a metodei (100%), însă a scăzut semnificativ acuratețea metodei la 94,19% şi sensibilitatea acesteia (1,2%) (tab. II). Aceste rezultate ce privesc sensibilitatea şi acuratețea nu sunt concordante cu specificitatea metodei, în situația studiată neexistând un compromis favorabil ce ar demonstra faptul că această metodă este performantă. O metodă prezintă o bună putere discriminatorie atâta timp cât atât sensibilitatea, cât şi specificitatea au valori mari, dar sensibilitatea nu atinge nivele maxime.

Asocierea vârstei materne ≥ 35 ani şi dublu test cu risc crescut ca metodă de predicție în aneuploidiile fetale

În lotul de studiu asocierea vârstă maternă ≥ 35 ani şi dublu test cu risc crescut a fost întâlnită în 4,75% din cazuri (n=67). Rata de detecție a anomaliei cromozomiale numerice în cadrul metodei de screening combinate (vârstă maternă ≥35 ani + dublu test cu risc crescut) a fost de 10,45% (n=7). Analiza gradului de asociere a rezultatelor pozitive (risc crescut) a testului combinat (vârstă+dublu test) cu prezența anomaliilor cromozomiale numerice a evidențiat (testul neparametric Yates) faptul că nu există o asociere semnificativă, aspect ce demonstrează faptul că această evaluare combinată nu este un factor de predicție semnificativ pentru prezența anomaliilor cromozomiale numerice (χ^2=1,18 r=0,32 p=0,103 95%CI) (tab. I). Evaluarea riscului relativ (RR=1,85 → 0,89-3,85; 95%CI) demonstrează prezența unui risc mic pentru prezența anomaliilor cromozomiale numerice în cazul asocierii vârstei materne peste 35 ani şi dublu test cu risc crescut (tab. III).

Studiul a relevat o valoarea mare a specificității (95,48%) acest fapt indicând că probabilitatea de a avea anomalie cromozomială numerică în contextul evaluării combinate date de vârsta maternă avansată şi dublu test cu risc crescut este de 95,48%. Rata rezultatelor fals pozitive este de 4,52% (tab. II).

TABELUL I

Prevalența factorilor de risc implicați în aneuploidiile fetale

Metoda de screening	Aneuploidie fetală		χ^2	r	p
	absentă	prezentă		*95%CI*	
Vârstă maternă (≥ 35 ani)	94,90%	**5,10%**	1,265448	-0,1295	0,26063
Translucența nucală (TN)	80,85%	**19,15%**	15,45634	0,6100402	0,00008
Dublu test	94,25%	**5,75%**	0,0065555	-0,014061	0,93547
Vârsta maternă + dublu test	89,55%	**10,45%**	2,202515	0,3212266	0,13779
Vârsta maternă + TN	60,00%	**40,00%**	21,17507	0,8354738	0,00000
Vârsta maternă + TN + dublu test	0,00%	**100,00%**	5,677814	0,8799	0,01718

TABELUL II

Performanța diferitelor metode de screening privind aneuploidiile fetale

Metoda descreening	Sensibilitate (%)	Specificitate (%)	VPP* (%)	VPN* (%)	RFP* (%)	Acuratețea (%)
Vârstă maternă (≥ 35 ani)	38,55%	55,12%	5,10%	93,49%	**44,88%**	54,15%
Translucența nucală (TN)	10,84%	97,14%	19,15%	94,57%	**2,86%**	92,06%
Dublu test	12,05%	87,65%	5,75%	94,10%	**12,35%**	83,20%
Vârsta maternă + dublu test	8,43%	95,48%	10,45%	94,35%	**4,52%**	90,36%
Vârsta maternă + TN	4,82%	99,55%	40,00%	94,36%	**0,45%**	93,98%
Vârsta maternă + TN + dublu test	1,20%	100%	100%	94,18%	**0%**	94,19%

** VPP – Valoarea predictiv pozitivă, VPN – Valoarea predictiv negativă, RFP – rata rezultatelor fals pozitive*

TABELUL III

Parametrii predictivi ai metodelor de screening privind aneuploidiile fetale

Metoda de screening	Raport de şansă		Raport de risc	
	OR	95%CI	RR	95%CI
Vârstă maternă (≥ 35 ani)	0,77	(0,48-1,24)	0,78	(0,51-1,20)
Translucența nucală (TN)	4,13	(1,78-9,29)	3,53	(1,88-6,61)
Dublu test	0,97	(0,46-1,99)	0,97	(0,51-1,85)
Vârsta maternă + dublu test	1,95	(0,78-4,61)	1,85	(0,89-3,85)
Vârsta maternă + TN	21,18	(2,59-45,75)	7,09	(3,22-15,61)
Vârsta maternă + TN + dublu test	-	-	17,20	(13,94-21,21)

Eficiența ridicată de screening pentru aneuploidii fetale realizat în primul trimestru de sarcină o deține metoda combinată (vârstă maternă peste 35 ani, prezența translucenței nucale crescute şi dublu test cu risc crescut) ce are rata de detecție de 100% şi o rată a rezultatelor fals-pozitive de 0%. Eficiența acestei metode este dată şi de riscul relativ ridicat (RR=17,2) şi specificitatea metodei (Sp=100%) (fig. 1).

Evaluând în funcție de rata rezultatelor fals pozitive din studiu reiese faptul că o metodă bună de evaluare a riscului pentru aneuploidii este evaluarea combinată a translucenței nucale crescute (TN) cu vârsta maternă peste 35 ani. Rezultatele indică pentru această metodă o specificitate de 99,5%, o rată de detecție de 40%, o rată a rezultatelor fals pozitive de 0,45% şi un risc relativ pentru prezența aneuploidiilor de 7,09 (RR=7,09).

Indicația unică având în vedere vârsta maternă avansată sau dublul test cu risc crescut reprezintă cele mai slabe metode de screening privind anomaliile cromozomiale numerice (Sp – vârstă maternă=55,12%, Sp – dublu test=87,65%).

Indicația unică privind translucența nucală crescută reprezintă o metodă de screening bună, având în vedere faptul că în lotul analizat specificitatea metodei a fost de 97,14%, rata rezultatelor fals pozitive a fost de 2,86% iar riscul relativ de 3,53 (RR=3,53) (fig. 1).

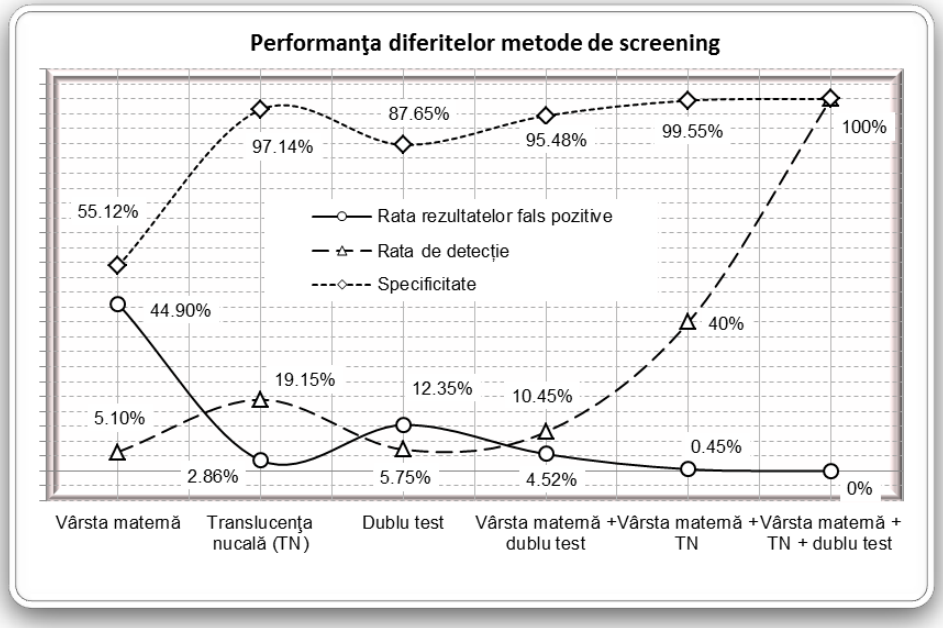

Fig.1. Performanțele diferitelor metode de screening privind aneuploidiile fetale

CONCLUZII

Pentru realizarea unui diagnostic prenatal corect şi creşterea eficienței metodei este necesară o selecție corectă a cazurilor cu risc de aneuploidie pe baza rezultatelor evaluării ecografice şi a celei biochimice (dublu test cu risc crescut) în corelație cu vârsta maternă avansată sau prezența în antecedente a unor copii cu aneuploidie (sindrom Down, sindrom Edwards, sindrom Patau, sindrom Klinefelter sau triplo X).

Alături de siguranță şi precizie, precocitatea reprezintă dezideratul unei metode de diagnostic prenatal fiabile.

În evaluarea performanței metodei de screening privind evaluarea combinată - vârstă maternă avansată, translucență nucală crescută şi dublu test cu risc crescut, numărul mic de cazuri din lotul de studiu a avut o influență semnificativă asupra rezultatelor. Astfel, prezența unui caz unic a determinat o specificitate mare a metodei (100%), însă a scăzut semnificativ acuratețea metodei la 94,19% şi sensibilitatea acesteia (1,2%). Aceste rezultate ce privesc sensibilitatea şi acuratețea nu sunt concordante cu specificitatea metodei, în situația studiată neexistând un compromis favorabil ce ar demonstra faptul că această metodă este performantă.

Evaluând în funcție de rata rezultatelor fals pozitive din studiu, reiese faptul că o metodă bună de evaluare a riscului pentru aneuploidii este evaluarea combinată a translucenței nucale crescute (TN) cu vârsta maternă peste 35 ani. Rezultatele indică pentru această metodă o specificitate de 99,5%, o rată de detecție de 40%, o rată a rezultatelor fals pozitive de 0,45% şi un risc relativ pentru prezența aneuploidiilor

de 7,09 (RR=7,09).

Indicația unică având în vedere vârsta maternă avansată sau dublul test cu risc crescut reprezintă cele mai slabe metode de screening privind anomaliile cromozomiale numerice (Sp – vârstă maternă=55,12%, Sp – dublu test=87,65%).

Indicația unică privind translucența nucală crescută reprezintă o metodă de screening bună, având în vedere faptul că în lotul analizat specificitatea metodei a fost de 97,14%, rata rezultatelor fals pozitive a fost de 2,86%, iar riscul relativ de 3,53 (RR=3,53).

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NOUTĂȚI

REGLAREA TRANSCRIPȚIEI GENICE PRIN INTERMEDIUL TFO

Oligonucleotidele formatoare de triplex (*Triplex-forming oligonucleotides* -TFOs) au rol reglator al expresiei genice prin inhibarea transcripției. Prin încorporarea situs-specifică a timidinei, TFO sunt inactivate şi formarea triplexului TFO:DNA este blocată. După expunerea de scurtă durată la radiații UV, timidina este îndepărtată, iar TFO sunt activate, ducând la inhibarea transcripției genice. În plus, încorporarea deoxicitidinei la nivelul secvențelor inhibitorului TFO permite dezactivarea indusă de lumină a TFO şi activarea fotochimică a a expresiei genice. Acestea sunt primele exemple de foto-reglare a TFO şi aplicațiile sale în activarea sau dezactivarea fotochimică a expreisie genice. (Govan J, Uprety R, Hemphill J, Lively MO, Deiters A. Regulation of Transcription through Light-Activation and Light-Deactivation of Triplex-Forming Oligonucleotides in Mammalian Cells. *ACS Chem Biol*. 2012 Apr 27. [Epub ahead of print]).

Teodora Vremeră

HEPATOCYTES' FUNCTION AND ADIPOKINES IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE DEPENDING ON THE ACE (rs4646994) AND PPAR-γ2 (rs1801282) GENES' POLYMORPHISMS

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HEPATOCYTES' FUNCTION AND ADIPOKINES IN PATIENTS WITH NON-ALCOHOLIC FATTY LIVER DISEASE DEPENDING ON THE ACE (rs4646994) AND PPAR-γ2 (rs1801282) GENES' POLYMORPHISMS (Abstract): Non-alcoholic fatty liver disease (NAFLD) is a pathology characterized by accumulation of fat (steatosis) found in at least 5% of hepatocytes, and is a multifactorial disease including a genetic component. **Aim:** To analyze the association of polymorphism of the ACE (I/D) and PPAR-γ2 (Pro12Ala) genes with functional activity of hepatocytes and adipocytokines content in patients with NAFLD. **Material and methods:** The study involved 96 patients with NAFLD and obesity (OB): men-41.67%, women-58.33%. Liver function was studied by the activity of organ-specific enzymes. The polymorphisms of PPAR-γ2 (Pro12Ala) and ACE (I/D) genes were studied by PCR. **Results:** The leptin plasma level was 1.74-2.39 times higher in women compared to men with development of leptin resistance (LR) regardless of NAFLD type or OB degree, especially in the D-allele of the ACE gene carriers by 22.19% and 28.73%. **Conclusions:** This study shows existing associations of changes in hepatic function, lipid metabolism, and adipocytokines activity in NAFLD with the ACE (rs4646994) and PPAR-γ2 (rs1801282) genetic polymorphisms. ACE DD-genotype and PPAR-γ2 gene's Ala-allele associate with higher conjugated bilirubin, transaminases, and leucocytes. Secretory activity of adipose tissue is significantly higher by the content of pro-atherogenic leptin, with the development of leptin resistance in women, regardless of the NAFLD type and OB degrees, especially in D-allele carriers of the ACE gene. NAFLD with OB is characterized by reliable increase in leptin concentration, especially in Pro12 genotype carriers of the PPAR-γ2 gene, with increase in LR and decrease of anti-atherogenic adiponectin. **Keywords:** NON-ALCOHOLIC FATTY LIVER DISEASE, GENETIC POLYMORPHISM, ACE (I/D), PPAR-γ2 (PRO12ALA), LEPTIN, ADIPONECTIN, OBESITY.

Non-alcoholic fatty liver disease (NAFLD) is the most common liver disease in developed countries with incidence of 24% in Europe (1). The disease is closely related to metabolic syndrome, obesity (OB), insulin resistance (IR), type 2 diabetes mellitus (DM), arterial hypertension, and hyperlipidemia (2-5). IR reduces the level of adiponectin in blood plasma, and increases concentration of leptin, which is also found in NAFLD (6). Reduced secretion of adiponectin, an anti-atherogenic, anti-inflammatory and anti-fibrotic agent, combined with increased secretion of leptin possessing opposite effects, promotes the progression of NAFLD into non-alcoholic steatohepatitis (NASH), as proved by multiple studies, including path morphology (7-10).

NAFLD is diagnosed in 20-35% of adult population in developed countries; in obese women, aged over 40, fatty liver dystrophy occurs in 75% of cases. Even among liver donors with normal levels of transaminases, steatosis is diagnosed in 20%. According to the National Health and Nutritional Examination Survey (NHANES III), the incidence of fatty liver dystrophy is met in up to 16% of patients with normal body weight and up to 76% in patients with obesity (11-14).

The clinical significance of NAFLD and the lack of effective drugs for limiting or reversing the progression of the disease in patients with NASH are of considerable interest and determine the need for further study of the main mechanisms involved in the development and progression of the disease. The accumulation of fat in liver occurs as a result of the imbalance between intake and excretion of triglycerides (15). However, genetic factors for the development of this pathology, which may serve as early markers of functional and organic hepatocyte cellular disturbances, have been still understudied (14, 16).

Therefore, *the aim of this study* was to analyze the associations of the ACE (I/D), PPAR-γ2 (Pro12Ala) genetic polymorphisms with functional activity of hepatocytes and adipocytokine content in patients with NAFLD.

MATERIAL AND METHODS

This study conforms to international *bioethical standards* (European Convention on Human Rights and Biomedicine, the WMA Declaration of Helsinki on ethical principles of scientific medical research involving human subjects, GCP, EUC directive #609) and approved by Commission for Bioethics in Research of the Bukovinian State Medical University, Ukraine. All patients signed written permissions and obtained full information about the study prior to participation. The study involved 96 patients with NAFLD, 56 (58.33%) women and 40 (41.67%) men. The mean age was 53.70±5.34 yrs. Compensated type 2 diabetes mellitus (DM 2), lasting from 2-7 years, observed in 44 (45.83%) patients. Existing literature data was used as control.

NAFLD *diagnosed* in accordance with the EASL-EASD-EASO Clinical Practice Guidelines for the management of NAFLD (8). The OB degree was determined by body mass index (BMI) $\geq$30 kg/m^2 (6, 8). During previous year the subjects had alcohol intake less than: for men >50 g C_2H_5OH per week, for women >30 g C_2H_5OH per week.

The *liver function* was studied by the activity of detoxification and protein-synthetic function of hepatocytes indices, by the presence or absence of cytolysis signs, mesen-

chymal inflammatory syndrome, or cholestasis syndrome. Leptin and adiponectin were measured in ELISA (DRG ELISA, Germany). Leptin resistance (LR) was calculated as the leptin/triglycerides ratio (>2.7). Results were interpreted according to ESC/ESH 2013 recommendations.

Polymerase chain reaction (PCR) was used to study the polymorphism of PPAR-γ2 (Pro12Ala, rs1801282) and ACE (I/D, rs4646994) genes. DNA was isolated from peripheral venous blood lymphocytes using a set of DNA-sorb-V reagents. PCR was performed using Taq DNA polymerase and specific primers for PPAR-γ2 (forward 5'-GAAACTGATGTCTTGACTCATGGGTG-3' and reverse 5'-CAACCTGGAAGA CAAACTACAAGAGC -3') and ACE (forward 5'-GCCGGGGACTCTGTAAGCCA CTGC-3' and reverse 5'-CCTTGTCTCGCCAGCCCTCCCA-3') (7). Allele discrimination of the PPAR-γ2 gene was performed using restriction endonuclease Cse I (HgaI) (Fermentas, Lithuania). The amplification products of of the I/D polymorphism of the ACE gene and those of the restriction of PCR PPAR-γ2 genes Pro12Ala polymorphism were separated in horizontal electrophoresis in 3% agarose gel concentrated with 4 μL of ethidium bromide for 45-60 minutes, visualized using transilluminator with molecular weights marker 100-1000 bp.

Statistical processing was carried out using the *MS Excel, Primer of Biostatistics 6.05* and *Statistica 7.0* software. Logic regression used for establishing genetic dependencies.

RESULTS

Liver protein synthesizing function did not suffer in observed patients, regardless of the analyzed genes' polymorphic variants (tab. I). In ACE gene's DD genotype, the level of direct bilirubin, as well as the activity of AST, ALT, and leukocyte content were higher than in II/ID-genotypes. Conjugated bilirubin was higher by 22.24% and 20.55% ($p<0.05$), AST – 2.12 and 1.71 times ($p<0.05$), respectively; for ALT – 1.32 times ($p<0.05$); leukocytes by 14.56% ($p<0.05$). Ala-allele carriers had higher rates of AST, ALT as well as leucocytes compared to Pro12-genotype of the PPAR-γ2 gene – by 35.21%, 38.64%, 29.81% and 12.95%, respectively ($p<0.05$). The fasting glucose level was higher in all patients regardless of type 2 DM, requiring further study.

Activity of adipose tissue by the content of adipocytokines in plasma, depending on the NAFLD type and OB degrees, is presented in tab. II. Leptin level is higher in women, regardless of NAFLD type and OB degrees by 1.74-2.39 times ($p<0.001$). In men with non-alcoholic steatosis of the liver (NASL) concentration of leptin was 25.39% higher than in men with non-alcoholic steatohepatitis (NASH), $p_{NASH}<0.05$. In case of 3rd degree OB, leptin content was higher than in 1st and 2nd OB degrees, regardless of gender: in men by 48.99% ($p_{OB1}=0.022$) and 43.55% ($p_{OB2}=0.034$); in women by 53.34% ($p_{OB1}<0.001$) and 50.98% ($p_{OB2}=0.002$).

The rate of leptin resistance (LR) increased by 22.22% ($p<0.05$) in NASL patients and by 46.15% in NAFLD ($p=0.035$) and combined with the 3rd degree OB by 42.79% ($p=0.044$). Concentration (tab. II) of adiponectin in patients with 2nd and 3rd degrees OB was lower than in those with the 1st degree by 5.73% and 5.91% ($p<0.05$). The single-factor dispersion analysis confirmed association of NAFLD with high leptin content in men ($F=236.86$, $p<0.001$) and LR ($F=283.94$, $p<0.001$).

TABLE I

Biochemical indices of hepatocytes function in NAFLD patients depending on the PPAR-γ2 (Pro12Ala) and ACE (I/D) genes' polymorphisms

Indices	ACE gene genotype			PPAR-γ2 gene genotype	
	II	ID	DD	12Ala, ProAla	Pro12
Total protein (g/L)	69.33±1.20	70.98±1.55	65.93±2.77	64.22±3.68	67.95±4.13
Total bilirubin (μmol/L)	15.28±1.35	16.92±2.43	16.17±1.23	16.24±2.32	16.09±1.25
Conjugated bilirubin (μmol/L)	5.07±0.86	5.18±0.59	6.52±0.44 $p_{II.ID}$<0.05	5.23±0.38	5.41±0.75
AST (U/L)	0.34±0.19	0.42±0.11	0.72±0.09 $p_{II.ID}$<0.05	0.71±0.08	0.46±0.10 p_{Ala}<0.05
ALT (U/L)	0.57±0.06	0.61±0.15	0.75±0.07 p_{II}<0.05	0.88±0.11	0.54±0.13 p_{Ala}<0.05
LDH (U/L)	317.40±19.43	320.51±17.0	334.82±34.6	349.08±26.85	313.99±30.64
Alpha amylase (U/L)	46.84±1.92	49.02±3.14	48.79±2.59	49.88±2.57	48.36±3.05
Alkaline phosphatase (U/L)	244.06±14.99	229.46±32.25	265.34±17.86	233.92±20.0	268.93±12.01 p_{Ala}<0.05
GGT (U/L)	38.98±3.74	43.56±4.27	44.08±2.62	41.72±5.11	43.95±6.44
Glucose (mmol/L)	6.33±0.75	7.13±0.62	7.45±0.47	6.93±0.77	7.25±0.48
Leucocytes (×10^9/L)	6.01±0.55	5.81±0.35	6.80±0.40 p_{ID}<0.05	6.64±0.43	5.78±0.29 p_{Ala}<0.05
Urea (μmol/L)	5.25±0.69	6.38±1.33	6.21±1.07	6.31±1.31	6.23±0.78
Creatinine (μmol/L)	75.58±5.13	84.77±10.21	85.83±8.69	88.47±6.54	85.36±11.0

p_{II}, p_{Ala} – reliability of differences between the values for a specific gene relative to the II homozygotes of the ACE gene and the carriers of the Ala-allele (12Ala+ProAla) of the PPAR-γ2 gene; p_{ID} – reliability of differences for a specific gene relative to ID-heterozygotes

TABLE II

Content of adipocytokines in patients considering the NAFLD type and degrees of obesity

Indices			Leptin, ng/mL	Adiponectin, ng/mL	Leptin resistance, U
NAFLD	NASH	M	30.38±3.92	75.88±2.50	20.09±2.37
		W	69.69±8.50 P_m<0.001		
	NASL	M	40.72±2.06 p_{NASH}<0.05	74.35±3.75	25.83±0,88 p_{NASH}<0.05
		W	71.01±9.21 p_m<0.001		
Obesity	OB I	M	22.23±3.77	77.88±1.81	17.92±2.31
		W	48.67±7.38 p_m<0.001		
	OB II	M	24.60±5.59	73.42±2.05 p_{OB1}=0.004	19.04±3.15
		W	51.13±6.44 p_m<0.001		
	OB III	M	43.58±7.66 p_{OB1}=0.022 p_{OB2}=0.034	73.28±2.53 p_{OB1}=0.018	33.28±6.07 p_{OB1}=0.035 p_{OB2}=0.044
		W	104.30±9.25 p_{OB1}<0.001 p_{OB2}=0.002 p_m<0.001		

OB I-III – obesity of the I-III degrees; M – men; W – women; p_{NASH} – reliability of differences between the indices relative to individuals with NASH by each separate index; p_{OB1} – reliability of differences between the indices relative to individuals with the first degree obesity by each separate index (including the content of leptin taking into consideration gender); p_{OB2} – reliability of differences between the indices relative to individuals with the second degree obesity by each separate index; p_M – reliability of differences between indices relative to men.

Obesity was associated with high leptin content in men (F=77.95, p<0.001) and women (F=341.43, p<0.001), with LR increase (F=103.17, p<0.001) and decrease of adiponectin (F=44.84, p<0.001).

In female D-allele carriers of the ACE gene, leptin level exceeded the one in individuals with II-genotype (tab. III) by 22.19% (p_{II}=0.048) and 28.73% ((p_{II}=0.036). In Pro12 genotype of the PPAR-γ2 gene, leptin level exceeded that of the Ala-allele carriers regardless of their sex (tab. III): in men 1.99 (p_{12Ala}=0.036) and 3.75 times p_{ProAla}=0.008), in women by 32.79% (p_{12Ala}=0.015) and 27.81% (p_{ProAla}=0.043). The rate of LR prevailed exclusively in male homozygous Pro-allele carriers, over Ala-allele carriers – 2.23 and 3.16 times (p<0.01), in contrast to women, where such dependences were not found, despite higher initial level of both leptin itself and the rate of LR.

TABLE III

The content of adipocytokines in NAFLD patients, depending on polymorphic variants of the ACE (ACE) and PPAR-γ2 (Pro12Ala) genes

Genotypes		Leptin, ng/mL		Leptin resistance, U		Adiponectin, ng/mL
Genotypes of the ACE gene	II	M	25.49±2.58	M	14.80±3.18	80.18±2.90
		W	28.93±3.88	W	20.05±5.16	
	ID	M	18.60±1.47 p_{II}=0.041	M	11.29±2.50	77.88±2.13
		W	37.18±3.55 p_{II}=0.048 P_m<0.001	W	23.03±4.47 p_m<0.01	
	DD	M	20.44±3.85	M	16.36±3.99	75.65±1.67
		W	40.59±4.98 p_{II}=0.036 P_m<0.001	W	24.17±3.81	
Genotypes of the PPAR-γ2 gene	12Ala	M	11.38±0.47	M	6.80±1.64	77.87±1.85
		W	27.48±1.39 P_m<0.001	W	27.91±4.38 p_m<0.001	
	ProAla	M	6.04±0.90 p_{12Ala}=0.004	M	4.79±0.73	77.65±1.49
		W	29.52±4.04 P_m<0.001	W	19.67±4.68 p_m<0.001	
	Pro12	M	22.65±3.05 p_{12Ala}=0.036 p_{ProAla}=0.008	M	15.14±3.69 p_{12Ala}<0.01 p_{ProAla}<0.01	76.88±2.11
		W	40.89±5.01 p_{12Ala}=0.015 p_{ProAla}=0.043 p_m<0.001	W	25.22±5.77 p_m<0.05	

M – men; W – women; p_{II} – reliability of differences between indices relative to individuals with genotype II; p_{ID} – reliability of differences between indices relative to individuals with genotype ID; p_{12Ala} – reliability of differences between indices relative to individuals with genotype 12Ala; p_{ProAla} – reliability of differences between indices relative to individuals with genotype ProAla; p_m – reliability of differences between indices relative to men.

DISCUSSION

Several studies focused on mechanisms of NAFLD development depending on polymorphisms of the genes involved in the regulation of lipids and carbohydrates metabolisms. Despite this fact, role of ACE and PPAR-γ2 genes in NAFLD remained unclear until recent times (3, 12).

Single-factor dispersion analysis confirmed association of I/D (rs4646994) polymorphism of the ACE gene with high leptin content and the LR rate both in men and in women, as well as reduction of adiponectin. Data concerning the ACE gene I/D polymorphism is similar to other European populations but present less I-allele compared to Asian populations. Similar associations of Pro12Ala polymorphism of the PPAR-γ2 gene were also established. Distribution of Pro12Ala polymorphism of the PPAR-γ2 gene in this study is similar to other European studies. In addition, Ala-allele was more frequent and Pro-allele less frequent than in both Asian and Equatorial races (7, 13, 17).

Similar data of adipose tissue secretory activity – the increased synthesis of leptin, increased LR, and decreased adiponectin in ProPro-genotype owners (PPAR-γ2 gene), were previously presented in several papers (7, 18). In our study, we found no association of the DM frequency depending on rs1801282 polymorphism of the PPAR-γ2 gene, unlike data found in Iranian population (19). While ACE is promising drug target for the management of NAFLD, no correlations of ACE gene polymorphism with risk factors and the disease severity in NAFLD were previously established (20), unlike our data.

CONCLUSIONS

This study shows existing associations of changes in hepatic function, lipid metabolism, and adipocytokines activity in NAFLD with the ACE (rs4646994) and PPAR-γ2 (rs1801282) genetic polymorphisms. ACE DD-genotype and PPAR-γ2 gene's Ala-allele associate with higher conjugated bilirubin, transaminases, and leucocytes. Secretory activity of adipose tissue is significantly higher by the content of pro-atherogenic leptin, with the development of leptin resistance in women, regardless of the NAFLD type and OB degrees, especially in D-allele carriers of the ACE gene. NAFLD with OB is characterized by reliable increase in leptin concentration, especially in Pro12 genotype carriers of the PPAR-γ2 gene, with increase in LR and decrease of anti-atherogenic adiponectin.

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THORACO-ABDOMINAL INJURIES: THE GENERAL SURGEON'S PERSPECTIVE

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THORACO-ABDOMINAL INJURIES: THE GENERAL SURGEON'S PERSPECTIVE (Abstract): **Backround:** Thoraco-abdominal traumatic injuries affect the inferior part of the thoracic cavity and the superior part of the abdominal cavity. This study summarizes five years of our surgical department's experience in the diagnosis and management of thoraco-abdominal trauma patients. **Methods:** We examined records from our surgical and emergency room departments from January 1996 to December 2000, and selected patients with thoraco-abdominal injuries. **Results:** Fifty-five patients were treated who suffered coexisting trauma of the thoracic and abdominal cavity. Males represented the majority of patients and mean age was 38.2 years. Traffic accidents were the major cause (55%) followed by criminal acts of violence (32%) and falls (13%). The most common thoracic injuries were rib fractures (40%) and simple lung contusions (35%) and the abdominal organs most commonly injured were the spleen (35%), liver (25%) and kidney (20%). Surgical interventions were performed in 68% of patients, whereas the remaining patients were treated conservatively. **Conclusions:** Thoraco-abdominal injuries are characterized by high heterogeneity and can provide significant decision-making challenges. The accurate diagnosis of all coexisting injuries is critically important, as the diagnosis will determine surgical or non-operative management of these injuries. **Key words:** THORACIC, ABDOMINAL, TRAUMA, DIAGNOSIS, MANAGEMENT.

The aim of this study is to present the five-year experience of our surgical department in diagnosis and management of thoraco-abdominal traumatic injuries that affect the inferior part of the thoracic cavity and the superior part of the abdominal cavity. These injuries are characterized by high heterogeneity due to the variety of injury mechanisms that can be involved and the anatomy of this body part. Thoraco-abdominal injuries can provide significant decision-making challenges and the surgeon should be able to consider all possibilities in order to diagnose life threatening injuries (1, 2).

The organs located in the superior part of the abdominal cavity are the liver, spleen, stomach and parts of the pancreas

and kidneys, which are protected by the inferior ribs. However, the abdomen is more vulnerable to injury than the chest because there is little bony structure below the ribcage to protect internal organs from anterior and lateral impacts. Due to the elasticity of the thoracic cage, blunt impact of the upper abdomen can compress and injure abdominal organs with minimal or no injury to the bones. Compression of the abdominal cavity against a fixed object such as a safety belt or steering wheel results in a rapid increase in intraluminal pressure, which may in turn cause bowel rupture and tears or hematomas of solid organs without rib fractures (2, 3).

MATERIALS AND METHODS

We present our experience with patients who have had coexisting trauma of the thoracic and abdominal cavity. For this retrospective study, the records of our surgical department and the emergency department were examined from January 1996 to December 2000 to flag patients with thoraco-abdominal injuries. A total of 55 patients were treated in our department during this time frame.

All patients admitted to the trauma center were resuscitated utilizing Advanced Trauma Life Support (ATLS) protocols. Resuscitation consisted of rapid infusion of warm fluids and blood. All patients had recordable vital signs on admission to the Emergency Room and had suffered concomitant thoraco-abdominal traumatic injuries affecting the lower part of the thorax and one or more abdominal organs (fig. 1).

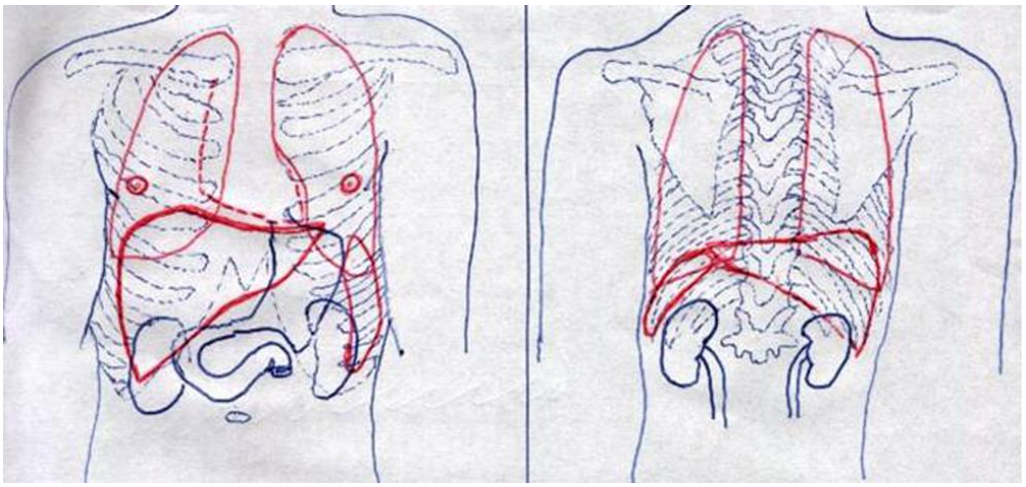

Fig. 1. Crude anatomy of the thorax and abdomen

The inclusion criteria for patients into our study were: 1) Injury of the lower chest (anterior ribs 5-10 and posterior ribs 9-12), including simple contusions of the thoracic wall, and 2) Simultaneous injury to an organ of the upper abdomen ranging from simple contusions to severe rupture of solid organs.

RESULTS

Males represented the majority of patients (85%) while females were less commonly represented (15%) with a male to female ratio of 5.7 to 1 (fig. 2). The patients ranged in age from 16 to 86 with a mean age of 38.2 years (fig. 3).

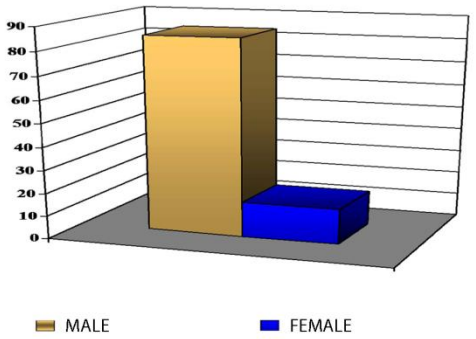

Fig. 2. Distribution of patients by sex

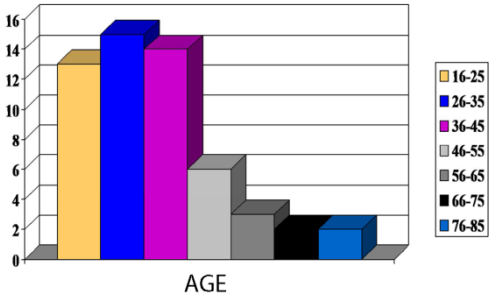

Fig. 3. Distribution of patients by age

Regarding the etiology of the injury, 55% of cases were caused by traffic accidents and 32% of cases were from criminal acts (of which 83% of these acts (15/18) involved injury by a lethal weapon, whereas the remaining 17% (3/18) were related to use of force). Falls accounted for the remaining 13% of total cases. Traffic accidents were further separated into car accidents (74%), bicycle accidents (16%) and pedestrians hit by automobiles (10%). In car accidents, about 2/3 of the patients were not using seatbelts.

The *mechanism* of *injury* is important in the management of a patient with a thoraco-abdominal injury. Three main groups of trauma mechanisms were recorded:

1. Blunt trauma of the inferior thoracic wall, where the kinetic energy of the power that injures the thorax is transmitted to the structures of the region (69% of cases),
2. Acute penetrating injuries (27% of cases), and
3. Combinations of both (4% of cases).

The injuries of the thoracic cavity were divided as follows: rib fracture (with or without hemothorax) (40%), rupture of chest wall (35%), and pulmonary fracture (with or without hemopneumothorax) (25%) (Table I).Injuries of the abdominal cavity were divided as such: spleen (35%), liver (25%), kidney (20%), diaphragmatic (6%) and combinationatorial injuries (14%) (i.e., diaphragm and inferior vena cava, diaphragm and stomach, or multiple injuries) (table I).

TABLE I

Thoracic injuries and abdominal injuries sustained by patients with thoraco-abdominal injuries.

Thoracic cavity	***Abdominal Cavity***
1. Rib fracture (with or without hemopneumothorax) (40%)	1. Spleen injury (35%)
2. Contusion of chest wall (35%)	2. Liver injury (25%)
3. Pulmonary contusion (with or without hemopneumothorax) (25%)	3. Kidney injury (20%)
	4. Diaphragmatic injury (6%)
	5. Combination (14%) - Diaphragm+vena vein - Diaphragm+stomach - Multiple injuries

Additionally, we recorded the following combinations: rib fracture with or without hemo/pneumothorax and spleen injury (26%), fracture of chest wall and liver injury (19%), rib fracture with or without hemo/pneumothorax and kidney injury (13%), fracture of chest wall and spleen injury (9%), fracture of chest wall and kidney injury (7%) and other combinations (26%) (table II).

TABLE II
Combined thoraco-abdominal injuries

Combination of thoraco-abdominal injuries
1. Rib fracture +/- hemo/pneumothorax & spleen injury (26%)
2. Contusion of chest wall & liver injury (19%)
3. Rib fracture +/- hemo/pneumothorax & kidney injury (13%)
4. Contusion of chest wall & spleen injury (9%)
5. Contusion of chest wall & kidney injury (7%)
6. Other combinations (26%)

Regarding the diagnostic modalities used for the patients with thoraco-abdominal injuries, we first used simple x-rays in 77% of cases, trauma exploration in 18% of cases and other tests (CT, U/S) for the remaining 5% of cases. As secondary diagnostic tests, we used U/S in 58% of cases, peritoneal lavage in 33%, CT in 5% and simple x-rays in 4% of cases. For tertiary diagnostic tests we used CT in 66% of cases, simple x-rays in 19% and other tests (angiography, urography, etc.,) in the remaining 15% of cases (table III).

Regarding trauma management, 68% of patients underwent exploratory laparotomy, or another major surgery. The specific interventions in the abdomen were: splenectomy (49% of cases), surgical hemostasis (38% of cases), nefrectomy (5% of cases) and diaphragm suturing (8% of cases). In the remaining 32% of cases, conservative management was chosen. The treatment of coexisting chest injuries was mostly conservative, since in only three cases (5%) a thoracotomy was required to control hemorrhaging of a large vessel in the chest. In 21% of patients (n = 12), Bülau drains were used for the treatment of hemopneumothorax (42%), pneumothorax (31%) and hemothorax (27%).

TABLE III
Diagnostic modalities used

1st diagnostic modality	
X-rays	77%
Wound exploration	18%
Others (US/CT)	5%
2nd diagnostic modality	
Ultrasound	58%
Diagnostic peritoneal lavage	33%
Computed tomography	5%
X-rays	4%
3rd diagnostic modality	
Computed tomography	66%
X-rays	19%
Others (angiography, urography etc)	15%

The average hospital stay was 13 days (min 6 days, max 27 days). Mortality rate was 5.5% (3 patients), which resulted from major injuries, such as rupture of the inferior vena cava or a section of the major hepatic vases.

DISCUSSION

Given our results over a five-year period, it is clear that thoraco-abdominal injury is a major challenge for the emergency trauma surgeon. Diagnostic errors often occur, as these injuries perplex even the most experienced trauma surgeons. An interesting observation of the current study is that car accidents are the major cause of thoraco-abdominal injury and are responsible for over half of the injuries, followed by acts of criminal violence, contrary to international experience, where the correlations are reversed (4).

The pre-hospital care, support of vital functions and hemodynamic stabilization of the patient have a crucial role in increasing survival for all trauma patients (2, 3). When the two largest cavities of the body are involved in trauma, this will lead the trauma surgeon to suspect serious abdominal injury, even in cases of simple contusions. Rapid assessment of physical signs of injury is a sine qua non, but it has been estimated that in many cases, clinical examination can be misleading, especially if patients are intoxicated, have neurological problems or require general anesthetics (5). Patients should be monitored closely for changes in vital signs as assessed by serial abdominal examinations (6, 7).

X-rays are necessary, but in most cases insufficient to complete the diagnosis (8). Ultrasound has proven to be an ideal recourse in the emergency evaluation of the trauma patient because it rapidly and accurately detects intra-abdominal fluid or blood and is easily repeatable. Ultrasonography provides valuable information that augments the surgeon's diagnostic capability but results in relatively decreased sensitivity for parenchymal intra-abdominal injuries (9, 10). Ultrasound sensitivity in the diagnosis of diaphragmatic rupture is approximately 89% (11). Diagnostic peritoneal lavage is an invasive treatment with a sensitivity of about 95% in detecting intra-abdominal bleeding with the quantitative analysis of the lavage fluid and is indicated in cases requiring quick decision-making (e.g., hemodynamic instability) (2, 3). Diagnostic peritoneal lavage is considered positive when there is 10 ml or more of frank blood or a red cell count over 100,000/mm3 (12).

The CT scan is a safe, fast and non-invasive procedure to accurately assess the two major body cavities but requires hemodynamically stable patients (13, 14). The CT scan is not only necessary for patients who are candidates for conservative treatment, but also for trauma patients with injuries involving the central nervous system. For penetrating injuries, wound exploration under local anesthesia is necessary. This procedure is usually performed in the operating room, but can be performed in the emergency department by some institutions. The wound is extended under local anaesthesia and the track followed through tissue layers (3). This practice solves many diagnostic problems, since it reveals whether there is a rupture of serosa membranes and the diaphragm. Finally, when the diagnosis remains inconclusive after these tests, other modalities such as angiography and intravenous urography are helpful alternatives.

A hemothorax found in association with a diaphragmatic injury in either a penetrating or blunt trauma may actually have its origin from an intra-abdominal source. Blood from injured abdominal organs may traverse a diaphragmatic rupture and enter the thoracic cavity. In cases of hemothorax with diaphragmatic injury, the trauma surgeon should strongly consider the possibility of intra-abdominal injury. The late compli-

cations of hemothorax, including residual clot, infected collections and trapped lung require additional treatment and, most often, surgical intervention (15, 16).

In cases of hemothorax with excessive bleeding that could not be controlled by Bülau placement (>500 ml upon placement and continuous bleeding) a thoracotomy and surgical hemostasis were performed. Concerning injuries of the abdominal organs, splenic injuries were treated via splenectomy (except for a single case), since splenic ruptures are dangerous even in hemodynamically stable patients. For patients with splenic parenchyma contusions conservative treatment was chosen. In 57% of patients with hepatic injuries, surgery was not necessary, and the patients were treated conservatively (as is the latest trend in surgery), although conservative treatment is not free of complications. Criteria for non-operative management of hepatic injuries include hemodynamic stability and lack of an associated injury requiring a celiotomy (16, 17, 18). We applied conservative treatment to most of the renal injuries considered minor in severity, except those patients who were hemodynamically unstable, or those with severe renal parenchyma fractures.

Injuries of the diaphragm are usually accompanied by other injuries, and they indicate that a more severe injury may have occurred, most of which are due to penetrating wounds and are called open thoraco-abdominal injuries. Diagnostic laparoscopy allows for the evaluation of trauma patients lacking clinical indications for a formal celiotomy. The clinical diagnosis of diaphragmatic rupture is difficult and is missed in anywhere from 7-66% of patients (19, 20). Thoracoscopy and laparoscopy are techniques that can accurately establish the diagnosis, but their role is limited to emergencies, whereas major surgical access is often more secured according to current data. Surgical repair is necessary (even for small ruptures) because the defect will not heal spontaneously and thoraco-abdominal pressure gradients will favor the enlargement of the defect with herniation of abdominal content (20).

CONCLUSION

It is evident that thoraco-abdominal injuries are heterogeneous in nature and difficult to categorize. All injuries that involve the chest and abdomen by the same projectile are true thoraco-abdominal injuries (21). Of critical importance is not to define exactly which organs were injured, but if a major surgery is necessary. The mortality rate of thoraco-abdominal injuries is about 5-10% (22, 23). Given this mortality rate, it becomes imperative to surgically explore any doubtful thoraco-abdominal injury, just as one would explore any questionable abdominal penetration. Taken together, unless one is certain that the lesion in question does not involve the abdomen, surgical exploration is mandatory.

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EVALUAREA MORTALITĂȚII ÎN PATOLOGIA CHIRURGICALĂ ABDOMINALĂ LA PACIENȚII PESTE 75 DE ANI. STUDIU RETROSPECTIV

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THE EVALUATION OF MORTALITY IN ABDOMINAL SURGERY OF PATIENTS AGED OVER 75. A RETROSPECTIVE STUDY ON A SERIES OF 111 CASES (Abstract). Abdominal surgery of patients aged over 75 is a real challenge, regarding the risk-benefit ratio, especially in emergency cases. The aim of the study was to assess the mortality in elderly patients admitted for abdominal surgical conditions. **Material and methods**. A retrospective study was carried out on a series of 111 patients aged over 75 that died during hospitalization for abdominal diseases in the 3rd Surgical Unit during the period 2007-2010. Data from the medical files, operating books and pathology reports were collected and analyzed. **Results**. Of the 61 cases admitted for benign conditions, 11 deceased prior to surgery due to high ASA risk. 50 patients underwent surgery, mainly on emergency basis (42 cases). Most of the patients in this group suffered from intestinal infarction (22), followed by abdominal sepsis (16), intestinal obstruction (12) and digestive bleeding (10). Resection followed by anastomosis was performed in 17 cases; of these, anastomotic fistula with sepsis developed in 7 cases (41 %). Malignant abdominal diseases were diagnosed in 50 patients, most frequently colorectal cancer (25) followed by gastric cancer (14), pancreatic cancer (5), billiary duct carcinoma (3), malignant ampuloma (2) and hepatoma (1). In this group, 46 patients were operated on, mainly in emergency (35). Of the 32 cases where resection was performed, anastomostic fistula with sepsis developed in 21 (65,6 %). **Conclusions**, the main cause of death was abdominal sepsis due to anastomotic fistula following resections, for both benign and malignant condition. **Key words**: MORTALITY, ABDOMINAL SURGERY, ELDERLY.

Patologia chirurgicală abdominală a pacienților peste 75 de ani ridică numeroase probleme de management din cauza prezenței aproape invariabile a comorbidităților de sistem, ce creşte riscul morbidității şi mortalității postoperatorii. Scopul studiului a fost de a evalua cauzele mortalității pacienților peste 75 de ani internați pentru o afecțiune chirurgicală abdominală.

MATERIAL ŞI METODE

Studiul retrospectiv se bazează pe analiza a 111 pacienți cu afecțiuni chirurgicale abdominale, decedați în timpul spitalizării în Clinica a III-a Chirurgie, pe o perioadă de 4 ani (2007-2010). A fost realizată o împărțire a lotului în relație cu tipul afecțiunii în două grupuri - 61 de cazuri cu patologie benignă, respectiv 50 de cazuri cu

afecţiuni maligne digestive.

A fost utilizată baza de date a Centrului de Statistică al Spitalului "Sf. Spiridon" precum şi informaţiile din foile de observaţie, protocoalele operatorii şi registrele de anatomie patologică. S-au exclus din studiu pacienţii decedaţi pentru o altă patologie decât cea abdominală. Factorii analizaţi au fost: vârsta, sexul, diagnosticul principal la internare, tipul de intervenţie chirurgicală, cauza de deces şi durata spitalizării.

REZULTATE

Pe fondul creşterii progresive a numărului internărilor în ultimii 4 ani s-a înregistrat şi o frecvenţă deosebită a pacienţilor vârstnici, cu excepţia ultimului an ce prezintă o uşoară scădere. Rata deceselor bolnavilor spitalizaţi în ultimii 4 ani a avut o medie anuala de 2%, iar cea a pacienţilor cu vârste de peste 75 de ani, din totalul internărilor de 6,9%. Numărul deceselor pacienţilor cu vârste de peste 75 de ani din totalul celor înregistrate în timpul spitalizării a atins un procent mediu anual de 24,6%, ceea ce reprezintă aproximativ 50% din totalul deceselor pacienţilor cu vârste peste 75 de ani internaţi pentru o afecţiune abdominală.

Caracteristicile generale ale lotului studiat

Vârsta pacienţilor a fost de 75-94 de ani, cu o medie de 80,4 ani, fără diferenţă semnificativă între lotul fără neoplazie şi cel cu neoplasm. Dintre cele 61 cazuri fără afecţiuni neoplazice, 37 (60,6%) au fost femei şi 24 (39,3%) bărbaţi. Ischemia mezenterică a fost cea mai frecventă afecţiune (22-36,06%) urmată de patologia septică (16-26,22%), ocluzivă (12-19,67%) şi hemoragică (10-16,39%). Durata medie de spitalizare în cadrul patologiei benigne a fost de 7,9 zile (fig.1).

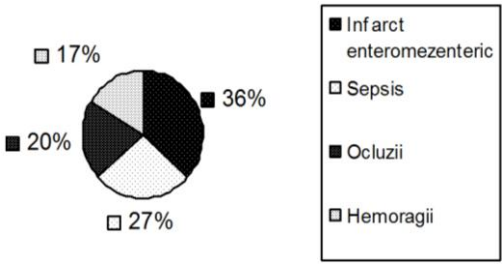

Fig. 1. Distribuţia lotului fără neoplazie pe variate afecţiuni

2. Infarctul entero-mezenteric a fost cauza principală de deces, la 11(50%) s-a efectuat doar laparotomie exploratorie, extensia totală/cvasitotală a infarctului entero-mezenteric fiind deasupra resurselor terapeutice chirurgicale, pacienţii decedând în medie la 48 de ore postoperator. La 8(36,36%) s-a practicat în urgenţă, excizia segmentului intestinal necrozat, însă toţi au evoluat septic, cu apariţia insuficienţei multisistemice de organ (MSOF). Majoritatea pacienţilor erau cunoscuţi cu patologie cardio-vasculară de tipul fibrilaţiei atriale, hipertensiunii arteriale şi insuficienţei cardiace, tratate şi controlate intermitent, fără terapie antitrombozantă. Trei pacienţi au decedat la câteva ore de la internare, iar cauzele infarctului entero-mezenteric au fost embolia arterială (50%), urmată de tromboza arterială mezenterica (30%) şi 20% tromboza venoasă.

3. Patologia septică care a inclus 5 cazuri de icter mecanic litiazic asociat cu angiocolită, 5 de colecistită acută gangrenoasă asociată cu peritonită biliară localizată şi/sau generalizată, 3 de apendicită acută gangrenoasă perforată asociată cu peritonită, un caz de ulcer perforat cu peritonită generalizată, unul cu abces hepatic şi un altul cu diverticulită acută perforată, cu peritonită stercorală. Cu excepţia cazurilor de icter mecanic litiazic, care au fost opera-

te electiv după încercarea eşuată de sfincterotomie endoscopică, în celelalte s-a intervenit de urgență. În trei cazuri de icter mecanic litiazic s-a practicat coledocolitotomia şi anastomoză bilio-digestivă iar în două coledocolitotomie cu drenaj Kehr. Cauza directă de deces a fost reprezentată de şocul septic cu MSOF, în două cazuri fiind evidențiată fistula bilio-digestivă. Cazurile de colecistită acută gangrenoasă asociată cu peritonită biliară, chiar dacă au fost operate în urgență, au evoluat nefavorabil, în ciuda tratamentului adecvat; gravitatea infecției la internare grevată pe un teren biologic tarat a făcut ca rezultatul postoperator să fie infaust. Într-un singur caz s-a documentat septicemia stafilococică, în rest infecțiile au fost cauzate de bacili Gram negativ.

4. Ocluzia intestinală a fost diagnosticată în 12 (19,67%), din care hernia inghinală/crurală ştrangulată în 6; sindrom aderențial, 3; un caz de hernie ombilicală nereductibilă şi un altul de stenoză pilorică. Cauzele directe de deces în aceste situații au fost: 5 fistule digestive cu sepsis agravant intraabdominal; o pneumonie de aspirație; o embolie pulmonară; un accident vascular cerebral (AVC) major şi 3 cazuri de şoc cardiogen. Exceptând stenoza pilorică, toate celelalte cazuri au fost operate în urgență.

5. Hemoragia a fost incriminată drept cauza inițială de deces în 10 cazuri, dintre care 8 cu ulcer peptic hemoragic; un caz de rectocolită ulcero-hemoragică şi altul cu hemoperitoneu prin ruptură de splină. Cauza directă de deces în aceste situații a fost pneumonia de ventilator (2 cazuri), infarct miocardic acut (2), sindrom de aspirație (1), AVC (1), embolie pulmonară (1) şi 3 cazuri de şoc hemoragic. În 2 cazuri de ulcer hemoragic s-a practicat intervenția chirurgicală de hemostază. Un singur caz de pancreatită acută necrotico-hemoragică formă gravă a decedat la câteva ore de la internare prin MSOF.

6. Neoplasmul colo-rectal s-a situat pe primul loc ca diagnostic principal de internare, înregistrând un număr de 25 (50%) din cazurile de neoplasm. Localizarea cea mai frecventă a fost distală: colon sigmoid (8), rect (5), joncțiune recto-sigmoidiană (3), colon descendent (4), colon transvers (3) şi colon drept (2). Numai 4 cazuri din 25(16%) au fost operate electiv, restul cu intervenție de urgență: ocluzie (19), perforație tumorală sau diastatică cu peritonită stercorală (7). Au fost practicate colectomii cu restabilirea continuității (13), operația Hartmann (8), amputație de rect (1), acolostomie laterală (1), ileotransversostomie (1). Un caz de neoplasm de rect hemoragic a decedat în perioada de pregătire preoperatorie secundar unui infarct de miocard acut. Cauzele directe de deces au fost şocul septic (18-36%), şocul cardiogen (3-6%), infarctul miocardic acut (2-4%) şi bronhopneumonia (2-4%). În 10 dintre cele 18 cazuri de şoc septic s-a evidențiat fistula anastomotică ce a contribuit în mare măsură la accentuarea şi agravarea stării septice.

7. Neoplasmul gastric a fost diagnosticat în 14(28%) cazuri. În 11 dintre acestea, s-a practicat gastrectomia totală sau subtotală, în două gastro-enteroanastomoza şi într-un caz jejunostomia de alimentație. Cauzele directe de deces au fost reprezentate de: şocul septic (7-14%), şocul cardiogen (2-4%), pneumonia de aspirație (2-4%), bronho-pneumonia (1), pneumonia stafilococică (1) şi infarctul miocardic acut (1). Şocul septic a avut drept cauză postoperatorie apariția fistulei anastomotice sau de bont duodenal.

8. Neoplasmul pancreatic s-a înregis-

trat în 5(10%) cazuri, toate fiind diagnosticate tardiv, complicate cu icter mecanic asociat cu angiocolită sau în stadiul de carcinomatoză peritoneală. Intervenţiile paliative de *by-pass* biliodigestiv s-au soldat, invariabil, cu fistulă anastomotică, ce a indus şocul septic ca şi cauză de deces.

9. Colangiocarcinomul a fost diagnosticat în 3(6%) cazuri, neoplasm al ampulei Vater în 2(4%) iar hepatom asociat cirozei hepatice într-un caz (2%). Durata medie de spitalizare este mai îndelungată în cazul patologiei maligne, fiind de 15,6 zile (fig. 2).

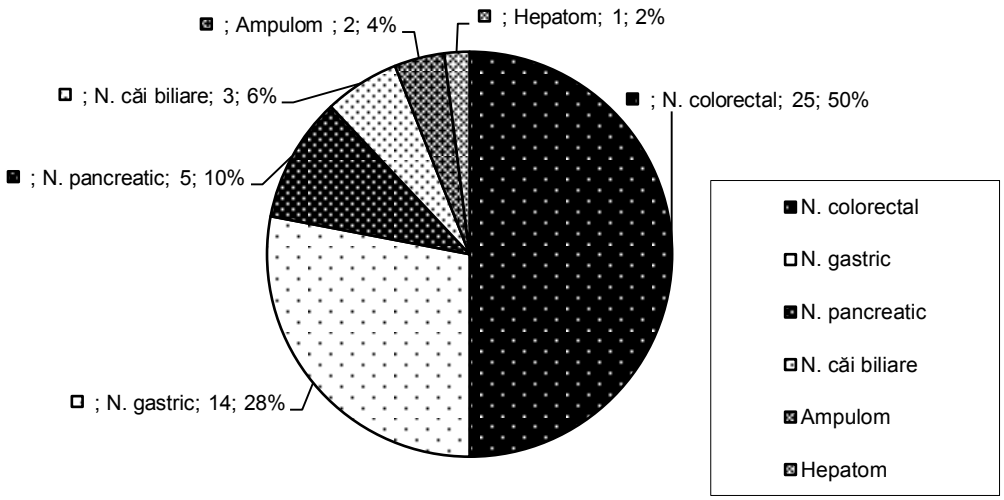

Fig. 2. Distribuţia cazurilor studiate pe categorii de patologie neoplazică

DISCUŢII

În general se admite că pacienţii vârstnici tolerează bine chirurgia abdominală, mai puţin complicaţiile postoperatorii. Rezultatele bune depind de o evaluare preoperatorie atentă, cântărind cu grijă riscurile şi beneficiile tratamentului chirurgical. Vârsta în sine nu este un factor de risc independent pentru morbiditate, mortalitate şi durata spitalizării, ci prezenţa comorbidităţilor majore ce cresc scorul ASA (American Society of Anesthesiology), întârzierea în sancţiunea chirurgicală şi situaţia locală a bolii care nu permite decât o paleaţie (1). O cauză importantă de deces este reprezentată de şocul septic, indiferent de patologia benignă sau malignă, mai ales în situaţiile de apariţie a unei fistule anastomotice. Infecţiile acute de organ reprezintă un diagnostic de internare ce are la vârstnic un potenţial de agravare dacă nu se intervine la timp (1, 10). În general factorii de risc pentru infecţii la un vârstnic includ subnutriţia cronică, reducerea masei musculare, dentiţia deficitară, şi diabetul zaharat. Reflexul de înghiţire deficitar poate conduce la favorizarea aspiraţiei conţinutului digestiv în arborele bronşic, cu apariţia sindromului Mendelson şi dependenţa de respiraţia mecanică. Cancerul este o altă cauză majoră de deces, mai ales pentru cazurile internate în stadii avansate, complicate, la care tratamentul chirurgical este paliativ. Deoarece cancerul colorectal şi cel gastric sunt pe primele două locuri în decesul vârstnicului, metodele de *screening* la

vârstnic sunt dezbătute în literatură în favoarea lor (1, 2, 9). Costa et al. (3) raportează o mortalitate postoperatorie în chirurgia abdominală de urgență la pacienții între 70-79 de ani de 19,7% şi pentru cei peste 80 de 26,2%, concluzionând că mortalitatea este strict dependentă de comorbidități. Tabloul clinic atipic al abdomenului acut la pacienții vârstnici şi tendința de a se prezenta târziu la medic duc la pierderea momentului terapeutic optim. Nu vârsta este un factor de risc ci întârzierea diagnosticului şi prezența comorbidităților severe (4, 5, 6). S-a constatat că există o atitudine de reținere pentru chirurgia radicală cu viză curativă la pacienții neoplazici vârstnici, bazată mai ales pe judecata subiectivă a chirurgului ce decurge din experiența personală (6, 7, 8).

CONCLUZII

Cea mai importantă cauză de deces a fost reprezentată de şocul septic indiferent de caracterul benign sau malign al afecțiunii abdominale. Numai 13% din cazurile cu afecțiuni benigne respectiv 42% din cele maligne s-au internat electiv, predominând în general caracterul de urgență a afecțiunii, ceea ce a contribuit la creşterea morbidității şi mortalității intra-spitaliceşti.

Cazurile la care s-a practicat o anastomoză digestivă s-au complicat cu fistule în proporție de 41% la lotul fără afecțiuni neoplazice şi de 65,6% la cel , fiind cauza princeps de şoc septic şi mortalitate.

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SPECTRUL ZGOMOTULUI CARE DETERMINĂ HIPOACUZIA PROFESIONALĂ ÎN INDUSTRIA GREA A JUDEŢULUI IAŞI

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INDUSTRIAL SOUND SPECTRUM ENTAILING NOISE-INDUCED OCCUPATIONAL HEARING LOSS IN IASI INDUSTRY (Abstract): In European Union every day millions of employees are exposed to noise at work and the risk this can entail. **Aim:** this study presents the sound spectrum in Iasi heavy industry: metal foundries industry, punching and embossing of metal sheets, cold and hot metal processing. **Material and methods:** it was used a type 2 Sound Level Meter (SLM) and the considered value was the average value over 10 test values in 10 consecutive days for each octave band in common audible frequency range. **Results and discussions:** It is obviously that the large values of sound intensities in the most of frequency octave band exceed maximum admissible and legal values. **Conclusions:** The study reveals the necessity of hardware, medical and managerial measures in order to reduce the occupational noise and to prevent the hearing acuity damage of the workers. **Key words**: INDUSTRIAL NOISE SPECTRUM, NOISE-INDUCED HEARING LOSS (NIHL), TEMPORARY THRESHOLD SHIFT, PERMANENT THRESHOLD SHIFT.

Zgomotul industrial distruge ireversibil celulele ciliate auditive din urechea internă, afectare care, în prezent, este una din cele mai răspândite boli profesionale cu consecinţe devastatoare asupra calităţii vieţii. Directiva europeană 2003/10/EC referitoare la zgomot stabileşte valoarea de 87 dB(A) ca limită maximă admisibilă (1), limită precizată şi de normele româneşti în vigoare (2). Aparatul auditiv nu are un mecanism care să limiteze suprasolicitarea. Expunerea la zgomotul industrial are efecte dăunătoare otice şi sistemice, încât controlul medical are o importanţă majoră şi se efectuează conform legislaţiei actuale (3) .

MATERIAL ŞI METODĂ

Măsurătorile presiunii sonore au fost realizate cu un aparat SLM de tip 2, dotat cu microfon dinamic pentru intensităţi mari, setat pe caracteristica A de referinţă a nivelului pragului minim audibil în câmp liber pentru urechea umană standard, caracteristica indicată la măsuratorile zgomotului industrial. Setarea "Slow" urmăreşte variaţia mediei temporale pe o durată corespunzătoare care este sub valorile instantanee de vîrf ale presiunii acustice; la setarea "Fast", mărimea constantei duratei de integrare este mai mică decît în cazul "Slow", valorile indicate prin medierea

temporală urmărind peak-urile presiunii sonore a zgomotului. Precizia asigurată de instrument este mai bună de ±1 dBA pe toată scala. Instrumentul a fost plasat la o depărtare de 0,5 m faţă de utilaj şi la 1,7 m deasupra nivelului podelei, direcţionat perpendicular pe planul vertical al maşinii, în felul acesta se măsoară cu precădere presiunea sonoră a undei directe, această poziţionare fiind similară celei în care se află uzual receptorul acustic uman în timpul muncii. S-au înregistrat valorile indicate de instrument pentru fiecare din cele nouă octave adiacente ale căror frecvenţe centrale au fost: 31.5, 63, 125, 250, 500, 1000, 2000, 4000 şi 8000 Hz.

Măsurătorile s-au efectuat în 12 zile, la ore diferite, pe parcursul a patru săptămâni, pentru a se diminua influenţa situaţiilor extreme. Pentru fiecare octavă, din cele 12 valori astfel măsurate au fost eliminate valoarea cea mai mică şi valoarea cea mai mare, apoi s-a calculat media celor 10 valori rămase ce au trasat caracteristicile spectrale.

REZULTATE

Spectrul sonor din secţiile de prelucrări la cald rezultă din zgomotele utilajelor din secţiile de forjă care sunt intens folosite. Acestea produc sunete cu nivele de presiune sonoră foarte ridicate şi nocive într-un domeniu foarte larg de frecvenţe.

Dată fiind plaja frecvenţială întinsă, măsurătorile au fost extinse şi pentru octava inferioară a cărei frecvenţă centrală este de 31.5 Hz.

S-au efectuat măsurători în toate cele 9 benzi frecvenţiale de octavă din domeniul audibil, în modul "Peak" şi în modul "Slow" şi s-au calculat mediile aritmetice ale valorilor obţinute (tab. I).

Pe baza valorilor medii s-a obţinut spectrul sonor existent în secţiile de forjă (fig. 1).

TABELUL I

Intensitatea sonoră măsurată în secţiile de forjă

Frecv [Hz]	**Măsurători în modul "*Peak*"**											**[dBA] "*Slow*"**
	Valorile intensităţii sonore măsurate în fiecare din cele 10 zile [dBA]										**Media "*Peak*" [dBA]**	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**		
31.5	77	75	73	78	80	77	76	74	75	74	75.9	72
63	84	82	80	83	86	78	81	82	88	84	82.8	76
125	90	89	94	87	91	94	88	87	93	91	90.4	78
250	95	97	92	96	99	91	89	98	96	95	94.8	85
500	103	101	105	99	98	107	105	106	98	103	102.5	88
1000	94	96	91	93	96	94	90	92	96	97	93.9	84
2000	98	100	98	95	97	102	99	100	97	96	98.2	86
4000	99	98	97	101	99	100	103	97	97	99	99	87
8000	90	87	91	93	89	94	88	87	92	88	89.9	79

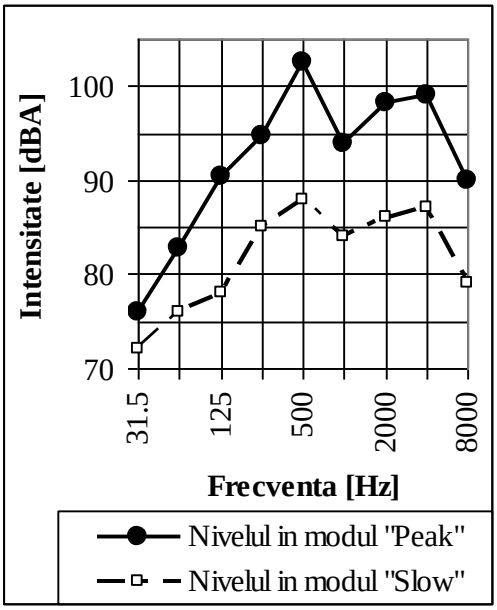

Fig. 1. Spectre sonore ale zgomotului în secţiile de forjă

Prelucrările semifabricatelor metalice la rece constau în cea mai mare parte în operaţii de ştanţare, tăiere şi îndreptare a tablelor groase şi profilelor grele, rezultând zgomote sacadate şi de intensitate deosebit de mare. Măsurătorile s-au efectuat în secţiile de prelucrări la rece cu instrumentul poziţionat în faţa utilajelor precum şi în lateralele acestora (tab. II şi tab. III).

TABELUL II

Intensităţile sonore din secţiile de prelucrări la rece în faţa utilajelor/preselor

Frecvenţa [Hz]	Mediile artimetice pe 10 zile pentru:		
	Zgomotul de fond (in repaus) [dBA]	Nivelul sonor în modul"Slow" [dBA]	Nivelul sonor în modul "Peak" [dBA]
31.5	-	-	-
63	78	80	87
125	76	78	93
250	79	82	92
500	80	83	98
1000	77	84	102
2000	74	86	108
4000	72	91	115
8000	71	94	118

TABELUL III

Intensităţile sonore din secţiile de prelucrări la rece lateral de prese

Frecvenţa [Hz]	31.5	63	125	250	500	1000	2000	4000	8000
Mod "Slow" [dBA]	0	77	78	82	88	88	92	94	96
Mod "Peak" [dBA]	0	88	91	95	103	105	119	122	122

Spectrul sonor în faţa şi lateral de presă indică valori mari (fig. 2 şi fig 3).

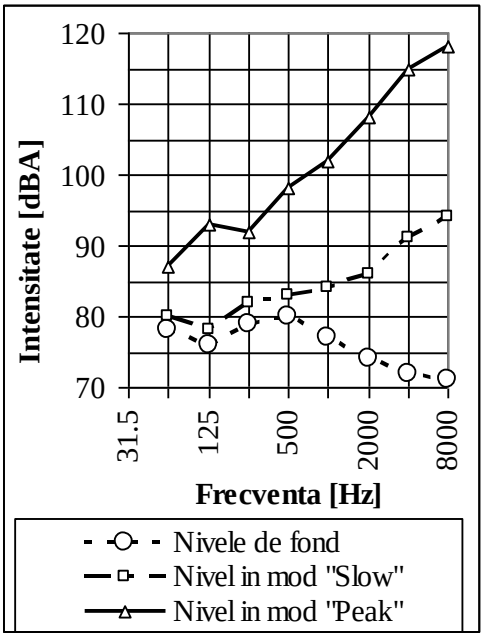

Fig. 2. Spectrul în faţa preselor

Măsurătorile se efectuează atât în faţa preselor, cât şi în lateral, pentru a pune în evidenţă proprietăţile de directivitate ale zgomotelor produse. Pentru că utilajele lucrează ritmat atunci când sunt folosite, s-au făcut măsurători atât în modul "*Peak*", cât şi în modul "*Slow*". Pentru a trasa şi spectrul zgomotului de fond, s-au făcut măsurători când utilajele sunt în aşteptare - "standby", adică sunt pornite, dar nu sunt în sarcină, cu instrumentul setat în modul "*Slow*".

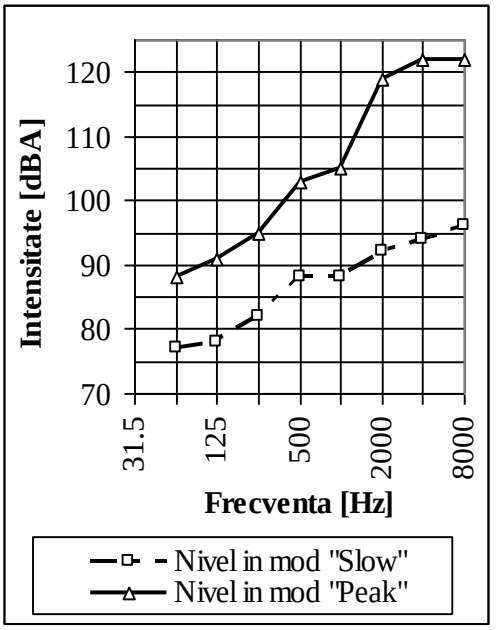

Fig. 3. Spectrul lateral de prese

DISCUŢII

În fiecare caz de reprezentare spectrală s-a calculat valoarea intensităţii globale la care şi-au adus contribuţia nivelele sonore medii ale tuturor celor 9 octave de frecvenţă de la 31,5 pîna la 8000 Hz. Bazându-ne pe însuşi principiul de măsurare care constă în filtrarea în benzi adiacente de frecventă, se poate considera ca fiecare din cele 9 nivele de intensitate sonoră se constitue în câte o sursă independentă de zgomot şi că toate sursele produc zgomot simultan. Având acest temei, pentru calculul valorilor globale ale nivelelor de zgomot, putem aplica algoritmul de compunere a nivelelor

de zgomot provenind de la surse independente (4), toate rezultatele fiind conrelate împreună cu duratele maxime de expunere la zgomot (tab. IV), admise pentru lucrătorii care îşi desfăşoară activitatea la respectivele locuri de muncă (5).

TABELUL IV

Valorile globale ale intensităţilor sonore corespunzătoare tuturor spectrelor sonore din secţiile de prelucrări metalice la cald şi la rece precum şi duratele maxime admise de expunere la zgomot pentru lucrători

	Niveluri sonore globale în modurile "Slow" şi "Peak"						
	În secţiile de prelucrări la cald		In secţiile de prelucrări la rece				
			În faţa presei			În laterala presei	
	"Slow"	"Peak"	Zg. fond *"Slow"*	"Slow"	"Peak"	"Slow"	"Peak"
dBA	99.7	126	85.6	97	121	99.7	126
Durata maximă de expunere	14 min	2 min	8 ore	30 min	7 min	15 min	2 min

Urmărind valorile intensităţilor globale se constată că numai zomotul de fond din secţiile de prelucrare la rece permite prezenţa lucrătorului pe o durată zilnică de 8 ore neîntrerupte, pe cînd, în toate celelalte cazuri, valoarea de 87 dBA menţionată mai sus (2) este considerabil depăşită şi că duratele maxime de expunere permise sunt foarte mici în comparaţie cu norma zilnică a duratei de muncă.

CONCLUZII

Studiul relevă faptul că în astfel de condiţii de zgomot prezentate lucrătorii sunt expuşi unei noxe sonore importante ce poate produce deteriorarea ireversibilă a organului auditiv. Acest fapt justifică implicarea medicului în consilierea managerilor şi lucrătorilor pentru luarea de măsuri tehnico-organizatorice de reducere a acestor noxe profesionale (6). De aceea, rolul medicului constă nu doar în monitorizarea şi tratarea celor afectaţi de această noxă profesională, ci şi în consilierea şi conştientizarea angajatorilor şi angajaţilor pentru prevenirea acestor îmbolnăviri.

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PROBLEME DE DIAGNOSTIC ÎN EXAMINAREA ECOCARDIOGRAFICĂ FETALĂ. OBSERVAȚII PE UN LOT DE STUDIU ÎN PERIOADA 2005-2010

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DIAGNOSTIC PROBLEMS IN FETAL ECHOCARDIOGRAPHIC EXAMINATION. OBSERVATIONS ON A LOT OF STUDY DURING 2005-2010 (Abstract) : Echocardiography is the key diagnostic method in the diagnostics of structural heart pathologies. **Aim** : To underline some possible situations that may cause errors in ultrasound examination of the fetal heart. **Material and method** : We examined a number of 39370 fetuses along a period of six years. The ultrasound scan was performed according with international standards of basic and extended cardiac scan. **Results** : 442 of them (1,12%) were diagnosed with cardiac malformations. **Conclusion** : Ultrasound examination errors can cause false positive, false negative or incomplete diagnosis. Limitations in fetal echocardiography are related to the examiner experience to do an accurate diagnosis. The difficulty of assessing complex congenital heart lesion is increased. The unsatisfactory study should be repeated and sometimes the exact diagnosis requires sequential procedures. The aim is to define fetal heart anatomy and adise the family, in collaboration with neonatologist. The final results ia an optimal behavior for the fetus and newborn. **Key words :** CARDIAC MALFORMATIONS, ECHOCARDIOGRAPHIC DIAGNOSIS, FALSE POSITIV

În general, malformațiile cardiace sunt bine tolerate intrauterin de către făt datorită particularităților anatomice ale cordului fetal, reprezentate de foramen ovale şi canalul arterial. Practic nu există semne clinice sau paraclinice care să indice sau să sugereze existența unei malformații cardiace fetale. Acestea pot fi diagnosticate doar prin examinare ecografică directă a cordului fetal în cadrul morfologiei fetale.

MATERIAL ŞI METODĂ

În studiu au fost incluse 39370 gravide cu vârste între 19 şi 42 ani, care au născut în spitalul nostru în perioada 1 ianuarie 2005-31 decembrie 2010. Parametrii urmăriți au fost: incidența malformațiilor cardiace raportată la numărul total de naşteri, prevalența acestora din totalul malformațiilor, vârsta gestațională maternă la momentul diagnosticului, sensibilitatea ecografiei ca metodă de diagnostic, atât global cât şi raportată la tipul şi severitatea malformației. Ecografiile au fost efectuate cu un ecograf Voluson ProV (cu sondă convexă de 3,5 MHz şi endovaginală de 7 MHz) de către medici specialişti şi primari cu competență în ecografie. De importanță majoră a fost ecografia de 20-24 săptămâni la care s-a efectuat examenul sistematic cardiac. Acesta a inclus obligatoriu pe lângă imaginea patru camere şi imaginea tra-

iectelor de evacuare ventriculară, secţiunea trei vase, trei vase şi traheea şi imaginile în axul lung al cordului inclusiv secţiunea arcului aortic şi a arcului ductal.

Defectele cardiace au fost împărţite în două grupe: minore şi majore. Au fost considerate majore anomaliile cardiace care au necesitat corecţie chirurgicală. La rândul lor defectele cardiace majore le-am împărţit în următoarele grupe:

– ale inimii stângi (sindrom de inimă stângă hipoplazică, coarctaţie de aotă, stenoză aortică valvulară)

– ale inimii drepte (stenoza sau atrezie pulmonară, atrezie tricuspidiană anomalie Ebstein)

– anomalii conotruncale (transpoziţie de vase mari tetralogie Fallot, ventricul drept cu dublă cale de ieşire)

– alte defecte (defecte complexe de sept atrioventricular, ventricul stâng cu dublă comunicare atrio-ventriculară, displazie tricuspidiană).

Nu au fost incluse în studiu anomalii cardiace minore imposibil de diagnosticat preanatal (defecte septale atriale minore, foramen ovale sau canal arterial persistent).

În cazul sindroamelor malformative, am inclus în statistică cazurile cu afectare cardiacă. Întrucât condicile de morbiditate şi mortalitate au inclus toate malformaţiile, am fost nevoiţi să le clasificăm în malformaţii diagnosticabile şi malformaţii nediagnosticabile ecografic. La cazurile de terminare a sarcinii, avort spontan sau moarte fetală sau neonatal s-a practincat examenul anatomopatologic sistematic al produsului de concepţie. Datele obţinute în urma necropsiei au fost folosite pentru verificarea acurateţii diagnosticului ecografic.

REZULTATE

În perioada 1 ianuarie 2005-31 decembrie 2010 în Spitalul Clinic de Obstetrică Ginecologie Cuza Vodă Iaşi s-au născut 1487 copii cu malformaţii, reprezentând 3,77% din numărul total de naşteri (39370). Din acestea 1174 (79%) au fost malformaţii diagnosticabile ecografic prenatal. Un număr de 800 paciente (53,8%) au provenit din mediul rural şi 687 paciente (46,2%) din mediul urban. Vârsta maternă a fost cuprinsă între 19 şi 42 ani, cu o medie de 31 ani. Prevalenţa pe grupe a defectelor cardiace a fost: 18 defecte ale inimii stângi, 67 defecte ale inimii drepte, 32 cazuri cu anomalii conotruncale, 16 cazuri sindrom Down cu afectare cardiac, 7 cazuri au prezentat alte anomalii genetice şi afectare cardiac. În 52 cazuri afectarea cordului a apărut în cadrul unui sindrom plurimalformativ. La 253 cazuri malformaţia cardiacă a fost complexă. Din 1174 paciente cu malformaţii diagnosticabile ecografic, 812 au efectuat cel puţin o ecografie prenatală (69,17%). Sensibilitatea globală a ecografiei a fost de 55%. Sensibilitatea maximă a fost înregistrată pentru diagnosticul sindroamelor plurimalformative (78%). În cazul malfomaţiilor cardiace complexe a fost dificilă stabilirea ecografică diagnosticului corect.

DISCUŢII

Ecografia prenatală este cea mai bună metodă pentru diagnosticul intrauterin al malformaţiilor fetale. Limitările examenului ecocardiografic ţin de vârsta sarcinii sub 18 săptămâni, rezoluţia echipamentului, caracteristicile programului de investigare şi experienţa ecografistului. Este crescută dificultatea de apreciere a leziunii în malformaţiile cardiace complexe. Cele mai bune imagini cardiace pot fi obţinute între 18 şi 24 săptămâni de amenoree. Condiţii tehnice ce limitează acurateţea imaginilor ecografice: oligoamnios, polihidranios, obezitatea maternă, poziţia fetală nefavorabilă, mişcările fetale excesive, hidrotoraxul, prezenţa herniei diafragmatice. Din experienţa noastră problemele de dia-

gnostic pot apare la cazurile cu:

Defect septal interatrial Foramenul oval (FO) prezintă o variație a dimensiunilor pe parcursul ciclului cardiac. Semne fals pozitive apar în examinările oblice (1). În incidența 4 camere sinusul coronar dilatat poate creea falsa impresie de canal atrioventricular parțial dar în acest caz, tricuspida şi mitrala se inseră la acelaşi nivel al septului interventricular.

Focarele ecogenice marginale Ocazional, un focar ecogen strălucitor este prezent la periferia miocardului ventricular sugerând o mică tumoră sau calcificare. Această imagine apare când planul de secțiune include porțiunea anterioră a unei coaste sau o parte de stern (fig. 2).

Defect septal interventricular Defectele adevărate au margini bine definite (fig.3A) la examinarea cu un fascicul de ultrasunete perpendicular pe septul interventricular. Rezultate fals pozitive apar când fasciculul de ultrasunete este paralel cu septul (fig.3B) şi în cazul examinării Doppler color cu velocitate neadecvată, sau în plan neadecvat (4).

Fig. 1. Imaginea evidențiază prezența unui focar hiperecogen la nivelul ventriculului stâng. În majoritatea cazurilor este uşor de diferențiat de o tumoră intracardiacă.

Fig. 2. Secțiunea longitudinală la nivelul unei coaste poate fi o capcană diagnostică pentru focarele hiperecogene localizate spre apexul cordului.

A

B

Fig. 3. Pseudo defect septal ventricular

A – Pe secțiune apicală 4-camere (când septul este paralel cu fasciculul de ultrasunete) defectul septal ventricular pare să fie prezent; B – Acest artefact poate fi evitat scanând aceeaşi porțiune a septul dintr-o orientarea diferită. În mod ideal fasciculul de ultrasunete trebuie să cadă perpendicular

pe septul interventricular.

Tetralogie Fallot Imaginea patru camere are aspect normal exceptând cazurile cu canal atrioventricular complet, agenezie de valvă pulmonară, dilatație de ventricul drept. În evoluție se observă îngustare infundibulară a ventriculului drept. Marile vase se încrucişează normal (5).

Stenoza valvulară aortică Formele uşoare de stenoză aortică nu pot fi diagnosticate. Îngroşarea valvei aortice şi dilatarea aortei ascendente pot ridica suspiciunea acestui diagnostic. Prin examinare Doppler color se evidențiază flux turbulent la nivelul valvei. Leziunea are un caracter evolutiv prin urmare, examinarea ecografică trebuie repetată.

Coarctație a aortei Apar frecvent rezultate fals pozitive şi negative. Se poate suspecta afecțiunea când există o discrepanță între calibrul arterial şi ventricular. Această discrepanță se evidențiază la 75% din fețiii afectați (6).

CONCLUZII

Limitările în ecocardiografia fetală țin de experiența ecografistului în stabilirea unui diagnostic fidel. Este crescută dificultatea de apreciere a leziunii în malformațiile cardiace complexe (7). Studiul nesatisfăcător trebuie repetat şi uneori, diagnosticul exact nu se poate da fără proceduri secvențiale (6).

Scopul examenului ecocardiografic fetal este definirea anatomiei cordului fetal, conferind obstetricianului în colaborare cu medicul neonatolog capacitatea de a furniza informații şi a sfătui familia în ceea ce priveşte defectul cardiac, anticiparea prognosticului, şi stabilirea conduitei optime privind fătul şi nou născutul. Această abordare multidisciplinară a îngrijirii materno-fetale este necesară pentru optimizarea naşterii (8).

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PELLAGRA, A SOCIAL DISEASE IN AN EASTERN EUROPEAN COUNTRY, AT THE END OF THE NINETEENTH CENTURY AND EARLY TWENTIETH CENTURY

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PELLAGRA, A SOCIAL DISEASE IN AN EASTERN EUROPEAN COUNTRY, AT THE END OF THE NINETEENTH CENTURY AND EARLY TWENTIETH CENTURY **(**Abstract): Pellagra appeared in Europe after Christopher Columbus brought maize to the continent, omitting however to bring with him the corn processing method of the Native Americans - which involved treating corn flour with lime water (calcium hydroxide) before thermal processing so as to partially release niacin from cellulose structure, which proved the decisive factor in the occurrence of the disease. It is an extremely rare disease nowadays, but it is the prototype of social disease in the late nineteenth and early twentieth century, affecting especially the rural one due to the poor economic condition of the Romanian peasant and their strong religious beliefs. Pellagra is one of the diseases whose development was directly correlated with socioeconomic status of the population in different regions of Romania, raising major issues of care and active intervention measures in health care. **Keywords**: PUBLIC HEALTH, ROMANIA, PELLAGRA, SOCIAL DISEASE, MAIZE, CORN.

Pellagra is a nutritional disease caused by a deficiency of the vitamin B_3 or niacin. An extremely rare disease in our days, it represents the prototype of the social disease at the end of the 19th and the beginning of the 20th century, its occurrence being linked to the existing economically poor conditions, which caused physical, mental and economic damage (1).

Historical aspects

At the end of the year 1492 Christopher Columbus returned to Spain after the discovery of America. He brought back with him golden beans of a Gramineae that was a cereal species unheard of in Europe. These had the advantage that they did not require more work than wheat and bore a hundred times more fruit. From these beans a sweet and tasty food could be cooked, with little effort and fire expense, and in much less time than bread. Little by little, corn bread replaced wheat bread on the peasant table (2). Moreover, all other foods were more expensive, because, according to Lombroso, the same amount of nutrients cost 3 times more if they were consumed in the form of potatoes or rice. Bread is 2 to 3 times more expensive, milk and meat 7-8 times (3).

Pellagra was first described in 1762 by Don Gaspar Casal, who observed the disease among farmers in the province of Asturias, where they called it “mal de la rosa”. First to describe the disease known as “pellagra”

was Frappoli Francesco from Milan in 1771, a name deriving from "pella-agra" with the meaning of "rough skin" (in Italian) (2).

Trouvenel was the first that signaled accurately the connection between maize and pellagra being considered the promoter of the "maidic" hypothesis.

In 1912 the disease reached an apogee in the U.S., with 25,000 newly diagnosed cases of pellagra in the past 5 years. This endemic development determined various institutions (like United States Public Health Service Investigations and Public Health Service) to engage in the study of pellagra. Joseph Goldberger, chief of the Public Health since 1914, concluded that pellagra was a nutritional disease. He relied on the number of cases of pellagra among patients of mental institutions and on the absence of this disease among nurses and caregivers. The difference between these two groups was in the diet, the latter ones having a more varied and improved diet. Also, Goldberger characterized pellagra as a rural disease associated with poverty. Without discovering the true cause in the diet which determined the occurrence of pellagra, he suggested that increasing the consumption of fresh meat, eggs and milk could act preventively. Seven years since the beginning of his research, Goldberger discovered that the factor which prevented pellagra was a protein, calling it the PP factor (factor that prevents pellagra). In 1926, Goldberger reached the conclusion that this factor was a part of the vitamin B complex, declaring that it was contained in large amounts in dry yeast. Only in 1937 the chemist Conrad Elvehjem found that nicotinic acid is the PP factor (4).

Sociological aspects

The relationship between the introduction of maize in Europe and the prevalence of pellagra can be more easily understood with the help of sociological theories of diffusion and adoption of innovations (5).

Applying this model, it follows that experienced poor agrarian populations in Europe (especially in the plains of SE Europe) easily took over the American maize cultivation practice because: it brought more advantages than cereal crops they were accustomed to, responding to acute needs of populations with poor diet for high-calorie food, the crops were cheap and easy to obtain, being easy to implement, with fast and visible results.

However, these populations did not adopt the practice of treating the corn with calcium hydroxide since they did not have in their culture the habit of undertaking similar actions, they did not realize the need and benefits of such a practice and implicitly they could not realize the ease with which one could apply this procedure. In addition, not knowing the potential dangers of untreated corn, they could not anticipate the immediate results that this process could have. The fact that the cultivation of corn was associated with increased incidence of pellagra predominantly in rural populations with poor nutrition style represents a systemic component of the model of innovation adoption, respectively the existence in the social system of certain norms and patterns of eating behaviors that created the fertile ground for the manifestation of the disease.

It should not be ignored that in certain contexts (such as rural Romania) the option of not cultivating or consuming corn by individuals (and thus avoiding the occurrence of pellagra) was non-existent because at systemic level corn was imposed by the ruling elite, and at community level by landowners. Hunger and poverty also encouraged its introduction in the diet.

Pellagra in Romania

Romania was predominantly an agricultural country. Corn cultivation started at the beginning of the eighteenth century, being introduced around the year 1710 by Nicolae Mavrocordat. The advantages of corn cultivation in parallel with the progressive impoverishment of the people by wars and the excessive taxation of the Phanariot period, was basically to prepare the ground for the emergence of pellagra (6).

The first cases of pellagra were described around 1830 in Moldova region, initially as isolated cases, being described as piles swelling or piles wound. In 1858 Dr. Iuliu Teodori first described the disease by the name of pellagra. Professor Felix described the disease in Walachia, in Muscel County, in 1889. In 1896, in "Gazeta de Transilvania" an article described pellagra as a disease unknown in these regions (2).

The first statistic of the population suffering from pellagra was made in 1882 by Dr. Felix, which counted about 4500 patients with pellagra (2, 6).

During the period 1884-1886, rural military ambulances provided ambulatory consultations, publishing in this matter statistical data regarding the morbidity; thus, 19,797 cases were reported in 1886.

In 1888 Dr. Sergiu, at that time general director of the Health Service, found that 10,626 people were suffering from pellagra in a population of 5,339,650 inhabitants of the Romanian Kingdom, which meant about 2% morbidity due to pellagra. According to their occupation, they were mostly peasant farmers, 7,572 (71.25%), or workers, 2,132 (20%), and only in an insignificant proportion held other jobs (artisan - 19 patients, trader - 28 patients, official - four patients, priest - 15 patients, man servant - 20 patients). It is to be noted the number of children between 1 and 12 years (83 patients). Pellagra affected in this sense the lowest classes (2, 6), dramatically ravaging the rural population (7).

Regarding statistical values about pellagra in Romania between 1888-1935 (6, 8), there are slight differences between the statistical values found in various documents, differences due to imperfect methods of recording. The evolution of these values was as following (fig. 1, 2) (6, 7, 9, 10).

Data reported were considerably smaller than the real number, many patients with a mild form opting not to consult a doctor, the erythema period being evanescent; in consequence those cases were not being considered during social surveys carried out (6).

Noting the differences between the numbers declared in those years, beginning with 1895 a census using nominal lists was established. Out of the 17,912 of patients registered in 1896, 10,173 (56.8%) were female and 7,739 were male (43.2%); almost all lived in rural communes-17,350 (96.8%), 112 lived in small urban communes and 450 in urban communes which were district capitals (11).

In years when we have statistics on gender, it appears that the female gender is predominant constantly sometimes the ratio being over 2:1 (fig. 3). Predominance of the female gender could be explained by organic overworking to which these were exposed, like pregnancy and lactation, while they had in care also the domestic activities, including participating in the agricultural work.

Between the years 1896-1899, looking for the etiology, sensing the connection of this disease with the alimentation, a study was carried out on the 779 pellagrous patients hospitalized in Roman, based on a questionnaire they completed. To ensure the needs of a family, this should have had to possess at least 4-5 hectares of land. Out of the respondents, 78.1 % had insufficient land areas in property to ensure their food needs.

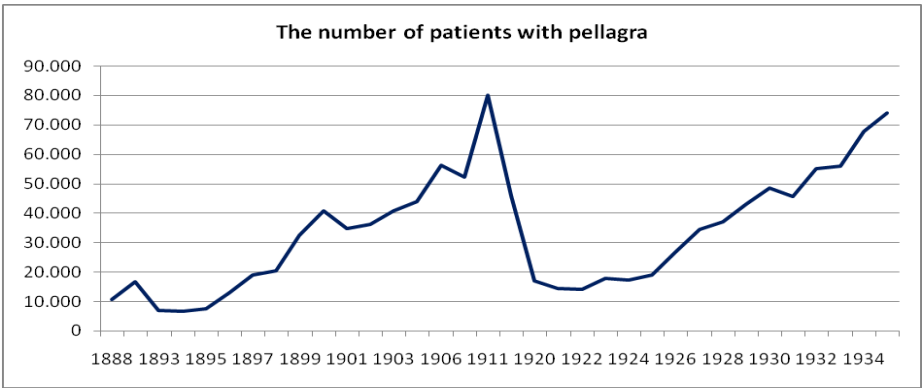

Fig. 1. Number of patients with pellagra in Romania between 1888-1935

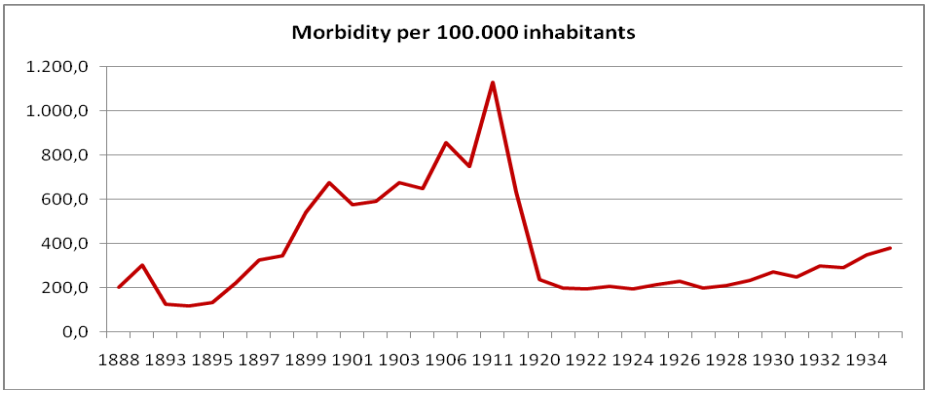

Fig. 2. Morbidity per 100,000 inhabitants at pellagrous patients in Romania between 1888-1935

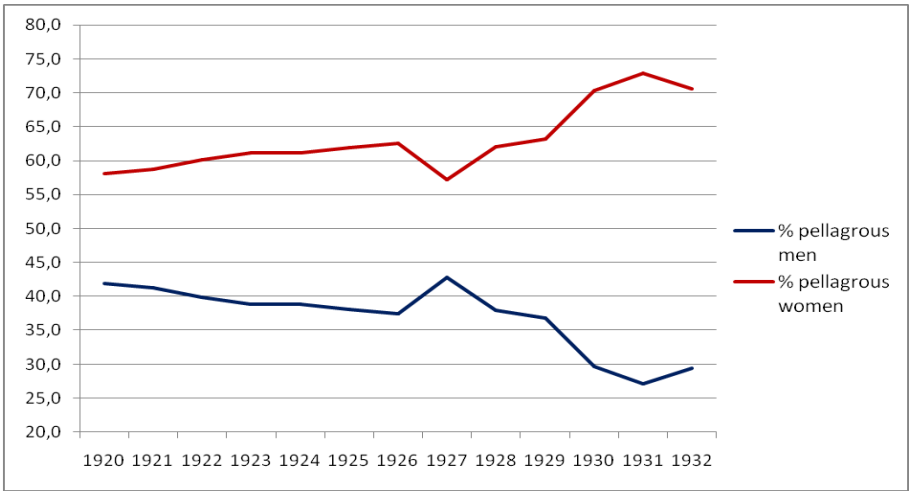

Fig. 3. Statistics by gender (male / female) from 1920-1932

Questioned on the crops grown, 651 of peasants cultivated corn, 164 wheat (which was kept especially for religious charity or to be sold), 112 rye, 493 beans, 67 lentils, 93 peas, 554 potatoes, 483 greengroceries (9). This was because landowners demanded that peasants should cultivate corn, because wheat required more time and they feared that the peasants would not do well their service jobs (12).

Also, of those questioned, only 347 had milk cows and 367 of them owned working cattle (9). The reason why the peasants did not have dairy cows, was because the feeding of a cattle for 10 months on the property of a squire cost 20-25 Lei and a peasant preferred to pay this amount for two plough oxen, with which he ensured the farm work, considering that the fate of the peasant was depending on the squire (13). Dairy cows were unkempt, did not have warm stables, being degenerate, giving very little milk so that the peasant consumed very little milk, cream and butter (7).

Even when the peasants had more substantial foodstuff like eggs, milk and / or poultry, they most of the time, preferred to sell them to other farmers or to townspeople. They were able, by this means, to satisfy their other needs. When these foods were not sold, they could be prohibited because of the fasting, which the peasant observed with the utmost strictness and from which prescriptions he wouldn't deviate, being convinced that violation of fasting caused great misfortunes. One couldn't even stand others to violate it. Peasants tied the occurrence of plagues to the fact that fasting had not been observed properly, accordingly to combat it, a greater sacrifice in observing the fasting period was needed (13). In the name of religion health was thus neglected. It was suggested that it would have been better if Orthodox religion found ways to enforce in believers' hygienic habits, reducing long and heavy fasting, especially during the heavy work. (7).

The years that followed World War I and the annexation of Transylvania to Romania are characterized mainly by a reduction in the number and the percentage of pellagrous patients.

This is explained by the fact that after the war, small property replaced estates, the peasant becoming the owner of the land he cultivated, which influenced his cultural and economic level. Contact with allied and enemy soldiers educated peasants in the sense of improving nutrition, while in the territories occupied by enemy, peasants were forced to sow large quantities of wheat.

Annexation of Transylvania, Bucovina and Basarabia decreased the percentage of the pellagrous in the Romanian population (11), where, apart from Basarabia, where there was widespread pellagra, other areas did not present the disease (6). In Transylvania there were also rare cases of pellagra because at least once a week bread was baked, and at least a stretch equal to the land cultivated with maize, was planted with wheat or rye (7).

Number of pellagra patients dropped probably due to typhus which afflicted the country at the time, but later their number increased again reaching before the war values.

At the same period, countries once afflicted by pellagra had managed, by socio-economic methods and the active involvement of the state, to eradicate this disease, so in the early '30s cases of pellagra were presented as a medical curiosity in the French press (7). Meanwhile, in Romania,

there was nothing happening, health inspectors were doing statistics centralized at the Ministry of Health and that was all. If for other social diseases were ensured funds, were set up committees and societies, for treating pellagra nothing was done, because it affected, in the clear majority, only the poorest strata of the population (14, 15).

The issue of health policy deficiencies addressed to the poor populations is not unique to the Romanian context, having been debated in the international literature for a long time. Profile global organizations have failed to solve these problems even today. It is estimated that only 10% of global health research aims at topics affecting 90% of the world population, specific diseases in poor communities of low-income countries being neglected by the global economic sector of research and development (16,17). In addition, the countries with low and middle income (category which includes Romania) choose to use the few resources they possess for projects that do not always coincide with the basic needs of the population (18). However, in recent decades, progress has also been made. Currently there is a consensus at the level of the scientific community and health activists on the fact that global improvement of the health of the poor (and implicitly diminishing the incidence of poverty and bad nutrition) depends primarily on the ability of public policies to reduce poverty, generating well-being and an improved quality of life (18) and also to educate the young generation for improving their life based by health promotion (19).

CONCLUSIONS

Pellagra, a disease extremely rare nowadays, is the prototype of social disease in the late nineteenth and early twentieth century, affecting in the period studied (1888-1935), the poor population, especially the rural one.

Pellagra appeared in Europe after Christopher Columbus brought maize to the continent, omitting however to bring with him the corn processing method of the Native Americans - which involved treating corn flour with lime water (calcium hydroxide) before thermal processing to partially release niacin from cellulose structure, which proved the decisive factor in the occurrence of the disease.

The emergence of pellagra in Romania predominantly in the rural area was due to the poor economic condition of the Romanian peasant (linked until World War I to the big landlords), but also to his strong religious beliefs. Thus, the Orthodox Church demanded severe fasting, even during the hard work periods, which peasants observed strictly, considering that plagues may thus be kept away.

Predominant presence of the disease in females (sometimes the ratio being more than 2:1) can be explained by organic overwork to which this gender was subjected by pregnancy and lactation. At the same time women in the period studied, also had to do the housework as well as participate in farm work.

The years that followed World War I were characterized mainly by a reduction in the number and the percentage of pellagrous patients, because on one hand big estates were replaced with small peasant properties, and due, on the other hand, to contact with other dietary habits brought by allied or enemy soldiers.

Regarding the Romanian state policy, implicitly of the Ministry of Health, although for other social diseases (alcohol-

ism, syphilis etc.) were assured funds, were set up committees and societies, for treating pellagra virtually nothing was done because this disease affected in the clear majority, only the poorest strata of the population.

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FLUIDITATEA MEMBRANARĂ – PARAMETRU BIOFIZIC ÎN RELAŢIE CU PROCESELE DE TRANSPORT MEMBRANARE

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MEMBRANE FLUIDITY - BIOPHYSICAL PARAMETER IN RELATION TO MEMBRANE TRANSPORT PROCESSES (Abstract) : Membrane fluidity is a complex parameter, influenced both through some biophysical (temperature, electrical charges, pH) and biochemical factors (protein/phospholipids ratio, phospholipids/cholesterol ratio, degree of fatty acids unsaturation). It is a parameter that reflects the main membrane characteristic organization (gel or liquid crystal structure). Experiments provide consistent data about membrane fluidity relations to various cellular processes, especially membrane processes. Passive transport is influenced directly by membrane lipid composition, and consequently, membrane fluidity, or by channels and carriers molecules interactions with adjacent membrane lipids. Membrane diffusion is related both to the hydrophobic nature of the particles and of the membrane environment. Depending on the mode of interaction with membrane proteins, lipids may be classified as non-annular lipids, annular and "mass" lipids. Different types of fatty acids affect the function of many channels, by stimulating or inhibiting them. These effects might be mediated through direct binding of the lipids to the channel or through indirect hydrophobic interactions. Active transport pumps conformation could be modulated, also, through interactions with adjacent lipids of the bilayer (especially cholesterol) and membrane micro-domains organization. In **conclusion**, membrane fluidity is close related both to passive and active transport. **Key words** : MEMBRANE FLUIDITY, ACTIVE TRANSPORT, PASSIVE TRANSPORT, LIPID BILAYER

Fluiditatea reprezintă o proprietate biofizică globală a membranelor celulare. Ea este consecinţa legăturilor slabe dintre moleculele componente ale membranei ce permit mişcări permanente ale moleculelor. Dinamica moleculară, în parte consecinţa agitaţiei termice cât şi a afinităţilor moleculare de ordin electrostatic (interacţiuni hidrofil-hidrofobe, legături prin punţi de hidrogen, legături van der Waals) determină o distribuţie asimetrică a moleculelor la nivelul membranei (atât transversal, între cele două straturi lipidice membranare, cât şi pe feţele membranare, în interiorul aceluiaşi strat fosfolipidic). Suplimentar, fluiditatea membranară este influenţată de coliziunile domeniilor extramembranare, modul de „adeziune" şi structura citoscheletului**.** Toţi aceştia sunt factori care restricţionează mobilitatea celor mai multe proteine şi lipide, cu alte cuvinte influenţează fluiditatea (1).

Înţelegerea acestui parametru biofizic membranar esenţial, fluiditatea, este în relaţie cu urmărirea evoluţiei modelului „mozaicului fluid" (fig.1.). La asemănarea dintre structura membranei celulare şi organizarea structurală adoptată de lipidele izolate într-o soluţie apoasă se adaugă rolul interacţiunilor hidrofile-hidrofobe în menţinerea statusului energetic inferior al membranei în mediu polar. Proteinele, la rândul lor, prezintă anumite segmente de natură hidrofobă (variabile de

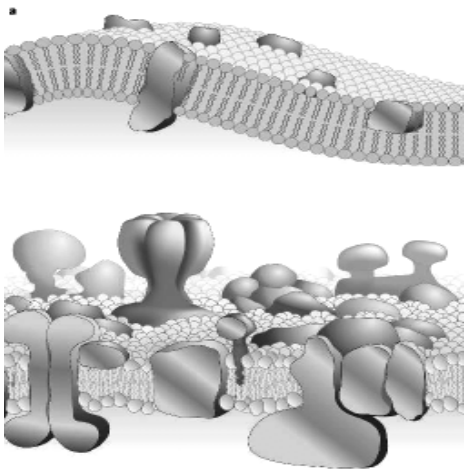

Fig. 1. a) Modelul Singer-Nicholson, b) Evoluţia modelui mozaicului fluid membranar - organizarea în microdomenii membranare (3)

la proteină la proteină) care se „potrivesc" lipidelor adiacente, perpendiculare pe planul membranei. Rigiditatea relativă a proteinelor şi dinamica lipidelor sugerează că, în principal, lipidele sunt cele care îşi modifică stereometria pentru a se „potrivi" proteinelor, deşi este posibil ca şi proteinele să îşi modifice stereometria în anumite cazuri de „nepotrivire" lipid-proteină (2). Modelul mozaicului fluid al membranei poate fi actualizat luând in considerare mai multe aspecte. Dacă lipidele sunt cele care se adaptează proteinelor, atunci raportul transmembranar lipide/proteine va fi unul variabil, ceea ce înseamnă că distribuţia transmembranară este asimetrică iar suprafaţa membranei poate fi „curbă". Aceasta dinamică moleculară membranară considerată, anterior, o consecinţă pasivă a activităţii celulare este privită, în prezent, ca un element activ menit să determine apariţia de domenii membranare (fig.1) implicate, se pare, în traficul celular (3). Tendinţa actuală sugerează un caracter mai mult de „*mozaic*" (de implicare activă în procesele celulare) în detrimentul celui de „*fluid*", în ceea ce priveşte organizarea membranei celulare.

În aceste condiţii fluiditatea membranei devine o proprietate biofizică esenţială a membranei, în relaţie cu numeroase funcţii celulare şi, îndeosebi, cu procesele de transport membranar pasiv şi activ.

FLUIDITATEA MEMBRANARĂ ŞI PROCESELE DE DIFUZIUNE

Transportul pasiv se referă la trecerea moleculelor fără consum de energie prin bistratul lipidic în sensul gradientului electrochimic. Se realizează prin difuziune simplă sau difuziune facilitată. Difuziunea simplă a unui solvit prin membrana celulară este limitată de agitaţia termică specifică moleculei respective şi, deasemenenea, este dependentă de coeficientul de permeabilitate membranară şi de solubilitatea solvitului respectiv.

Studii efectuate pe embrionul de găină au pus în evidenţa faptul că, pe măsură ce embrionul creşte, apar modificări ale fluidităţii membranare. Aceste modificări sunt determinate de schimbarea tipurilor de fosfolipide membranare şi pot fi corelate cu o scădere a permeabilităţii membranare pentru uree şi glucoză (5). Difuziunea ionilor de Na^+ în sensul gradienţilor electrochimici, esenţială pentru menţinerea celulelor în stare funcţională, este dependentă de prezenţa colesterolului în membrană. Apa se organizează în mici bariere hidrofile membranare atât ca urmare a interacţunii inelelor colesterolice între ele cât şi prin împachetarea lipidelor membranare cu partea hidrocarbonată spre centrul bistratului (6). Acestea sunt o argumente care susţin ipoteza conform căreia difuziunea prin membrana celulară depinde de compoziţia lipidică şi de modul de organizare a lipidelor membranare.

FLUIDITATEA MEMBRANARĂ ŞI TRANSPORTUL PRIN CANALE IONICE

Fluiditatea membranară poate influenţa transportul prin canalele ionice în principal prin interacţiunea proteinelor ce au funcţie

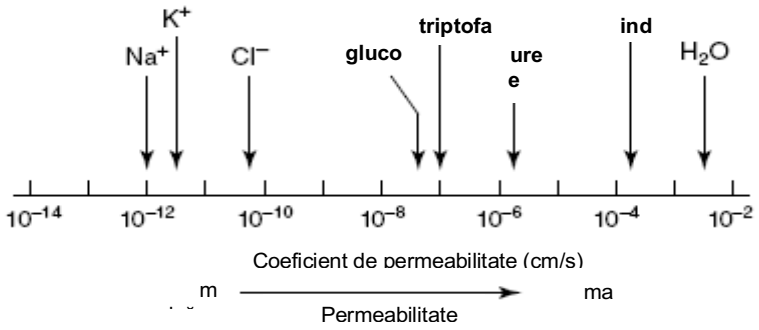

Fig. 2. Reprezentarea grafică a procesului de difuziune membranară în funcţie de coeficientul de permeabilitate pentru diferiţi solviţi (4)

de canal ionic cu fosfolipidele dar şi cu sfingolipidele sau colesterolul. Această interacţiune depinde de localizarea canalului ionic (distanţa dintre lipide şi proteine) dar şi de tipul de lipid (fosfolipid, colesterol, sfingolipid) şi de „disponibilitatea" de interacţiune a proteinei canal cu aceste lipide. In funcţie de modul de interacţiune cu proteinele membranare, lipidele se pot clasifica în lipide non-inelare, lipide inelare şi lipide „de masă". Unele proteine interacţionează specific cu anumite lipide, acestea fiind esenţiale pentru activitatea proteinei şi având rol de co-factor (7). Lipidele cu rol de co-factor se găsesc, de obicei, în interiorul proteinei, între α-helixurile transmembranare sau la interfata proteină-proteină, în cazul unor subunităţi proteice, şi constituie lipidele non-inelare. Lipidele aflate în jurul unor proteine, care sunt într-un status fizic particular şi care au un efect specific asupra acestora au fost numite lipidele inelare. Lipidele membranare care nu sunt în contact cu o proteină sau pentru care efectul proteinelor din jur este limitat sunt numite generic lipide „de masă" ; acestea au aceleaşi proprietăţi cu lipidele dintr-un bistrat lipidic simplu.

În ceea ce priveşte lipidele inelare acestea sunt implicate fie în mod indirect, prin geometria structurii lor specifice, fie în mod direct, prin interacţiunea cu proteina canal.

Cuplarea de tip hidrofob dintre segmentele α-helix intramembranare şi lipidele din jur influenţează conformaţia membranei. Proteinele au o dinamică conformaţională în relaţie cu modificările reversibile locale ale bistratului. Costurile energetice totale ale deformărilor suferite de lipide vor influenţa şi modificările conformaţionale proteice (fig.3).

Canalul MscL, de exemplu, un canal mecanosenzitiv (*mechanosensitive channel of large conductance*), este sub controlul diferenţei de potenţial membranare care depinde de energia liberă asociată moleculelor membranare, aceasta depinzând, la rândul ei, de curbura spontană a membranei şi, deci, de modul de aşezare al lipidelor respectiv al proteinelor în membrană (9).

În cazul canalelor voltaj dependente, modul în care senzorul voltaj-dependent interacţionează cu mediul lipidic membranar nu este cunoscut cu certitudine. Un canal intens studiat a fost canalul de potasiu voltaj dependent, K_v. Structura canalelor potasiu (K_v), deşi cunoscută, nu explică modul în care domeniul S_4 (senzorul cu sarcini pozitive - VS) estet capabil să se rotească în bistratul lipidic. Prin simulare moleculară pe calculator s-a studiat izolarea domeniului VS din structura canalului K_v de la *Archebacterii*, în mediul zwitterionic şi anionic al bistratului lipidic, urmărindu-se interacţiunea VS-lipide. În urma simulării s-a pus în evidenţă pătrun-

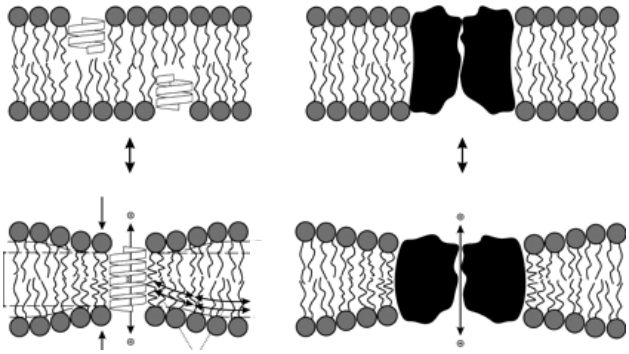

Fig. 3. Cuplarea de tip hidrofob între modificările de conformaţie ale unui canal şi deformările la nivel membranar : a) apariţia unui canal format din doi monomeri implică compresiuni şi modificări de curbură membranară. b) Cuplarea de tip hidrofob dintre membrană şi canal şi modificările de conformaţie ale canalului sunt condiţionate de tipul şi aranjarea lipidelor din bistratul înconjurător (8)

derea moleculelor de apă spre centrul VS şi în bistrat (10). De asemenea, s-a pus în evidenţă o deformare locală semnificativă care apare ca urmare a interacţiunilor dintre gruparea fosfat a fosfolipidelor şi segmentul care conţine reziduurile de arginină (Arg) de la nivelul S4 (fig. 3).

Organizarea membranei celulare depinde, printre alţi factori, şi de conformaţia geometrică a acizilor graşi, conformaţie care este dependentă de gradul de saturare/nesaturare al acestora. Putem spune, în aceste condiţii, că influenţa lipidelor membranare asupra canalului ionic este în relaţie cu gradul de organizare al membranei (ordonată - cristal lichid sau dezordonată - gel), adică şi cu fluiditatea membranei. Unii acizi saturaţi, de exemplu acidul linoleic, au rol în activarea canalelor de K^+ dependente de Ca^{2+} din mioblastele de pui, determinând hiperpolarizarea membranei prin influxul de Ca^{2+} şi fuzionarea celulelor (12). Acidul arahidonic şi oleic scad reversibil conductanţa canalelor de Na^+ voltaj dependente din muşchiul striat şi din miocardul de şobolan, determinând hiperpolarizarea membranei. Alţi acizi graşi saturaţi, de exemplu acidul palmitic, nu au efect asupra canalului (13). Acidul arahidonic inhibă reversibil activitatea canalelor de K^+ dependente de ATP din membrana apicală a celulelor canalului colector renal. Efectul nu se datorează metaboliţilor acidului arahidonic, nici modificării fluidităţii membranei. Mai mult, el apare numai dacă acidul gras se află în stratul lipidic intern al membranei, determinând o interacţiune directă cu canalul ionic (14). Acidul arahidonic şi alţi acizii graşi liberi nesaturaţi cu legături duble *cis* (oleic, linoleic, linolenic, eicosapentaenoic, eicosatetraenoic) activează canalele de Ca^{2+}, dependente de K^+ (canale BK) din celulele GH3, pe când acizii graşi saturaţi (stearic) şi nesaturaţi cu duble legături *trans* (elaidic, linol-enelaidic) nu au efect asupra activităţii acestor canale. Deoarece în acest caz nu s-a detectat o modificare paralelă a fluidităţii membranei, se sugerează de asemenea o interacţiune directă (15).

Nu doar fosfolipidele sunt în relaţie cu fluiditatea membranară ci şi restricţiile impuse de interacţiunea sfingolipidelor cu proteinele membranare. Canalul ionic format de alamethicin în bistraturi lipidice plane de difitanoil-fosfatidilcolină prezintă o activi-

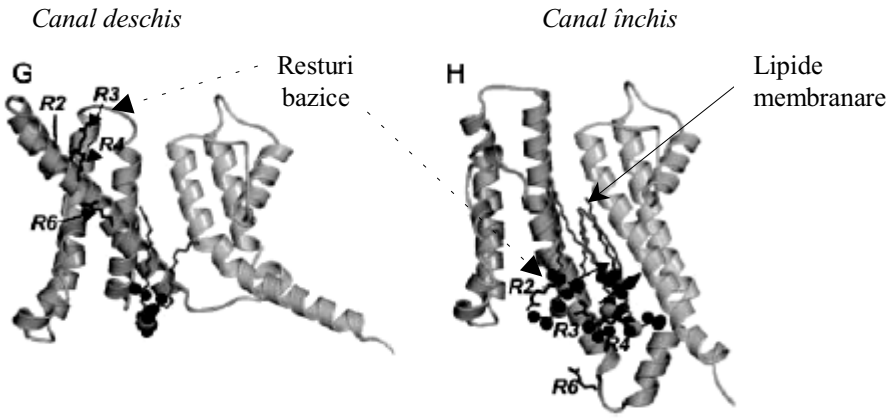

Fig. 4. Senzorul VS (segmentul G şi H) în cazul Kv şi interacţiunea sa cu bistratul lipidic. Segmentul S4 ce conţine resturi bazice (R_2- R_6) interacţionează cu lipidele membranare mult mai evident în conformaţia *închis* (11).

tate maximă dacă se adaugă şi gangliozide (sialoglicosfingolipide : CD1a, un disialoganliozid mai puţin polar, sau GQ1b, un tetrasialogangliozid polar). Autorii nu exclud nici unul din cele două mecanisme : interacţiunea directă sau modificarea microviscozităţii membranei, prin declanşarea unei tranziţii de fază(16).

Canalele ionice cu poartă dependentă de nucleotide ciclice CNG (*cyclic nucleotide--gated*) sunt implicate în semnalizarea fotoreceptorilor, respectiv a neuronilor senzitivi olfactivi. Semnalizarea prin nucleotide ciclice este necesară deoarece asigură o activare rapidă şi eficientă a acestor canale neselective de cationi. *Raft-urile* sunt acele domenii membranare specializate, îmbogăţite în colesterol şi sfingolipide implicate în multe căi de semnalizare cu punct de plecare membranar. S-a raportat că CNGA2, un tip al canalului CNG de tip olfactiv, este asociat în realizarea de *raft-uri* membranre la nivelul epiteliului olfactiv. Acest tip de canal CNGA2 nu este legat de în mod direct de caveolină, iar localizarea membranară se potriveşte doar parţial cu repartizarea caveolinei la nivelul celulelor renale embrionare HEK29. Tratarea celulelor HEK293 cu un agent care reduce colesterolul (metil-ciclodextrină), a dus la inhibarea activităţii canalelor CNGA2 exprimate la nivelul celulelor HEK239. S-a pus în evidenţă de asemenea că depleţia colesterolului duce la scăderea afinităţii CNGA2 pentru AMPc. Ca urmare canalele olfactive CNG îşi pierd activitatea dacă se alterează structura *raft-ului* membranar (17).

În ceea ce priveşte efectul colesterolului asupra canalelor ionice, ne putem referi la modul în care acesta participă la realizarea edificiului membranar, respectiv, prezenţa colesterolului în diferite *raft-uri* membranare, la nivelul cărora se găsesc anumite canale ionice. Efectul colesterolului şi al lipidelor anionice (DOPA-acid dioleil-fosfatidic) în menţinerea conformaţiei receptorului nicotinic (nAChR) a fost studiat prin spectroscopie IR, prin urmărirea modificărilor moleculare ale receptorului în prezenţa, respectiv în absenţa unui agonist al nAChR, reconstituit într-o membrană artificială (PC--din ou (EPC)/DOPA/Col = 3 : 1 : 1). Abilitatea acestor lipide de a modula statusul funcţional al nAChR, sugerează că lipidele prin efectul indirect, sunt esenţiale în funcţionarea şi stabilizarea nAChR. Astfel, putem spune că fluiditatea membranară reglează statusul funcţional sau de repaus al nAChR şi că modificări structurale minore ale fos-

folipidelor anionice sunt esenţiale pentru activitatea maximă a receptorului(18). De asemenea, s-a pus în evidenţă implicarea colesterolului în activitatea canalelor de calciu de la nivelul celulelor musculare netede. Efectul colesterolului asupra activităţii canalelor de Ca^{2+} este în relaţie cu rolul său în organizarea *raft-urilor* membranare. Acest fapt a fost studiat prin administrarea de β-ciclodextrina (un agent depletant de colesterol), în prezenta şi în absenţa colesterolului exogen. Se pare ca β-ciclodextrina afectează vasoconstricţia dependentă de presiune, în timp ce excesul de colesterol atenuează acest efect (19).

În ansamblu, activitatea canalelor ionice este dependentă de compoziţia lipidelor membranare, colesterol, sinfingolipide, fie prin interacţiunea directă cu acestea, fie indirect prin alterarea structurii *raft-urilor* membranare la nivelul cărora se găsesc şi canalele ionice.

FLUIDITATEA MEMBRANARĂ ŞI ACTIVITATEA TRANSPORTORILOR

Rezultatele obţinute cu privire la efectul fluidităţii membranare asupra activităţii transportorilor sunt relativ heterogene. Acestea depind pe de o parte de acţiunea directă a lipidelor membranare şi de modificarea fluidităţii în funcţie de gradul de nesaturare al acizilor graşi precum şi de raportul sfingomielina/colesterol membranar, iar pe de alta parte, indirect, de activarea unor enzime sau prin prezenţa unor metabolitţi sau medicamente.

De exemplu, lanţurile lungi ale acizilor graşi nesaturaţi (LCPUFAs) cresc fluiditatea membranară şi cresc numărul receptorilor de insulină, inhibă TNF-alfa, IL-6 şi MIF (*macrophage migration inhibitor factor*), în timp ce sinteza de leptină creşte numărul de receptori GLUT4 (20).

Sfingomielina este şi ea implicată în semnalizarea insulinică prin intermediul GLUT4, neexistând însă o relaţie bine stabilită între GLUT1 şi transportorul bazal (insulino-independent). S-au verificat efectele asupra GLUT1, prin modificare conţinutului de sfingomielină (prin adăugare de sfingomielină exogenă sau de glutation-inhibitor de sfingomielinază). Captarea bazală de glucoză a fost corelată cu conţinutul de colesterol (CL), sfingomielină (SM) şi raportul SM/CL. Expunerea preadipocitelor la sfingomielinază (SMase) duce la o creştere semnificativă a captării glucozei, fluiditatea membranară şi conţinutul în sfingomielină şi colesterol scăzând la 60 de minute după administrarea de SMase. Nu s-a observat, în acest context, o creştere a numărului de GLUT1 la suprafaţa membranară, fluiditatea membranară, conţinutul în colesterol şi sfingomielina revenind la valorile iniţiale după aproximativ 2 ore. Captarea bazală de glucoză a fost corelată negativ cu conţinutul de colesterol şi pozitiv cu raportul SM/CL (21).

Cotransportorul de Na^+/glucoză, cu expresie crescută la nivelul intestinului subţire, tubului proximal renal (ce are un rol important în absorbţia glucozei şi galactozei din filtratul glomerular) este reglat de cantitatea de AMPc intracelular, respectiv de activitatea PKA care poate creşte rata transportului de glucoză cu 30 % şi PKC care poate scădea rata transportului de glucoză cu până la 60 %. Sinteza de AMPc poate fi influenţată de molecule amfifile precum clorpromazina care poate duce la o creştere a fluidităţii membranare şi, implicit, la creşterea transportului de glucoză (22).

Cotransportul Na^+/glucoză duce şi la un transport al apei, în prezenţa unui gradient osmotic. Astfel factorii care modifică fluiditatea la nivelul interfeţei fosfolipide – apă, duc la alterarea echilibrului de legare al sodiului, prin inducerea unor modificări conformaţionale, prin alterarea afinităţii de legare a glucozei şi prin modificarea translocării glucozei la nivel membranar (23).

Ipotetic, toţi transportorii membranari pot fi afectaţi de interacţiunea cu lipidele (Tabelul I), dar este posibil ca nu în toate

TABELUL I
Efectul fluidităţii membranei asupra transportorilor (23)

Transportorul	Tipul celular utilizat	Factorul utilizat	Fluiditatea membranei	Activitatea transportorului
Glucoză (Na-dependent)	Membrane apicale intestinale şi corticale	CL/fosfolipide Fluidizator citofacial Clorpromazină Gentamicină Diabetzaharat (streptozicin)	↓ ↑ ↑ ↓ ↓	↓ ↑ ↑ ↓ fără corelaţie
Glucoză (Na-independent)	Eritrocite umane Fibroblaste 3T3 Osteocite	Depletia colesterolului Adaugarea colesterolului Benzilalcool	↑ ↓ ↑	↓ V_{max} ↑ V_{max} ↓
Serotonină	Endoteliu pulmonar	Colesterol Acid cis-vaccenic Hipoxie Hiperoxie VitaminaE	↓ ↑ ↑ ↓ ↓	↓ ↑ ↑ ↓ ↓
Tirozină	Sinaptozomi	Dieta fără proteine	↑	↓
Glutamină	Sinaptozomi Neuroblastom M1 Retinoblastom Y79	Dietă fără proteine Acizi graşi 18:2, 18:3 Acizi graşi 20:4, 22:6	↑ ↑ ↑	Fără modificare ↑ K_m şi V_{max} Fără modificare
Alanină	Celule tubulare renale Hepatocite	Benzil alcool Consum etanol	↑ ↑	Fără modificare Fără modificare
Fenil-alanină	Celule Ehrlich	Acizi graşi polinesaturaţi	↑	Fără modificare
Alfa-amino-izobutirat	Celule Ehrlich Retinoblastom Y79	Acizi graşi polinesaturaţi Retinoblastom Y79	↑ ↑	↓ K_m Fără modificare
Taurină	Neuroblastom M1 Retinoblastom Y79	Acizi graşi 18:2, 18:3 Acizi graşi 20:4, 22:6	↑ ↑	↑ K_m şi V_{max} ↓ K_m
Colină	Retinoblastom Y79	Acizi graşi 18:3, 20:4, 22:6	↑	↓ K_m şi V_{max}
Glicină	Retinoblastom Y79	Acizi graşi 18:2,18:3,20:4, 22:6	↑	↑ K_m şi V_{max}

experimentele a fost atins pragul critic care să afecteze transportul. O altă ipoteză ar fi aceea că numai transportul substanţelor vitale pentru funcţiile vitale celulare este modulat pozitiv de fluiditatea membranei, în timp ce transportul substanţelor care ar trebui să rămână extracelular nu este afectat sau este chiar inhibat (24).

FLUIDITATEA MEMBRANARĂ ŞI ACTIVITATEA POMPELOR IONICE

Modificarea fluidităţii membranare este în relaţie cu modularea unor enzime membranare, cum sunt Na^+/K^+-ATPaza, acetilcolinesteraza şi captarea glutamatului. Administrarea de compuşi de amoniu la şoareci a dus la reducerea sfingomielinei şi a colesterolului de la nivelul membranelor celulelor neuronale, crescând nivelul de fosfatidilserină, în timp ce nivelul de fosfatidilcolină şi fosfatidiletanolamină au rămas nemodificate. Nu s-au pus în evidenţă modificări ale fluidităţii membranare în relaţie cu lipidele „de masă" (evaluare prin metoda de polarizare a fluorescenţei-*DPH*) ci mai degrabă o scădere a fluidităţii membranare în relaţie cu lipidele inelare (evaluare cu ajutorul fluorescenţei cu *pyren*). De asemenea, scăderea tiolilor şi creşterea malondialdehidei indică prezenţa

stresului oxidativ ca posibil factor ce ar modifica fluiditatea membranei, influienţând, secundar, transportul membranar (25). Pentru a putea determina posibila relaţie dintre fluiditatea membranară şi Na^+/K^+-ATPaza în reglarea funcţiilor membranare în hipertensiune, s-au analizat efectele oubainei asupra fluidităţii membranare eritrocitare la şobolani cu hipertensiune esenţială (SHR). Rezultatele au arătat o scădere semnificativă a fluidităţii faţă de lotul martor şobolani Wistar-Kyoto (WKY). De asemenea, s-a constatat că oubaina induce o scădere semnificativ mai mare la SHR faţă de WKY, concluzia fiind că fluiditatea membranei este în strânsă legătură cu activitatea Na^+/ K^+-ATPazei în cazul SHR (26). Recent s-a pus în evidenţă ca activitatea Ca^{2+}-ATPazei membranare plasmatice depinde de gradul de ordine al lipidelor membranare. Astfel, în cazul reticulului sarcoplasmatic, activitatea pompelor de Ca^{2+} (SERCA) este optimă în condiţiile în care mediul lipidic membranar este mai puţin ordonat, în comparaţie cu membrana plasmatică. În schimb activitatea pompei de Ca^{2+} la nivelul membranei plasmatice (PMCA) depinde de caracterul ordonat al bistratului membranar, activitatea PMCA fiind mai crescută cu cât procentul de acizi nesaturaţi este mai mic (27).

Un aspect, cel puţin interesant, care poate pune în relaţie fluiditatea membranei, tratamentul cancerului şi condiţiile în care acesta este administrat, este în cazul pompelor de tip ABC (ATP binding casette). Glicoproteina P joacă un rol esenţial în farmacocinetica rezistenţei la tratament, prin împiedicarea acumulării intracelulare a unor medicamente antineoplazice. Aceasta funcţionează ca o pompă dependentă de ATP, făcând parte din familia transportorilor ABC. Pentru a clarifica efectul terapeutic a hipotermiei moderate asupra distribuţiei medicamentelor via MDR1 (*multi drug resistance protein*, sinonim cu glicoproteina P) s-au urmărit la diferite temperaturi pe culturi de celule. MDR1 se exprimă şi la nivelul barierei hemato-encefalice, limitând efectul blocanţilor de calciu şi al antiaritmicelor (prin efluxul acestora). Digoxinul a fost ales ca marker al transportului via MDR1. Astfel transportul mediat de MDR1 a fost scăzut la temperaturi scăzute, cel mai probabil prin modificări ale fluidităţii membranare. La 32ş C în schimb, nu s-a gasit nici o modificare semnificativă, sugerându-se ca difuzia pasivă nu se modifică în timpul hipotermiei moderate (28).

În ansamblu, factorii care reduc fluiditatea (incorporarea de colesterol în membrane şi creşterea presiunii intramembranare) scad şi activarea ATP-azei, pe când cei care cresc fluiditatea (incorporarea de acizii graşi polinesaturaţi, benzil alcoolul) stimulează activitatea pompei. În unele stări patologice cronice (alcoolism, diabet zaharat, HTA, obezitate) deşi fluiditatea membranei a fost găsită redusă faţă de normal, nu s-a putut stabili o corelaţie clară cu activitatea Na^+/ K^+-ATPazei, aceasta fiind de multe ori neschimbată semnificativ faţă de normal. Acest fapt s-ar putea datora fenomenelor de adaptare ale funcţionării pompei la noile condiţii de fluiditate redusă (fenomen de adaptare homeoviscoasă).

CONCLUZII

Fluiditatea este un parametru membranar complex dependent atât de factori biochimici cât şi de cei biofizici. Factorii biochimici sunt în general reprezentaţi de : raportul lipide membranare/proteine, raportul acizi graşi saturaţi/acizi graşi nesaturaţi, procentul de colesterol membranar şi prezenta unor sfingolipide. Factori biofizici cel mai adesea implicaţi sunt : potenţialul electric membranar şi temperatura.

Se poate pune în relaţie cu funcţia de transport membranar şi fluidiatea membranei. Relaţia este dictată de modul în care sunt implicate componetele membranare în interacţiunea cu structurile de transport. Acestea

pot interacţiona, direct, în funcţie de complemetaritatea electrică-chimică şi sterică, fie indirect, prin modificarea gradului de organizare membranar.

Modul în care fluidiatatea membranei influenţează transportul membranar este în relaţie cu modificarea factorilor biochimici şi biofizici. Fluidiatea membranei poate fi, cel mai probabil, pusă în relaţie şi cu alte funcţii celulare importante.

Pentru a avea o imagine de ansablu asupra influenţei fluidităţii membranei asupra funcţiilor celulare ar trebui realizată o corelaţie şi cu celelate funcţii membranare şi anume de recepţionare şi de transducere a semnalelor.

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EMBRYO SELECTION ON MORPHOKINETIC CRITERIA THROUGH TIME-LAPSE TECHNOLOGY ALONG WITH PREIMPLANTATION GENETIC TESTING (PGT) IN A COUPLE WITH RECURRENT PREGNANCY LOSS. CASE REPORT

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EMBRYO SELECTION ON MORPHOKINETIC CRITERIA THROUGH TIME-LAPSE TECHNOLOGY ALONG WITH PREIMPLANTATION GENETIC TESTING (PGT) IN A COUPLE WITH RECURRENT PREGNANCY LOSS. CASE REPORT (Abstract): In this paper we present the case of a couple with a history of recurrent pregnancy failure in the first trimester, a balanced Robertsonian translocation (45,XY,der(13;14)(q10;q10)) being identified in the male karyotype. The couple was referred to genetic counseling and underwent an in vitro fertilization (IVF) cycle associated with preimplantation genetic testing. The embryos were cultured in a time-lapse system and the morphokinetic parameters were assessed daily. From the five embryos which were appropriate from embryo biopsy and freezing, only one embryo was genetically suitable for embryo transfer. The frozen embryo transfer resulted in a pregnancy and successful birth of a female baby. In the era of human assisted reproduction, state of the art biotechnologies such as time-lapse embryo monitoring and PGT should be used in synergy in order to complement the overall picture and enhance the process of decision making in the quest of obtaining a healthy offspring. **Keywords:** IVF, PGT, TIME-LAPSE, MORPHO KINETIC PARAMETERS, EMBRYOSELECTION, RECURRENT PREGNANCY LOSS.

Recurrent pregnancy loss (RPL) may be defined by two or more failed clinical pregnancies confirmed by ultrasound or histopathologic examination. This entity affects 2-5% of the couple trying to conceive, genetic abnormalities being among the most common causes of first trimester pregnancy loss, identified in more than 50% of the cases (1;2). In almost 4% of the cases of recurrent pregnancy loss, the chromosomal abnormalities are determined by unbalanced Robertsonian translocation, a chromosomal anomaly involving 2 acrocentric chromosomes (3). One of the most frequent subtype present in clini-

cal practice, strongly associated with male infertility, severe oligoasthenozoospermia and recurrent pregnancy loss is Robertsonian translocation (45,XY,der(13;14)(q10;q10)), and for those couples trying to conceive and have such a genetic disorder, an IVF cycle associated with preimplantational genetic testing (PGT) may be the solution (4).

This case report aimed at presenting the case of a couple dealing with infertility, with an history of recurrent pregnancy loss in the first trimester, most likely due to a meiotic malsegregation of derivative chromosome in a man with a balanced Robertsonian translocation identified in the male karyotype.

CASE PRESENTATION

We present a case of a couple, a 35 years female, respectively 37 years old male patient, with a history of infertility of almost 15 years. The anamnesis revealed that patients had recurrent pregnancy losses in the first trimester, thereby a series of specific investigations, including genetic karyotyping were recommended. A balanced robertsonian translocation (Tr crs 13,22) 45, XY, der (13,22) (q10; q10) was identified in the male karyotype. In light of this information, patients were referred to genetic counseling and were guided to follow an in vitro fertilization procedure associated with preimplantation genetic testing (PGT).

Ovarian stimulation was started on 2nd day of the menstrual cycle, using GnRH antagonist ganirelix (Orgalutran, Organon, Oss), after a previous ultrasound assessment and hormonal blood work. Subsequently, hMG 375 IU per day (Menotropine-Menopur, Ferring Pharmaceuticals) was administered for the following 9 days. Final oocyte maturation was triggered by administration of 0.2 mg triptorelin (Diphereline; Ipsen). Accordingly, the oocyte retrieval was carried out 37 hours after the administration of the trigger and resulted in 20 eggs (17 M II).

The oocytes were evaluated and inseminated using intracytoplasmic morphologically selected sperm injection (IMSI) and the resulting embryos were cultured and monitored until day 5 using a time-lapse system (EmbryoScope, Vitrolife, Denmark).

Further on, daily information regarding the embryos was provided by the Embryoscope software by monitoring the morphokinetic parameters and the images of the embryo's development, without removing the cell from controlled environment. Based on the information provided by the time-lapse system, the following parameters were determined and expressed as hours: tPnf; t2; t3; t4; t5; t8; tEB (tab. I) for the embryos that reached blastocyst stage.

TABLE I

Morpho kinetic parameters of embryos cultured in time lapse system

Embryo	t Pnf	t 2	t 3	t 4	t 5	t 8	t EB
W2	26.80	30.30	41.80	42.56	50.7	63.75	135.75
W4	25.31	29.20	43.22	46.11	57.21	72.74	120.74
W5	27.06	28.81	43.57	47.43	56.94	72.49	121.50
W7	27.32	30.32	43.07	44.32	59.19	63.77	128.51
W10	28.33	30.33	43.59	45.33	60.96	63.61	110.85

*t 2 = time to 2 cells; t 3 = time to 3 cells; t 4 = time to 4 cells;
t 5 = time to 5 cells; t 8= time to 8 cells; t EB= time to expanded blastocyst

Additionally, the length of the third cell cycle (CC3=t5-t3) and the interval t5-t2, were also calculated, based on a model proposed previously by Basile *et al.* (5), according to which these two variables, t5-t2<20.5 hours, respectively 11 hours<CC3 <18 hours may be used to predict the probability of selecting chromosomally normal embryos using morphokinetic parameters.

A number of 16 embryos achieved 2 PN stage, however, only 5 blastocysts had the proper quality to be biopsied and frozen. The embryo biopsy was performed by removing cell from the inner cell mass.

TABLE II

Parameters with defined optimal ranges for ploidy prediction and results from the genetic screening for the biopsied embryos

	Morph kinetic parameters with defined optimal ranges			Aneuploidy Screening
Embryos	t5 (47.2-58.2)	CC3 (11.7-18.2)	t5-t2 (<20.5)	
W2	50.7	12.2	20.39	Arr9p11.2p21.2 (31,127,576-44, 059, 743) x 3
W4	57.21	13.99	28.01	Trisomy 13, trisomy 16, trisomy 21
W5	56.94	13.37	28.12	Trisomy 19, trisomy 22
W7	59.19	16.12	28.87	Trisomy 16
W10	60.96	17.37	30.63	Multiple aneuploidy

The genetic material was amplified with the PicoPLEX® WGA Kit (Rubicon Genomics Rubicon Genomics) as instructed by the manufacturer. Array Comparative Genomic Hybridization (aCGH) was performed on a blade with 8x60,000 (60K) samples covering the whole human genome with a spatial resolution of ~ 5 Mb DNA (G5963A, design ID: 067559, UCSC hg19, GRCh37, February 2009, Agilent). The files obtained from the scan were interpreted with Cyto Genomics software v3.0 from Agilent, using standard cell parameters. The analysis revealed that four of the embryos were abnormal, their transfer being contraindicated. One of the embryos presented a duplication on the region 9p11.2p21.2, as compared to the female reference (tab. II). Since the duplication was small, it was considered to be non-pathogenic.

A hormone replacement therapy was used for frozen embryo transfer, consisting in the administration of oral estradiol valerate (Cyclo-Progynova; Bayer, Germany) 6 mg/ t.i.d, from the first day of menstrual cycle. The first transvaginal ultrasound was performed in day 10 of the treatment when endometrial thickness was 10 mm and from day 11 natural micronized progesterone (Utrogestan; Lab. Besins Int., France) was started at a dose of 600 mg/day. Embryo transfer was performed in day 5 of progesterone administration.

The euploid blastocyst wasted, evaluated and transferred with a soft catheter, under ultrasound guidance. The biochemical pregnancy was confirmed by beta-hCG determination 12 days after the transfer and clinically, by ultrasound, 21 days post transfer. The pregnancy evolved without obstetrical complications and the baby was

delivered at 38 weeks through C-section, with a 9 APGAR score and weighting 3050 grams. The following neonatal assessments did not reveal any development problems.

DISCUSSION

Recurrent pregnancy loss (RPL) is a multifactorial disorder that causes significant physical and psychological distress to patients and challenges medical providers engaged in the management of this reproductive health issue (6,7).

According to the guideline elaborated by ESHRE (8), patients with abnormal karyotype should receive genetic counseling. A study conducted by Ikuma *et al*. (9) showed that miscarriage rates may be lower when preimplantation genetic testing is used along with IVF. The introduction of a new method of embryo selection, called time-lapse monitoring, has been long awaited. Unlike the conventional routine embryo evaluation, the time lapse monitoring of embryos provides a stable microclimate for embryo culture, certain potentially stressful factors such as variation in temperature or pH being avoided because the embryos are cultured continuously, without being removed from the incubator for daily routine evaluation (10, 11).

A study conducted by Rubio *et al.* (12), showed that embryos cultured in the Embryo Scope® system have a higher implantation potential and are associated with lower rates of early pregnancy loss, one of the possible explanations being the more stable culture environment, which ensures a better protection against oxidative stress. Furthermore, a study conducted by Campbell *et al*., (13), Basile *et al*. (5), Chawla *et al*. (14), concluded that the kinetic pattern differs among chromosomally normal and abnormal embryos, euploid embryos having a more defined pattern. Additionally, a study conducted by Del Carmen Nogales *et al*. (15) showed that the subtypes of chromosomal abnormalities such as complex aneuploidy exhibit modified morpho kinetics parameters (t3 and t5).

In our case study, only the morphokinetic parameters registered for the embryo selected for transfer (W2), were in the optimum interval proposed by Basile (5). However, due to the limited number of analyzed embryos, no statistical analysis was performed to reveal any significant statistical differences as compared to the other embryos which were identified as aneuploid with PGT. Nevertheless, there is evidence showing that morphokinetic parameters may reflect the implantation potential of embryos, significant increased pregnancy and live birth rate, as well as reduced early pregnancy loss being observed when embryos classified as low risk were transferred (16-19).

Embryo-selection by morphokinetics criteria-time lapse imaging, may be considered as a complementary method and an option for those couples with multiple implantation failure, especially in those clinics where preimplantation genetic testing is not available, the procedure is forbidden by law or to reduce costs associated with this procedure.

Concluding, in the era of human assisted reproduction, state of the art biotechnologies such as time-lapse embryo monitoring and PGT should be use in synergy in order to complement the overall picture and enhance the process of decision making in the quest of obtaining a healthy offspring.

INFORMED CONSENT

Informed written consent was obtained from the patient for publication of this case

report and accompanying images.

CONFLICT OF INTERESTS

No conflict of interests was declared by the authors.

FINANCIAL DISCLOSURE

The authors declared that this study has received no financial support.

LIST OF ABBREVIATIONS

IVF: in vitro fertilization

IMSI: intra cytoplasmic morphologically selected sperm injection

PGT: preimplantation genetic testing

RPL: Recurrent pregnancy loss

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NEWS

LIQUID BIOPSIES COULD REVOLUTIONISE CANCER MEDICINE

Tumor biopsies are the current standard for cancer diagnosis and the preferred method for molecular testing to guide the selection of precision therapies. Liquid biopsies, mostly those involving cell-free DNA (cfDNA) from plasma, are rapidly emerging as an important and minimally alternative approach. Liquid biopsy is becoming a valuable tool for molecular testing for new insights into tumor heterogeneity, and for cancer detection and monitoring. Although liquid biopsy has most often referred to the analysis of cfDNA from peripheral blood, this term also refers to the isolation and analysis of tumor derived material from blood or other bodily fluid like: cerebrospinal fluid, saliva, pleural fluid, ascites, stool and urine. Although more prospective studies are needed liquid biopsies have the potential to revolutionize cancer medicine (Corcoran RB, Chabner BA. Application of cell-free DNA analysis to cancer treatment. *N Engl J Med* 2018; 379(18): 1754-1765).

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CERCETĂRI ASUPRA FUNCŢIEI PLACHETARE ÎN CONDIŢIILE HEPATOPATIEI EXPERIMENTALE ACUTE ŞI CRONICE

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PLATELET FUNCTIONS IN ACUTE AND CHRONIC EXPERIMENTALLY INDUCED HEPATOPATHIA (Abstract) : It's well known the coagulation damage in chronic hepatitis and hepatic cirrhosis. The aim of our study was to quantify platelet dysfunction in acute and chronic toxic hepatitis. **Material and method** : We determined adhesivity, aggregability and icosanoids production in rats with acute and chronic hepatopathia induced by CCl_4 administration. **Results** : Our data shows that platelet adhesivity and aggregability are affected in chronic toxic affectation but also in acute intoxication ($p < 0.05$). Icosanoids production (expressed like MDA levels are significantly decreased only in chronic CCl_4 intoxication ($p < 0.001$). Significantly correlation appears between aggregability and adhesivity in control group ($r = 0.66$) and between aggregability and MDA ($r = -0.78$) and adhesivity and MDA ($r = -0.57$) in lot C. **Conclusion** : Primary haemostasis and platelet dysfunction are involved in coagulation dysfunction in hepatic diseases and the modifications of platelet parameters also appeared in acute hepatic affectation. **Key words** : HAEMOSTASIS, PLATELET DYSFUNCTION, HEPATIC DISEASE

Afectarea coagulării în condiţiile cirozei hepatice este binecunoscută. În etiologia acestei hipocoagulopatii este implicat în general deficitul de sinteză hepatică a unor factori plasmatici ai coagulării. Unele studii atestă însă şi deficite ale hemostazei primare asociate mai ales cu un deficit funcţional plachetar. Date preliminare sugerează că afectarea funcţionalităţii plachetare ar exista şi în unele hepatopatii acute. Scopul urmărit a fost evaluarea funcţiei plachetare la şobolani cu afectare hepatică toxică (produsă prin administrarea de tetraclorură de carbon CCl_4) acută sau cronică.

MATERIAL ŞI METODE

Partea experimentală a fost realizată pe şobolani rasa Wistar masculi, de sex masculin, cu greutatea cuprinsă între 216-300 grame. Aceştia au primit hrană şi apă la discreţie şi au fost menţinuţi în condiţii standard de laborator la o temperatură de 20 ± 2^0C cu un ciclu de 12 ore lumină, 12 ore întuneric. S-au asigurat de asemenea condiţiile de ventilaţie ; au fost găzduiţi în cuşti de inox, de dimensiuni potrivite conform normelor în vigoare.

Diagnosticul de afectare acută respectiv cronică a ficatului a fost pus prin evaluarea modificărilor morfologice hepatice comparativ cu lotul de referinţă. Modificările morfologice s-au manifestat atât la nivel celular cât şi tisular, prin alterări în histoarhitectonica hepatică.

Investigarea funcţionalităţii plachetare în condiţiile hepatopatiei indusă experimental s-a realizat prin determinarea agregabilităţii plachetare (1), a adezivităţii plachetare, prin metoda colorimetrică a lui Bellavitte şi colab. (2) şi sintezei de icosanoizi plachetari, prin dozarea MDA (3).

Animalele au fost divizate în trei loturi de studiu, după cum urmează :

- M – lotul martor, format din şase şobolani masculi, rasa Wistar, de crescătorie, cu greutăţi cuprinse între 236 şi 300 de grame ;
- A – lotul cu afectarea acută hepatică, format din şapte şobolani masculi, rasa Wistar, de crescătorie, cu greutăţi cuprinse între 216 şi 274 de grame ; au primit CCl_4, în doză unică, 0,01 ml/kgc în 0,5 ml ulei vegetal administrat intraperitoneal. Animalele au fost sacrificate la 48 de la injectare.
- C – lotul cu afectare cronică hepatică, format din şase şobolani masculi, rasa Wistar, de crescătorie, cu greutăţi cuprinse între 220 şi 258 de grame ; au primit CCl_4, 0,008 ml/kgc în 0,5 ml ulei vegetal, intraperitoneal, de două ori pe săptămâna, timp de 8 săptămâni pentru inducerea hepatopatiei.

Animalele au fost sacrificate la 48 de ore de la ultima injectare după anesteziere cu tiopental, în doză de 1 ml/100 g din soluţia 0,01% tiopental. S-au recoltat fragmente de ficat pentru evaluare anatomo-patologică a hepatopatiei precum şi sânge din cord pentru determinarea parametrilor funcţionali plachetari.

S-a făcut standardizarea tehnicii de lucru, în toate cazurile respectându-se anumite condiţii de recoltare şi prelevare.

În ceea ce privesc probele de anatomie-patologică :

- aceeaşi persoană a efectuat recoltările ;
- s-a utilizat acelaşi instrument de prelevare ;
- aceleaşi zone topografice de prelevare ale fragmentelor ;
- fixarea imediată a fragmentelor tisulare şi evitarea manipulării manuale ;
- aceeaşi persoană a făcut prelucrarea pentru examenul microscopic ;
- acelaşi mod de prelucrare pentru examenul microscopic.

Fragmentele hepatice au fost fixate în formol 10% incluse în parafină, secţionate şi colorate standard, hematoxilină-eozină şi Van Gieson. Tehnica uzuală histologică care s-a folosit pentru toate preparatele a fost completată cu tehnici de prelucrare şi colorare specifice anumitor structuri modificate de procesul patologic studiat.

Recoltarea sângelui. După anestezierea animalului cu tiopental, s-a deschis cutia toracică şi s-a puncţionat cordul cu un sistem de recoltare tip Vacuette®, în vacutainere de 2,5 ml, conţinând ca anticoagulant citrat de sodiu 3,8%. Proporţia finală, sânge/citrat este cea clasică de 9/1. Vacutainerele au fost centrifugate ulterior pentru obţinerea de plasmă bogată în plachete (PRP). Parametrii plachetari determinaţi în acest experiment au fost :

- **Agregabilitatea plachetară**, prin micrometoda pe cititorul TECAN, după Beddnar B (1), modificată de Robert C. Chadderon şi Michael Cappello (4) ;
- **Adezivitatea plachetară**, după metoda descrisă de P. Bellavite et al. (2) ;
- **MDA plachetar**, după metoda lui Enache, 1998, modificată (3).

Datele au fost încărcate şi prelucrate cu ajutorul funcţiilor statistice din *EXCEL* şi *EpiInfo* folosindu-se testul *t-Student* (pragul de semnificaţie unanim acceptat este de 95% adică $p=0,05$) şi testul γ^2. Corelaţia liniară este corelaţia dintre două variabile ale căror valori formează o dreaptă. Intensitatea legăturii dintre variabile se măsoară cu ajutorul coeficientului de corelaţie Pearson (r), care trebuie să ia valori între $-1 \leq r \leq 1$, cu cât este mai apropiat de valoarea 1 cu atât corelaţia este mai puternică, fie directă, fie in-

versă dacă r = -1.

REZULTATE

Examenul anatomopatologic al fragmentelor de ficat recoltate a relevat aspecte normale ale morfologiei hepatice la lotul martor. La lotul cu afectare acută hepatică au fost evidenţiate anomalii morfologice hepatice specifice hepatitelor acute toxice : ficat cu structură histologică păstrată, degenerescenţă hidropică şi steatoza hepatocitară, hepatocitoliză, degenerescenţă hidropică. În cazul lotului C, cu afectare cronică, examenul anatomopatologic efectuat pe fragmentele hepatice recoltate evidenţiază în unele cazuri alterarea arhitectonicii ficatului, cu apariţia de noduli de regenerare (semn de ciroza hepatică-CH), infiltraţii şi fibroză în spaţiul port, mitoze, steatoză macroveziculară si chisturi lipidice (leziuni caracteristice toxice). Rolul examenului anatomopatologic a fost de a confirma apariţia leziunilor hepatice.

Examenului sângelui recoltat pe citrat de sodiu pentru a evalua funcţia plachetară a relevat următoarele valori ale adezivităţii, agregabilităţii şi MDA plachetar.

Agregabilitatea plachetară. Analizând loturile studiate pentru parametrul agregabilitate plachetară se observă diferenţe statistic semnificative între martor şi toate celelalte loturi cu excepţia lotului LA. Vizibilă şi în tabel, diferenţa de agregabilitate plachetară între lotul martor şi cele cu hepatopatie este mare cu un prag de semnificaţie de peste 95%.

Adezivitatea plachetară. Folosind testul *t-Student* pentru comparaţia parametrului adezivitate în loturile de studiu sunt puse în evidenţă diferenţe statistic semnificative faţă de lotul martor.

MDA plachetar. Studiind valorile MDA plachetar se observă că nu există diferenţe semnificative statistic între lotul martor şi cel cu hepatopatie acută (A) dar se remarcă semnificaţii statistice semnificative pentru MDA între lotul martor si cel cu afectare cronică hepatică.

Evidenţierea unor posibile corelaţii între parametrii de investigat. În ceea ce priveşte cei trei parametrii funcţionali plachetari am încercat să observăm eventuale corelaţii în cadrul fiecărui lot în parte şi să evidenţiem dacă există sau nu un paralelism între funcţiile plachetare în condiţii normale şi/sau patologice. Am considerat r > $\pm$0.5 corelat, iar r > $\pm$0.6 semnificativ corelat. Sintetizând, tabelul IV evidenţiază coeficienţii de corelaţie înregistraţi între parametrii investigaţi. Se observă că, în lotul martor singura corelaţie semnificativă, directă există între adezivitate şi agregabilitate, nici un fel de corelaţii nu există între alte două funcţii. Acest tip de corelaţie nu se menţine la loturile cu afectare hepatică acută respectiv cronică probabil datorită prăbuşirii agregabilităţii comparativ cu adezivitatea, parametru care comparativ nu scade într-o manieră atât de importantă. În ce priveşte corelaţia dintre agregabilitate şi MDA aceasta are valori scăzute în lotul martor şi în cel cu afectare acută dar la lotul cu afectare cronică există o corelaţie inversă, importantă (r=-0,78) ceea ce arată

TABELUL I

Semnificaţii statistice pentru agregare între loturile de studiu faţă de martor

Martor	Lot	Media ± DS	*t-Student*	p
22,44 ± 15,43 (n=6)	**C (n=6)**	**1,33 ± 0,61**	**3,35**	**p < 0,01**

TABELUL II

Semnificaţii statistice pentru adezivitate a loturilor de studiu faţă de martor

Martor	Lot	Media ± DS	*t-Student*	p
52,49 ± 1,35 (n=6)	**C (n=6)**	**16,93 ± 6,56**	**13,01**	**p < 0,001**

TABELUL III
Semnificaţii statistice pentru MDA a loturilor de studiu faţă de martor

Martor	Lot	Media ± DS	*t-Student*	p
9,07 ± 2,11	**C (n=6)**	**3,94 ± 1,64**	**4,70**	**p < 0,001**
(n=6)	A (n=7)	7,99 ± 2,09	0,92	NS

TABEL IV
Valori ale coeficientului de corelaţie Pearson „r"

Coeficient de corelaţie Pearson „r"	LOT M	LOT A	LOT C
Agregabilitate-adezivitate	0,66	0,38	0,07
Agregabilitate - MDA	-0,32	-0,06	-0,78
Adezivitate - MDA	-0,02	-0,34	-0,57

că diminuarea agregabilităţii şi a sintezei de icosanoizi plachetari, evidenţiabilă prin valoarea MDA, în condiţiile afectării cronice hepatice. Intre adezivitate şi MDA există o există o corelaţie inversă semnificativă doar în lotul cu afectare cronică hepatică (-0,57).

DISCUŢII

Administrarea de medicamente, radiaţiile ionizante, substanţele poluante din mediu înconjurător cu potenţial pro-oxidant induc formarea de radicali liberi de oxigen (ROS). Peroxidarea lipidică indusă de ROS este considerată a fi nocivă pentru membranele celulare şi stă la baza unor numeroase situaţii patologice. Leziunile hepatice în experimentul nostru s-au realizat folosind CCl_4, un solvent industrial şi un hepatotoxic cunoscut. Este cunoscut faptul că CCl_4 determină afectare hepatică dar şi renală la om dar şi la animalele de experienţă. Prin activarea metabolică, dependentă de citocromul P_{450} a CCl_4 rezultă radicalii liberi CCl_4 respectiv Cl care iniţiază peroxidarea lipidică (5). Vitamina E are efect protector asupra lezării hepatice cu CCl_4 ceea ce confirmă ipoteza stresului oxidativ în acest model (6). La animalele de laborator la care s-a administrat CCl_4 este afectată capacitatea metabolică a mitocondriilor din hepatocit, confirmând ipoteza stresului oxidativ (7). Administrarea pe termen lung de CCl_4 provoacă afectare cronică hepatică, cu necroză, infiltrat inflamator, degenerescenţă hidropică şi fibroză care pot duce în final spre ciroză hepatică, fapt confirmat de numeroase studii (4,8,9). Moartea celulară se produce în final prin necroză şi apoptoză, în proporţii variabile (10).

Numeroase studii au confirmat afectarea toxică a ficatului sub acţiunea CCl_4 administrată intraperitoneal, gavaj sau vapori. Unii autori susţin că după 8-12 săptămâni CCl_4 produce ciroză hepatică (7), alţii indică doar semne de fibroză hepatică (11). Mecanismul prin care apare afectarea funcţiei hepatice şi respectiv fibroza este mult discutat. Acesta este extrem de important pentru studiul nostru, deoarece, aşa cum arătat anterior fibroza hepatică reprezintă un element cheie în funcţionalitatea plachetului (12) şi invers trombocitul, prin factorii de creştere joacă un rol în apariţia şi dezvoltarea fibrozei hepatice. Totuşi afectarea funcţiei plachetare ca urmare a unui deficit funcţional hepatic apare şi în alte tipuri de hepatopatii induse experimental (13, 14). Krahenbuhl et al. (7) realizează un model de ciroză hepatică experimentală la şobolani, prin expunere la CCl_4 şi dovedeşte, prin metode biochimice şi analiza sterologică a ficatului că leziunile hepatice s-ar datora afectării funcţiei mitocondriilor, afectare evidenţiată prin scăderea activităţii unor enzime mitocondriale, prin scăderea formării hipuratului din benzoat şi a metabolizării palmitatului. Acelaşi studiu susţine că, deşi

apar modificări, acestea sunt în special de natură metabolică şi greutatea ficatului nu se modifică (fapt observat şi de noi în studiul efectuat). Deşi studiul lui Krahenbuhl (7) nu abordează direct problema funcţiei plachetare câteva date sunt extrem de interesante şi anume : afectarea metabolismului acizilor graşi cu lanţ lung, afectare care ar putea explica defectele de agregabilitate plachetară obţinute şi de noi.

În determinarea cauzelor scăderii agregabilităţii plachetare la cirotici ipoteza modificării compoziţiei lipidelor membranare, ca urmare a funcţionării defectuoase a ficatului lansată în urmă cu peste 20 de ani este din ce în ce mai incriminată în ultimii ani (15,16,17,18,19). Pe de altă parte tot acest studiu confirmă că defectele mitocondriale evidenţiate pe modelul cu tetraclorură de carbon nu sunt valabile şi pe modelul de ciroză biliară unde din contră, metabolismul benzoatului este crescut ; acest lucru este corelat şi cu funcţia plachetară deoarece şi alte studii arată că, în ciroza biliară există o tendinţă la hiperfuncţionalitate plachetară şi chiar la tromboze (20). Creşterea concentraţiei de acizi graşi mononesaturaţi, ca urmare a unui deficit de metabolizare este evidenţiat de Koike et al. (21) într-un studiu efectuat atât pe subiecţi cu hepatită cronică cu virus hepatitic C cât şi pe şoareci transgenici ; mai mult acesta susţine că, creşterea concentraţiei de acizi graşi mononesaturaţi ar duce la o creştere consecutivă a fluidităţii membranei hepatocitare ceea ce duce la o amplificare a ratei de diviziune celulară şi creştere a riscului de apariţie a cirozei hepatice şi a carcinomul hepatocelular. În acest context, studiul lui Manibusan (22) sugerează că PRP ar putea favoriza carcinogeneza la şobolani la care s-a indus hepatopatie cu CCl_4 ; de altfel şi în studiul nostru am obţinut, la analiza histologică în lotul cu CCl_4 imagini sugestive pentru carcinogeneză. Alţi autori consideră că în hepatopatiile cronice cresc nivelele de acid oleic, palmitic şi stearic şi scad nivelurile de acizi graşi polinesaturaţi (linoleic, arahidonic şi docosahexanoic), precursori de icosanoizi, ceea ce ar putea explica deficitul funcţional plachetar prin alterarea transmiterii mesajului biologic. Studii mai vechi (23) sugerează că defectul plachetar este legat mai mult de factori plasmatici decât de cei intrinseci plachetari (ipoteză negată de Laffi, 24) şi că numai normalizarea funcţiei hepatice este cea care îmbunătăţeşte funcţionalitatea plachetului, ipoteză susţinută şi de noi. Unele experimente au încercat îmbunătăţirea funcţiei plachetare la hepatici prin aport alimentar de acizi graşi (15).

CONCLUZII

Este cunoscut faptul că CCl4 determină o afectare hepatică dar şi renală, atât la om cât şi la animalele de experienţă.

Mecanismele patogenice implicate sunt stresul oxidativ şi modificarea compoziţiei membranare în acizi graşi.

Dintre funcţiile plachetare scăzute semnificativ, în studiul nostru sunt agregabilitatea şi adezivitatea.

Sinteza de icosanoizi plachetari este semnificativ scăzută in lotul cu afectare hepatică cronică dar nu şi la lotul cu la lotul cu hepatopatie acută faţă de lotul martor.

Corelaţiile puternic semnificative apar doar între agregabilitate şi adezivitate la lotul martor şi între agregabilitate şi MDA plachetar la lotul cu afectare cronică hepatică, ceea ce ar putea sugera legătura dintre deficitul de agregabilitate şi compoziţia membranară în acizi graşi.

Studiul nostru confirmă numeroase alte studii care susţin că deficitul hemostazei în hepatopatii are drept cauză şi alterarea funcţiei plachetare.

Trombopatia apare şi în condiţiile unei hepatopatii acute toxice experimentale, nu doar în formele cronice aşa cum descriu cele mai multe studii.

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PSYCHOLOGICAL EFFECTS AND ETHICAL IMPLICATIONS OF THE PRENATAL DIAGNOSIS OF SEVERE MALFORMATION IN THE FETUS

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PSYCHOLOGICAL EFFECTS AND ETHICAL IMPLICATIONS OF THE PRENATAL DIAGNOSIS OF SEVERE MALFORMATION IN THE FETUS (Abstract): Considered from the standpoint of the parents' and medical team's rights, the prenatal diagnosis of severe inborn malformations is an ethical and legal must, which carries a twofold meaning: recognition of the women's right to abortion and fetus protection. The purposes of our paper are: to reveal the psychological effect of antenatal diagnosis in case of fetal malformation; to point out a series of ethical implications of elective abortion of fetuses or discontinuation of the treatment of newborns suffering from multiple malformations; and to draw attention on the low antenatal diagnosis rates in our country. **Material and methods:** In Romania, on a 23-year-long study conducted over 115 neonates suffering from gastroschisis and on 105 newborns with omphalocele, the rate of antenatal malformation diagnosis was very low, and implicitly leaded to a high malformation and chromosomal aberration incidence and hence to a high neonatal mortality rate. Also, the birth of these babies set a considerable financial burden on pediatric surgery wards for the somewhat hopeless treatment. **Results:** Most of the times, making abortion decisions, caring for or discontinuing the treatment of newborns suffering from multiple malformations also have a negative impact on the family. Whichever the case, any choice made about an abnormal fetus should be respected and protected by the family, the laws in effect and the country's culture and social structures. **Conclusions**: It is the parents who should decide the future of the pregnancy or, when there was no antenatal diagnosis, of the neonate; the decision must be made after having been well informed by a multidisciplinary medical team. **Keywords**: ABORTION, NEWBORN, MALFORMATIONS, CHROMOSOMAL ABERRATIONS, ANTENATAL DIAGNOSIS.

An increasingly higher number of newborns with anterior abdominal wall malformations have benefitted from antenatal diagnosis. Therefore, whenever the obstetrician detects any type of fetus abnormality by ultrasound scanning, the parents ought to be prenatally advised by a multidisciplinary medical team (1). Despite the fact that isolated omphalocele and gastroschisis have better prognosis than other inborn malformations detected prenatally, such a diagnosis may have an unfavorable

psychological impact on parents and even an unfortunate influence on their future reproduction decisions (2). Should other associated abnormalities be detected, or should the malformation be classified as part of a particular genetic syndrome, the fetal prognosis may be poor, and the parents may consider an elective termination of pregnancy (3). The debate may be extended to include the case of newborns suffering from multiple malformations, for which "postnatal abortion" may be considered (4, 5). Considering that abortion (therapeutic or elective) is both moral and legally accepted in most of the countries, does this implicitly mean that these neonates may be condemned to non-treatment (namely "postnatal abortion")? The purposes of our paper are: to reveal the psychological effect of antenatal diagnosis in case of fetal malformation requiring surgical therapy; to point out a series of ethical implications of elective abortion of fetuses or discontinuation of the treatment of newborns suffering from multiple malformations and chromosomal aberrations; and to draw attention on the low antenatal diagnosis rates in our country.

MATERIAL AND METHODS

Unfortunately, in Romania, the prenatal malformation diagnosis rate is very low, which implicitly leads to a high malformation and chromosomal aberration incidence in newborns, and hence to high neonatal mortality rates (6, 7). Therefore, a 23-year-long study (1990-2012) conducted over 115 neonates suffering from gastroschisis and on 105 newborns with omphalocele, who were hospitalized in the Pediatric Surgery Department of the "Sfânta Maria" Clinical Emergency Children's Hospital of Iaşi, revealed prenatal diagnosis rates of only 11.4% for gastroschisis and 13.3% for omphalocele, whereas in most European countries these rates exceed 70-80% (8, 9). The studies that considered the socio-economic factors showed that the omphalocele and gastroschisis risks were higher among underprivileged mothers, which may probably be accounted for by the tender age of the mothers, by the mothers' drug and tobacco abuse, by the absence of any family planning within this population, by their single status or their low education level (10, 11). According to our findings, 40% of the mothers whose babies were diagnosed with gastroschisis and 22% of those whose newborns were diagnosed with omphalocele were single, whereas 15% of the parents with babies suffering from gastroschisis and 32% of those whose neonates had omphalocele were alcohol and tobacco abusers. The mothers' education level was slightly higher for omphalocele (27% illiteracy or only primary education versus 40%). On the other hand, they were also older (27.0 years on the average versus 20.6 years). As concerns the gastroschisis newborns, we should also point out that almost half (41.2%) of the mothers were very young, i.e. 20 years or younger, and 65.7% of them were primigravida, primipara. The babies diagnosed with gastroschisis and omphalocele came mostly from the rural areas (68.5% and 62% of the cases, respectively). Urban newborns enjoy prenatal diagnosis rates that are two times higher as compared to rural neonates, which is statistically significant. All these socio-economic factors had a negative influence on the number of mothers with anterior abdominal wall defect babies who came to be seen by a doctor and also on the antenatal diagnosis rate, respectively. In its turn, this also had negative repercussions

on the future course of the disease. Therefore, the mortality rates were extremely high in our study, i.e. 70.2% for gastroschisis and 54.3% for omphalocele, as compared to 0-10% rates in developed countries (9, 12). In our country, high antenatal diagnosis rates of severe chromosomal aberrations or inborn malformations associated with anterior abdominal wall defects (27.6% of the omphalocele cases also suffer from chromosomal aberrations) may result into a therapeutic abortion decision meant to protect the families involved against the unwanted psychological effects of giving birth to a baby suffering from multiple malformations, which would put a considerable financial burden on pediatric surgery wards for the somewhat hopeless treatment of these babies. This would remove these patients with guarded prognosis from future studies and thus improve survival rates (abortion being presented as treatment) (6). In our study, due to the small number of cases diagnosed prenatally (13 cases of gastroschisis and 14 cases of omphalocele) and also due to the retrospective nature of the study, as the mothers came to our clinic one or two days after baby delivery, we were unable to quantify the full extent of the psychological impact of the prenatal diagnosis of abdominal wall defect, although, according to their statements, it had been a strongly negative one.

RESULTS AND DISCUSSION

The purpose of any antenatal examination in case of inborn abnormality or malformation detection is to provide information on the fetus' evolution and quality of life, on any possible ante or postnatal procedures, on adequate management and type of delivery, and on the baby's long-term prognosis. Several meetings between the parents to be and the multidisciplinary medical team monitoring the malformed fetus will most probably be necessary in order to tackle all these issues (1). The team should include an obstetrician, an ultrasound scanning specialist, a neonatologist, an anesthesiologist, a pediatric surgeon, a geneticist and a psychologist. The influence of a prenatal surgical examination on the mortality rates and on the perinatal management of anterior abdominal wall defects, as well as the psychological effects of antenatal parent counseling have been thoroughly described in literature (13), but the number of counseling sessions required to decrease the parents' anxiety has not been determined yet (14). Should other associated abnormalities be detected, or should the malformation be classified as part of a particular genetic syndrome, the fetal prognosis may be poor, and the parents may consider an elective termination of pregnancy (3). This decision varies greatly depending on the antenatal diagnosis team, on the parents' attitude, on the fetus' state and also on the laws regulating therapeutic abortions in effect at that time. Therapeutic abortion is the abortion induced legally by a qualified physician because the fetus is severely malformed or to safeguard the health of the mother. In some cases, the parents' religious beliefs or their socio-economic and educational status may have considerable influence on their making a therapeutic abortion decision. Especially these parents should be given the possibility to talk about their fetus' prognosis with the members of the neonatal team and with a specialized psychologist, in order to get full grasp of the actual situation and to make the last feelings of fear or guilt disappear before making their final decision. Given all these factors, a homo-

geneous set of therapeutic abortion assessment criteria is hard to define and to harmonize worldwide, which also accounts for the very diverse and somewhat contradictory data provided by literature.

A European study conducted in 2001 reveals a therapeutic abortion rate amounting to 37% for omphalocele associated with other malformations or chromosomal aberrations, and to 29% for gastroschisis (8). Although most researches acknowledge a positive prognosis of these cases after their surgical repair, the high rate (16-17%) of abortions performed in Europe in cases of planned pregnancies with isolated anterior abdominal wall defects, with no other associated diseases, proves that parents still do not trust the specialists' ability to rule out any malformations and abnormalities associated with omphalocele and gastroschisis prenatally, which leads too easily to the parents' making a decision of termination of pregnancy (8). Similar conclusions were noted in a recent study, which pointed out that one in three mothers chose abortion when their fetus was diagnosed with isolated omphalocele (15). That is why sometimes, if the parents' decision is clearly against the best interest of the child, it is ethically permissible and legally required to disqualify them.

There are currently many papers assessing the psychological impact of antenatal diagnosis on the expectant parents (1, 2, 13-16). Both family planning and pregnancy are normally considered happy times for the family, but when a fetal condition is detected prenatally, the time left until the baby's delivery is one of stress and uncertainty for the couple. The parents' anxiety is not connected to their age or social status, and their apprehension is significantly reduced after surgical examination and adequate antenatal counseling. Moreover, there has been noted no statistically significant difference between the extent of the parents' anxiety about the various groups of inborn malformations (13). In other words, the level of anxiety, both when the diagnosis was initially set and when the baby was born, was independent of the severity of the abnormality detected. Just like Drotar pointed out, this finding may reflect the state of shock suffered by the parents when given the inborn malformation news, which news will cause a "crisis" situation in the lives of the expectant parents (17). Marteau suggests that immediate and long-term suffering caused by the occurrence of a fetal malformation may be reduced by the parents' adequate preparation before the test, and especially by support, by post-test counseling and the provision of information as complete as possible (18). Although anterior abdominal wall anomalies have better prognosis than other inborn malformations detected prenatally, such a diagnosis may have an unfortunate influence on their future reproduction decisions. In a research conducted on 18 parents with omphalocele and gastroschisis babies, 9 of the 14 mothers that initially wanted to keep having children before the delivery of the malformation baby decided to stop trying to have children, whereas the others deferred making any such decision. Nevertheless, 6 of the 7 mothers of babies with omphalocele and 10 in 11 mothers of babies with gastroschisis retrospectively admitted that the antenatal diagnosis was useful, as it provided them with the time required for the parents' psychological preparation for making the best possible decisions related to birth planning (2). Prenatal diagnosis influenced the clinical prevalence of anterior abdominal wall ab-

normalities both by the therapeutic termination of problematic pregnancies and by better delivery and pre and postnatal conduct planning. Therefore, Forrester shows that 87% of the prenatally diagnosed omphalocele cases and 37% of the gastroschisis cases underwent at least one procedure during pregnancy, namely a fetal karyotype test (41%) or therapeutic abortion (59%) for omphalocele and successive ultrasound scans (59%) of *"in utero"* transportation to a specialized center (63%) for gastroschisis (19). Invasive antenatal diagnosis procedures are carried out in pregnancies with high risks of chromosomal aberrations detected by screening, as well as if the couples wish an absolute guarantee of a normal fetal karyotype, regardless of the aneuploidy risk calculated by screening.

Considered from the viewpoint of the rights of the parties involved, prenatal diagnosis is a legal must, caring a twofold meaning: recognition of the women's right to abortion and, on the other hand, recognition of the fetus' right to life and protection. The extraordinary development of medical techniques and devices that we have witnessed these last decades should serve the patient's best interests; more precisely, it should support the improvement of the patient's health and even save his/her life. As far as ultrasound scanning of a pregnant uterus is concerned, when severe fetal malformations are diagnosed, to the healthcare system the fetus becomes a new patient. This new patient will be subjected to repeated ultrasound scanning examinations, biochemical tests will be carried out on him/her (amniotic puncture, umbilical cord puncture) and he/she may even undergo surgical procedures (vesical-amniotic shunt for posterior urethra valve, ventriculo-amniotic shunt for hydrocephaly, trachea obstruction for congenital diaphragmatic hernia, silo-bag insertion for gastroschisis). However, when the malformations detected are varied and severe (unfortunately, though, sometimes even for isolated surgically rectifiable abnormalities (8)), their antenatal diagnosis could lead to abortion, i.e. virtually to killing the patient, in which case the breakthroughs of medical science and technology are a disservice to the patient.

Abortion for genetic reasons has been considered a discriminative action against disabled people for a long time, as numerous cultural and social factors are involved in the decision of termination of an abnormal pregnancy (20). Thus, during its General Conference of 1997, UNESCO forbade any form of discrimination based on genetic characteristics, whereas the Catholic and Orthodox Churches recommend that human life be respected and protected in an absolute manner since the very conception of the baby, and the products of conception diagnosed with various genetic abnormalities should be entitled to a social environment adapted to their human development (21). In any event, the choice made by the family about a pregnancy with impaired fetus should be respected and protected by the family, the laws in effect and the country's culture and social structures. The couple and not the doctors should decide the future of a pregnancy (autonomy principle) (20). Nevertheless, since the prenatal diagnosis is set and the associated conditions are revealed at increasingly lower gestational ages, which are even considered "legal" for elective termination of pregnancy, matters related to the definition of normal/abnormal fetuses or to the possibility of selection of the "ideal baby" have been tackled in literature lately. Therefore,

Gauderer, in a research conducted on 139 fetuses with congenital malformations (18 of whom had anterior abdominal wall defects or other gastro-intestinal anomalies), pointed out a fact which is difficult to reveal by statistical analysis and hard to accept from an ethical point of view, namely that too few couples decide to go on with a pregnancy with an "imperfect" fetus, as two pregnancies with surgically rectifiable anterior abdominal wall anomalies and with no other associated malformations were electively terminated, despite proper antenatal counseling (22). On the other hand, the pregnant woman's 'altruistic' position in relation to the health and wellbeing of the fetus is more often a problem in the obstetric practice (23). Almost every woman would go through a great deal and put up with discomfort, pain and even risks to their own health in order to protect the fetus, and that is why sometimes they would say no to therapeutic abortion even in the worst-case scenario (plurimalformative syndrome, chromosomal aberrations). Moreover, killing a human being who is not an aggressor is a terrible deed, which is legally and morally inacceptable in any community, regardless of its religion or social development status (24). The question is at what point during their human development from egg, embryo, fetus or newborn, be they malformed, human beings earn their right to live (divergent views were apparent in relation to if, or when the fetus might be regarded as a person, starting with the view that the fetus became a person from conception; some perceived the point of viability as crucial, or when the nervous system is ready to function). And does anyone have the right to suppress the future of a human being, who is either "perfect" or "imperfect", regardless of his/her embryo, fetus or malformed newborn status?

On the other hand, there is the principle of "respect for persons", which presumes recognizing the women's autonomy and capacity for decision-making and as full participants in the medical decisions of abortion. The principle of respect for autonomy requires that the views of those who are capable of deliberation about their personal goals be sought and respected. As the foetus lacks such capabilities, the question of foetal autonomy does not arise. Another leading concern of bioethics is justice, which requires a fair distribution of family planning methods, including access to safe abortion in cases of contraceptive failure (25, 26). Unfortunately, these problems do not have a certain answer and, although many countries have legalized abortion, the issue of active or passive neonatal euthanasia is still open to debate, as is the case for neonates with inborn anterior abdominal wall defects associated with chromosomal aberrations or other inborn malformations.

The debate may be extended to include the case of newborns suffering from multiple malformations, for which "postnatal abortion" may be considered (4, 5). If abortion (therapeutic or elective) is morally and legally accepted, does this implicitly mean that these newborns may be condemned? In Netherlands, according to the Groningen Protocol, active neonatal euthanasia would be morally acceptable in particular circumstances (27), but in other European countries it is illegal, yet sometimes tacitly accepted and practiced (28), and the exclusive assurance of life support and physical comfort to terminal patients or to very premature infants (below 22 weeks of gestation) is widely practiced (29, 30). Although the principle of nonmaleficence or "primum non nocere"

requires physicians to avoid harm, and withholding a beneficial treatment is likely to have the consequence of producing harm (passive euthanasia), no clear-cut legal support exists in all these cases. Nevertheless, from a moral point of view the feeling of compassion and the intent to put an end to the suffering of a newborn without real chances to a happy future are expected. This is requested either by the parents or by the attending physician, with or without a prior talk with the parents.

If no abnormalities and malformations are associated, over 90% of the babies with anterior abdominal wall defects will enjoy normal physical, mental and intellectual development, and will be perfectly integrated in society as adult, so they may be declared "cured and healed". Healing was associated with themes of wholeness, narrative, and spirituality and was defined in terms of developing a sense of personal wholeness that involves physical, mental, emotional, social and spiritual aspects of human experience. "If you become whole again," Kubler-Ross observed, "you're healed" (31). But if the neonate is plurimalformated, could anyone know and measure his suffering, how could anyone be certain that the case is truly terminal or that there may be a little hope of recovery and what would a happy future mean to a newborn suffering from multiple malformations? The principle of beneficence obligates people to try to bring about more beneficial consequences than harmful ones. Both the medical team and the parents have the obligation to consider and protect "the child's best interest", which involves all those actions that need to be taken for a better life: physical and emotional integrity, family, access to education and healthcare, social integrity. Philosophically and ethically speaking, promoting "the child's best interest" means doing one's best, using the resources available at a given moment, in order for the child "to feel good", to have a "good life" (32). In children, however, this calls for delicate action both from the medical team and from the parents, who ought to take into consideration the patient's state and life quality. From a strictly surgical viewpoint, we reckon that the life quality of a child suffering from multiple congenital malformations and chromosomal aberrations, who needs many surgical procedures and whose life expectancy is several years at the most (this is the case with children with trisomy 13 or 18 who, in addition to their anterior abdominal wall defects, may also suffer from porencephaly, hydrocephalus, myelomeningocele with double incontinence, cardiac malformations, limb anomalies, cleft lip and palate, internal organs malformations) is extremely poor. According to the definition of World Health Organization (WHO), health refers to physical, mental and social integrity and well-being, as well as the patients' ability to fulfill their regular tasks in their everyday life. Life quality is a subjective concept, as each patient experiences his or her own feelings; what is unconceivable for some may be acceptable to others, and the parent-child relationship is even more difficult to handle as it involves feelings of love, pity, responsibility, protection. More often than not, making medical decisions related to the care or treatment discontinuation of such children has an important psychological impact on the family members. Who is in a position to make the final decision, the parents or the medical team, considering that abuse and conflicts may arise from both sides? Both the parents' autonomy as regards the discontinuation of their child's medical treatment, and the responsibility of

the medical team when non-treatment is the only alternative to preserve the pediatric patient's best interest are matters that are insufficiently dwelt on and poorly regulated in our country's laws. As there is no clearly defined legislative framework regulating fetuses in our country, newborns suffering from multiple malformations and pediatric patients, the whole responsibility and ensuing emotion are transferred to their families, tutors and members of the medical team. The ethical principles, which the medical decision-making process relies on, more precisely the principle of patient autonomy respect, the doctor's duty not to act to the detriment of the patient and to promote the latter's best interest, a fair assignment of resources and non-discrimination, require adaptations and specification in pediatric medical practice (25, 26).

CONCLUSIONS

When parents have to face an antenatal diagnosis of congenital malformation, a multidisciplinary team should provide adequate counseling, considering both fetus evolution and prognosis, and the psychological effects of parents. The counseling should be done with honesty, compassion and integrity, with the observance of ethical principles such as autonomy, freedom of reproduction, beneficence and justice principle. Unfortunately, there are still many instances where principles, guidelines, rules or laws applied for the benefit of one part may restrain autonomy, beneficence and justice done to another. The latest developments in medical sciences and the implementation of the most advanced techniques of resuscitation and intensive care make it possible for newborns with severe congenital malformations to survive. Nevertheless, the use of this technology sometimes makes one wonder whether this is in the child's best interest, considering the poor life quality it provides.

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NOVEL THERAPEUTIC OPTIONS FOR OLD DRUGS

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NOVEL THERAPEUTIC OPTIONS FOR OLD DRUGS (Abstract): Introducing a new drug on the market requires a long time and costs. That is why discovery of new uses for existing drugs offers the advantage of delivering drugs already tested over time, this being for the benefit of the patients. This article aims to review some of the drugs whose initial use was different from the current one, analyzing their therapeutic course, from the discovery to the present. **Keywords**: OLD DRUGS, POTENTIAL USES, BENEFIT OF THE PATIENTS.

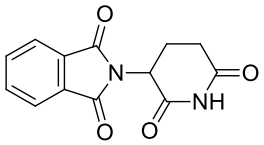
Since decades, the rational discovery of new medicines has depended on the modification of molecular structures. In recent years, there have been major changes in the identification and development of new drugs at the industrial level; it has been estimated that it takes about 15 years to bring a drug to the market and the pharmaceutical industry is investing tens of billions of dollars annually (1).

From the statistical data provided by pharmaceutical companies and research laboratories, it was found that from about 5,000 preclinical candidates, only five are clinically tested and only one will receive marketing approval. So, discovery of new uses of already existing drugs on the market, whose tests and side effects are well known, can be beneficial in the treatment of some of the diseases of the present century. Many examples can be quoted to prove its benefits. For example, aspirin, approved by the *Food and Drug Administration* (FDA) as a pain killer, was used to reduce the mortality rate among heart attack victims; the drug mitomycin, approved for the treatment of gastric and pancreatic cancers, has been found to be useful in the treatment of lung, bladder, breast and cervical cancers, as well as in certain forms of leukemia; trimethoprim, a drug approved to treat pneumonia, has been proved useful as an AIDS management (2).

The new uses of the old drugs therefore may provide cost-effective treatment, especially in developing countries with limited resources. In the present review, we are presenting the novel potential uses of drugs that are approved by FDA for important clinical conditions.

Thalidomide

Thalidomide is a glutamic acid derivative originally developed as a hypnotic in the 1950s and taken off the market because of its teratogenic effects (3).



2-(2,6-dioxopiperidin-3-yl)isoindoline-1,3-dione

Fig. 1. Thalidomide

Because thalidomide causes drowsiness, it has also been used as a sedative, being considered a safe and effective medicine that causes a deep sleep. It has begun to be quickly manufactured and sold around the world under various trade names; because of its effectiveness in improving morning sickness, it has become extremely consumed among pregnant women. Between 1957 and 1960 there was an unusually high increase in babies born in Germany, Australia and the UK with severe deformities of limbs and internal organs, an increase that initially produced confusion and anxiety among doctors and researchers. In 1961 a German doctor, Widukind Lenz, and an independent doctor in Australia, William McBride, expressed suspicion that the terrible malformations were related to the ingestion of thalidomide by mothers during pregnancy. Thus, at the end of 1961, Lenz suggested a possible connection between the occurrence of these congenital anomalies and the use of thalidomide during pregnancy (4). The substance not only caused limb deformities but also affected other organs such as ears, eyes, heart, kidneys, nerves, genital organs and other internal organs.

Because of these observations thalidomide was quickly retired from the market in Germany and England, and later in several other countries. Until August 1962, a significant decrease in the births of children with limb malformations was reported, but at least 10,000 affected children were already born worldwide (5).

New therapeutic uses of thalidomide

Thalidomide and its derivatives are known as immunomodulating agents, known for their sedative, teratogenic, antiangiogenic and anti-inflammatory properties (6). They are commonly used in the treatment of various types of cancer, such as multiple myeloma or myelodysplastic syndrome (MDS), but also in the treatment of inflammatory skin pathologies associated with leprosy or Hansen's disease (7).

The anti-inflammatory and immunomodulatory effect of thalidomide

Thalidomide has been reintroduced in therapy as an anti-inflammatory agent, with remarkable efficacy in the treatment of erythema nodosum leprosum (ENL) and subsequently in the treatment of multiple myeloma (7). ENL is an inflammatory and debilitating complication of leprous, infectious disease caused by *Mycobacterium leprae*. The anti-inflammatory action of thalidomide is explained by the mechanism discovered by Sampaio *et al.* (8) and by Moreira *et al.* (9). They argue that by inhibiting TNF-α production in monocytes and macrophages, the inflammatory response is regulated by inducing the production of a wide range of cytokines, such as interleukins and interferon-d, in response to injuries.

As for the immunomodulatory effect thalidomide revealed some of its effects on the immune system. In 1984, Barnhill *et al.* (10) showed that thalidomide is an inhibitor of phagocytosis in polymorphonuclear leukocyte cultures and a chemotaxis inhibitor in cultured monocytes. They suggested that these results help explain the anti-inflammatory properties of thalidomide.

Anti-proliferative effects of thalidomide

Both thalidomide and its structural derivatives can reduce the proliferation of cancer cells that have developed resistance to standard chemotherapy, being successfully used in both the United States and Europe for the treatment of multiple myeloma and other cancers; their use are prolonging patients' lives. Anti-tumoral effects of thalidomide have been attributed to the increased degradation of mRNA for both FGF and VEGF and a decrease of adhesion receptors (11). Myeloma cells are affected both by its

antiangiogenic action and by the ability of thalidomide to enhance the immunomodulatory response.

Antiangiogenic action of thalidomide

In 1994, D'Amaro *et al.* (12) has been discovered another property of thalidomide: it has been found to be a potent inhibitor of angiogenesis (formation of new blood vessels) on a rabbit cornea model. They have suggested its use in diseases where this effect may be desirable, diseases such as diabetic neuropathy, macular degeneration, or in solid tumors. Other researchers have suggested the use of thalidomide in serious cases of infantile hemangiomas (13).

Uses of thalidomide in dermatology

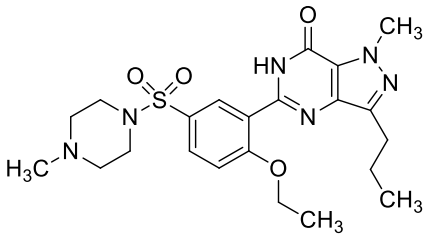
Thalidomide is an effective second-line treatment against foot-and-mouth stomatitis and is the only one that has shown a certain consistency in efficiency (despite the more suppressive than curative effect.) However, the use of thalidomide is limited by adverse effects, which are often dose dependent. Although the efficacy of thalidomide on recurrent stomatitis is recognized, the literature has less or no information on the efficacy, tolerance, safety and long-term use posology in current practice. Many patients achieve complete long-term remission with a low maintenance dose and high tolerance (14).

Similar results were also obtained when thalidomide was used in Behcet's treatment. Behcet's syndrome is an inflammatory autoimmune disease characterized by a systemic vasculitis whose main symptoms are oral and genital ulceration and eye, joint, blood vessel and central nervous system injuries.

Another use of thalidomide is in the treatment of prurigo nodularis, a disorder characterized by extremely pruritic nodules, mainly located at the extremities of the body (13). Most of the therapies were unsatisfactory, but thalidomide administration in doses ranging from 100-300 mg/day was found to be quite useful in these patients.

Sildenafil

Sildenafil was introduced into the pharmaceutical market in 1998 as sildenafil citrate in the VIAGRA™ pharmaceutical product. This is a highly selective inhibitor of the phosphodiesterase (PDE5) enzyme, widely used as a treatment for erectile dysfunction (15).



5-(2-ethoxy-5-((4-methylpiperazin-1-yl)sulfonyl)phenyl)-1-methyl-3-propyl-1*H*-pyrazolo[4,3-*d*]pyrimidin-7(6*H*)-one

Fig. 2. Sildenafil

Although initially sildenafil was used in the treatment of angina pectoris and more erectile dysfunction, today the compound appears to have therapeutic effect in disorders of the respiratory system and central nervous system. On the central nervous system, it can have neuroprotective effects in multiple sclerosis as well as significant

memory enhancement effects (16).

Sildenafil and pulmonary arterial hypertension

Pulmonary arterial hypertension is defined as a group of diseases characterized by a progressive increase in pulmonary vascular resistance, leading to right ventricular failure and premature death (17). Pathobiological mechanism of the disease includes pulmonary endothelial dysfunction, which leads to impaired production of vasodilators, such as nitric oxide and prostacyclin, and overexpression of vasoconstrictors, such as endothelin-1 (18).

Alkhayat *et al.* (19) investigated in a study the efficacy and tolerability of sildenafil 20 mg given orally three times daily in patients with secondary pulmonary arterial hypertension. In this placebo-controlled trial, sildenafil significantly improved exercise capacity, as assessed according to the six-minute walking test, in patients with pulmonary arterial hypertension. Their findings show that there is a symptomatic benefit associated with the inhibition of phosphodiesterase type 5 in patients with pulmonary arterial hypertension improvement in exercise capacity with sildenafil treatment, regardless of demographic or disease characteristics or other baseline variables. Sildenafil also significantly improved cardiopulmonary hemodynamics. In conclusion, this study demonstrates the efficacy and safety of sildenafil in the treatment of patients with symptomatic pulmonary arterial hypertension secondary to COPD (19).

The use of sildenafil in systemic sclerosis

Systemic sclerosis (SSc) is a connective tissue disease that is characterized by inflammation, vasculopathy and fibrosis (20). Tissue fibrosis directly contributes to increased mortality and decreased quality of life (21). However, an effective therapy for tissue fibrosis has not been established.

Higuchi *et al.* demonstrated the antifibrotic effects of sildenafil on cultured SSc fibroblasts. Sildenafil is a PDE5 inhibitor and a standard therapeutic agent for pulmonary arterial hypertension due to its strong vasodilatation effects (22).

Recently, it has been demonstrated that an increase in intracellular GMPc level can improve fibrosis levels in various diseases. Thus, in a recent study, the phosphodiesterase inhibitor, sildenafil increased the intracellular GMPc level in cutaneous fibroblasts in a dose-dependent manner and also significantly decreased the expression of profibrotic factors such as TGF-β1, whose level shown to be increased in skin fibroblasts of systemic sclerosis (22).

Therefore, various PDE5 inhibitors such as sildenafil and tadalafil have been investigated and developed, demonstrating excellent results in the treatment of systemic sclerosis and digital ulcers (22). These results suggest that PDE5 inhibitors may have notable effects on vasculopathy and systemic sclerosis associated fibrosis, and sildenafil may be considered a promising treatment for fibrotic disorders such as systemic sclerosis.

Sildenafil in the prevention of fetal cardiovascular dysfunctions

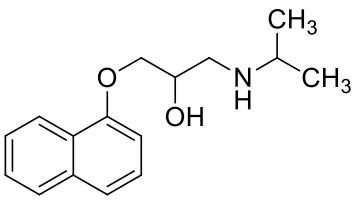
Frequent complications occurring during pregnancy, complications such as fetal hypoxia, may trigger various cardiovascular dysfunctions during uterine life, and may also lead to the appearance of extrauterine cardiovascular disease. Using the chicken embryo model, Itani *et al.* (23) demonstrated in 2017 that sildenafil treatment directly protects the fetal cardiovascular system during hypoxia, its protective mechanisms including reducing oxidative stress and increasing the availability of nitric oxide.

The effects of sildenafil have been ana-

lyzed on the fetal cardiovascular system of chicken embryos, as this is the only established animal model on which direct effects on the heart and fetal circulation can be studied, independently of the effects on the mother and / or placenta (23). Therefore, the data obtained from this study demonstrated that sildenafil can have direct benefits on the fetal cardiovascular system in case of hypoxia during intrauterine life, thus proving to be a good antioxidant that can prevent cardiovascular dysfunction of fetal origin.

Propranolol

Propranolol is a non-selective beta-adrenergic antagonist used in the treatment of hypertension, angina pectoris, anxiety, cardiac arrhythmia, essential tremor and also prophylactic against migraine, varicose vein and myocardial infarction (24).



1-(isopropylamino)-3-(naphthalen-1-yloxy)propan-2-ol

Fig. 3. Propranolol

In addition, data from clinical trials suggesting the use of propranolol in several diseases such as hemorrhages, septicemia, hypermetabolic syndrome associated with severe burns (25), Alzheimer's disease-associated acne or psychosis (26) and aggression associated with lesions or cerebral diseases (27).

The antimigraine action of propranolol

Propranolol has been prescribed for migraine prophylaxis since 1966, when Rabkin *et al.* (23) have occasionally discovered their effectiveness in migraine headache in patients who were treated for angina pectoris. There are several research reports illustrating the effect of propranolol as a β-adrenergic blocker on the expansion of cortical depression, demonstrating that treatment with it blocked CSD in rats suffering from ischemic stroke without altering regional brain flow. Ayata *et al.* in 2006 demonstrated that chronic administration of several drugs, including propranolol, inhibited the generation of CSD (cortical depression) (28).

The antitumor effects of propranolol

The use of β-blockers in the cancerous process has been extensively studied in breast cancer, prostate, ovarian, melanoma, and colon cancer. Beta-blockers have been shown to reduce metastatic recurrence and reduce mortality in most studies, although there were, of course, studies in which the results were irrelevant (29).

Propranolol is a non-specific β-adrenergic antagonist, well-known and widely used, with a range of actions of interest in the oncology context. Propranolol has shown effects on cell proliferation and invasion, on the immune system, on the angiogenic cascade, and on the sensitivity of tumor cells to existing treatments. Furthermore, there is evidence that it acts at several points in the metastatic cascade, especially

in the context of the postoperative wound response (30).

Use of propranolol in the treatment of burns

Burns are traumatic tissue injuries, accompanied by systemic nervous, vascular, metabolic and humoral reactions, heat, chemical or microbial lesions. Burns are among the worst forms of injuries, ranked fourth among the causes of injuries after car accidents, falls and violence, with about 90% of cases occurring in middle-income countries (31).

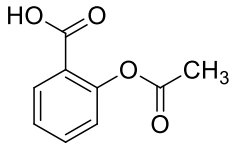
Recent studies have reported that the administration of propranolol to children with severe lesions for one year significantly improved energy consumption at rest as well as cardiac function. Attenuation of cardiac sequelae occurred at dose level, with the most favorable results being observed at a dose of 4 mg/kg body weight/day. Peripherally, propranolol has been shown to decrease the blood flow of the lower limbs by increasing vascular resistance in adults with severe burns. The induction of peripheral vasoconstriction by propranolol has led to the successful implementation of β-blocker in the management of infantile hemangiomas, varicose bleeding and recurrent epistaxis (31).

Propranolol administration at a dose that reduces heart rate by an average of 20% in adult patients with burns to a body surface area of ≥ 30% of the total area has been shown to diminish blood loss during skin grafting procedures and improved the lesion healing rate on average by one week. This is a tremendous therapeutic benefit, as patients with burns require many special surgical interventions, where limitation of blood loss is more than necessary (32).

Recent studies have shown the efficacy of propranolol in *hyperthyroidism*. The level of thyroid hormones in the circulation is increased in hyperthyroidism and this can lead to tachycardia, palpitations, tremors, and anxiety. So, propranolol has been widely used to treat these beta-adrenoreceptor effects (33).

Acetylsalicylic acid

Acetylsalicylic acid, which has been used for over a century as an analgesic, is used in the last several years for its antiplatelet activity in cardiovascular disease (34).



2-acetoxybenzoic acid

Fig. 4. Acetylsalicylic acid

In 1853, salicylic acid was synthesized in its pure form by a French pharmacist Henri Leroux and an Italian chemist Raffaele Piria, and it was observed that this substance produced gastrointestinal irritation; these problems were solved by replacing a hydroxyl group of salicylic acid with an acetyl group (35).

In 1897, a German chemist Felix Hoffman developed and patented an acetylsalicylic acid synthesis process, patented under the name Aspirin™ on February 27, 1900, which is also the time when the name "aspirin" was born (35).

Currently, aspirin or acetylsalicylic acid is one of the most commonly used drugs worldwide, producing around 40,000 tonnes per year. In addition to the well-known analgesic, antipyretic and anti-inflammatory effects, aspirin are used in the secondary prevention of cardiovascular events, where benefits are clearly demonstrated, but also in primary prevention, where studies continue to evaluate benefit-risk ratio (36).

Aspirin in the primary prevention of cardiovascular events

Cardiovascular diseases represented by ischemic heart disease and stroke (AVC) are the leading causes of mortality in the world, according to the World Health Organization (37). Acetylsalicylic acid at a low dose of 75-100 mg inhibits irreversibly acetylation, cyclooxygenase (COX), thus blocking the production of thromboxane (TXA2) and inhibiting platelet aggregation. Thus, aspirin is the most widely used drug in the prevention of cardiovascular disease, proving its effectiveness in the secondary prevention of vascular atherothrombotic disorders (non-fatal myocardial infarction, non-fatal stroke) in patients with known cardiovascular disease (38).

The role of aspirin in the prevention of preeclampsia

Preeclampsia is defined as the presence of excess hypertension and protein in the urine after the 20th week of pregnancy in a woman who previously had a normal blood pressure. Untreated, the disease can lead to serious complications for the mother and the fetus, sometimes even to death (39).

Meta-analyzes of different randomized clinical trials have suggested that lower-dose aspirin before or at 16th week of pregnancy is associated with a significant reduction in the risk of preeclampsia.

Based on the studies studied, early administration of aspirin (≤ 16 weeks) was associated with significant reductions in preeclampsia, while its administration at 16 weeks later had no significant effect on preeclampsia or fetal growth restriction (39). In conclusion, low doses of aspirin (50-150 mg/day) may be a promising prophylactic treatment for pregnant women at high risk of preeclampsia or other placental disorders when given early.

The role of aspirin in chemoprevention

One of the most extraordinary new perspectives for the use of aspirin in therapy is in preventing cancer. In 1971 was made the first study showing the existence of a relationship between aspirin and chemoprevention, but over the last two decades many publications have suggested that aspirin can substantially reduce the risk of a person to develop cancer and in the same time can have a beneficial effect on its survival (40).

Rothwell *et al.* published in 2010 a 20-year follow-up of a study supporting the role of a low dose of aspirin in reducing the incidence and mortality of colorectal cancer, with the greatest benefit being shown in the case of proximal cancers. Thus, administration of aspirin to the subjects for 5 years or longer has reduced the risk of colorectal cancer on the right side by 70% and also the associated risk of rectal cancer. According to the published information, there was no benefit increase for doses greater than 75 mg per day (41).

In 2015, Cuzick *et al.* (40) indicated that the effects of aspirin on the prevention of colorectal cancer are beginning to be observed about 3 years after the start of use, and the low doses between 75 and 325 mg/day appear to be most effective, the toxicity increasing with the increasing of the dose. Therefore, the use of aspirin is still associated with hemorrhagic events, both cardiovascular and gastrointestinal, when increasing doses are administered over a prolonged period (42).

Therefore, aspirin, one of the most long-acting therapeutic agents, manages to remain constantly in the news by the variety of actions and benefits it demonstrates. The use of cardiovascular events in secondary prevention is well established, and its effectiveness in the medium-risk population will continue to be investigated, also considering the increased risks of gastrointestinal and hemorrhagic side effects. At the same time, there is increasing evidence for the benefits of using low-dose aspirin in reducing the risk of can-

cer, especially colorectal cancer, benefits highlighted in many studies about 5 years after administration (43).

CONCLUSIONS

Both for the substances presented in the present paper and for many other drugs whose therapeutically value can be revalued, time is the most important factor that will prove their evolution in therapy.

The rediscovery of an old drug is a challenge the old therapeutic agents can become valuable targets in research and development of new drug therapies.

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THE REASONS FOR CHOOSING THE PROFESSION OF DENTIST AMONG THE STUDENTS OF THE FACULTY OF DENTAL MEDICINE - IASI, ROMANIA

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THE REASONS FOR CHOOSING THE PROFESSION OF DENTIST AMONG THE STUDENTS OF THE FACULTY OF DENTAL MEDICINE-IASI, ROMANIA (Abstract): The profession of dentist, as any other medical profession, is a challenging one. **Aim**: To determine the reasons and factors that are influencing the choice of Dental Medicine as profession among the students of the Faculty of Dental Medicine from "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania. **Materials and methods:** The data were collected through redesigned questionnaire containing 33 questions. Career aspirations questionnaire were distributed among dental students of the 2nd year and 3rd year, Faculty of Dental Medicine Iasi. The collected data were analyzed using the *SPSS 20.0* software. For the age variable, were determined the average, median, and standard deviation. The Pearson chi-square correlation test was used in order to evaluate the correlations of the results. The confidentiality threshold was set at 5%. **Results:** Most of the participants consider that dental careers offer a stable job (82.5%), this profession is better paid (80%) and offers a better status (85%). Rural subjects are more insecure as regards their future career in dentistry than those from the urban areas (35.7%). Male subjects are more uncertain about their future in the field of dental medicine in comparison with female. The influence of the environment, especially of the entourage, represented an important factor in choosing a profession, along with the family (the parents). **Conclusions:** The reasons for choosing the profession of dentist differ from one person to another and are influenced by the educational background, socio-economic status and cultural history. **Keywords**: DENTAL STUDENTS, DENTAL SCHOOL, CAREER CHOICE.

Investigating the point of views and perspectives of medical students contributes to a better understanding of the way in which academic studies are coordinated, how they plan their career, how they shape interpersonal relationships, and how they shape their role in society. In a broader sense, such investigations can be useful for planning and evaluating the educational process by influencing curricula, the learning process and the profile of future professionals.

Students' motivation in choosing careers in the medical field is of particular importance to professors and admissions committees (1). Studies conducted so far in many countries have "examined" the reasons for why the people are choosing the profession of dentist (2-4). At some point,

dentistry was a predominantly a male occupation, but women gained ground over time (5). External social factors determine students to shape their future dentist career based on models to which they can identify and confirm professionally (3).

The profession of dentist, as any other medical profession, is a challenging one, is a profession which requires the pursuit of the principles of bioethics. Therefore, the dental treatment in its complexity (orthodontic, pedodontics, prosthetic, endodontic, etc.) must follow the ethical and bioethical principles, as "*Bioethics brings hope of respect and permanent revival of human values, of spiritual revival of man as a unique being*" (6).

The reasons for choosing the dental profession, as well as the requirements and selection processes, vary from one country to another or even within the same country (2-4). Several studies have been conducted on the socio-economic profile, career choices and professional profiles of dental students. In general, the results indicate that career choice is motivated by vocation and status, most students focusing on technical issues and professional status rather than on their role in society (7, 8).

Understanding the reasons for choosing this profession is important because this decision involves setting future goals that may affect career choices. While some individuals choose knowledgeable professions with realistic goals, others can choose a profession not knowing in which direction they are heading. Some make career choices by choosing the most convenient path, such as choosing the profession that one or both parents practice. The aim of the study was to determine the reasons and factors that are influencing the choice of Dental Medicine as profession among the 2nd and 3rd year students of the Faculty of Dental Medicine from "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania.

MATERIALS AND METHODS

The data were collected through redesigned questionnaire containing 33 questions related to subjects' age, sex, background and reasons for choosing the profession of dentist, what influenced the choice of this profession, and questions about the expectations in the relation with the future profession.

Career aspirations questionnaire were distributed among dental students of the 2nd year and 3rd year, Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iasi, Romania. Approval of the questionnaire and research methodology was received from the college ethics board. A pilot survey was conducted (n=20) where the validity and reliability of the questionnaire was ascertained.

The collected data was analyzed using the *SPSS version 20.0* software. For analyzing the data collected, distributions and percentages were determined. Average, median, and standard deviation were determined for the age variable. In order to evaluate the correlations of the results was applied the *Pearson X-square* correlation test. The test was considered to have a statistical significance for a p value < 0.05. The confidentiality threshold was set at 5%.

RESULTS

The study group consisted of 394 students (167 female and 127 male) (tab. I), 214 students being enrolled in the second year of studies and 180 students in the third year at the Faculty of Dental Medicine,

"Grigore T. Popa" University of Medicine and Pharmacy. The distribution of subjects by the residence was as follows: 89% of the subjects were from urban area and 11% of the subjects from rural area (tab. I).

The average age in the study sample was 22.32 years (minimum 20 years, maximum 42 years) (fig. 1).

TABLE I.

Demographic characteristics in the study sample

Characteristics	2nd year		3rd year	
	N	%	N	%
Students	214	54%	180	46%
Sex				
• Female	124	57.9%	115	63.9%
• Male	90	42.1%	65	36.1%
Residence				
• Urban	194	90.7%	158	87.8%
• Rural	20	9.3%	22	12.2%
Family socio-economic status				
• High level	159	74.3%	136	75.6%
• Medium level	49	22.9%	42	23.3%
• Low level	6	2.8%	2	1.1%

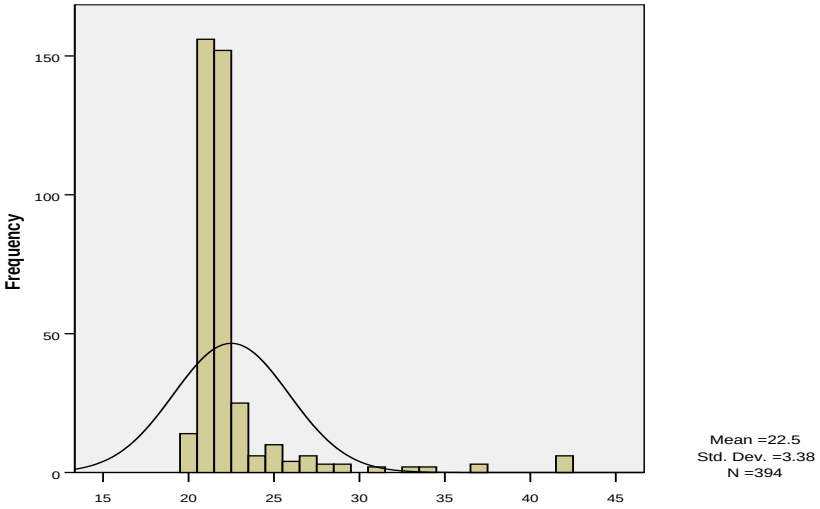

Fig. 1. Participants average age.

To the question "*Is it easy for a dentist to find a job*?" 38% of the students responded negatively, 37% positively and 25% "*do not know*". The distribution of answers to this question according to the residence had a higher value for the urban subjects who responded positively in a proportion of 38.4%. Rural subjects are more insecure as regards their future career in dentistry than those in urban areas (35.7%). Male subjects are more uncertain about their future in this area, as 32.3% of them responded "*do not know*" and 46.4% of female subjects' responded negatively (tab. II).

TABLE II

Repartition of the answers to the questionnaire

		Sex			*Residence*		
		Male	*Female*	*p*	*Urban*	*Rural*	*p*
It is easy for a dentist to find a job ?	Yes	43.9%	34.3%	.000	38.4%	35.7%	.168
	No	23.9%	46.4%		38.6%	28.6%	
	Don't know	32.3%	19.2%		23.0%	35.7%	
I would like to make more money	Yes	74.2%	81.6%	.213	80.1%	66.7%	.006
	No	17.4%	12.1%		14.2%	14.3%	
	Don't know	8.4%	6.3%		5.7%	19.0%	
Careers in dentistry offers a stable job	Yes	85.8%	87.9%	.652	86.6%	90.5%	.699
	No	4.5%	5.0%		4.8%	4.8%	
	Don't know	9.7%	7.1%		8.5%	4.8%	
Dentistry is better paid than other professions	Yes	85.2%	83.3%	.675	83.2%	90.5%	.101
	No	7.7%	7.1%		7.1%	9.5%	
	Don't know	7.1%	9.6%		9.7%	0.0%	
Dentistry provides a high professional status	Yes	85.2%	89.1%	.087	87.2%	90.5%	.827
	No	9.0%	3.8%		6.0%	4.8%	
	Don't know	5.8%	7.1%		6.8%	4.8%	
I can work in several specializations of dentistry without having that specialization	Yes	36.8%	40.2%	.041	39.2%	35.7%	.574
	No	43.9%	32.2%		37.2%	33.3%	
	Don't know	19.4%	27.6%		23.6%	31.0%	
I can make my own schedule	Yes	87.7%	79.5%	.047	82.4%	85.7%	.109
	No	10.3%	13.8%		13.4%	4.8%	
	Don't know	1.9%	6.7%		4.3%	9.5%	
I like the idea of being my own employer	Yes	87.7%	91.6%	.449	89.5%	95.2%	.434
	No	9.7%	6.7%		8.2%	4.8%	
	Don't know	2.6%	1.7%		0.3%	0.0%	
Dentistry is an exciting and challenging career	Yes	89.0%	94.1%	.008	93.2%	83.3%	.052
	No	7.1%	5.9%		5.7%	11.9%	
	Don't know	3.9%	0.0%		1.1%	4.8%	
I like to interact with other people	Yes	96.8%	97.5%	.199	97.4%	95.2%	.000
	No	1.3%	0.0%		0.0%	4.8%	
	Don't know	1.9%	2.5%		2.6%	0.0%	
I like helping people	Yes	96.8%	97.9%	.211	97.2%	100.0%	.542
	No	1.3%	0.0%		0.6%	0.0%	
	Don't know	1.9%	2.1%		2.3%	0.0%	
I like to work manually	Yes	91.0%	91.0%	.022	87.8%	85.7%	.319
	No	7.7%	7.7%		7.7%	4.8%	
	Don't know	1.3%	1.3%		4.5%	9.5%	

Statistical analysis revealed that female subjects chose this professional career to earn money in a bigger proportion (81.6%) than the male subjects (74.2%), those from the urban area being more motivated by this statement than those from rural ones (80.1% *vs.* 66.7%). The majority of the participants considered that the dental careers offer a stable job, this profession being better paid and offering a better social status.

Since the residency exam is not an obligation in Romania, we can consider this statement as one of the reasons for choosing Dentistry at the expense of General Medicine. The difference between positive and negative responses at the question concerning the fact that dentists can work in several specializations of Dental Medicine without having a specific specialization was small (39.2% and 35.7% respectively), urban subjects responding positively in a bigger proportion than those from the rural areas, with more female subjects (tab. II).

Other reasons underlying the choosing of this profession among the dental school students was the possibility "*to make their own schedule*" (84%) (male subjects (87.7%) and the one from rural area (85.7%) responded predominantly positive at this question) and "*to be their own employer*" (88%) (predominantly female subjects (91.6%)). From all the questioned subjects, 90% considered that being a dentist represents as an exciting and challenging career. Other reasons that may influence the choice of the dentist profession were represented by the desire to work with people (to interact with other people) (96%), to help people (97.5%) and to work manually (86.0%) (tab. II).

The influence of the environment, especially of the entourage, represented an important factor in choosing a profession. To this assertion, about 40% of the study sample subjects responded affirmatively, more being male subjects (39.37%) and from the urban area (38.06%). Over 55% of respondents answered this question negatively.

Another important factor in choosing the profession was represented by the family and especially by the influence of the parents. At the question concerning the family and friends encouragement to be a dentist, the subjects responded 69.7% affirmatively, mostly being male subjects (74.8%) from the rural area (74.1%). To a lesser extent, subjects were influenced by their own dentist (32.73%), these categories of students being in majority male subjects (44.5%) and from rural areas (40.5%) (tab. III).

The affirmation *"I have been studying / working in the past in a field related to dentistry*" is suited more to the older subjects who attend dental medicine as a second faculty, previously working as dental assistants or dental technicians. Thus, 30.3% of respondents to this assertion were males from the urban area (22.7%).

The analysis of the results showed a reduced influence from the parents, the mother being more influential than the father (18.3% and 16% respectively), the relatives (11.4%) or other persons from the entourage (the school professors 4.8%, the dentist 9.1%, the family dentist 9.4%). 66.8% of the subjects responded that they have chosen this profession for their own reasons (fig. 2).

"*What influenced the choice of this profession*?". Figure 3 highlights the future career options: 54.35% of the participants want to become specialists after finishing the faculty while 30.9% are satisfied with the status of dentist.

TABLE III

Factors that have influenced the choice of the dental profession

		Sex			*Residence*		
		Male	*Female*	*p*	*Urban*	*Rural*	*p*
I've heard about dentistry in high school	Yes	43.9%	41.8%	.002	44.6%	26.2%	.073
	No	49.0%	57.3%		52.3%	69.0%	
	Don't know	7.10%	0.80%		3.10%	4.80%	
Family and friends encouraged me to be a dentist	Yes	74.8%	69.5%	.084	74.1%	50.0%	.003
	No	23.9%	30.5%		25.3%	50.0%	
	Don't know	1.30%	0.00%		0.60%	0.00%	
The family dentist encouraged me to choose this career	Yes	44.5%	27.6%	.002	33.5%	40.5%	.470
	No	54.2%	70.3%		64.5%	59.5%	
	Don't know	1.30%	2.10%		2.00%	0.0%	
I have been studying / working in the past in a field related to dentistry	Yes	30.3%	17.6%	.011	22.7%	21.4%	.821
	No	67.1%	81.2%		75.3%	78.6%	
	Don't know	1.90%	1.30%		1.70%	0.00%	

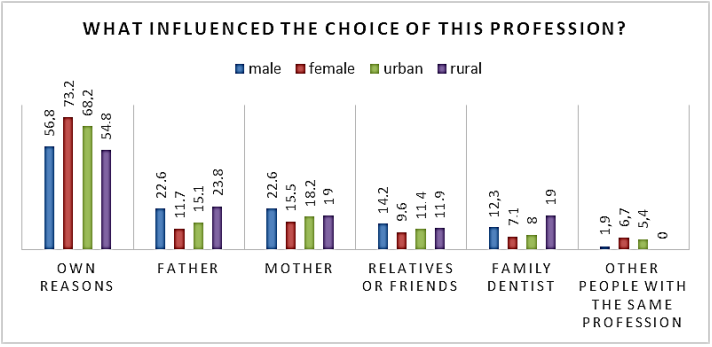

Fig. 2. Distribution of responses to the statement

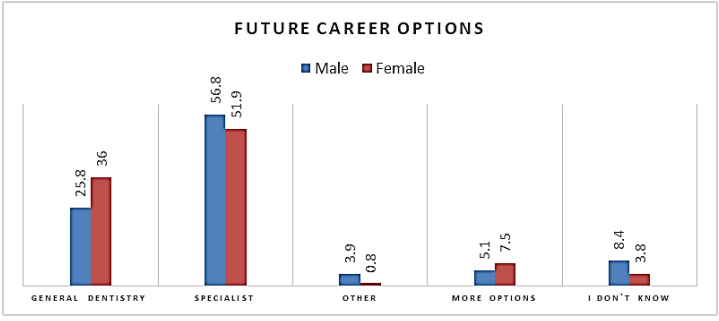

Fig. 3. Distribution of responses to the statement *"Future career options".*

Given the fact that students are at the begging of their studies in the area of dentistry being in the first years of college, it is expected that most of them to be undecided (39.3%) with regard to the specializations they will choose. However,

among the chosen answers, we can see that the options are directed towards orthodontics (21.8%), oral surgery (20.1%) and even specialist/researcher, with most of the responses in female subjects from the urban area (fig. 4).

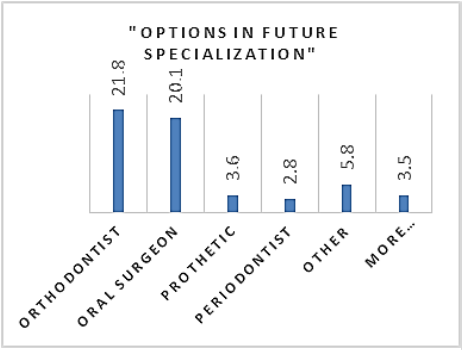

Fig. 4. Distribution of the responses to the question "*Options in future specialization"*

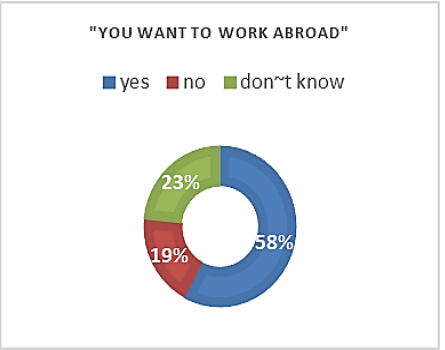

Fig. 5. Distribution of responses to the question "*You want to work abroad ?"*

Regarding the option to work abroad, in other country than Romania, more than half of the subjects (58%) responded affirmatively, followed by those who are undecided (23%). The main reason the participants want to leave the country is the financial one and the uncertainty of finding a job for young graduates (fig. 5). The highest frequency of affirmative responses was recorded in female subjects (65.91%) from the urban area (61.07%).

DISCUSSION

Career choice is a critical decision that can affect the progress of the young, so it is important to understand what motivates young people to pursue their careers, but also what they want to do after completing medical studies (9, 10).

Dentistry, among other professions, remains one of the most sought-after professions among young graduates, with dentists having an important position in society as health insurers. Nowadays, health promotion represents a global problem that requires as a must, much attention from both the health professionals and the public. Over centuries, health promotion has been regarded as an effective strategy to improve the nation's health and to attain a high level of well-being for the individuals (11). Therefore, the young people choose dentistry as their future career having in their mind that the oral health promotion will decrease the disease, and in the same time will enhance the well-being of a person/community.

The factors that can influence the choice of this profession may vary from socio-economic to emotional factors (being motivated by own dental experiences) (12, 13). The choice and the way the individual engages in the profession are influenced by economic and social factors. Various studies among students showed that financial stability and social status are the most relevant factors influencing the choice of this profession (14-16). The results of this

study indicate that choosing the profession of dental practitioner is mainly influenced by the socio-economic factor by having a stable job and a consistent financial gain. These results are supported by studies conducted so far, where job stability was the main factor influencing choice, followed by the "regular working hours", the fact that this profession is recognized as important and also because "they like to work with people"(17). In female subjects, the percentage was higher in the perception that "they can run a business" compared to male subjects, the same ratio being kept when referring to "the desire to work with people".

In our study, thee desire to work with people was the second motivating factor in choosing this profession. This situation was also found in other studies as a primary or secondary factor in choosing the profession of dentist (17, 18). Our findings on the reasons for choosing a physician profession are supported by the results of studies in other countries (19). Students generally choose this career because they perceive dentistry as a challenging profession.

Unlike other studies in America and Sweden showing parental influence in choosing the profession, in our study the subjects chose this path for "own reasons", with parental influence being reduced (about 14%). Increased influence is found in families where one or both parents are dentists (1, 19).

Other important elements in the future professional evolution of dental students are represented by the desire to pursue postgraduate studies after completion of house job and the possibility to work abroad. The results of the present study shows that 53.5% of the graduates wanted to pursue postgraduate studies following medical studies. The results are much lower compared to other studies conducted therefore it is important to increase the effort needs to be made to encourage graduates to pursue this field (20, 21).

CONCLUSIONS

The reasons for choosing this profession differ from one person to another and are influenced by the background, socio-economic and cultural history. The financial factor is one influencing the choice of this profession. It is important to increase the efforts needs to be made to encourage graduates to pursue postgraduate studies.

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NEW ORIGINAL *IN VITRO* METHOD TO ASSESS CHOLINESTERASE REACTIVITY IN ORGANOPHOSPHATE POISONING

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NEW ORIGINAL *IN VITRO* METHOD TO ASSESS CHOLINESTERASE REACTIVITY IN ORGANOPHOSPHATE POISONING (Abstract) : Assessment of organophosphate poisoning could benefit from a safe, non-expensive, easy to perform, quick (< 1 hour) test, which evaluates the level of cholinesterase activity "in vitro" regarding to the capability of oximes to reactivate OF-blocked cholinesterase. In the proposed protocol, 0.5 mL of sample serum is incubated, prior to the evaluation of level of cholinesterase activity, with 5 μL of a Toxogonin® dilution (0.125 mg) for 30 minutes at 37°C. For the standardization of the newly proposed protocol, several important issues were documented in the present article. The new original method of assessing cholinesterase reactivability will consist in an advantage for the diagnosis, prognostic evaluation and therapeutic orientation in OF intoxication. **Key words** : ORGANOPHOSPHATE, CHOLINESTERASE, TOXOGONIN

Organophosphate (OF) poisoning continues to represent an important medical issue through its high prevalence and severity among toxic pathology. Despite the existence of efficient antidotes, the prognosis of this intoxication remains reserved.

Diagnosis in OF poisoning is based on the assessment of plasma cholinesterase (ChEs) activity, this being the most constantly affected enzyme in OF poisoning (1,2). A method limitation results from a very wide interval of normal values for ChEs activity. Due to this, in medical practice, a relatively low value may represent the normal, while a so-called normal value may be detected when inhibition of ChEs activity is present (3,4,5,6).

Reversibility of cholinesterase inhibition after Toxogonin® (Tox) administration characterizes, with few exceptions, OF poisoning. The reactivation through Tox consist in the release of ChEs from the complex with OF, with the consequent regain of its enzymatic capabilities on choline compounds, expressed by an increase in the ChEs level of activity towards normal values. Thus, in case of suspected OF poisoning, evaluation of reactivability of ChEs activity levels will add important new perspectives for the diagnosis, compared with only the simple detection of the plasma ChEs activity.

In a specific "in vitro" protocol of testing, adding Tox to the serum sample will generate a recovery of the ChEs activity only in circumstances of acute OF poisoning. ChEs activity will not be significantly influenced if there is no previous blockage

of ChEs by OF. Tox-sensibilization method is based on the reactivatory effect of Tox, which increases the level of ChEs activity when the enzyme is blocked by OF. This effect is possible only in the initial period after intoxication (generally the first 48-72 hours). Then, the enzyme becomes irreversibly blocked by OF, called "ageing process" (a dezalkylation of the complex ChEs–OF). During this interval, obidoximes are efficient in recovering ChEs from their complexes with OF. This period can be longer, if the absorption still continues (i.e. additional ignored sites where absorption of poison persist, such as head hear, fat tissue, enteroenteric circuits etc.), or shorter (depending on the type of OF involved, different ways the poisoning was produced etc.) (7,8,9).

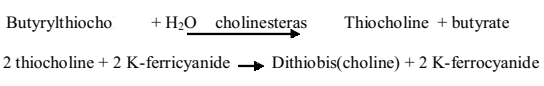
Another advantage of this test is the possibility to assess if the enzyme is still responsive to Tox, and sustains the continuation of Tox treatment, an expensive and even harmful treatment if the ChEs complex is „aged" (instead of unbinding the OF from the enzyme by linking with it, Tox will bind the enzyme, decreasing even more its level of activity) (10).

The aim of this study is to propose and standardize an original laboratory test, helpful for diagnosis with high sensitivity the OF poisoning, allowing a more orientated therapeutic approach.

MATERIAL AND METHOD

Serum samples from 23 consecutive patients, admitted in "Sf.Ioan" Emergency Clinic Hospital of Iasi, during 2009-2010, were used in the study. Inclusion criterion was the diagnosis of acute OF poisoning. Exclusion criteria were: age less than 18 years, mixed poisonings, refuse of patient or patient's family to sign the informed consent, associated chronic therapies with either: ibuprofen, procainamide, phenazopyridine, L-Dopa (which are known to interfere with the spectrophotometric method for detection ChEs levels of activity used in the study). Reference serum samples were analyzed from patients free from pesticide exposure, as follows: 23 patients with severe liver disease, and 23 patients without hepatic disorders.

The method used to determine the level of ChEs activity is a colorimetric technique, and relay on a Vitros System Chemistry 5,1/FS in which 11 ěL drop of sample is deposited on a dry, multilayered, analytical element coated on a polyester support type of slide and evenly distributed by the spreading layer to the underlying layers. ChEs brought in the reaction from the sample will hydrolyze butyrylcholine to thiocholine. The liberated thiocholine will reduce potassium hexacyanoferrate II, as it is shown below:



The rate of color loss is monitored by reflectance spectrophotometry and is proportional to the amount of ChEs activity present in the sample. Reference interval is: 4.65 – 10.44 U/mL (females), and 5.90 – 12.22 U/mL (males).

The idea of the research was to determine the level of ChEs activity using two samples of 0.5 ml serum obtained from the same specimen of blood, one analyzed without any preparation, using the conventional protocol, and the second one, after a preliminary incubation (half an hour at 37°C on an water thermostat – to reproduce, at least at minimum, the conditions within the human body) with Toxogonin® (active ingredient - obidoxime chloride, 1 mL ampoule, 250 mg/mL, manufactured by Merck Pharma GmbH). The blood samples were centrifuged immediately after collection. Each determination used 0.5 mL of serum, which was separated from the clot right after centrifugation (technical specifications of the kit producer being an interval of

maximum 4 hours), and processed after a maximal period of 24 hours (the kit producer recommendations are less than 7 days) while stored in refrigerator (at 2-8°C, never frozen), (11-13).

Several phases of the research were considered necessary, due to the need of obtaining answers on several issues regarding the new test.

I. Adding Tox, "in vitro", to a serum sample collected from a patient with acute OF poisoning, will generate different results for the levels of ChEs activity than by testing only the simple serum of the same patient ?

For this, blood samples were collected from patients admitted with acute OF intoxication and centrifuged as described. 0.5 mL from that serum was directly analyzed to determine the level of ChEs activity, and another 0.5 mL from the same sample was first incubated with Tox, being analyzed for the same parameter after the incubation. In the event of increasing values for the level of ChEs activity, we can consider that we have an "in vitro" effect of reactivation of the blocked enzyme through contact with Tox.

II. Are these results (increased values for the level of ChEs activity when is performed an initial incubation with Tox), the simple consequence of a colored substance insertion (Tox) in a colorimetric spectrophotometric working analyzer ?

For this, the new method was tested in patients without OF exposure : some associating liver diseases and some without any hepatopathy. Those samples were analyzed as simple serums and also, after an initial incubation period with Tox – with the new protocol. A lack of increase in ChEs activity for the samples associating incubation with Tox, both in patients with initially normal values of ChEs (subgroup non-intoxicated, without hepatopathy), or with initially decreased values (due to an impaired synthesis of ChEs secondary to liver disorders), can support the idea that the newly proposed test is characteristic for OF poisoning.

III. Which dilution of Tox has to be used for the serum incubation ?

Despite the temptation for using high concentration of Tox in order to obtain a maximal effect on ChEs reactivation, the risks for bias will increase with the use of large volumes or quantities of Tox. For example, the case of ibuprofen (mentioned in the technical references from the cholinesterase kit producer) is very suggestive : therapeutic serum concentration of 4 mg/dL associate no bias, at values of less than 20 mg/dL the interference is negligible (bias < 0.3 U/mL), but at concentrations over 40 mg/dL the error exceed 2.3 U/mL (14).

Thus, the dilution better to be used was estimated in relationship with the therapeutic level for Tox used in medical practice. The treatment is generally started with 2 ampoules of 250 mg Tox, for a total volume of blood of 5000 – 5500 mL, resulting in 0.1 mg / ml of blood. For a volume of 6 ml of blood collected in a vaccuette, results a need of 0.6 mg of Tox / vaccuette correspondent to an average of 2 ml of serum obtained by centrifugation from one vaccuette. A draft calculation associates 0.3 mg Tox for 1 mL serum, which signifies 0.15 mg Tox for the volume of 0.5 mL serum requested by the method. If we dilute the content of one ampoule of Tox (1 mL, containing 250 mg obidoxime) in proportion of 1/10 (0.5 mL Tox + 4, 5 mL NaCl 0.9%), 5 μL of this dilution will contain 0.125 mg.

In order to determine which dilution is the best to be used, 4 concentrations of Tox were tested for the smallest volume (5 μL) that was possible to be manipulated in the hospital laboratory : undiluted - for the amount of 1.25 mg/5 μL ; 1/10 - for the amount of 0.125 mg/5 μL ; 1/20 - for the amount of 0.0625 mg/5 μL ; 1/5 - for the

amount of 0.25 mg/5 μL.

IV. Is there any influence from the yellow color of the Tox used in the test, on the results offered by the spectrophotometer ?

For this, instead of serum, samples of NaCl 0.9% (without any ChEs) were investigated without and with a preliminary incubation with Tox, for spectrophotometric results on the level of ChEs activity. Ten samples were analyzed and the average difference between obidoxime incubated samples and those with only NaCl was considered as colorimetric influence of Tox, value that had to be subtracted from every result obtained for the reactivability of ChEs.

V. Is it necessary to constitute every time a fresh dilution for Tox, or is it possible to use a previously constituted dilution for many days (the whole surveillance period of an intoxicated patient), without interfering the rigourosity of the method ?

For this, in three of the patients included in the study, at different moments (a total of 19 comparative tests), the blood samples were comparatively assessed by incubating 0.5 mL of serum with 5 μL of Tox from a dilution instantly prepared on the moment of determination, and another 0.5 mL from the same sample of serum with Tox from a dilution prepared in the same week. Due to the known influence of sunlight on Tox, the prepared dilution was stored in a brown glass bottle.

All aspects of the clinical study were made with regard to the bioethics rules endorsed by European Union legislation, as well as the Romanian regulations for good clinical practice and clinical trials. The study protocols have the approval of the Bioethics Commission of the "Gr.T. Popa" University of Medicine and Pharmacy Iaşi.

Statistical analysis was carried out using SPSS (Statistical Package for the Social Sciences) 17 software for Windows 7.0. Comparisons of ChEs levels of activity at each time between the two methods used were assessed with unpaired *t*-test. For all tests, *p*-value less than 0.05 was considered significant.

RESULTS

Issue I. Values obtained for the level of ChEs activity determined using Vitros System Chemistry 5,1/FS on different samples of serum from 4 organophosphates poisoned patients, with the conventional method and a new method that involve a preliminary incubation of the serum (0.5 mL) with 5 μL of Tox (1,25 mg) for 30 minutes at 37°C are presented in Table I.

Increased values for the level of ChEs activity were considered as indicative for "in vitro" effect of reactivation of the blocked enzyme through contact with Tox.

Issue II. Values obtained for the level of ChEs activity on different serum samples from : A - patients without OF intoxication but associating liver disease (23 cases), and B - patients without any hepatopathy or insecticide exposure (23 cases), using the conventional method and a new method are presented in Table II.

Issue III. Comparative results for the level of ChEs activity determined with the conventional method and variations of the new methodology involving the preliminary incubation (30 minutes at 37°C) of

TABLE I
ChEs activity on different serum samples from OF poisoned patients.

Test: level of ChEs activity (U/mL)	Sample I	Sample II	Sample III	Sample IV
Conventional method	0.4	0.3	0.8	0.5
Tox – serum incubation method*	4	6.2	4.5	3.9

(*p value 0.0058)

TABLE II
Level of ChEs activity in patients without organophosphate poisoning

Test: level of activity of ChEs (U/mL)*		Conventional method	Tox – serum incubation method**
A.	Patients associating liver diseases	1.96±0.40	2.06±0.20
B.	Patients without hepatopathy or OF exposure	5.36±0.76	5.40±0.62

(*values expressed as mean ± standard deviation ; ** p value 0.47)

TABLE III
Level of ChEs activity on serum samples from patients with and without OF poisoning using different Tox dilutions

Level of ChEs activity (U/mL) Dilution of Toxogonin:	group	Conventional method*	Tox – serum incubation method
Undiluted for the amount of 1.25 mg/5 µL	1**	0.5±0.21	5.9
1/10 for the amount of 0.125 mg/5 µL			4.9
1/20 for the amount of 0.0625 mg/5 µL			4.7
1/5 for the amount of 0.25 mg/5 µL			5.2
Undiluted for the amount of 1.25 mg/5 µL	2***	5.31±0.72	5.1
1/10 for the amount of 0.125 mg/5 µL			4.3
1/20 for the amount of 0.0625 mg/5 µL			4.5
1/5 for the amount of 0.25 mg/5 µL			4.9
Undiluted for the amount of 1.25 mg/5 µL	3#	1.92±0.41	2.1
1/10 for the amount of 0.125 mg/5 µL			1.6
1/20 for the amount of 0.0625 mg/5 µL			1.7
1/5 for the amount of 0.25 mg/5 µL			1.9

(*mean ± standard deviation ; **p value 0.0007 between two methods used ; *** p value 0.83 ; #p value 0.059 between methods used)

TABLE IV
Level of ChEs activity with NaCl 0.9 % to replace the serum

Test: level of ChEs activity (U/mL)	No. samples	Conventional method	No. samples	Tox - serum incubation method*
NaCl 0.9% 0.5 mL	10	< 0.2	10	0.56±0.10

* p value 0.057

the serum (0.5 mL) with 5 µL of Tox from different dilutions (undiluted, diluted 1/5, 1/10 and 1/20) on samples of serum from : 23 patients intoxicated with organophosphates (1), 23 patients without any hepatopathy or insecticide exposure (2), and 23 patients without OF intoxication but associating liver disease (3) are presented in Table III.

Issue IV. Values obtained for the level of ChEs activity using NaCl 0.9 % to replace serum in the test methodology, determined with conventional method and with the new proposed method using Tox (0.125 mg – dilution 1/10), in order to test the influence of the Toxogonin® yellow color on the results offered by the spectrophotometer are presented in Table IV.

Issue V. Values obtained for the level of ChEs activity determined on serum samples from OF poisoned patients, using the new method that involves a preliminary incu-

TABLE V
ChEs activity using 1/10 Tox dilution fresh and previously constituted

Level of ChEs activity (U/mL) Tox – serum incubation method	Time elapsed from the moment of constitution of the dilution																		
	Sample 3 (16 h)		Sample 4 (24 h)		Sample 7 (48 h)		Sample 10 (72 h)		Sample 11 (96 h)		Sample 12 (120 h)			Sample 13 (144 h)			Sample 14 (168 h)		
	Subject		Subject		Subject		Subject		Subject		Subject			Subject			Subject		
	D*	G	D	F	F	G	F	G	F	G	D	F	G	D	F	G	D	F	G
Fresh Tox dilution	0.4	4.1	0.5	3.9	2.6	0.9	2.8	0.9	2.9	1.2	3	3.2	1	3.4	3.1	1	3.8	3.2	1.2
Previously-constituted Tox dilution**	0.4	4	0.6	3.9	2.6	0.9	2.6	0.8	2.8	1.2	3.1	3.2	1	3.5	3	1	3.7	3.2	1.3

* Letters D, G, F - were designated to poisoned patients, as they were included in the study;

** p value 0.42.

bation of the serum (0.5 mL for 30 minutes at 37°C with 5 μL of Tox (0.125 mg) 1/10 dilution, prepared in two manners: fresh, just before incubation with serum, and constituted previously with the occasion of the first determination are included in Table V.

DISCUSSION

The results obtained from analyzing issues I and II have supported our hypothesis that, in acute OF poisoning, additional information can be obtained using a laboratory method that test the effect of an "in vitro" incubation with Tox to generate a possible recovery in the level of ChEs activity. Results from Table II showed that in group A, level of ChEs activity is almost equal with both methods, because there is a decreased amount of enzyme synthesized by affected liver, while in group B, where ChEs activity is not influenced by anything, the differences between values obtained with both methods are inconclusive, with no statistical significance.

Technical aspects regarding the methodology of the test were assessed in issues III, IV and V revealing the best dilution of Tox to be used, which is 1/10, containing 0.125 mg / 5 μL (conclusion from issue III).

The presence of Tox in the sample (0.125 mg added to the 0.5 mL of serum) result in an artifact of 0.5 U/mL false increase in the level of ChEs activity which has to be subtracted from the values obtained with the newly proposed protocol (from those of samples incubated with obidoxime) - conclusion from issue IV, but this value was not statistically significant. Once constituted, the dilution of Tox can be safely used for at least one week, if stored in brown glass bottles (conclusion from issue V), because differences in the two dilutions used (fresh and previously prepared) is not statistically significant in patients analyzed.

CONCLUSIONS

Analyzing "in vitro" the Tox effect on restoring the level of ChEs activity resulted in a safe, non-expensive, easy to perform, quick (< 1 hour) test capable to offer new, interesting perspectives on OF intoxications. It clearly consists in an advantage for the diagnosis, prognostic evaluation and therapeutic orientation in organophosphate poisoning. These aspects are now evaluated in a prospective study, which will be finalized in May 2012. Finally, the desire of the authors is that professionals involved in the management of organophosphate poisonings will start to use the new proposed method, and communicate their personal considerations on this topic.

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INVESTIGATING MEDICAL STUDENTS' HEALTH CONCERNS DURING THEIR ACADEMIC STUDIES

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INVESTIGATING MEDICAL STUDENTS HEALTH CONCERNS DURING THEIR ACADEMIC STUDIES (Abstract): **The aim** of the study is to identify medical students' opinion regarding their health and thoughts related to their physical health in relation to their clinical practice. **Material and methods**: medical students enrolled in all years of study (from 1 to 6) answered to an online questionnaire. Socio-demographic, family, academic and health-related data were gathered. A series of items focused on students' opinion about physical health in relationship with the completion of their practical stages in different clinics. Dietary habits and lifestyle data were also collected. Statistical data were analyzed using *SPSS v.21*. **Results**: 220 students aged M = 21.92 ± 1.96 (19 to 32 years old), 2/3 living in urban area. 9.5% of them had a chronic disease. In the case of 5% of students, both parents were working abroad. 5% of respondents declared that they consumed drugs or psychotropic substances. 1/5 are smokers and 1/3 drink coffee daily. Students reported an average of 6.73 (±1.09) hours of sleep per night. Almost half of them were tense during clinical hours (48.2%). 20% of the students strongly agreed with the fact that they were always preoccupied with their health. 53,2% used supplements seasonally to improve their health. More than half of the students were afraid of developing cancer (53.2%), followed by diabetes (6.8%) and 58.6% claimed that they always have hand sanitizer on them. **Conclusions**: Medical education is a long and demanding academic training when different aspects influence students' psychological, physical and social health .The contact with patients, the knowledge about illness, suffering and dying, determines the individuals to deal with objective or imaginative fears and explaining this phenomenon has positive consequences for the personal and professional life of the future medical doctor. **Keywords**: MEDICAL STUDIES, STUDENTS, DIETARY HABITS, DISEASE, PHYSICAL HEALTH, MEDICAL STUDENT SYNDROME.

During their academic years, medical students are taught about diseases, how to identify symptoms and to give a correct diagnostic. It is proved that students can suffer from diseases they learn about and it is speeded among students who are trained in Psychology or Medical Sciences. When studying a psychological disturbance, students may experience more psychological distress regarding the members of their families or their own psychological health. Also, while learning about a physical disease, students can interpret a physical disturbance as a sign of a serious illness.

By misinterpreting their own physical symptoms, medical students could experi-

ence the fear that they suffer from a disease. Some studies showed that this kind of beliefs (about having a severe disease) is persistent in time, despite medical examination or laboratory analysis and it is a temporary kind of hypochondria. (1-2) This situation is like hypochondria and could be related to Medical Student Syndrome (MSS). Other used terms were nosophobia, hypochondriasis of medical students or medical student it is, a mild form of health anxiety or transient hypochondriasis.

In the case of hypochondria, the patient always seeks a medical diagnosis, but in case of MSS no increase in the number of medical consultations is revealed. So, students identified with MSS will not look more frequently to have a medical check but the belief or the fear that they were having a disease is present. This is a unique type of hypochondriasis which can lead to health anxiety in strong relationship with the diseases medical students focus their attention on during medical training.

The rates of MSS among medical students vary from 5% to 70% (3-5). Consequently, scientific reports regarding the effects of progressing through medical education on health anxiety are scarce and contradictory. Studies show that the fear of having a disease may persist over the years during medical studies (6-8) and that some factors influence the level of fear: personality traits, fantasy proneness, clinical year, thought suppression (9). Nevertheless, results focusing on medical students' stress, fear of illness or burnout present contradictory data when compared to non-medical students (10).

The aim of the study is to identify medical students' thoughts regarding their health and behaviors related to maintaining physical health.

MATERIAL AND METHODS

Participants and Instruments

The study was developed between January and March 2019. The research targeted medical students from all years of study and was divided in three parts: the first one collected socio-demographic, medical and academic data. The second part of the questionnaire consisted of a number of self-rated items developed with the purpose of identifying aspects related to students' health. The third part included items targeting their health-related behaviors regarding physical health or limiting exposure to diseases during practical stages and worries about catching a disease. The participants had to rate their responses on a Likert-like scale from 1 to 5 (*1 – strongly disagree; 2 – disagree; 3 – neutral; 4 – agree; 5 – strongly agree).*

A number of 220 medical students provided online answers to the questionnaire. Google Forms was used due to its familiarity with students.

Socio-demographic data (sex, age, year of study, environment, living arrangements), medical (chronic disease) and anthropometrical data (weight, height) were collected. Supplementary items were gathered related to academic results or family situation (siblings, level of education for both parents and having at least one parent working abroad).

Statistical analysis of data

Collected data were analyzed using IBM SPSS Statistics, version 23. Means and standard deviations were used for the descriptive analysis of socio-demographic, academic results and anthropometric data. Frequencies were used for analyzing the distribution of answers for the rest of the items. Independent Sample T-test were

used for the comparative analyses.

RESULTS

Demographic, academic and family-related data

All the participants were domestic students enrolled in the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi. Almost half of them (46.8%) were in their third year of study, followed by second year (15%), fourth year (12.7%), first and fifth year (both of them 8.2%) and sixth year (9.1%).

Most of the participants were female students (82.5%), with age ranging from 19 to 32 years old (M = 21.92 ± 1.96). This gender majority is specific to medical students. More women are usually enrolled in medical faculties.

Regarding their living environment, 78% of the participants declared that they were living in urban areas. Most of the students lived with their families and in rental accommodation or dorm rooms (33.6%, 38.6% respectively); a smaller percentage lived either alone (12.7%) or with their life partner (15%).

The admission process in the university is based on a final mark (minimum 5, maximum 10) resulted from a written exam with multiple answers, covering items from different disciplines (biology, chemistry and physics) and the mark from the baccalaureate exam. The mean admission mark for the researched sample is 8.76 (± 0.85). For the baccalaureate exam the mean was 9.26 ± 0.58.

One item investigated whether respondents have at least one parent working abroad. In case of 5% of the students, both parents were working abroad at the time of the investigation and another 14,1% declared that one parent is working in another country (9.1% for fathers and 5% for mothers).

Anthropometric, dietary habits, and health-related data

Students had to declare if they suffer from a chronic disease and 9.5% of them claimed that they had a chronic disease. As anthropometric data, it was obtained an M = 63.29 (± 12.26) for weight and M = 168.19 (± 8.22) for height.

Regarding the consumption of alcohol, tabaco, coffee, carbonated/energizing drinks or psychoactive substances, the first table presents the frequency of answers. Almost a fifth of the students (20.9%) smoke daily, 13.1% of the students drink alcohol monthly or weekly, almost half of them (45.9%) drink coffee every day, 51.8% occasionally drink carbonated drinks and 34.1% occasionally drink energizing drinks. Most students claimed that they never used ethnobotanicals (95.9%) or drugs (94.1%) and only a small percentage revealed that they have used them occasionally (3.6%, 5% respectively). So, around 5% of respondents declared that they consumed drugs or psychoactive substances (tab. I).

TABLE I

The consumption tobacco, drinks or drugs (%)

Frequency of use	Tobacco	Alcohol	Coffee	Carbonated drinks	Energizing drinks	Ethno-botanics	Drugs
Never	60	15	11.8	9.5	62.3	95.9	94.1
Occasionally	15.9	71.8	22.7	51.8	34.1	3.6	5
Monthly	0.9	7.3	1.4	12.3	1.4	0.5	0.5
Weekly	2.3	5.9	18.2	21.8	2.3	0	0.5
Daily	20.9	0	45.9	4.5	0	0	0

Students were also asked about their sleeping and dietary habits. Respondents reported an average of 6.73 (±1.09) hours of sleep per night, with a minimum of 3 and a maximum of 11. Also, 13.1% of the students reported that they wake up every night, while 59.5% of them wake up occasionally.

The students had a rich diet in fruits and vegetables (43.2%), sweets (25.9%) and fat (16.8%). Only 10.5% of the students self-rated as having a healthy and balanced diet.

Health-related thoughts and behaviors

Students were asked to read five statements and decide to what extent they agreed with them, in order to identify their concern about their physical health. Almost 20% of the students strongly agreed with the fact that they were always preoccupied with their health. Also, 11.1% of the students in our sample agree or strongly agree that people around them paid little attention to their health.

While consulting patients, 7.7% of the students are very concerned they could have the same disease. A small percentage (4.1%) strongly believe they have a disease the doctors haven't discovered yet. Finally, 21.9% of the students agree or strongly agree with the statement that there is something wrong with their bodies. The frequency of the answers is presented in second table.

We were also interested in students' feelings during their practical stages. Almost a quarter of students (22.7%) were scared for no apparent reason or they felt it was hard for them to calm down (21.9%).

TABLE II

The frequency of answers (%)

ITEMS	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I am very preoccupied with my health.	1.8	18.6	30.5	29.5	19.5
People around me pay little attention to my health.	33.2	36.4	19.4	9.7	1.4
While I consult patients, I am afraid I could have the same disease.	33.2	32.3	15.5	11.4	7.7
I think I have a disease the doctors haven`t discovered yet.	74.1	9.5	5.9	6.4	4.1
I always have the feeling there is something wrong with my body.	30.0	31.8	16.4	11.4	10.5

TABLE III

The distribution of answers to items regarding the feeling during practical stages (%)

ITEM	Strongly disagree	Disagree	Neutral	Agree	Strongly agree
I was scared for no apparent reason.	23.6	25.9	17.7	19.1	13.6
It was hard for me to calm down.	34.1	24.1	20.0	11.4	10.5
I had a hard time swallowing.	41.8	26.4	12.3	11.4	8.2
I lacked enthusiasm.	12.3	26.4	25.9	18.6	16.8
I was afraid I did not have enough time for all my assignments.	13.2	21.4	20.9	26.8	17.7
I felt tense.	8.2	22.7	20.9	26.8	21.4
Life doesn`t have a meaning.	39.1	17.3	12.7	13.2	17.7

Students agreed or strongly agreed that they had a hard time swallowing (19.4%), they lacked enthusiasm (35.4%), they were afraid they did not have enough time for all the assignments (44.5%), they were tense during clinical hours (48.2%) and, more importantly, they felt life didn't have a meaning (30.9%). The frequency of answers is presented in Table III.

Behaviors regarding physical health

More than one quarter of students (30.9%) believe they lose more hair than others; more than half of them carry hand sanitizing with them (58.6%), rarely take medication without medical prescription (69.5%), use dietary supplements seasonally to improve their health (53.2%) and never bit their nails (65.9%). 15.5% of the students in our sample think very frequently that they could have a serious affection.

Half of the students declared they were healthy (50.9%), while the rest declared various affections; the most common were digestive (15.5%), genital (7.3%) and endocrinological (5.9%) problems. More than half of the students were afraid of developing cancer (53.2%), followed by diabetes (6.8%).

Comparative analysis showed a significant difference between students with and without parents working abroad regarding the admission t (199) = 2.48, p = 0.002 and baccalaureate means t(207)a = 4.43, p = 0.004. The results showed that students with parents working in another country obtained lower marks for both baccalaureate (M*with* = 9.35, M*without* = 8.92) and faculty admission exams (M*with* = 8.46, M*without* = 8.83).

DISCUSSION

The present paper offers some preliminary results from a larger research focusing on medical students preoccupation for their health, especially during their medical training. It was pointed out by the scientific literature that students enrolled in Psychology or Medical sciences courses are more prone to experience fear of having a disease (physical or mental illness) than students enrolled in other specialties. The results of some studies showed contradictory rates concerning two aspects: the level of fear of having a disease or the tendency to identify themselves as having the signs of a studied physical or mental illness. With such rates ranging from 5 to 70%, various researchers tried to identify variables linked to this tendency (3, 5, 10).

Our study revealed that more than half of the respondents are afraid of developing cancer (53.2%), a major fear also identified by other studies led on medical students (9). Studies focusing on medical students revealed that 70% of them have groundless medical fears during training. On the contrary, some other studies found no difference regarding health-seeking behaviors and fear of illness between medical and non-medical students (19, 20).

Training in the medical sciences is an important source for medical knowledge. Due to the large amount of information and hard work, students tend to diagnose themselves or to self-administrate dietary supplements with no medical prescription in order to improve their physical and mental power. The rates for this study correspond to results obtained by other studies focusing on medical students: more than 50% in USA (11), 37.4% in Italy (12) or 76.1% in Saudi Arabia (13). Even if some studies point out that there is no significant difference between students with and without medical knowledge, other findings show that there are significant differences in the knowledge of health sciences and non-

health sciences students pertaining to health benefits and safety of the consumption of dietary supplements (14).

The present study identified that 1/5 of the students smoke and then 1/3 of them drink coffee daily. Also, 35% of the students drink energizing drinks. The use of alcohol, coffee and energizing drinks is also comparable with other studies. Aljaloud (2016) identified that 71.5% of students use energizing drinks (15). In a study conducted by Casuccio *et al* (16) it was revealed that 22% of medical students were regular consumers of energizing drinks. Moreover, the consumption of these drinks was found to be associated with a high rate of alcohol consumption and 45% of the students declared that they felt side-effects like insomnia, palpitations and irritability after the consumption of energizing drinks. Some other authors presented higher reports: 73,8% in Canada (17). Regarding the consumption of tobacco, studies show that the rates are decreasing, but this was associated with a worldwide phenomenon. With a percentage of smokers of 40, this study reveals an increased rate, compared to studies on medical students from Europe (19%), Africa (19%) or SUA (21%) (18).

CONCLUSIONS

Medical education is a long and demanding academic training. Apart from psychological aspects or social characteristics, other aspects influence students' psychological, physical and social health during their academic years. The contact with patients, the knowledge about illness, suffering and dying determines the individuals to deal with objective or imaginative fears. Even if studies prove that the fear of illness is not always a characteristic of medical students' life, medical teachers should consider this when new topics are presented. Also, explaining this phenomenon has positive consequences for the personal and professional life of the future doctor.

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This paper presents a part of a larger research focusing on medical students and the influence of medical knowledge on students' behavior regarding the interest for their physical and mental health. Some of the results were presented as an oral presentation at International Conference for Medical Students - *CONGRESSIS 2019*, Iasi, Romania.

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NEWS

ARE PETS A SOURCE OF ANTIBIOTIC RESISTANT INFECTIONS?

Macrolide-resistant Streptococcus pyogenes is an emerging problem with a great public health concern throughout the world. A study was carried out in order to investigate the possible role of pet animals in the epidemiology of such pathogen. For this purpose, nasal or oral swabs were collected from 115 pets (40 dogs and 75 cats) with respiratory illness. The collected swabs were cultured for isolation and identification of S. pyogenes. Of the 115 examined pet animals, S. pyogenes was recovered from 11 (9.6%), from which, the isolation rates among dogs and cats were 15% and 6.7%, respectively. Macrolide-resistant S. pyogenes was isolated from dogs and cats in the following rates 10 % and 5.3 %, respectively (Ahmed Samir, Khaled A. Abdel-Moein, Hala M. Zaher. Emergence of penicillin-macrolide-resistant Streptococcus pyogenes among pet animals: An ongoing public health threat. *Comp Immunol Microbiol Infect Dis* 2019; 12(68): 101390).

THEORETICAL AND PRACTICAL ASPECTS OF ^{18}F-FDG PET

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THEORETICAL AND PRACTICAL ASPECTS OF ^{18}F-FDG PET (Abstract): The incidence of oncological diseases has become very high in the last decade, whether we refer to more or less developed countries. The incidence of the oncologic disease continue to increases in spite of substantial progress in the development of medicine and medical technologies. At the same time, an efficient treatment depends on an accurate and timely diagnostic. A solution for precise diagnostic is provided by nuclear medicine through the positron emission tomography (PET) technique, which allows an accurate evaluation of metabolism in different organs and tissues. PET permits timely identification of functional disturbances underlying the development of the disease. In the past decade's positron emission (PET) ^{18}F-fluorodeoxy-glucose (^{18}F-FDG) has been used to evaluate tumor patients. Evaluation of distribution, spread and proliferation of cancerous cells is considered to be useful for therapeutic guidance and therapeutic effect assessment. Thus, radiolabeled molecules, such as the [^{18}F]-2-Fluoro-2-deoxy-β-D-glucopyranose has been developed. Fluorine-18 is a short-lived positron emitting isotope which finds a massive use as a label for radiotracers used with the molecular imaging technique of PET. The Quality Control (QC) process concerns all the mechanisms, actions and tools developed to detect the presence of errors in a production batch. This review focuses on the importance of ^{18}F-FDG and our goal was to present some theoretical and practical aspects of the ^{18}F-FDG synthesis and what parameters and how should they be evaluated in QC laboratories. **Keywords***:* POSITRON EMISSION TOMOGRAPHY, ^{18}F-FDG SYNTHESIS, QUALITY CONTROL.

WHY [^{18}F]-FDG?

The [^{18}F]-FDG is a generic tumor-seeking radiopharmaceutical and it is widely used for the diagnostics of malignant tumors. Chemical name of [^{18}F]-FDG is [^{18}F]-2-Fluoro-2-deoxy-β-D-glucopyranose, identified by Chemical Abstract Service with registry number 63503-12-8, but more commonly referred to as ^{18}F-Fluorodeoxyglucose or simply FDG. This compound is a radioactive derivative of 2-deoxy-D-glucose labelled with

positron-emitting isotope ^{18}F in the position 2 of the glucose core structure as shown in the Figure 1. The radioactive fluorine undergoes beta decay through the emission of positrons, followed by their annihilation and subsequent emission of two 511 keV gamma photons. The longer half-life of ^{18}F, of almost 110 minutes, has allowed the commercial distribution of radiotracers to PET centers placed outside of clinical locations.

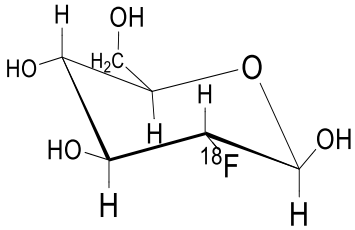

Fig. 1. The [^{18}F]-FDG structure

CHEMICAL ASPECTS BEYOND GLUCOSE METABOLISM

Most living organisms have as a primary source of energy a common monosaccharide found in nature, such as glucose. Glucose homeostasis plays an essential role in most fundamental aspects of life, and its dysregulation is associated with many important human diseases such as cancer (6). In the course of cancer progression, even in the availability of oxygen, there is a change in glucose metabolism from mitochondrial oxidative phosphorylation to a glucose dependent glycolytic pathway. To sustain the high energy demand required for rapid proliferation, cancerous cells increase their glucose uptake as well as their glycolytic rate, which can be up to tens or hundreds of times greater than that of normal cells (7). This atypical metabolic process is known as the Warburg effect. This metabolic phenomenon has been recognized as a hallmark of cancer and serves as a promising target for diagnosis and therapy of cancer (8).

The applicability of given radiopharmaceuticals in oncological diseases is based on the process of intensified glycolysis in neoplasms, described in 1926 by Otto Warburg (9). The transport of [^{18}F]-FDG in malignant tumor cells is due to the expression of a class of uniporter proteins, called glucose-transporters (GLUT1–4), the most active of them being GLUT1. The kinetics of glucose transport via GLUT1 has been explored since radioisotope use has become available (10). In the cell, the radiopharmaceutical is subjected to phosphorylation under the influence of the enzyme hexokinase, with the generation of [^{18}F]-FDG-6-phosphat which, due to its properties, is not involved in the tricarbonic acid cycle and is not ejected from the cell. In tumorous cells, the process of dephosphorylation is decreased due to the low activity of the enzyme phosphorylase (11). Thus, conditions arise for heightened FDG accumulation in malignant tumor cells. This phenomenon has been called the "metabolic trap" and the level of [^{18}F]-FDG accumulation in tumors does not depend on their microcirculation, since the radiopharmaceutical is actively transported to cells (12). Furthermore, the [^{18}F]-FDG is excreted by the kidneys and consequently a part of the radiopharmaceutical is inevitable physiologically absorbed in the urinary system.

METHODS OF OBTAINING [^{18}F]-FDG FOR PET

The [^{18}F]-FDG production is a multistep process that begins with a particle accelerator (by far the most efficient, useful and economically viable method) which produces the [^{18}F]-fluoride radionuclide through proton irradiation of oxygen-18

enriched water in a small enclosed volume, usually 0.5–2.5 mL (13). For obtaining radioactive fluorine, over 20 nuclear reactions are known, of which the most effective and widely used in the world is irradiation of oxygen-18 enriched water which provides fluoride [^{18}F] of high radioactivity under optimized conditions, achieved at cyclotron, even with low energy, in less than 1 hour of irradiation time (14). Nowadays most of the radiopharmaceutical laboratories use the nucleophilic substitution method for FDG production, introduced into practice by Hamacher and his collaborators in 1986 (15) while the European Pharmacopoeia still mentions the electrophilic route for FDG production (the precursor and possible impurities) as the alternative routes.

The ^{18}F-FDG was synthesized for the first time by Pacák and his colleagues in 1969 and it was evaluated as a PET tracer in the early 1980s (16, 17). Ido and his team exploited the high reactivity of molecular fluorine in the production of FDG by electrophilic fluorination and they did the first PET images in human (17). The significant differences between these two forms are the specific activity (radioactivity/mole) of the produced ^{18}F isotope. Nucleophilic ^{18}F-fluoride is produced by the $^{18}O(p,n)^{18}F$ nuclear reaction to give a high amount of radioactivity (>370 GBq/batch). Nucleophilic [^{18}F]-fluoride is produced with specific activity in the range of 10^2 GBq/μmol (12). Electrophilic [^{18}F]-F_2, on the other hand, has a much lower specific activity (100−600 MBq/μmol) because fluorine [^{19}F]-F_2 gas must be added as a carrier to extract the [^{18}F]-F_2 from the target (14). The specific activity becomes essential when working on ligand−receptor binding because the addition of a carrier ([^{19}F]-F_2) leads to increased mass of the final radiopharmaceutical, which may disturb any signal received by PET from radiopharmaceutical binding and may also cause pharmacological effects (14, 18). Because nucleophilic fluorination is the most important route for obtaining ^{18}F-labeled compounds, we will go directly to dealing with practical aspects of nucleophilic FDG production method.

After the required irradiation time (typically not more than one hour at 35 μA current) the radioactive [^{18}F]-fluoride is transferred to a radiopharmaceutical production laboratory for further conversion into [^{18}F]-FDG suitable for injection. The [^{18}F]-fluoride is collected inside an FDG synthesizer, placed in a hot cell. The radiochemical synthesis of 2-^{18}F-fluoro-2-deoxy-D-glucose can be carried out by means of an automated module leading to a product which becomes an injectable solution subjected to sterilizing filtration. The module uses a disposable cassette which contains the necessary reagent kit with the chemicals required for [^{18}F]-FDG synthesis. The reagent kit includes eluent solution, acetonitrile, precursor, ethanol, sodium hydroxide solution, buffer solution, an anion exchange column, a reverse phase and alumina N cartridges and sterile water for injection. The use of a module with a disposable cassette makes the duration of the synthesis process of about 30–35 min. In most cases, [^{18}F]-FDG synthesis is performed according to the two-stage method of Hamacher (15). The first steps in radiochemistry with [^{18}F]-fluoride ion are almost invariably the removal of bulk [^{18}O] water and the solubilization in an organic solvent. This is commonly achieved by trapping the [^{18}F]-fluoride ion onto an ion exchange resin, to allow the recovery of the expen-

sive [^{18}O]-water, followed by the elution of the [^{18}F]-fluoride ion in a small volume of an aqueous weak base (usually potassium carbonate). Water is then removed by two or three successive cycles of azeotrope evaporation with acetonitrile (at temperature >85°C for ~10 minutes). The addition of a Kryptand, such as Aminopolyether 2.2.2 (K2.2.2) or tetra butyl ammonium carbonate (TBA), during this process enables the [^{18}F]-fluoride ion to be subsequently solubilized in a polar aprotic solvent (such as acetonitrile) (13).

The precursor molecule for the radiochemical synthesis of [^{18}F]-FDG through nucleophilic substitution is 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-b-D-manno-pyranose. It is commonly named mannose triflate and it is a sugar molecule containing a proper leaving group for a facile nucleophilic reaction, trifluoromethanesulfonyl group (usually called triflate). In order to achieve radiolabeling of glucose at the C_2 atom with radioactive [^{18}F]-fluoride through nucleophilic substitution, the other possible reaction centers are protected with four tetra-acetyl groups (named protecting groups). During the nucleophilic substitution reaction (~5 min at 80-90°C using anhydrous acetonitrile), the leaving group, triflate, is displaced by radioactive [^{18}F]-fluoride (13).

Removal of protective groups (the four acetyl protecting groups) in the synthesis of FDG is easily accomplished through acidic or basic hydrolysis. Both methods are equally effective but require different conditions, each with its advantages and disadvantages (a high temperature or the probability of obtaining by-products). However, the alkaline hydrolysis is the most commonly used method in commercial synthesis modules since it requires less time and lower temperatures (19). In this case to remove the acetyl groups, the [^{18}F]-labelled intermediate with intact acetyl groups is treated with mild base (1-2 M NaOH) at room temperature (may be performed directly on a C-18 cartridge) (20). However if alkaline hydrolysis is performed at higher temperatures the [^{18}F]-FDG has a potential for epimerization into [^{18}F]-Fluorodeoxymannose (FDM). The FDM should be treated as a radiochemical impurity that should be controlled in the FDG manufacturing process and also quantified during the quality control tests (21).

Purification of the final product can be performed by passing it through a series of anion exchange columns, a C-18 reverse phase column and an alumina column. This process will remove impurities, as well as unhydrolyzed and partially hydrolyzed intermediates, unreacted fluoride but will also help with neutralization and pH adjustment.

A final step in the synthesis of [^{18}F]-FDG is sterilizing filtration in which the purified [^{18}F]-FDG solution is passed through a vented 0.22 μm filter. Sterilization with steam may also be performed, but it is not required by European Pharmacopoeia (22). The filtered product (final [^{18}F]-FDG) is held in quarantine until it receives the final approval for patient use from the quality control department.

The FDG may be dispensed either into unit doses or in multi-dose vials using automated systems. This process must be done in aseptic conditions, in a *class A* environment, following the applicable *Good Manufacturing Practice* (GMP) regulations. The product vial and the outer lead shielding should be affixed with a label containing the relevant product information, such as: product name, total activi-

ty at reference time, expiration time.

The technology has also focused on increasing the efficiency of chemical labelling by reducing the time of synthesis and increasing the efficiency of the individual steps. The result is that, in a relatively short period of time, the yields of a single [^{18}F] production of FDG have increased from a few hundred to several thousand mCi (23). However, the problem with higher yields and higher specific activities has potentially serious implications for the radioactive stability of many biological drugs, such as [^{18}F]-FDG. These drugs degrade by radiolysis when exposed to large quantities of ionizing radiation with high linear energy transfer values (24). When the activity concentration of [^{18}F]-FDG is increased to hundreds of mCi/mL, [^{18}F]-fluoride is the main active product of auto radiolysis and becomes the most important radiochemical impurity.

QUALITY ASSURANCE AND QUALITY CONTROL GUIDELINES

In the clinical application of PET radiopharmaceuticals, it is important to assure the safety of the product. Many guidelines for the production of PET radiopharmaceuticals have been published in each country or region. A pharmacopeia is a regulatory source for the quality assurance (QA) of any drug product in the world. In Europe, the European Pharmacopeia (EP) is recognized as the official collection and is referenced in various forms (22). Precise procedures for the production of PET radiopharmaceuticals and their Quality Assurance (QA) / Quality Control (QC) are described in these guidelines. Thus the EP contributes to the quality of medicines and the protection of public health and also includes QA standards in the form of monographs for several PET drug products. Because [^{18}F]-FDG injection is the most commonly used PET radiopharmaceutical, the discussion of QA procedures in this article focuses on this drug. A careful examination of official sources, such as The European Pharmacopoeia (EP) and International Atomic Energy Agency (IAEA) for information on ^{18}F-FDG revealed the QA standards for PET drugs (13).

Quality control guarantees that the necessary and suitable tests are carried out and that the raw materials, packaging materials and products are released for use or sale, only if their quality is adequate. Quality control is not limited to laboratory operations but must be integrated into all activities and decisions concerning the quality of the radiopharmaceutical (25). A quality control laboratory has two primary functions: to test radiopharmaceutical and to test and analyze the raw materials and supplies used in the production of that radiopharmaceutical.

The [^{18}F]-FDG is an injectable radiopharmaceutical, and consequently each production unit must be manufactured accordance with the *Good Manufacturing Practice* (GMP) regulations. Documentation is an integral component of GMP and the quality management system. All processes must be documented in the form of *Standard Operating Procedures* (SOPs), and all personnel must follow these official procedures during the manufacturing and testing of [^{18}F]-FDG and all associated records must be collected and properly filed. The entire [^{18}F]-FDG manufacturing process may be confined to the cleanroom environment or within controlled zones to help ensure the pharmaceutical quality of the finished product. A clean and aseptic

environment are essential requirements in [^{18}F]-FDG manufacture, especially when the final injectable product is not subject to steam sterilization (26).

Quality control testing of [^{18}F]-FDG batches is done according to the guidelines outlined in the European Pharmacopoeia. Parameters given by this regulatory source, such as chemical identity and purity, radionuclidic and radiochemical identity and purity, have to be checked before releasing the product for human use (22). These tests can be accomplished using different types of radiation measurement equipment. Because of their short half-life, some radiopharmaceuticals may be released before completing all European Pharmacopeia recommended tests. The release of the product is not conditioned upon completion of all the tests as specified in [^{18}F]-FDG individual monographs, but they must be performed later, and the results recorded. It is a process that ensures compliance with product specifications regulated in the Pharmacopeia.

Daily QC testing comprises a visual inspection, as the product must be clear, colorless and free of particulates. The test should be performed before the drug product is released. The pH is determined on the final product of ^{18}F-florodeoxyglucose using pH paper and as acceptance criterion it must be between 4.5 and 8.5. This test must also be completed before the drug product is released.

In order to identify a radionuclide from a given sample, activity measurement are performed. The half-life is a measurable parameter and it is a characteristic of each radionuclide, allowing its identification (for [^{18}F]-FDG the half-life must be 105–115 minutes). PET drugs like [^{18}F]-FDG are based on [^{18}F] which is a positron emitter. The interaction with the surrounding electrons produces an annihilation reaction that releases two anti-collinear high energy photons (26). Each photon produces one current pulse proportional to its energy, which is amplified by the detector's electronics and produces a signal on the corresponding channel. A gamma spectrum is used in order to determine the radioisotopes present in a sample, as all PET tracers should produce a Gaussian bell curve centered in 511 keV, corresponding to the positron-electron resting mass energy (28).

Kryptofix (amino polyether) concentration for [^{18}F]-FDG approval has a limit because its presence over a certain concentration causes certain side effects, like apnea and convulsion (28). Although all automatic synthesis modules have multiple removal steps, the final [^{18}F]-FDG products must be tested before final release. The amino polyether test is performed by a spot test using a TLC silica gel plate. The central part of the spot due to the test solution should be more intense than that of the reference solution (2.2 mg/V, where V is the maximum recommended dose in milliliters) (22).

Different impurities may appear in the final radiopharmaceutical solution, coming from the manufacturing, cleaning or any other process during synthesis. These impurities are classified in the European Pharmacopoeia, which establishes that chromatographic techniques (such as TLC or HPLC) are suitable for estimation of their concentration (22). Different compounds will have different affinities for the mobile and stationary phases which in turn affect their migration speed expressed in the Retention Factor (RF) or Retention Time (RT) of radiotracer and reference standard match (30). The current European

Pharmacopoeia stipulates the minimum radiochemical purity for [^{18}F]-FDG should be no less than 95% of the total radioactivity while [^{18}F]-FDM fraction should not exceed 10% (22).

The endotoxin detection is based on spectrophotometry of a chromogenic substrate released in contact with lipopolysaccharides from the external membrane of different types of Gram-negative bacteria. According to the European Pharmacopoeia, the final radiopharmaceutical may be released before endotoxin testing is completed but could be immediate accomplished with a PTS system or later using a gel-clot test.

Sterility test is performed to detect the presence of viable microorganisms in the decayed sample. This control test is made once the radiopharmaceutical is fully decayed. Due to the short half-life of any radionuclide used in PET, this test is performed after the final product it was completely administered to patients.

CONCLUSIONS

The incorporation of fluorine-18 into a radiotracer combines the biochemical specificity of the tracer molecule with the high sensitivity of radioactivity detection. An example of the former is the incorporation of fluorine-18 into 2-deoxy-2-glucose to give [^{18}F]-2-fluoro-2-deoxy-2-glucose ([^{18}F]-FDG) as a radiotracer of glucose utilization. [^{18}F]-FDG, originally developed in the late 1970s, is now very widely used as a clinical research tool and as a diagnostic agent in oncology and neurology.

Positron emission tomography has advanced to become an important clinical diagnostic tool, but also a valuable technology used in research and drug finding and development. Increased demand for 2-deoxy-2-[^{18}F]-fluorodeoxyglucose for PET clinical investigations, has headed to the continuous optimization of the synthesis methods, leading to the compound being produced reliably, in the desired quantities and with higher purity.

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CONTRIBUŢII LA AMELIORAREA EFICIENŢEI INTERVENŢIILOR PREVENŢIONALE PENTRU REDUCEREA RISCULUI ENDOMIO-PARAMETRITELOR ŞI AL INFECŢIILOR PLĂGII OPERATORII

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CONTRIBUTION TO IMPROVE THE EFFICIENCY OF PREVENTIONAL INTERVENTIONS IN ORDER TO REDUCE THE RISK OF ENDOMIOPARAMETRITES AND SURGICAL WOUND INFECTIONS (Abstract): There are few new methods conferred an efficient prevention in order to reduce the incidence of endometritis after cesarean section. The study aim was to compare two techniques using in post partum post-cesarean endometritis prevention: local (vaginal) intra-partum application of 0.25% Clorhexidine solution during elective cesarean deliveries vs intraoperative cesarean section antibioprevention with 2g second generation cephalosporin administered by intravenous injection after clamping the umbilical cord. **Key words**: PUERPERAL FEVER, VAGINAL ANTISEPSIS, INTRACESAREAN ANTIBIOPREVENTION, ENDOMETRITIS

A RAPID, SIMPLE AND SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NO FROM NO DONORS

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A RAPID, SIMPLE AND SENSITIVE SPECTROPHOTOMETRIC METHOD FOR THE DETERMINATION OF NO FROM NO DONORS (Abstract): **Aim**. To develop and validate a spectrophotometric UV-VIS method for the determination of nitric oxide (NO) released from NO donors, such as nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs), with poor solubility in aqueous medium. **Material and methods**: The method is based on the nitric oxide-mediated stress (nitrosative stress) through sulfanilamide that reacts with *N*-(1-naphthyl)ethylenediamine dihydrochloride to form a pink-red azo dye with maximum peak at 540nm. The method was validated in term of linearity, detection limit, quantification limit, precision, and accuracy. **Results**: The linearity of the method was proved for range of 0.78-100µM $NaNO_2$ with a correlation coefficient of 0.9998. The accuracy of the method was checked by recovery experiment performed at three concentrations, *i.e.*, 20 µM, 40 µM and 60µM $NaNO_2$ and the recovery (%) was found to be in the range of 98.34% and 101.96%. The low values of relative standard deviation (RSD) are indicative of the accuracy and reproducibility of the method. The precision of the method was studied as intraday and interday variations. An RSD less than 2% indicates that the method is precise. **Conclusions**: This research offers all the proofs that recommend the use of this method for the determination of NO released from NO donors, such as NO-NSAID, using a hydroalcoholic medium, as it has good sensitivity, precision, and accuracy. **Keywords:** NO-NSAID, GRIESS ASSAY, SPECTROPHOTOMETRY.

Nitric oxide-releasing nonsteroidal anti-inflammatory drugs (NO-NSAIDs) are a new class of anti-inflammatory drugs developed with the aim to reduce gastrointestinal side effects through NO release (1). NO is one member of a new class of signaling molecules with key role in a variety of biological processes including immune defense, inflammation, and neurotransmission. It is involved in regulating all aspects of our lives from waking, digestion, sexual function, perception of pain and pleasure, memory recall and sleeping (2, 3).

NO-NSAIDs are synthesized by the ester linkage of a NO-releasing moiety to conventional non-steroidal anti-inflamma-

tory drug (NSAID) (4) (fig. 1). Along with prostaglandins, NO released from NO-NSAIDs reduces the gastrointestinal injury by maintaining the mucosal blood flow, optimizing mucus and bicarbonate gastric secretion, and inhibiting activation of pro-inflammatory cells. As a result, NO may neutralize the detrimental effects of COX (5).

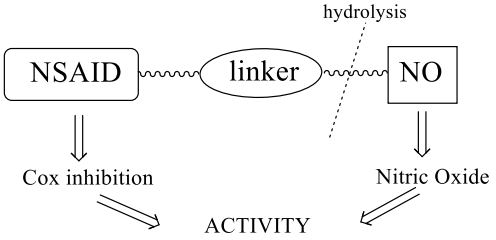

Fig. 1. General representation of NO-NSAIDs

For the detection and quantification of NO in complex biological systems and in physiological conditions, different methods can be used such as: chemiluminescence detection; fluorescence detection using 4.5-diaminofluorescein; conversion of Fe(II)-/oxyHb to metHb; electrochemical detection (amperometric NO-specific and porphyritic electrodes); Griess assay of NO_2^-, NO_3^-, and immunoassays using anti-nitrotyrosine antibodies. Many of these methods are indirect and have a low specificity for NO, because they interfere with other components of biological systems (6, 7).

Griess colorimetric assay is the most commonly used method for the measurement of NO due to the low cost and simple operation (8). It has been used extensively in the analysis of several biological samples such as plasma (serum), urine, saliva, and cell culture media. The method is based on indirect quantification of NO *via* spectrophotometric measurement of its stable NO_2^-, using aqueous medium. Decomposition product NO is rapidly oxidized to NO_2^- by oxygen. The half-life of NO in biological matrix is very short, ranging from less than 1 sec, in the presence of hemoglobin, to ~30 sec. This method involves two steps: firstly NO_2^- reacts with sulphanilamide in acidic media to produce a diazonium ion, which in the next step reacts with *N*-(1-naphthyl)ethylenediamine to form a pink-red azo dye (fig. 2). The absorbance of this adduct at 540 nm is linearly proportional with NO_2^- concentration in the sample (6, 7).

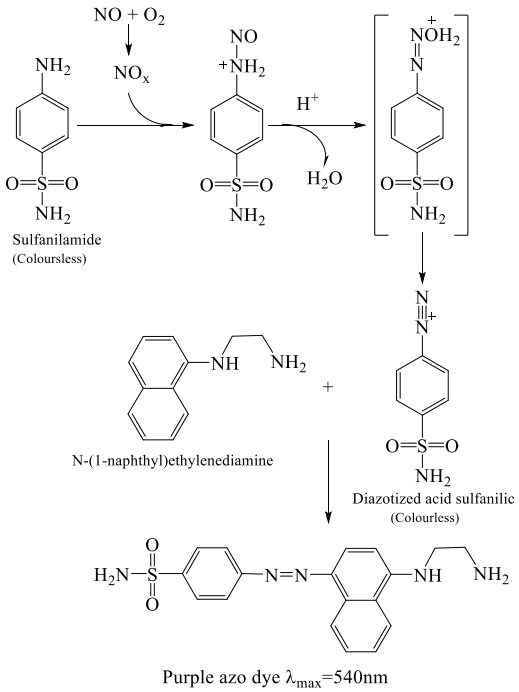

Fig. 2. Quantification of free nitric oxide using Griess colorimetric assay

In continuation of our efforts to discover new NO donors, new NO-NSAIDs have been synthesized by our research group, starting with indomethacin as conventional

NSAID. The aim of this study was to develop and validate a modified Griess assay which to increase the method sensitivity in hydro alcoholic medium and to be used for NO-NSAIDs that have a poor solubility in aqueous medium.

MATERIAL AND METHODS

Reagents and equipment

All used reagents and solvents were purchased from Sigma Aldrich and were of analytical reagent grade: sodium nitrite (>99% reference substance), phosphoric acid (≥85 wt. % in H_2O), sulfanilamide (SULF) (≥98%), *N*-(1-Naphthyl)ethylenediamine dihydrochloride (NED) (≥98%).

To measure the absorbance of the samples a micro plate reader (Tecan Sunrise Remote Microplate Reader TW/ML-Abbott F039306) was used, and the pH of phosphate buffer was measured with a Hanna Model Pack pH 21 digital pH-meter, calibrated with standard buffer solutions.

Preparation of standard stock solution

A fresh stock aqueous solution (0.1 M sodium nitrite in distilled water) was prepared by dissolving 6.9692 g of the reference substance in distilled water and diluting to 1,000 mL. This stock solution was standardized using the standard procedure reported in the *European Pharmacopoeia* and *Romanian Pharmacopoeia*, 10th edition (real molarity=0.0999, molarity factor=0.999). From this, a solution of 100 µM sodium nitrite was prepared by diluting 1 ml of the stock solution to 1000 ml with phosphate buffer (PBS) of pH = 7.4 (20mM in MeOH/H_2O=1/1 (*v/v*)).Working solutions, containing different sodium nitrite concentrations (100µM, 50µM, 25µM, 12.50µM, 6.25µM, 3.125µM, 1.56µM and 0.78µM) were prepared by dilution of solution of 100 µM sodium nitrite in PBS.

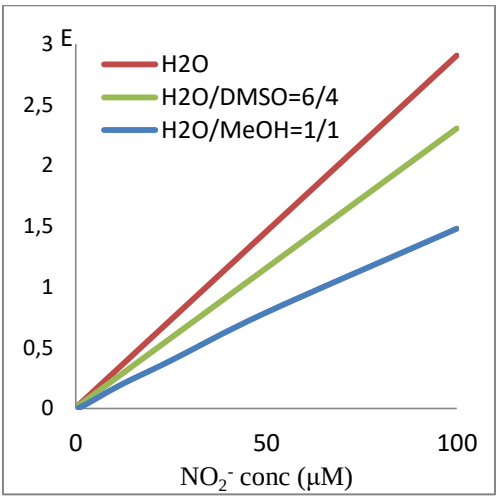

Fig. 3. Nitrite detection by the Griess assay using different solvents

Preparation of Griess reagents

The modified Griess reagent consists of NED solution (0.34%, *w/v*) in distilled water and SULF solution (3.4% *w/v*) in 10% (*w/v)* phosphoric acid.

Method validation

The developed method was validated by studying the following parameters: linearity, detection limit, quantification limit, precision, accuracy, in accordance with the guidelines on bioanalytical method validation (9-11).

Preparation of sodium nitrite standard curve

To avoid the precipitation of NO-NSAID derivatives and to allow hydrolysis of NO-releasing moiety different co-solvents like DMSO and MeOH were used, which were added to water. As noted in figure 3, the presence of the co-solvents interferes with the measurement of absorbance for the final azo dye compound and decreases the slope of the calibration curve for NO_2^-. Given the shorter assessment time, stability of the final azo dye, low cost of solvents, solubility of the NO-NSAID in a wide range of concentrations, we chose to

validate the modified Griess assay using MeOH as co-solvent.

In a micro plate reader, an aliquot of 170µL from the working sodium nitrite solution (in the range of 0.78-100µM) was added to 50µL SULF solution. After 10 min, 50µL NED solution was added and the absorbance of the formed pink-red azo dye was measured at λ_{max}=540 nm, after 20 min. A blank sample was prepared under the same conditions (170µL PBS, 50µL SULF and 50µL NED). The experiment was repeated 5 times for each concentration and the average absorbance was calculated. The calibration curve was constructed by plotting the average absorbance value *vs.* the corresponding nitrite concentration.

Precision

The precision of the method was studied as intraday and interday variation under optimal working conditions, by measuring the absorbance of the sample in triplicate. Three different concentrations of sodium nitrite (10µM, 25µM and 50µM), as reference substance, were used. The intraday precision was determined by absorbance measurements of each sample three times during the same day, while the interday precision was determined by absorbance measurements of the same sample for 3 days over a week.

Accuracy

In order to establish the accuracy of the developed method, different sodium nitrite stock solutions (20µM, 40µM and 60µM) were analyzed. For each sample4 determinations were performed.

Sensitivity

The sensitivity of the method developed for the measurement of $NO_2^-$was analyzed in terms of the limit of quantification and limit of detection.

RESULTS AND DISCUSSION

Linearity

Linearity was studied over a concentration range of 0.78-100 µM sodium nitrite. The average absorbance value was calculated for each concentration (tab. I) and was graphically represented vs. concentration (fig. 4). Statistical analysis of the experimental data leads to regression line equations. The linear regression data for the calibration curve showed good linear relationship over the concentration range. Linear regression equation was found to be f(x)= 0.0159*x + 0.0491.The standard error (SE) of regression curve and the *Pearson* correlation coefficient (r2) were also calculated.

TABLE I

Absorbance values of the samples, at different sodium nitrite concentrations

Concentration (µM)	Sample absorbance					
	Series 1	Series 2	Series 3	Series 4	Series 5	Average
100	1.64125	1.64325	1.643	1.64275	1.64	1.64205
50	0.8525	0.85375	0.854	0.85425	0.85625	0.85415
25	0.44975	0.45	0.45	0.45075	0.45025	0.45015
12.5	0.259	0.25925	0.259	0.25925	0.259	0.2591
6.25	0.1505	0.1505	0.1505	0.1505	0.15075	0.15055
3.125	0.0935	0.094	0.094	0.094	0.09475	0.09405
1.5625	0.06825	0.068	0.068	0.0685	0.06925	0.0684
0.78125	0.057	0.057	0.057	0.057	0.05775	0.05715
	Range 0.781-100 µM, Pearson coefficient (r^2)=0.99988					

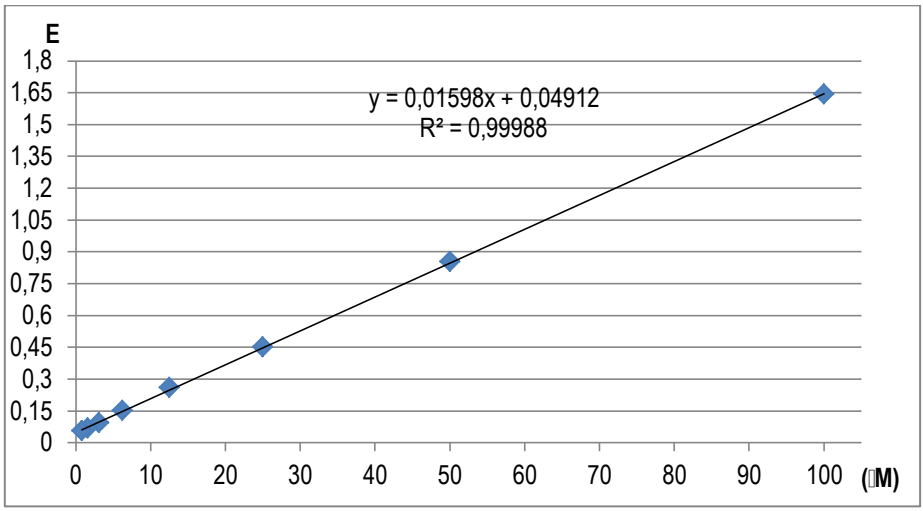

Fig. 4. Calibration curve for NO_2^- detection

Limit of quantification and detection

The limit of detection (LOD=3.3 x Standard error/Slope)was calculated to be 1.32 µM and the limit of quantification (LOQ=10 x Standard error/Slope*)* was 4.02 µM, meaning that our results are in agreement with other studies that used the calibration curve for quantitative measurements(12).

Accuracy

Sample concentration was calculated based on the experimental absorbance data and the regression curve equation, the results being presented in Table II. It was noticed that recovery was between 98.34% and 101.96% (100±2%) for the studied concentration range, and the coefficient of variation <2. The relative standard deviation (RSD) was also calculated. The results proved that the developed method has a high degree of accuracy.

TABLE II

The results related to the accuracy of the developed method

Concentration (µM)	Absorbance value	Calculated concentration (µM)	Recovery (%)	RSD (%)
20	0.375	20.393	101.96	1.38%
	0.365	19.767	98.84	
	0.371	20.143	100.71	
	0.367	19.892	99.46	
40	0.701	40.793	101.98	1.50%
	0.685	39.792	99.48	
	0.679	39.417	98.54	
	0.693	40.293	100.73	
60	1.017	60.568	100.95	1.53%
	0.992	59.004	98.34	
	0.999	59.442	99.07	
	1.023	60.944	101.57	

Precision

The precision could be expressed as the coefficient of variation (CV) or the relative standard deviation (RSD), and the results are presented in Table III. The obtained results support the reproducibility of the method. The RSD (%) values were less than 2%, confirming that the method has a high degree of accuracy and precision and thus can be used for the quantitative measurements of NO from different NO donors, such as NO-NSAIDs.

TABLE III

The results related to the precision of the developed method

Theoretic concentration (μM)	Intra-day precision		Inter-day precision	
	Concentration found (μM)	RSD (%)	Concentration found (μM)	RSD (%)
10	10.12	1.37%	9.87	1.69%
	9.95		10.2	
	9.85		9.97	
25	24.81	0.79%	24.76	0.80%
	24.96		24.88	
	25.2		25.15	
50	49.01	0.90%	48.95	0.75%
	48.85		49.62	
	49.68		49.55	

CONCLUSIONS

The developed UV-Vis method is quite simple, cheap, rapid, precise, accurate, reproducible, and sensitive for the determination of nitrite from different samples. It could be successfully used for the determination of NO released from new NO hybrid donors like NO-NSAIDs with high molecular weight and poor solubility in aqueous medium.

ACKNOWLEDGEMENTS

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NEWS

IRON DEFICIENCY AND ANEMIA AFTER BARIATRIC PROCEDURE - ROUX-EN-Y GASTRIC BYPASS

Obesity is a complex, multifactorial, and largely preventable disease, affecting, along with overweight, over a third of the world's population today. Treatment of obesity can be with diet, exercises and surgical treatment of obesity that involves restrictive surgical interventions like gastric sleeve, Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion. Bariatric procedure RYGB reduce capacity for food intake and absorption of energy from the food. The downside is that reduce the main sites for absorption of essential vitamins and minerals, like folate, vitamin B12, calcium and iron. Iron deficiency and anemia are common in the long run after RYGB. Major falls in iron stores appeared more than 2 years after surgery, when many patients are no longer followed. Individualized advice on iron supplements and access to intravenous iron treatment when iron stores were depleted seemed to reduce the frequency of anemia. These patients need for lifelong individualized iron supplements. A better access to intravenous iron treatment can contribute to improve health after RYGB. (Sandvik J, Hole T, Klockner CA, *et al*. Intravenous Iron Treatment in the Prevention of Iron Deficiency and Anemia After Roux-en-Y Gastric Bypass. *Obes Surg* 2020; 30(5): 1745-1752).

DESIGN, CHARACTERIZATION AND BIOCOMPATIBILITY EVALUATION OF POLYMERIC NETWORKS AS CARRIERS FOR INDOMETHACIN MODIFIED RELEASE

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DESIGN, CHARACTERIZATION AND BIOCOMPATIBILITY EVALUATION OF POLYMERIC NETWORKS AS CARRIERS FOR INDOMETHACIN MODIFIED RELEASE (Abstract): Pharmaceutical nanotechnology's progression expands drug versatility, utilizing carrier systems to efficiently deliver active ingredients to targeted tissues for controlled release within effective concentrations. Integrating nonsteroidal anti-inflammatory drugs (NSAIDs) into nano-systems holds potential for enhancing pharmacokinetic properties and diminishing adverse effects. **Aim**: Our study concentrated on formulating nanoparticles embedding indomethacin (IND), characterizing them, exploring drug release dynamics, and assessing their biocompatibility in rats. **Materials and methods:** IND was loaded within copolymeric networks comprising poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5.5]-undecane) and poly(aspartic acid) (PAS) as a protective colloid, employing a dispersion polymerization approach. Fourier transform infrared spectroscopy (FT-IR) characterized the copolymeric matrices, and spectrophotometric analysis via dissolution method evaluated in vitro IND release. In vivo biocompatibility was gauged by monitoring hematological, biochemical, and immune parameters in rats. **Results:** Our developed nano-systems effectively loaded IND within polymeric matrices. The kinetics of IND release were influenced by copolymer composition, with lower comonomer concentrations extending release duration. The investigated copolymer networks incorporating IND did not elicit significant hematological, biochemical, or immune changes when administered to rats. **Conclusions:** the studied polymer samples exhibited promising in vivo biocompatibility, positioning them as potential candidates for IND modified-release systems with prospective biomedical applications. **Keywords:** INDOMETHACIN, COPOLYMERS, MATRIX, IN VIVO BIOCOMPATIBILITY, RATS.

Nanotechnology is the scientific domain focused on manipulating matter at an almost atomic level to create fresh structures, materials, and devices. This technology holds the potential for significant advancements across various sectors, including medicine, consumer products, and industry. Nanomaterials typically range in size from 1 to 100 nanometers (1), and at this scale, they exhibit distinct properties that profoundly affect their physical, chemical, and biological characteristics (2). Pharmaceutical technology is constantly evolving, engaging in activities such as the preparation and manufacture of medicines to enhance pharmacological properties and minimize adverse effects. This aims to improve patient compliance with therapy and overall satisfaction with medical care (3).

Nanoparticles exhibit a higher cellular uptake efficiency compared to larger micro molecules, making them attractive candidates for efficient transport and delivery systems. In therapeutic contexts, drugs can be either incorporated within the particle matrix or affixed to the particle's surface. An effective drug targeting system should have the capacity to regulate the drug's behavior within the biological environment, particularly its pharmacokinetics (4,5).

Inflammatory diseases impact a considerable global population, and NSAIDs are frequently used in their treatment. In recent years, the utilization of nano-systems incorporating NSAIDs has shown promise in amplifying anti-inflammatory effects while reducing adverse effects (6). However, conventional NSAID treatments often entail weeks of administration, leading to undesired side effects and necessitating repeated dosing. NSAIDs function by inhibiting cyclooxygenase enzymes responsible for converting arachidonic acid into prostaglandins (7). Due to their short half-life and high protein binding, substantial doses of NSAIDs are required for efficacy, leading to adverse effects such as an increased risk of gastrointestinal and cardiovascular complications (8, 9).

In order to address these limitations, there is a growing interest in utilizing systems with dimensions ranging from a few tenths to several hundred nanometers. Nowadays, it is feasible to create biocompatible formulations with precise control over their shape, size, and surface charge, and to obtain innovative drug carrier for modified the NSAIDs release (10-14).

IND is an indole derivative NSAID, possessing anti-inflammatory, analgesic, and antipyretic properties. These effects are achieved by reducing the production of prostaglandins through the inhibition of cyclooxygenases. Notably, it exerts a more pronounced inhibition of COX-1 compared to the COX-2 enzyme (15).

Due to its strong anti-inflammatory properties and moderate analgesic effects, indomethacin is approved for use in both articular and extra-articular inflammations (such as pericarditis, pleurisy, and uveitis), dysmenorrhea, chronic pelvic pain, migraines, persistent arterial canal in newborns, and managing fever from Hodgkin's disease (7, 8). Literature indicates that indomethacin suppresses small intestinal motility and can inhibit stretch-dependent responses in visceral smooth muscle (16, 17), particularly in the gastrointestinal tract, mediated by mechanosensitive interstitial cells of Cajal located in the circular and longitudinal muscle layers (18). Indomethacin has also demonstrated beneficial effects in COVID-19 patients by counter-

acting the virus's mechanisms that exacerbate the disease. These mechanisms involve the inhibition of cyclooxygenases, regulation of the renin-angiotensin system, blocking of pro-apoptotic proteins, reduction of the excessive inflammatory response due to elevated bradykinin levels, and antiviral activity (19, 20)

Indomethacin belongs to Biopharmaceutics Classification System class II, characterized by its low solubility and high permeability (21). Given the limited utility of IND due to its adverse effects, particularly gastric irritation (22), integrating it into nano-systems and optimizing its release could yield several pharmacological advantages. This approach holds promise for reducing gastrointestinal side effects and fine-tuning its anti-inflammatory and antinociceptive effects following administration in laboratory animals (15).

Our **objective** was to develop polymeric networks capable of loading IND as carrier systems to facilitate modified drug release.

MATERIALS AND METHODS

Our research team synthesized matrices through copolymerization of poly 2-hydroxyethyl methacrylate (p-HM) with varying proportions of other polymers. We utilized poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5,5]-undecane) to create carrier systems for IND, incorporating three different concentrations of undecane (IND-U-1.75, IND-U-3.5, IND-U-10), effectively embedding IND (10 wt% of the copolymer weight, dissolved in ethanol/phosphate buffer solution at pH = 7.4). These macromolecular systems were synthesized via dispersion polymerization using 4,40-azobis (cyanopentanoic acid) as a radical initiator, alongside sodium lauryl sulfate as a surfactant and poly(aspartic acid) as a water-soluble condensation biocompatible and biodegradable polymer, which acted as a colloidal protector (23). This addition enhanced the absorption capacity of the network, bolstered its stability, and conferred resistance to chemical degradation, owing to the highly cross-linked nature of poly(aspartic acid), characterized by an amine-terminated aliphatic polyurea structure. These copolymer systems, incorporating IND, underwent physicochemical characterization. The presence of IND within the copolymer matrices was assessed using FT-IR. The in vitro drug release profile was determined spectrophotometrically via the dissolution method.

The biocompatibility of these nano-systems was examined in vivo by assessing their impact on various hematological, biochemical, and immune parameters in rats. Four groups, each comprising five healthy, non-genetically modified white male Wistar rats, were utilized. These rats were administered the test substances orally in a single dose using an eso-gastric tube. The groups included: C (control, receiving distilled water at a dosage of 0.1 ml/100 g body weight); p-HM (receiving p-HM); IND-U-1.75 (receiving IND-U-1.75); and IND-U-10 (receiving IND-U-10). One day and one week following the administration of the substances, 0.3 mL of blood was drawn from the lateral tail vein for laboratory analyses. The following determinations were conducted: leukocyte formula (including polymorphonuclear - PMN, lymphocytes - Ly, eosinophils - E, monocytes - M, basophils - B), serum levels of aspartate aminotransferase (AST),

alanine aminotransferase (ALT), urea, creatinine, complement, and the phagocytosis capacity of peripheral blood polymorphonuclear neutrophils (PMN), assessed through the nitroblue tetrazolium reduction (NBT) test. These investigations aimed to evaluate the impact of the tested substances on liver and kidney function, as well as the immune defense capacity of the laboratory animals.

The *in vivo* experiment protocol adhered to the guidelines set forth by the Ethics Commission of the "Grigore T. Popa" University of Medicine and Pharmacy from Iasi, aligning with current European ethical regulations (24).

RESULTS

Polymeric networks containing IND were synthesized earlier with varying ratios of comonomers (23). We selected two of these networks for examination concerning their in vivo biocompatibility in rats. The aim is to utilize them subsequently to explore their impacts on inflammatory responses in acute and subacute inflammation models, which are experimentally induced in the laboratory.

Polymeric networks loaded with IND have been synthesized and characterized. FT-IR spectroscopy confirmed the presence of both comonomers, the conformation of the bioactive compound, and the loading process of IND onto the polymeric matrix. The absorption bands at 532 cm^{-1} and 927 cm^{-1}, alongside the shifting of major peaks of the polymers, verified the uptake of the drug by the two distinct networks (fig. 1).

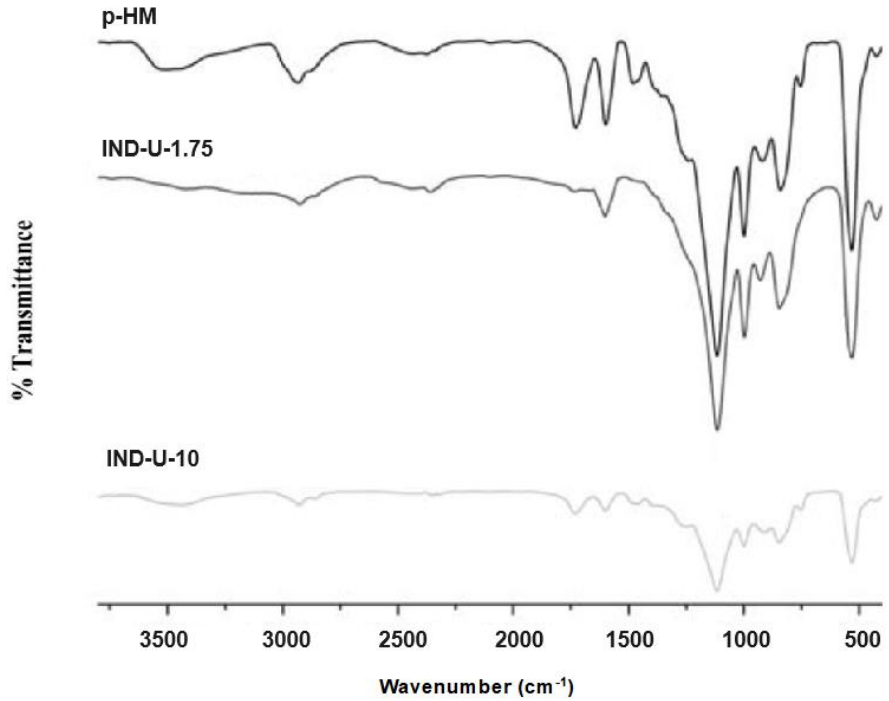

Fig. 1. FT-IR spectra of the copolymeric networks incorporating IND

DISCUSSION

Examining the in vitro kinetic profile of IND reveals that the percentage of undecane significantly impacts the drug release rate from the polymer matrices. Specifically, nearly 60% of the IND quantity is released from the IND-U-1.75 copolymer within the initial 120 minutes, and over 90% within 500 minutes, reaching a release plateau after 750 minutes. Conversely, in the system with a higher concentration of undecane (IND-U-10), the drug is released at a notably slower rate over time, with no observed release plateau even after 2500 minutes (fig. 2).

After one day, as well as after seven days in the experiment, no significant differences were observed in the percentage values of PMN, Ly, E, M, and B between groups C, p-HM, IND-U-1.75, and IND-U-10 (tab. I).

No notable changes were observed in the activity of ALT, AST, urea, and creatinine serum levels among the groups of animals studied. This indicates that the administration of the tested substances did not evidently impact the liver and kidney function at any of the assessment time points (tab. II).

Serum complement activity and phagocytosis capacity of PMN remained relatively stable after both 24 hours and one week in all studied groups. This implies that the investigated nano-systems had minimal impact on the immune defense capacity of the rats (tab. III).

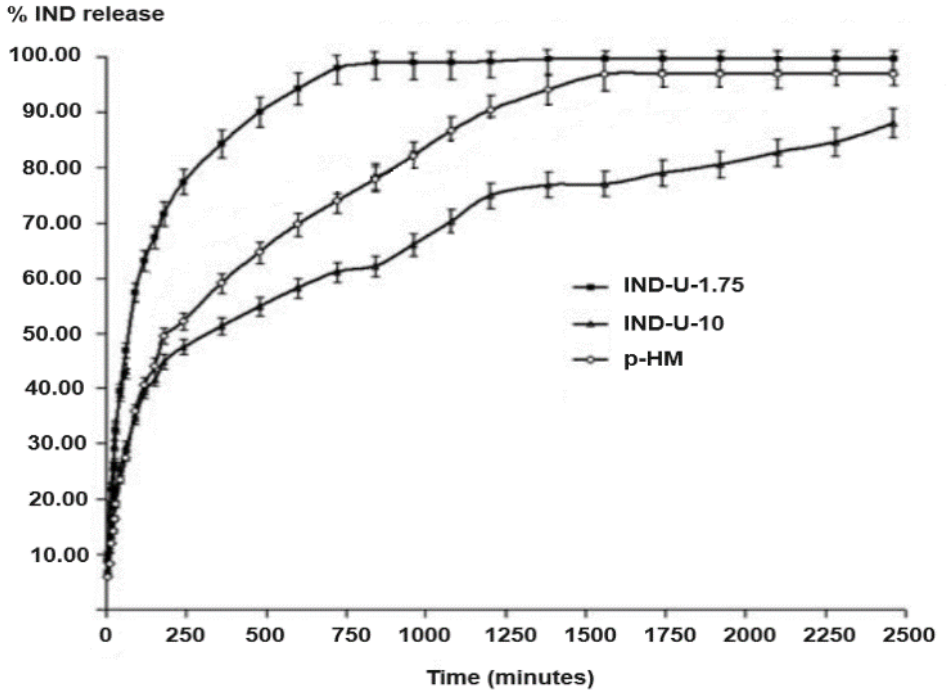

Fig. 2. The in vitro kinetic profile of IND from copolymeric carriers

TABLE I.

Evaluation of the polymeric networks-loading IND influence on the percentages of leukocyte formula elements

		White blood count (%)				
		PMN	Ly	E	M	B
C	1 day	26.32±6.5	67.55±9.1	0.4±0.03	5.34±0.5	0.1±0.01
	7 days	27.16±5.1	66.42±9.5	0.3±0.05	5.38±0.3	0.2±0.01
p-HM	1 day	27.06±5.9	67.38±8.7	0.3±0.05	5.44±0.5	0.2±0.01
	7 days	27.23±5.7	66.46±9.3	0.3±0.03	6.06±0.5	0.2±0.03
IND-U-1.75	1 day	26.59±6.3	67.23±9.5	0.4±0.03	5.23±0.5	0.1±0.01
	7 days	27.63±6.1	67.16±9.1	0.3±0.03	5.32±0.3	0.2±0.03
IND-U-10	1 day	26.38±5.5	67.44±8.7	0.3±0.05	5.46±0.7	0.2±0.01
	7 days	27.47±5.3	66.63±9.3	0.4±0.03	5.14±0.5	0.1±0.01

TABLE II.

Evaluation of the polymeric networks-loading IND influence on the blood values of ALT, AST, urea and creatinine

		ALT (U/ml)	AST (U/ml)	urea (mg/mL)	creatinine (mg/dL)
C	1 day	44.42±5.5	95.52±8.3	38.44±3.5	0.34±0.01
	7 days	44.38±5.3	95.33±9.5	39.14±3.3	0.33±0.02
p-HM	1 day	44.63±5.7	96.48±9.7	39.23±3.7	0.34±0.02
	7 days	45.06±6.1	95.63±9.5	39.63±2.9	0.35±0.01
IND-U-1.75	1 day	44.63±5.3	96.16±8.9	38.63±4.3	0.35±0.01
	7 days	44.87±6.3	96.44±9.7	40.48±3.3	0.36±0.01
IND-U-10	1 day	44.46±5.5	95.59±9.3	39.23±3.7	0.35±0.02
	7 days	45.23±5.7	96.38±9.1	40.06±3.5	0.35±0.01

TABLE III.

Evaluation of the polymeric networks-loading IND influence on the serum complement activity and the phagocytic capacity of PMN

		NBT (%)	Complement (UCH50)
C	1 day	14.33±3.3	56.76±6.37
	7 days	14.59±2.7	57.63±7.29
p-HM	1 day	14.48±3.5	58.44±9.17
	7 days	14.23±3.1	58.16±8.83
IND-U-1.75	1 day	14.06±2.9	57.33±8.29
	7 days	14.63±3.7	58.42±8.32
IND-U-10	1 day	14.16±2.3	57.59±7.22
	7 days	14.42±3.5	58.38±8.47

Considering the restricted application of IND owing to its adverse effects, notably gastric irritation, incorporating it into nano-systems and refining its release mechanism could offer several pharmacological benefits. This strategy shows potential for mitigating gastrointestinal side effects and optimizing its anti-inflammatory and antinociceptive effects upon administration in laboratory animals. Literature sources have revealed diverse strategies for developing nano-formulations incorporating IND, employing either preformed polymers or lipids (25).

Over time, a variety of strategies have been employed to develop nano-systems for the transport and release of IND, including:

- incorporation of phospholipids or lipid emulsions with IND, utilizing lipid, surfactant, and co-solvent mixtures (26,27);
- formulation of liposomal IND, encompassing plurilamellar monophasic vesicles with components such as stearylamines, cholesterol, or chitosan, as well as sterically stabilized liposomes (28,29);
- development of bioconjugates of IND, including triacylglycerides containing indomethacin, bioconjugates with phosphatidylcholine, palmitic acid, and stearic acid (30,31);
- development of enteric nanoparticles containing IND, utilizing materials such as EUDRAGIT® L100, polyethylene glycol, and polysorbate 80 (32);
- preparation of amorphous nanosuspensions containing IND stabilized with polyvinylpyrrolidone, achieved through the aqueous wet milling method (33).
- obtaining of chitosan nanoparticles with IND through the ion gelation method by adding an aqueous solution of tripolyphosphate anions (34).

CONCLUSIONS

Polymeric matrices composed of poly(2-hydroxyethyl methacrylate-co-3,9-divinyl-2,4,8,10-tetraoxaspiro [5,5]-undecane) and poly(aspartic) acid were synthesized as platforms for the controlled release of IND. The kinetic profile of IND is contingent upon the copolymer composition, wherein a lower concentration of comonomer leads to a prolongation in drug release duration. Treatment with both types of copolymer networks incorporating IND did not induce significant hematological, biochemical, or immune alterations, indicating favorable in vivo biocompatibility. This suggests that these polymeric systems hold potential for controlled drug release, with potential applications in the biomedical field.

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QUALITATIVE ASSESSMENT OF PRE- AND PRO-PROSTHETIC REHABILITATION OUTCOMES IN EXTENSIVE PARTIAL EDENTULISM

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QUALITATIVE ASSESSMENT OF PRE- AND PRO-PROSTHETIC REHABILITATION OUTCOMES IN EXTENSIVE PARTIAL EDENTULISM (Abstract): **Aim** of this study was to evaluate the outcomes of the pre- and post-prosthetic rehabilitation of the prosthetic field status in patients with extensive partial edentulism. **Materials and methods:** This prospective research was conducted on a cohort of 194 patients (mean age 56.46 years ± 0.738 years). The study group included patients with extensive partial edentulism candidate to removable prosthetic treatment or implant-prosthetic therapy. The evaluation of the prosthetic field status during the pre-treatment stage and at the completion of the pre- and post-prosthetic stages was conducted by quantifying the variables associated with the following clinical-biological indices: general status; dental support; periodontal support; bone support; mucous support; occlusion support; mandibulo-cranial support. **Results:** Highly significant differences were recorded between primary and secondary clinical-biological indices after pre- and pro-prosthetic treatment. In the study group, 57.7% of cases remained steady, while 34.5% improved by 25%, and 7.7% by 50%. No cases reached a 75% improvement. **Conclusions:** Significant improvements were observed across most indices, particularly in secondary scores. Clinical indices, such as occlusion and mandibulo-cranial relationships, are the most responsive to pre- and post-prosthetic interventions. **Keywords:** EXTENSIVE PARTIAL EDENTULISM, PRE-PROSTHETIC, PRO-PROSTHETIC, REHABILITATION.

INTRODUCTION

Edentulism is recognized as a major public health issue, considering projections that the proportion of the population over 65 years old will significantly increase to 26% by 2030 (1, 2). The complications of extensive partial edentulism include dental migrations, extrusions, facial changes, and temporomandibular disorders (3). A successful prosthetic treatment must focus on the possibilities to improve all components of stomatognathic system muco-osseous addressing the common complications of the partial extensive edentulism (such as malocclusion, mandibular latero-deviations, TMJ pain, muscular disorders, abnormal cranial-mandibular relationships (3). Thus, pre- and pro-prosthetic stages are essential for correcting the unfavorable clinical-

biological indices of the prosthetic field, considering the inter-connection of the components of the stomatognathic system (4). Thus, optimal biomechanical stability of the prosthetic restorations can be reached only with favorable clinic-biological indices of the prosthetic field. Pre-prosthetic and pro-prosthetic procedures must address the potential etiologies from different perspectives (5), while the dental practitioner must have a comprehensive understanding of the status prosthetic field components status, including remaining teeth, periodontal indices, mucosal-osseus support, occlusion, as well as cranio-mandibular relationship (6). Alveolar bone resorption and unregular residual ridge are the most important complications of extensive partial edentulism. Also, occlusion and cranio-mandibular relationships are directly influenced by edentulism complications such as temporo-mandibular joint (TMJ) pathology (7). Systemic status (neuromuscular control, psychological state, overall health resilience) (8), local factors such as number and position of missing teeth, mandibular movement patterns, as well as financial criteria or patient' preferences must be considered in the planning of the prosthetic treatment (9).

AIM OF STUDY

The study aimed to evaluate the outcomes of the pre- and post-prosthetic rehabilitation of the prosthetic field status in patients with extensive partial edentulism.

MATERIALS AND METHODS

The prospective research was conducted on a cohort of 194 patients (age: mean age 56.46 years ± 0.738 years, range 41-78 years; gender: 105 men, 89 women). The study included patients with extensive partial edentulism candidate to removable prosthetic treatment in Clinical Base of Faculty of Dental Medicine, "Grigore T. Popa" University of Medicine and Pharmacy Iasi.

The evaluation of the prosthetic field status during the pre-treatment stage and at the completion of the pre- and post-prosthetic stages was conducted by quantifying the variables associated with the following clinical-biological indices:

- General status (GSI);
- Dental support (DSI);
- Periodontal support (PSI);
- Bone support (BSI);
- Mucous support (MSI);
- Occlusion support (OSI);
- Mandibulo-cranial support (MCSI).

The clinical-biological indices were measured using the clinical scoring scale developed within the Department of Extensive Partial Edentulism and Removable Restorations, Faculty of Dental Medicine, "Grigore T. Popa" U.M.Ph. Iași:

- 1 (low),
- 2 (medium),
- 3 (good),
- 4 (very good).

The status of the prosthetic field was evaluated during the pre-treatment stage (primary indices) and upon completion of the pre- and post-prosthetic stages (secondary indices). The quantification of clinical-biological indices during the initial stage outlines the therapeutic project framework, which serves as the basis for selecting non-specific and specific prosthetic field preparation procedures. The statistical analysis of the collected data was performed using *SPSS version 27.0*. The Marginal Homogeneity Test was used to compare primary

and secondary indices and assess statistical significance.

RESULTS

The table 1 presents the distribution of scores across different indices (primary and secondary), highlighting changes in general status, dental support, periodontal support, mucosal support, bone support, occlusion, mandibulo-cranial relationship, and total score. The results are analyzed using the Marginal Homogeneity Test, with significant differences noted for most indices.

General Status Indices (GSI):

-For the primary index, 90.2% of cases scored as "Very Good," while 9.8% scored as "Good."

-For the secondary index, 91.8% of cases scored as "Very Good," and 8.2% as "Good." No significant difference was observed when it was compared with primary GSI ($p = 0.083$).

Dental Support Indices (DSI):

-In the primary index, the scores were distributed as 10.3% "Very Good," 61.3% "Good," and 28.4% "Medium."

-For the secondary index, 45.4% scored as "Good" and "Very Good," respectively, with 9.3% scoring "Medium." A significant difference was observed when it was compared with primary DSI ($p < 0.001$).

Periodontal Support Indices (PSI):

-For the primary index, 57.7% of cases were "Very Good," 34% "Good," and 8.2% "Medium."

-The secondary index scored as 82.5% "Very Good," 16% "Good," and 1.5% "Medium." The difference was highly significant when compared with primary index ($p < 0.001$).

Mucosal Support Indices (MSI):

In the primary index, 28.9% scored as "Very Good," 59.8% as "Good," and 11.3% as "Medium."

For the secondary index, 35.6% scored "Very Good," 63.4% "Good," and 1% "Medium," with significant differences between primary and secondary indices ($p < 0.001$).

Bone Support Indices (BSI):

Primary scores were 38.7% "Very Good," 48.5% "Good," and 12.9% "Medium."

Secondary scores showed 46.9% "Very Good," 44.3% "Good," and 8.8% "Medium."

The differences were significant between primary and secondary indices ($p < 0.001$).

Occlusion support indices (OSI):

-For the primary index, 84% were classified as "Low," 6.7% as "Medium," 7.7% as "Good," and only 1.5% as "Very Good."

-Secondary scores shifted notably, with 44.3% as "Low," 15.5% as "Good," and 34% as "Very Good." This change was highly significant ($p < 0.001$).

Mandibulo-Cranial Relationship indices (MCRI):

-In the primary index, 33% scored "Very Good," 10.8% "Good," 7.7% "Medium," and 48.5% "Low."

-Secondary scores showed a significant improvement, with 57.7% as "Very Good," 8.8% as "Good," and only 23.2% as "Low" ($p < 0.001$).

Total Score:

-The primary index recorded 9.3% "Very Good," 69.6% "Good," and 21.1% "Medium."

-Secondary scores showed a shift, with 45.4% "Very Good," 47.4% "Good," and 7.2% "Medium," with highly significant difference when compared to primary index ($p < 0.001$).

TABLE I.

Qualitative scores of the prosthetic field (pre- *vs.* post-treatment)

SCORES		Low		Medium		Good		Very good		Marginal Homogeneity test
		N	%	n	%	n	%	n	%	
General status	Primary					19	9.8	175	90.2	p = 0.083
	Secondary					16	8.2	178	91.8	
Dental support	Primary			55	28.4	119	61.3	20	10.3	p < 0.001**
	Secondary			18	9.3	88	45.4	88	45.4	
Periodontal support	Primary			16	8.2	66	34.0	112	57.7	p < 0.001**
	Secondary			3	1.5	31	16.0	160	82.5	
Mucous support	Primary			22	11.3	116	59.8	56	28.9	p < 0.001**
	Secondary			2	1.0	123	63.4	69	35.6	
Bone support	Primary			25	12.9	94	48.5	75	38.7	p < 0.001**
	Secondary			17	8.8	86	44.3	91	46.9	
Occlusion	Primary	163	84.0	13	6.7	15	7.7	3	1.5	p < 0.001**
	Secondary	86	44.3	12	6.2	30	15.5	66	34.0	
Mandibulo-cranial relationship	Primary	94	48.5	15	7.7	21	10.8	64	33.0	p < 0.001**
	Secondary	45	23.2	20	10.3	17	8.8	112	57.7	
TOTAL SCORE	Primary			41	21.1	135	69.6	18	9.3	p < 0.001**
	Secondary			14	7.2	92	47.4	88	45.4	

The distribution of the clinical biological indices changes after the pre- and post-prosthetic stages is exposed further (Table 2; Fig.1). For DSI, 48.5% of cases maintained a steady state, while 49% exhibited a 25% improvement, and 2.6% showed a 50% improvement. No cases achieved a 75% improvement. For PSI, 70.6% of cases remained steady. Improvements were observed in 27.3% of cases at 25%, and 2.1% at 50%. No cases reached a 75% improvement. For MSI, 84.5% of cases remained steady, while 13.9% showed a 25% improvement, and 1.5% demonstrated a 50% improvement. There were no cases with a 75% improvement. For BSI, majority (88.7%) showed no change, while 10.3% experienced a 25% improvement, and 1.0% achieved a 50% improvement. No cases reached 75% improvement. Occlusal indices showed more dynamic changes: 52.1% remained steady, while 7.7% improved by 25%, 16.0% by 50%, and 24.2% by 75%. This index demonstrated the highest rate of significant improvement. For mandibulo-cranial relationships, 64.9% of cases showed no change, with 13.9% improving by 25%, 4.6% by 50%, and 16.5% achieving a 75% improvement. In the study group, 57.7% of cases remained steady, while 34.5% improved by 25%, and 7.7% by 50%. No cases reached a 75% improvement.

TABLE II.

Qualitative scores changes during pre- and pro-prosthetic treatment

SCORES CHANGES	Steady		Improvement 25%		Improvement 50%		Improvement 75%	
	n	%	n	%	n	%	n	%
General status	191	98.5	3	1.5				
Dental support	94	48.5	95	49.0	5	2.6		
Periodontal support	137	70.6	53	27.3	4	2.1		
Mucous support	164	84.5	27	13.9	3	1.5		
Bone support	172	88.7	20	10.3	2	1.0		
Occlusion	101	52.1	15	7.7	31	16.0	47	24.2
Mandibulo-cranial relationships	126	64.9	27	13.9	9	4.6	32	16.5
SCOR TOTAL	112	57.7	67	34.5	15	7.7		

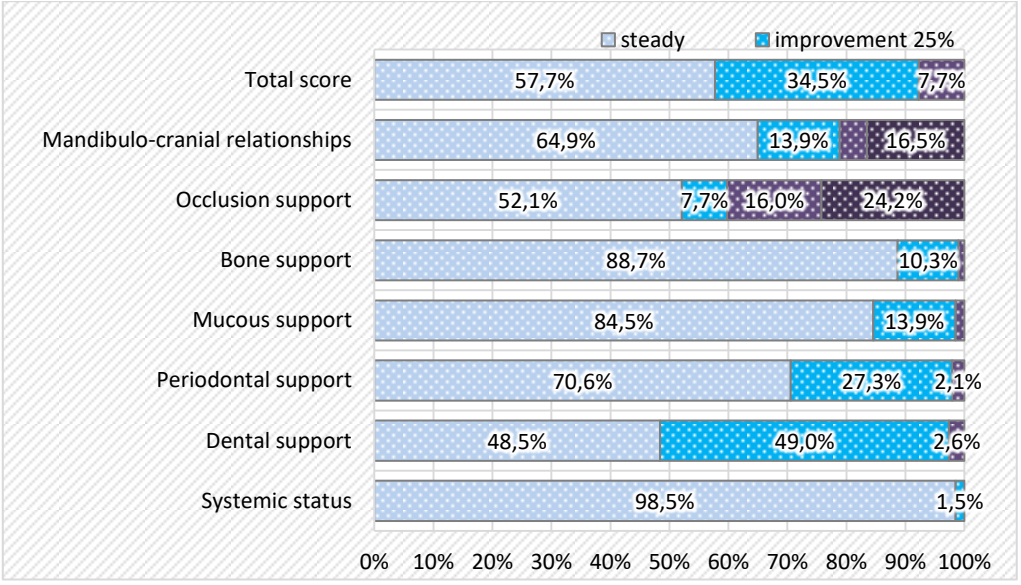

Fig. 1. Changes of clinical biological indices of prosthetic field after pre- and pro-prosthetic stages

DISCUSSION

Various studies have analyzed the prevalence of extensive partial edentulism and its implications, but a review highlighted a lack of research exploring the correlations between edentulism and biological factors (10). The evaluation of the prosthetic field involves important anatomical and physiological aspects, along with the identification of optimal strategies for the pre- and post-prosthetic stages. Ensuring the stability of the prosthetic field in edentulous patients before implementing prosthetic treatments is

essential (11).

In this context, the aim of our research was to use of a qualitative scoring system developed at the Faculty of Medicine, "Grigore T. Popa" U.M.Ph. Iași, in the evaluation of the outcomes of pre- and post-prosthetic preparation of the prosthetic field. A significant improvement in odontal support indices can be achieved through the treatment of carious lesions, non-carious lesions, and crown reshaping or resurfacing (12). Similarly, significant improvement in periodontal support indices is achieved through non-surgical therapy for periodontal disease, sometimes combined with specific surgical procedures during the post-prosthetic stage (flap surgeries, grafts, or guided tissue regeneration techniques) (13). Improvements in mucosal and bone support indices can be achieved through mucosal grafting interventions and bone augmentation procedures (14). Diagnosing occlusal and mandibulo-cranial relationship disorders is essential for prosthetic treatment planning and ensuring a favorable prognosis for future prosthetic restorations (15). Interventions aimed at improving occlusal indices, combined with transitional prosthetics, have allowed normalization of mandibulo-cranial relationships. When mandibular-cranial misalignment occurs, dysfunctions of the stomatognathic system are exacerbated, restricting therapeutic options (16). Improving occlusal indices is also necessary to avoid instability, aesthetic compromises, and inadequate contours of future dentures (17).

The relevance of clinical and biological indices as tools for planning prosthetic treatment in patients with extensive partial edentulism has been highlighted by similar studies utilizing expert systems (18-20). The scoring system used in this study can be further validated and refined in future research with larger study groups and various types of edentulism and prosthetic therapeutic solutions. Qualitative scoring systems can also be incorporated into expert systems or AI-based applications to optimize long-term outcomes of prosthetic treatments.

CONCLUSIONS

The qualitative scoring system allows the planning of the pre- and pro-prosthetic interventions requested to improve unfavorable prosthetic field of the edentulous patients as well as the evaluation of the outcomes of pre-prosthetic and pro-prosthetic therapeutic procedures in the rehabilitation of the prosthetic field for patients with extensive partial edentulism. Significant improvements were observed across most indices, particularly in secondary scores. Clinical indices, such as occlusion and mandibulo-cranial relationships, are the most responsive to pre- and post-prosthetic interventions. These changes reflect advancements in prosthetic rehabilitation outcomes, with notable improvements in periodontal, mucosal, and bone support, occlusion, mandibulo-cranial relationships, and overall scores.

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PATIENT RELATED PROGNOSTIC FACTORS IN HEAD NECK CANCERS: COMORBIDITIES AND AGE - FOCUS ON TUBERCULOSIS AND ON GERIATRIC ONCOLOGY

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PATIENT RELATED PROGNOSTIC FACTORS IN HEAD NECK CANCERS: COMORBIDITIES AND AGE-FOCUS ON TUBERCULOSIS AND ON GERIATRIC ONCOLOGY (Abstract): Head and neck cancers are often diagnosed in the late stages, the evolution being often unfavorable even if the multimodal treatment is administered correctly. In principle, it can be considered that there are three categories of factors involved in prognosis: patient, tumor and treatment related-factors. The **aim** of the study was to highlight the involvement of patient-related factors in the prognosis of locally advanced, recurrent and metastatic head and neck cancers. **Materials and methods:** After the initial selection, including the identification of cases treated in the Oncology Clinic and the Oncology outpatient clinic, the study group included 190 patients. Among them, after applying the general inclusion and exclusion criteria, 149 cases of head and neck cancers were selected. Patients admitted to the oncological record in the period 2002-2021 with all anatomical locations of head and neck cancers, stage III and IV: nasopharynx, oropharynx, larynx, hypopharynx, oral cavity, glands salivation, nasal cavity and paranasal sinuses, cervical lymph node metastases of unspecified starting point - Cancers with Unknown Primary (CUP). **Results:** The presence of comorbidities in the case of patients with head and neck cancer, but especially pulmonary tuberculosis, active, reactivated or sequelae, is associated with an unfavorable prognosis. Studies are needed to analyze in more detail the implication of the association between pulmonary tuberculosis and each subtype of head and neck cancer. **Conclusions:** There is an increased incidence of neurological pathologies, especially stroke compared to the results published in the literature. In our study, age >65 years was not identified as a significant prognostic factor, but a tendency to reduce survival to more than 2 years in the case of geriatric patients should be mentioned. **Keywords:** HEAD AND NECK CANCERS, PROGNOSTIC, OS, COMORBIDITIES, TUBERCULOSIS, GERIATRIC.

INTRODUCTION

Head and neck cancers are often diagnosed in the late stages, the evolution being often unfavorable even if the multimodal treatment is administered correctly. In principle, it can be considered that there are three categories of factors involved in prognosis: patient, tumor and treatment

related-factors. If the histological tumor type, TNM staging, the anatomical tumor site, the biological and genetic profile and the type of treatment are factors recognized as being involved in the prognosis, the characteristics of the patient including age, gender, and comorbidities are less evaluated, without a consensus in the reported results. These factors could be directly involved in the prognosis but could also limit the option of oncological active therapies. If the impact of age is currently the subject of studies promoted by the Geriatric Oncology Society (SIOG), there seems to be a mutually reinforcing relationship between tuberculosis and head and neck cancer. Comorbidities are evaluated by doctors within prognostic scores and less individually (1-4).

The aim of the study was to highlight the involvement of patient-related factors in the prognosis of locally advanced, recurrent and metastatic head and neck cancers.

MATERIALS AND METHODS

After the initial selection, including the identification of cases treated in the Oncology Clinic and the Oncology outpatient clinic, the study group included 190 patients. Among them, after applying the general inclusion and exclusion criteria, 149 cases of head and neck cancers were selected.

Inclusion criteria

- Patients admitted to the oncological record in the period 2002-2021 with all anatomical locations of head and neck cancers, stage III and IV according to the International Classification of Diseases (ICD) version 10 of 2019: nasopharynx, oropharynx, larynx, hypopharynx, oral cavity, glands salivation, nasal cavity and paranasal sinuses, cervical lymph node metastases of unspecified starting point - Cancers with Unknown Primary (CUP);
- Patients for whom there was histopathological confirmation of the disease;
- Patients for whom the date of death is known;
- Patients staged according to AJCC TNM version 6;
- Patients who have undergone at least one type of active oncological treatment for curative or palliative purposes;
- Synchronous and metachronous cancers provided that the first location is in the head and neck area.

Exclusion criteria

- Cases declared oncological for objective reasons without histopathological confirmation;
- Patients alive or lost from evidence at the time of analysis;
- Patients for whom there are no data on the oncological therapies administered or who have only benefited from symptomatic palliative treatment;
- Patients with CUP with metastases in other anatomical regions than the cervical one;
- Patients diagnosed with another malignancy prior to the diagnosis of head and neck cancer;
- Patients with skin carcinomas including malignant melanoma in the head and neck region;
- After applying the general inclusion and exclusion criteria in the study group which included 147 cases, the mean and median age of the patients was 69.1 years, respectively 62 (32-88) years.

RESULTS

Median overall survival (OS) in the study lot was 10 months (1-192) (figs. 1, 2).

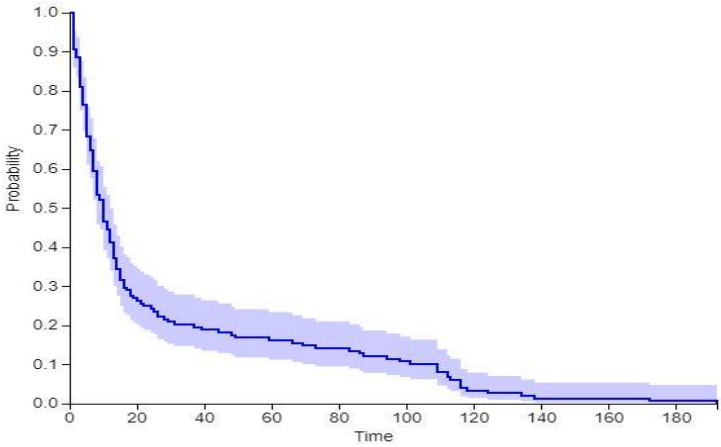

Fig. 1. Kaplan-Meier curves analyzing survival in head and neck cancers

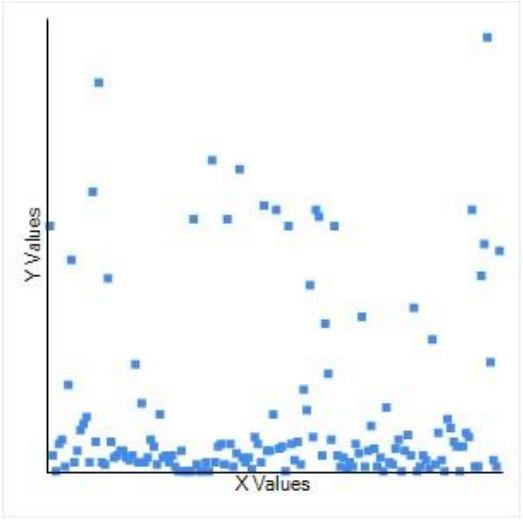

Fig. 2. Relationship of age (horizontal axis) with survival expressed in months (vertical axis)

The male gender was predominant in the study group, 129 cases (86.57%) being men and 19 (12.75%) females. The mean and median age of male patients was 61.4 and 62 (32-81) years, respectively. Median survival for the male patient group was 10 (1-192) months. Mean and median age of female patients was 65.1 and 63,5 (45-88) years, respectively, and median survival was 5 (1-113) months (fig. 3).

Only one case (0.7%), 66 years old, in the study group was diagnosed and treated for a nasopharyngeal neoplasm, with a median survival of 39 months. The second least common type of head and neck neoplasm was hypo-pharyngeal cancer, diag-

nosed in 17 cases (11.5%). The mean and median age was 63 and 61.5 (41-82) years, respectively, and the median OS was 5(1-112) months. CUP with cervical metastases were diagnosed in 18 cases (12%). The mean and median age was 63 and 61 (41-82) years, respectively, with a median survival of 6 (1-124) months in this cancer subtype. A number of 43 cases representing 28.9% of the total were located in the oral cavity, the mean and median age being 62.3 and 61 (32-88) years, respectively, and the median survival of 8 months (1-116) months. In the case of oropharyngeal cancers, the median OS of 10 (1-192 months) was identified in the group of 25 patients (16.8%) with mean and median age of 60.1 and 60 (44-79) years, respectively. The use of at least one of the treatments with a potential curative purpose (surgery, chemotherapy, radiotherapy) was a mandatory inclusion criterion.

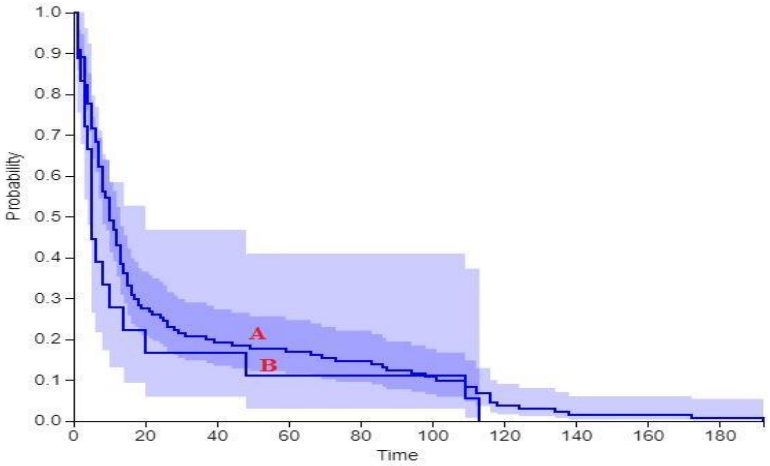

Fig. 3. Kaplan-Meier curves comparing survival in head and neck cancers by gender: A - men, B - female

The comorbidities reported (declaratively or based on the records of the medical documents presented by the patient) were identified in 62 cases (41.6%), 87 of the patients (53.38%) not declaring the presence of known pathologies. With a P=0.427 value, the association of comorbidities with median survival does not show statistical significance. In the group of patients without comorbidities, the median survival was 10 (1-138) months, and in the group of patients with comorbidities median OS was 11 (1-192) months. Hypertension, ischemic heart disease, type II diabetes mellitus (DM), and history of stroke were the most frequently reported associated diseases (figs. 4, 5). The presence of pulmonary tuberculosis either in medical history, as a disease reactivated during multimodal treatment, or identified as imaging sequela has been evaluated as a predictor of survival in head and neck cancers. Even though a negative correlation of pulmonary tuberculosis with prognosis was identified, it was not considered statistically significant (P=0.55). Active pulmonary tuberculosis or sequela of tuberculosis were identified in 13 cases (8.72%), the remaining 136

(91,27%) being cases without other comorbidities other than tuberculosis. The mean and median age of patients diagnosed with tuberculosis was 55,8 and 55 (32-71) years, respectively, and that of patients without a history of tuberculosis or active disease of 62,4 and 62 (41-88) years, respectively (figs. 6, 7).

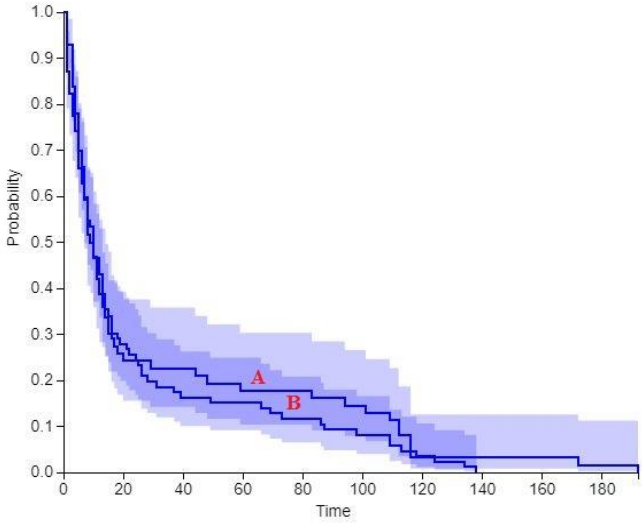

Fig. 4. Kaplan-Meier curves comparing survival in head and neck cancers according to the absence (A) or presence (B) of comorbidities

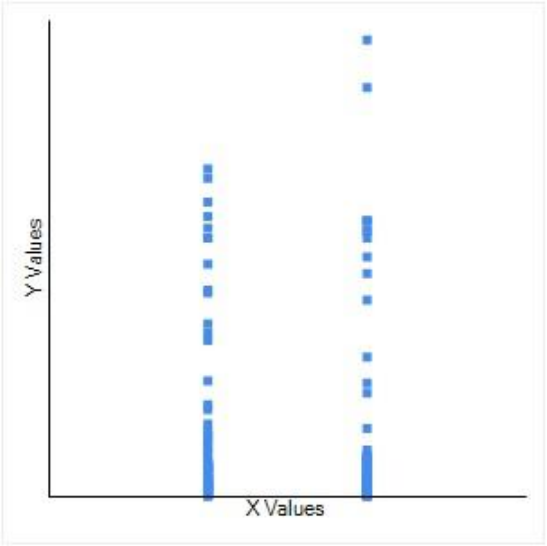

Fig. 5. Correlation of the presence of comorbidities (horizontal axis: left – no declared comorbidities, right – one or more comorbidities present) with survival expressed in months (vertical axis)

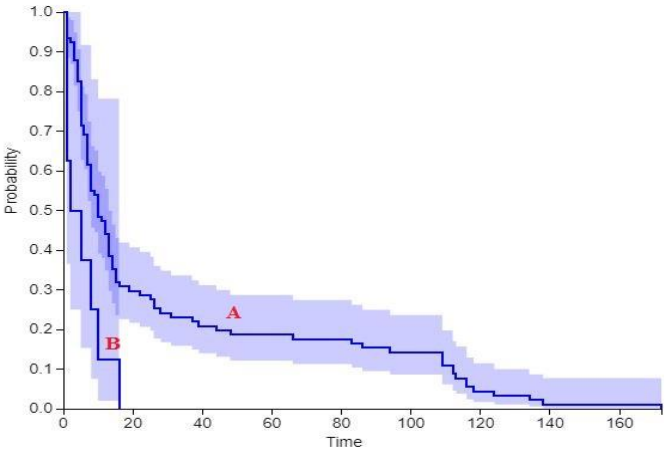

Fig. 6. Kaplan-Meier curves comparing survival in head and neck cancers according to the absence (A) or presence (B) of tuberculosis (active disease, reactivated or sequelae) after oncological diagnosis

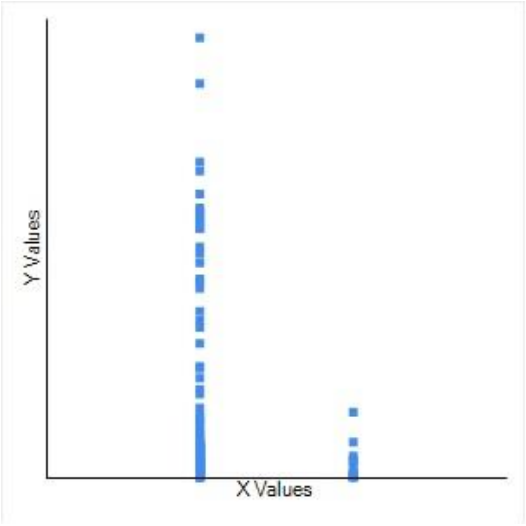

Fig. 7. Correlation of active disease, reactivated or sequelae of pulmonary tuberculosis (horizontal axis: left – no tuberculosis, right – tuberculosis) with OS in months (vertical axis)

For the correlation of age at diagnosis with the prognosis, the median OS was comparatively analyzed in the group of patients aged ≤65 years and >65 years. Even though a negative correlation was identified between advanced age and median survival, a p=0.4 value did not reveal a conclusive statistical significance. A number of 96 (64.4%) patients included in the study were aged ≤65 years, the mean and median age in this group being 56,4 and 57 (32-65) years, respectively. Median survival for patient

≤65 years was 9 (1-192) months. A number of 53 (35.6%) cases were diagnosed at ages >65 years. In this group, the mean and median age was 72.1 and 71 (66-88) years, respectively, and the median survival was 11 (1-134) months (figs. 8, 9).

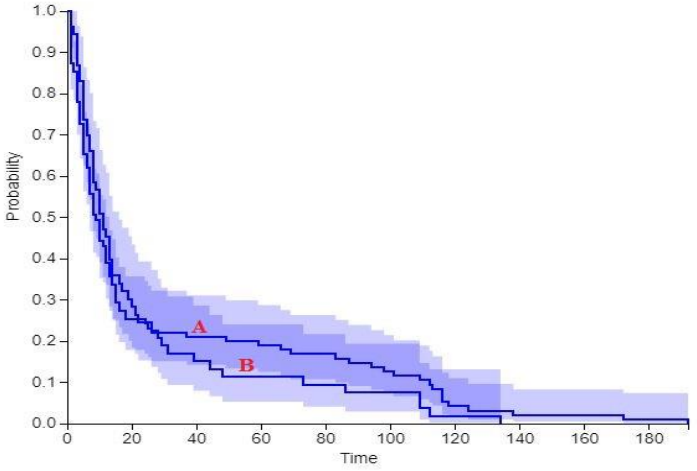

Fig. 8. Kaplan-Meier curves comparing survival in head and neck cancers by age: A ≤65 years, B>65 years

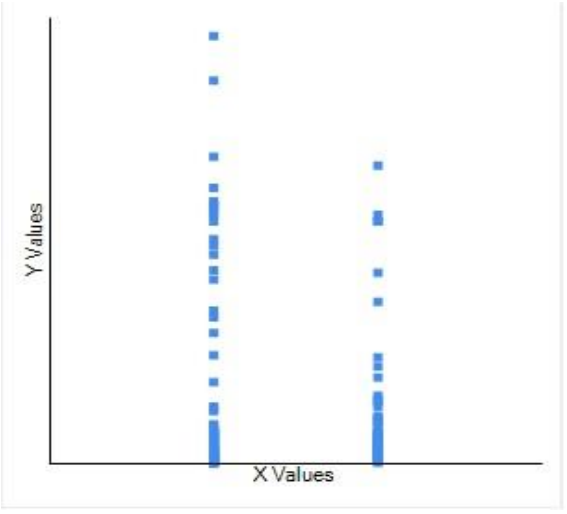

Fig. 9. Correlation of age (horizontal axis: left - age: ≤65 years, right - age >65 years with survival expressed in months (vertical axis)

The co-occurrence of comorbidities is identified as an unfavorable prognostic factor both in our study and in the literature. In the proposed study, hypertension and cardiovascular diseases, diabetes and neurological diseases are the most fre-

quently reported comorbidities in association with head and neck cancers. The advantage in terms of median OS in the group of patients without reported comorbidities is one month. Chronic obstructive pulmonary disease and dyslipidemia are reported in the literature as being among the most common pathologies associated with head and neck cancers, but are not found in the proposed analysis. It is possible that these diseases are underdiagnosed, and the identification of comorbidities based on medical records and patient statements is another possible source of error. It should be noted the high rate of chronic neurological pathologies associated with head and neck cancers, especially the sequelae of stroke, as possible consequences of the therapeutic neglect of hypertension and diabetes. The presence of comorbidities is associated with an increased risk of mortality 30 days after completion of treatment, and the possibility of survival at 1 year in the case of patients with severe comorbidities is considered below 50%. Active pulmonary tuberculosis, sequelae of tuberculosis or disease reactivation during oncological treatment is identified in 8.72% of head and neck cancers. Even if the negative correlation of the association of the two diseases with the prognosis was not statistically significant, lower survivals are noted in the group of patients who associate tuberculosis and head and neck cancer. The relationship between the two diseases is considered bidirectional, the presence of the disease in the antecedents favoring carcinogenesis. A cancer diagnosis can increase the risk of pulmonary tuberculosis by 2.9 times compared to the general population. The risk of tuberculosis in the case of patients with head and neck cancer is considered to be higher in the first 6 months after treatment, but remains high afterwards. The mechanism of this association is not elucidated, but it is assumed that nutritional status and immunosuppression associated with oncological therapies increase this risk. Referring to subjects without any cancer, the adjusted hazard ratio (HR) for pulmonary tuberculosis for patients with head and neck cancer not receiving chemotherapy and radiotherapy is 1,26, but it is 3,74 for patients with head and neck cancer receiving chemotherapy or radiotherapy. However, there are no consistent data to analyze each anatomical location of head and neck cancers (5-11).

Cases of multiple myeloma, leukemia, esophageal cancer, lung cancer, pancreatic cancer are also associated with an increased risk of developing primary or reactivated tuberculosis. In an endemic area of Korea, a 10-time higher incidence of tuberculosis is reported in patients diagnosed with cancer than in the general population. According to Preda *et al*. carcinogenesis induced by M. tuberculosis infection could be associated with some factors including immunosuppression, pro-inflammatory cytokines related to infection, and DNA damage (12, 13).

The study by Matsuo *et al*. mentions higher values of the risk of associating tuberculosis in patients with head and neck cancers, the authors considering that the risk being as much as 16 times higher than in the general population. In this context, the authors consider prophylactic therapy appropriate, especially for patients who require long-term treatment under continuous hospitalization, the risk being both for patients and for the community. Wang and colleagues consider the relationship be-

tween tuberculosis and cancer to be symbiotic, with both diseases having similarities in their effect on the immune system and pathogenesis (14-16).

In our study, using the threshold of 65 years to define geriatric patients, 35.6% of cases fell into this category. Median survival is superior (11 months) both for patients older than 65 years, as well as for those aged up to 65 years, in relation to the values obtained for the general group. Even though approximately 30% of head and neck cancers are elderly (>70 years), this subcategory of geriatric patients is underrepresented in clinical trials. However, there is a tendency to reduce survival rates after the first 2 years of follow-up. According to the MARCH-NC meta-analysis, only 5% of elderly patients are included in the database. Mere assessment of eligibility for maximal multimodality treatment based on age or Eastern Cooperative Oncology Group (ECOG) and Karnofsky performance indices cannot identify those patients for whom standard treatment may be detrimental. The use of scales and instruments that evaluate comorbidities, social, nutritional status and poly-medication confirms that applying the principles of geriatric oncology could limit acute and late adverse effects by identifying cases that require de-escalation of treatment. Thus Zhou *et al.* proposed a nomogram with prognostic purpose for elderly patients with cancers of the oral cavity. Hemoglobin level, neutrophil-to-lymphocyte ratio (NLR), body mass index (BMI) and nodal tumor invasion status are included in this nomogram. The Geriatric Oncology Society (SIOG) proposes, along with the G8 geriatric scale, the comprehensive geriatric assessment (CGA) for the pre-therapeutic identification of fragile patients. A G8 score $\leq$ 14 can be considered suggestive both for the prognosis and for the radiotherapy discontinuation rate and for a prolonged postoperative recovery. The Chemotherapy Risk Assessment Scale for High-Age Patients (CRASH) can be used to predict the adverse effects of chemotherapy. Evaluation using the G8 questionnaire includes 8 items (diet, weight loss, mobility, neuropsychiatric impairment, polymedication, age and self-reported health status) and is easier than CGA, De-escalation of radiotherapy treatment including both dose reduction total, the dose per fraction and the number of fractions, but also the reduction of target volumes are concepts addressed in geriatric oncology of head and neck cancers. At least 4 scales Charlson Comorbidity Index (CCI), Osaka head and neck comorbidity index (OHNCI), the Adult Comorbidity Evaluation-27 (ACE-27) Kaplan Feinstein Comorbidity Index (KFI) are currently used to evaluate comorbidities and their involvement in prognosis. However, as demonstrated by Kallogjeri *et al.* the use of scales such as ACE-27 is easy, but useful in predicting the impact of comorbidities on survival, especially in the case of more lethal cancers such as breast cancer and prostate cancer. Geriatric Depression Scale (GDS), Cumulative Illness Rating Scale-Geriatric, Mini-Nutritional Assessment, Functional Assessment of Cancer Therapy-General (FACT-G) are evaluation scales used to quantify some characteristics of geriatric patients, being useful in defining the administration criteria of active oncological therapy and especially tolerance to chemotherapy. The geriatric frailty present more intensely in the case of these patients can be explained by

the history of smoking and alcohol consumption, but also of cardiovascular and metabolic comorbidities. Being less represented in clinical trials, elderly patients with head and neck cancers must be included in trials to accurately identify prognostic factors and optimal therapeutic management (17-23).

CONCLUSIONS

The presence of comorbidities in the case of patients with head and neck cancer, but especially pulmonary tuberculosis, active, reactivated or sequelae, is associated with an unfavorable prognosis. Studies are needed to analyze in more detail the implication of the association between pulmonary tuberculosis and each subtype of head and neck cancer. There is an increased incidence of neurological pathologies, especially stroke compared to the results published in the literature. In our study, age >65 years was not identified as a significant prognostic factor, but a tendency to reduce survival to more than 2 years in the case of geriatric patients should be mentioned.

ACKNOWLEDGMENTS

All the authors contributed equally to this study.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding regarding this scientific research.

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INSIGHTS ON THE ESTIMATE OF COSTS IN ROAD TRAFFIC ACCIDENTS

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INSIGHTS ON THE ESTIMATE OF COSTS IN ROAD TRAFFIC ACCIDENTS (Abstract): Traffic road accidents represent a major challenge for public health at world level regarding the morbidity, mortality, but also the economic implications. This study is a systematical literature review with the objective to synthesize the knowledge relating the estimate of the road traffic accidents cost, identifying, selecting and evaluating the studies carried out up to the present time to reach a consensus on this matter. When we speak about the total cost of road accidents, we should be considered four main categories: costs in the event of death, costs in the case of non-fatal injury, costs generated by the material damage and administrative costs. This study has conducted an analysis of all components of the total cost of road traffic accidents and enabled a methodological framework for conducting accurate estimates in future studies. The need for studies on this subject is given by the necessity of ways to create, precedence and verification of the methods of road traffic accidents prevention and the traffic participants protection. Finally, a true picture of the economic impact of road traffic accidents allows a complex contouring of this public health problem. **Keywords**: ECONOMIC BURDEN, ESTIMATION, ROAD TRAFFIC ACCIDENTS

Traffic road accident is a fortuitous event, unpredictable, produced from the guilt of one or more participants in road traffic, which causes damage to the vehicles involved as well as the injury, mutilation or even death of the persons involved (1).

Traffic road accidents represent a major challenge for public health at world level regarding the morbidity, mortality, but also the economic implications. Following World Health Organization (WHO), all over the world are produced approximately 1,250,000 of deaths and between 20 and 50 million people are injured in road accidents. In the 2015 WHO's report, Romania appears with some 1861 deaths in 2013, representing a ratio of 8,7 to 100,000 inhabitants, in a slight decrease compared with previous years. These data point out that in comparison with the Western European states, in Romania, the mortality rate is still much higher, even though it is one of the countries which belong to the „Road Safety Program 2011-2020". The most affected category of road users is that of pedestrians, followed by drivers (2).

The economic consequences of this true traumatic outbreaks are hard to assess, at

the property damage is added the efforts to save the casualties, medical assistance granted to the survivors and economic loss by cutting off their productive activity or their invalidity (3-5).

Up to the present, in Romania has not been carried out an estimate of the total costs of road accidents, there isn't a methodological framework for achieving this. This study analyzes data from scientific literature regarding the calculation of these costs and proposes to create a foundation of the methodological framework stated above.

METHODOLOGY

This study is a systematical literature review with the objective to synthesize the knowledge relating the estimate of the road traffic accidents cost, identifying, selecting and evaluating the studies carried out up to the present time to reach a consensus on this matter. Data collection was carried out by searching the electronically databases platforms: PUBMED, RESEARCHGATE, WEB OF SCIENCE and GOOGLE ACADEMIC using combinations of keywords such as: „cost, traffic accidents, estimation, injury, death, economic burden". In this article, there were included studies containing information on the estimation and cost calculation methods of road accidents, the categories of costs which make up the total cost, the types of data, their origin and the way they have been analyzed and the problems encountered in collecting the data.

RESULTS AND DISCUSSION

When we speak about the total cost of road accidents, we should be considered four main categories: costs in the event of death, costs in the case of non-fatal injury, costs generated by the material damage and administrative costs (6-9).

Costs in the event of death

The costs in this category include: costs generated by receiving proper medical up to death, the costs relating to the handling of the corpse after death, the losses suffered by the family and the community, recouping the loss of life, recouping the lost productivity (9-11).

The costs generated by medical care

This category includes costs from pre-hospital emergency medical cares and intra-hospital emergency compartments medical care's costs (10). Most of the victims of road accidents benefit from these medical cares, and the costs they involve are related to the ambulance service (dispatching rooms, transport, medical cares at the spot) and to the emergency compartments (consult investigations, treatment) (12). Regarding intra-hospital medical treatment, in addition to expenditure related to the diagnostic and treatment, costs of hospitalization (accommodation and meal) should be considered and. Given the complexity of these medical cares, their costs are much higher in comparison with other subcategories (10, 13).

The costs relating to the handling of the corpse after death

These costs include the expenses which shall begin immediately after death: corpse transport, autopsy, beautifying, funerals (11, 14). Road traffic accidents are causing violent deaths, and this is the reason for which it is appropriate to carry out forensic autopsies of the victims. Even if this activity costs are related to the category for administrative costs, we decided to include it here to have a full frame of the costs related to the death of persons involved in a road traffic accident.

The losses suffered by the family and the community

This category refers to the loss for the community and family of the activities the deceased carried out on his free-time which include domestic activities (such as cleanliness, cooking, the care of children or other persons) or voluntary activities. All of this can be financially quantified with the rates of the persons who provide these services counter-cost (15, 16).

Recouping the loss of life

Recouping the loss of life is the capitalization of the pain, suffering, and mourning generated by the death of a person. Mishan, Miller, and Viscusi, have used three distinct approaches to assess the economic loss of life: "the court-award", "the injunction public" and "the willingness-to-pay". In Romania, these approaches may be assimilated with the decisions of the claims because of a penal lawsuit, respectively a civil lawsuit (16-21).

Recouping lost productivity

Krupp *et al* they suggested approaches for recouping lost productivity, as follows: "The gross-loss-of output", "the net-loss-of-output" and "value of time" (22). But for the study of the costs of road accidents, most of the authors have used the first approach, being considered the most simple and eloquent in this case (21, 23, 24). This approach calculates the costs relating to the age of the victim and estimated gains in the future. In Romania, the age at which begins productivity is 15 years, and the ending is 64 years (25). Also, it must be considered the unemployment rate, additional revenues included in the gross salary and it should not be forgotten the percentage of people who also work on the black market.

Costs in the case of non-fatal injury

In this category come the costs related to the: medical cares, temporary incapacity for work from the period of convalescent and permanent incapacity for work (16).

The costs generated by the medical care

In addition to the costs generated by pre-hospital emergency and intra-hospital emergency compartments medical cares described above, it adds the costs of primary, specialized long-term and recovery medical care. Primary medical care generates costs related to consulting, investigations and the treatment of the patients with less severe injuries, produced in road traffic accidents, which shall be made directly to the general practitioner. For calculating the costs of long-term specialized and recovery medical cares, it must be considered the periodic checks, home cares, the visits to the general practitioner, therapeutic reinterventions, and healing procedures (10, 26-28).

Costs due to the temporary incapacity for work

Temporary incapacity for work brings additional costs that include costs from adaptation to disability, the allowance of sick leave, loss of productivity and health or life insurance. For calculating the costs related to the adaptation to the disability, it should be considered the changes in the level of dwelling/motor vehicles and the medical devices (wheelchair, crutches, etc.) required for daily activities (22, 29). Per the laws of Romania, any person with a temporary incapacity for work receives an allowance for the sick leave arising because of the multiplication of the daily average calculation basis and the number of working days of sick leave (30). During the

last period, more people in Romania purchases life and private health insurance contracts, because of which the insurance companies, in the case of a temporary incapacity for work, are obliged to pay compensation to the insured (31).

To quantify loss of productivity due to the temporary incapacity for work it shall be considered both loss of productivity at the place of work, which is estimated by multiplying the days of sick leave with the daily gross earnings, and loss of productivity for family and community described earlier in the case of the costs related to death (32).

Costs due to the permanent incapacity to work

Estimating these costs implies: adaptation to disability, disability pension/compensation, lost productivity and health or life insurance. Depending on the degree of working capacity reduction, compensation can be paid monthly, monthly complementary personal budget and accompanying allowance (15, 22, 33). The loss in productivity is also, estimated based on the degree of working capacity reduction, starting from a total loss, in which case it is directly correlated with the loss of productivity in the event of death, described above, goes to the partial loss of working capacity, in which case the number of working hours decreases, the calculation of costs being made taking account the number of working hours lost. In terms of costs related to health or life insurance, compensation for permanent incapacity to work will be higher than if the temporary and will be directly proportional to the deficit (34-37).

Material damage costs

Costs generated by material damages include, in addition to repair costs of vehicles, private properties (houses, shops, fences etc.) or public properties (streets, indicator, guard rails, etc.) and the costs relating to the period in which those mentioned above cannot be used. Here we are talking about the costs of hiring/purchase another vehicle for personal use, public transport costs, the costs of the taxis use; also in the case of scrapping of vehicles for goods or persons transport, its owner will lose his daily income (24, 38).

Administrative costs

Administrative costs cover everything that is related to the road accident from the moment in which it takes place until the file is closed and includes the costs of firefighters and police interventions, the costs of medico-legal (autopsy, medico-legal certificate) and judiciary expenditure (33).

DISCUSSION

Proposal concerning the sources and methods of gathering data to estimate the total cost of traffic accidents in Romania

The conducted literature review allows us to establish a methodological framework for data collection required for the estimation of the total cost of road traffic accidents in Romania.

Having regard to the categories of costs resulting from the studied literature, we appreciate that to estimate the total cost of road traffic accidents it is necessary to collect data from a variety of sources and through different methods such as: analyzing databases of the Ministry of the Interior, Ministry of Health, Ministry of Labor, Family and Social Protection for the Elderly and the Ministry of Justice, analyzing

databases of insurance companies, interviews and questionnaires.

Data relating to the number, type, the severity of accidents, the number of injured or deceased victims, intervention duration, resources used (human and material), the types of material damage, etc. can be collected from the office of the Traffic Police (audits, expense reimbursements). To calculate the costs of injuries caused by traffic accidents, data (such as pre-hospital resources used, type, number, severity, and prognosis of injuries, treatment, investigations, intra-hospital resources used, accommodation and meals costs, etc.) are necessary-these data can be collected from the Ambulance Service, hospitals, general practitioner offices, by analyzing observation sheets, pre-hospital emergency records, Emergency unit records, consultation records and expense reports attached to them.

Calculating the costs related to handling corpses will be done by correlating the number of people killed with the fare imposed by the Forensic Medicine Services on autopsy and embalming corpses and tariffs of the companies that provide funeral services.

For the estimation of the costs of the losses suffered by family and community, it is necessary to use questionnaires to collect information on household and volunteer activities, duration and their frequency, because this information is then correlated with the rates charged by professionals for such activities. To capitalize loss of life, it is necessary to consult the court decisions regarding compensation in cases of road accidents. The costs related to the insurance (health, life and of the material property) will be estimated using data collected from insurance companies and will represent the sum of all compensation in case of road traffic accident considering the type of insurance and the type of damage.

Regarding the costs of adapting to disability, it is necessary to apply a questionnaire to disabled individuals and families of those with disabilities, showing the value of these costs or expenses. For loss of productivity, it is necessary to know the number of fatalities (Office of the Traffic Police), the number of persons with temporary incapacity for work and the period of incapacity and the number of those with permanent incapacity for work and the degree of loss of it and the number of reduced working hours, these figures can be collected from the Bureau of Medical Expertise and Recovery of work capacity.

Finally, in collecting the data shown above, consideration must be given to the following points: limited/restricted access to documents, documents may contain drafting error or may be incomplete, these problems must be identified, corrected and removed. Also, it must be taken into consideration the possibility that a part of the road accidents could not be declared to the police and it could be found only in the databases of the insurance companies.

CONCLUSIONS

When an estimate as accurately as possible of the total cost of road traffic accidents is desired, bear in mind that it is made up of a multitude of components, which requires a thorough analysis and, any difficulties which may arise during the data collection, should be considered, each time.

This study has conducted an analysis of all components of the total cost of road

traffic accidents and enabled a methodological framework for conducting accurate estimates in future studies.

The need for studies on this subject is given by the necessity of ways to create, precedence and verification of the methods of road traffic accidents prevention and the traffic participants protection. Last but not least, a true picture of the economic impact of road traffic accidents allows a complex contouring of this public health problem.

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TRENDS IN PREMATURE MORTALITY: UNCONDITIONAL PROBABILITY OF DYING BETWEEN AGES OF 30 AND 70 FROM CARDIOVASCULAR DISEASE, REPUBLIC OF MOLDOVA

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TRENDS IN PREMATURE MORTALITY: UNCONDITIONAL PROBABILITY OF DYING BETWEEN AGES OF 30 AND 70 FROM CARDIOVASCULAR DISEASE, REPUBLIC OF MOLDOVA, 2003-2015 (Abstract) **Aim:** To estimate the burden of premature mortality from major cardiovascular diseases among adults in the Republic of Moldova and to evaluate National target achieving of 1.5% annual relative reduction from 2013-2020. **Material and methods:** We conducted a population-based study. To assess the premature mortality impact was calculated the probability of dying between ages of 30 and 70 from major cardiovascular diseases in the Republic of Moldova following adjusted World Health Organization's methodology. **Results:** Along with the mortality chance up to the age of 70 years for people at the age of 30 years old because of cardiovascular diseases determined to be in decrease trend for the period 2003-2015 from 21.2% to 15.9% ($p<0.001$) is find a rising percentage change of +3.7% of unconditional probability of dying from cardiovascular disease between ages of 30 and 70 in the Republic of Moldova from 2013-2015 instead of planned reduction of -3%. **Conclusions:** These data can be used to inform development of policies that address the observed disparities of national target achieving. **Keywords:** CARDIOVASCULAR DISEASE, PREMATURE MORTALITY, PROBABILITY OF DYING.

Nowadays, cardiovascular disease (CD) is univocally acknowledged as the most serious challenge of the 21st century for public health on global and national level. Such declaration was achieved because of its first place in mortality and general disability ratings, clear loss of life quality and excessive costs. Therefore, CD prevention and control became one of the key elements for the strategy to fight against noncommunicable diseases, which were elaborated in recent years. According to statistics of World Health Organization (WHO), on global level noncommunicable diseases are responsible for a lot more deceases, compared to all other deceases in a sum, having a growth forecast from 38 million up to 52 million of deceases for the 2012-2030-year period (1). A major percentage of deceases (82%) caused by noncommunicable diseases, are due to four specific diseases: cardiovascular disease, cancer, respiratory chronic diseases and diabetes. As a result, they were named major noncommunicable diseases. From the total deaths caused by major noncommunicable CD has a highest proportion of 46.2% versus cancer (21.7%), chronic respiratory diseases (10.7%) and

diabetes (4%) (1).

For the year 2015 in Republic of Moldova major noncommunicable diseases were demonstrated the following distribution: CDs-58%, malignant tumors-15.2%, digestive system diseases-2% and diabetes-0.8%. Therefore, the burden of CD is a big challenge in Republic of Moldova too, having a stable proportion over than 55% for the last decade.

From those 38 million deaths caused by noncommunicable diseases in the year 2012, around 42% were registered as premature deaths, affecting the population of age younger than 70 years old. In 2012, CDs were responsible for the largest proportion of noncommunicable diseases deaths under the age of 70 (37%). At the same time, 48% of premature deaths caused by noncommunicable diseases, happened in countries with low and medium income, and 28%-well developed countries. It is very important to mention that a quite large part of premature deaths is apriority evitable. Deaths caused by CDs were lowered at quite a high rate in countries with a high income, thanks to governmental politics, which contributes for a healthy life style and providing an accessible and affordable medical assistance (1, 2).

The "epidemiological transition" phenomenon from infectious diseases to noncommunicable diseases was caused by the globalization effects, fast urbanization and population aging-low controlled factors by an individual, as well by the health system.

According to WHO declarations (1), the decrease of economical productivity caused by premature deaths, individual and national costs with a reference to noncommunicable diseases serves as an important barrier in poverty decrease and durable development of the country, specifically for countries with a low and medium income. Therefore, the decrease importance of premature mortality caused by noncommunicable diseases is undeniable, being set as the no. 1 global objective from the nine objectives established by global plan of actions for prevention and control of noncommunicable diseases. In this context, the nominalized document foresees a relative decrease of 25% of premature mortality cause by CDs, cancer, chronic respiratory diseases and diabetes until the year 2025, therefore being name as "25x25" objective.

Actual approaches for measuring of mortality phenomenon impact through the prism of premature deaths events, which "changes the accent from the classic focus upon deaths occurrence to centralization upon lost products because of these deaths" (3), offers the evaluation possibility for population's health in the context of life prolonging. Confirmed by the study of global burden of diseases in 2010 (GBD-2010), which estimated that in the period 1990-2010 simultaneously with the lightly decreasing trend for major CDs can be also seen an increase of 17-28% for years of potential life lost caused by ischemic heart disease and cerebrovascular disease, especially stroke (4).

Premature mortality represents the major criteria in the impact evaluation of noncommunicable diseases among the population (1). Therefore, ensuring the achieving of the No.1 global objective ("25x25") is a priority.

MATERIAL AND METHODS

The main study objective was to identify the evolution of premature mortality from CD in the Republic of Moldova for the period 2003-2015 and to determine the National target achieving of 1.5% annual

relative reduction in mortality from CD starting with 2013 year. This research is a population-based descriptive study.

To achieve the aim of the study was applied a descriptive method of investigation using the data and methodology provided by the WHO (1) and statistical data of the National Center of Health Management of the Republic of Moldova (2003-2015) (5).

To assess premature mortality from CD was calculated indicator of unconditional probability of dying between ages of 30 and 70 which is one of the 25 indicators recommended by WHO as monitoring measures for the prevention and control of noncommunicable diseases (1).

The study applied the WHO methodology of calculation to find out the probability of dying between ages of 30 and 70 from CD. The unconditional probability of dying between exact ages 30 and 70 from CDs for each year from the period 2003-2015 was estimated using age-specific death rates (in 5-year age groups).

The International Classification of Diseases codes used are: I00-I99.

First were calculated five-year death rates ($_5^*M_x$) using the equation (1):

$$_5^*M_x = \frac{\begin{matrix}Total\ deaths\ from\\ cardiovascular\ diseases\\ between\ exact\ age\ x\ and\ age\ x{+}5\end{matrix}}{\begin{matrix}Total\ population\ between\\ exact\ age\ x\ and\ age\ x{+}5\end{matrix}} \quad (1)$$

The probability of death for each five-year group ($_5^*q_x$) was calculated using the following formula (2):

$$_5^*q_x = \frac{_5^*M_x * 5}{1 + {}_5^*M_x * 2.5} \quad (2)$$

Unconditional probability of dying between ages of 30 and 70 from cardiovascular disease was calculated using the formula (3):

$$_{40}^*q_{30} = 1 - \prod_{x=30}^{65}(1 - {}_5^*q_x) \quad (3)$$

When between-group differences were compared the summary, percentages were shown with 95% confidence intervals. The significance of the differences between groups was estimated by z-test to compare two proportions.

RESULTS

WHO (1, 6) has established the core objective to reduce the premature mortality cause by CD, cancer, diabetes and chronic respiratory diseases by 25% until the year 2025 (referred as 25x25).

According to WHO strategies, Republic of Moldova has established a national intermediary objective of premature mortality reduction with in part specifications for each of the five priority noncommunicable diseases in the country: cardiovascular disease, cancer, digestive diseases, chronic respiratory diseases, and diabetes. Therefore, reduce percentage for premature mortality until the year 2020, having as a reference year 2013, has constituted for: CD-10%, malignant tumors-7%, digestive diseases, respiratory chronic diseases and diabetes by 10% (7, 8). The premature mortality from CD National Target is a 10% period reduction from 2013-2020 with a 1.5% annual reduction (9, 10).

The probability of dying between ages of 30 and 70 from major noncommunicable diseases was established by the WHO as a monitoring indicator of progresses for objectives achieving in the set amount of time.

The unconditional probability of dying from CD between ages of 30 and 70 by gender in the Republic of Moldova in 2003 and 2015 is shown in (tab. I).

TABLE I

Unconditional probability of dying between ages of 30 and 70 from cardiovascular disease by gender, Republic of Moldova, 2003-2015

Probability of dying 30-70, %		CI, 95%	Difference, (CI, 95%) *p value (z-test)*
2003 (year)			
Male	25.9	25.8, 26.0	8.5 % (8.4, 8.6) p<0.001, z=134.1
Female	17.4	17.3, 17.5	
Total	21.2	21.1, 21.3	
2015 (year)			
Male	21.8	21.7, 21.9	10.8% (10.7,10.9) p<0.001, z=201.9
Female	11.0	10.9, 11.1	
Total	15.9	15.8, 15.9	

In 2003, a 30-year-old individual male had a 25.9% (95% CI 25.8, 26.0) chance of dying from CD before the age of 70.

The study results found a chance of 17.4% (95% CI 17.3, 17.5) in case of 30-year-old individual female of dying from cardiovascular disease before her 70th birthday. In 2003, the unconditional probability of dying from cardiovascular disease between ages of 30 and 70 for males is greater than for females (p<0.001, z=134.1).

In 2015, a 30-year-old individual male had a 21.8% (95% CI 21.7, 21.9) and individual female had an 11.0% (95% CI 10.9, 11.1) chance of dying from CD before age of 70. This represents a greater chance of dying from cardiovascular disease a 30-year-old in 2015 individual male than individual female before his/her 70th birthday (p<0.001, z=201.9), as well as in 2003 (tab. II).

TABLE II

Unconditional probability of dying between ages of 30 and 70 from cardiovascular disease and noncommunicable diseases by gender, Republic of Moldova, 2003-2015

Probability of dying 30-70, %			Difference, (CI, 95%) *p value (z-test)*
	2003	2015	
Cardiovascular disease			
Total	21.2	15.9	5.3% (5.2, 5.4) p<0.001, z=96.5
Male	25.9	21.8	4.1% (4.0, 4.4) p<0.001, z=63.1
Female	17.4	11.0	6.4% (6.3, 6.5) p<0.001, z=127.7
Noncommunicable diseases			
Total	37.0	31.0	6.0% (5.9, 6.1) p<0.001, z=89.7
Male	44.6	40.4	4.2% (4.1, 4.4) p<0.001, z=55.7
Female	30.5	22.6	7.9% (7.8, 8.0) p<0.001, z=123.8

The individual males of 30-year-old had a decreasing trend for chance of dying before age of 70 by CD from 25,9% (95% CI 25.8, 26.0) in 2003 to 21.8% (95% CI 21.7, 21.9) in 2015 ($p<0.001$, $z=63.1$). The decreasing trend of unconditional probability of dying from CD in case of 30-year-old individual female was found to be from 17.4% (95% CI 17.3, 17.5) in 2003 to 11.0% (95% CI 10.9, 11.1) in 2015 ($p<0.001$, $z=127.7$).

The unconditional probability of dying between ages 30 and 70 from CD in the Republic of Moldova had a decreasing trend from 21.2% (CI 95% 21.1, 21.3) in 2003 to 15.9% (CI 95% 21.1, 21.3) in 2015 ($p<0.001$, $z=96.5$). In case of noncommunicable diseases together the individual males of 30-year-old had a decreasing trend for chance of dying before age of 70 from 44,6% (95% CI 44.5, 44.7) in 2003 to 40.4% (95% CI 40,3, 40.5) in 2015 ($p<0.001$, $z=55.7$).

The probability of dying before age of 70 from noncommunicable diseases had a decreasing trend in case of 30-year-old individual female from 30.5% (95% CI 30.4, 30.6) in 2003 to 22,6 % (95% CI 22.5, 22.7) in 2015 ($p<0.001$, $z=123.8$).

The unconditional probability of dying between ages 30 and 70 from noncommunicable diseases in the Republic of Moldova had a decreasing trend from 37,0% (95% CI 36.9, 37.1) in 2003 to 31.0% (95% CI 30,9, 31.1) in 2015 ($p<0.001$, $z=89.7$). Percentage change in unconditional probability of dying between exact ages 30 and 70 from CDs was related to the period of whole investigation from 2003 to 2015. To monitor progress in attaining the National target reducing premature mortality from CD by 2020, percentage change in unconditional probability of dying between exact ages 30 and 70 from cardiovascular diseases was related to the period from 2013 to 2015 as well (fig. 1).

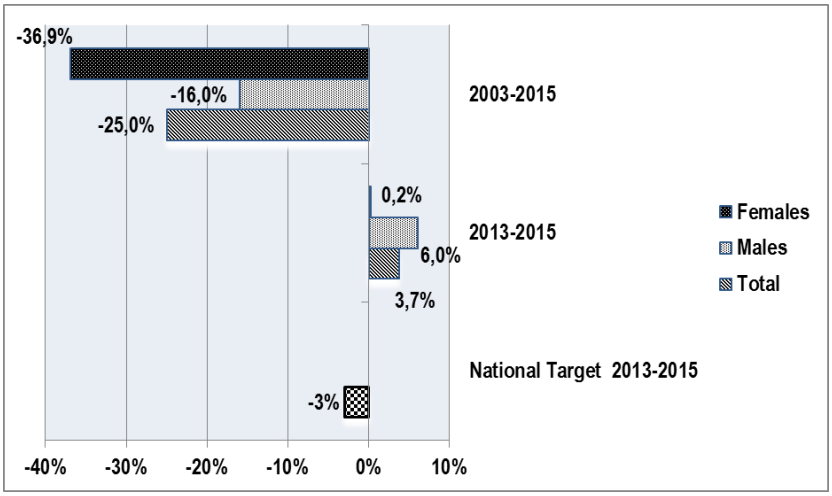

Fig. 1. Percentage change in probability of dying between exact ages 30 and 70 from cardiovascular disease, 2003-2015 and 2013-2015, Republic of Moldova

Change of the probability of dying from CD between ages 30 and 70 from 2003-2015 in the Republic of Moldova represented a 36.9% reduction for males and

16% reduction for females. The chance of dying from cardiovascular disease a 30-year-old individual before age 70 from entire population were found to have a decreasing change of 25%.

The premature mortality from CD national target is a 10% period reduction from 2013-2020 with a 1.5% annual reduction. According this, in 2015 referred to 2013 the expected achieving in premature mortality from CD is 3% reduction.

Despite this, change from 2013-2015 produced the adverse increasing trends comparing to decreasing change 2003-2015. The percentage change of unconditional probability of dying between ages of 30 and 70 by major CDs was estimated as following: +6.0% for males, +0.2% for females, and +3.7% for whole investigated population.

DISCUSSION

According the World Health Organization Global status report on noncommunicable diseases 2014 (1) in 2012, unconditional probability of dying from one of the main noncommunicable diseases between ages of 30 and 70 years was 19%. This, probability varied by region, from 15% in the region of Americas to 25% in the South-East Asia Region. WHO have determined dispersion by country, from greater than 30% especially in low- and middle-income countries to less than 10% in seven countries (Australia, Israel, Italy, Japan, Republic of Korea, Sweden and Switzerland).

In 2012, a 30-year individual from the Republic of Moldova had a 30.7% chance of dying from major noncommunicable diseases before his or her 70th birthday which is the one of the highest level around the world.

In the frame of 2013-2020 national targets achieving the differences over time in describing evolution from 2003-2015 and increasing evolution from 2013-2015 of percentage change of unconditional probability of dying between age 30 and 70 from CD are of great interest. That the adverse upward changes from 2013-2015 must be taken in account to turn trends into decreasing direction again to achieve 2020 National Target of reducing premature mortality from CD.

CONCLUSIONS

1. During the investigation period of 2003-2015, men at the age of 30 years compared to women of the same age identify superior level of frequency for probability of dying until the age of 70 because of a major CD in Republic of Moldova ($p<0.001$);

2. The mortality chance up to the age of 70 years for people at the age of 30 years old because of CDs, as well as for major noncommunicable diseases decrease for the period 2003-2015, respectively 21.2%-15.9% ($p<0.001$) and 37.0%-31.0% ($p<0.001$);

3. Percentage changes (2013-2015) of +3.7% for unconditional probability of dying from CD of a 30-year-old person up to the age of 70 years referred to 2013 year versus those planned as a decrease of 3% for the same period be a warning for nation objective achieving 2013-2020.

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OPEN RADIOCARPAL DORSAL FRACTURE-DISLOCATION ASSOCIATED WITH CHRONIC DISTAL RADIOULNAR JOINT DISLOCATION: A CASE REPORT

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OPEN RADIOCARPAL DORSAL FRACTURE-DISLOCATION ASSOCIATED WITH CHRONIC DISTAL RADIOULNAR JOINT DISLOCATION: A CASE REPORT (Abstract): Radiocarpal dislocations are rare clinical entity often caused by high-energy trauma. According to Dumontier are classified into type I: pure radiocarpal dislocations and type II: fractures-dislocations or associated with other injuries. Treatment of these lesions is still controversial in terms of reduction of dislocation and fixation: orthopedic/surgical, K-wires/ external fixator ± ligament reconstruction. We present the case of a 32-years-old male patient, presenting a radiocarpal dislocation Dumontier II, with volar soft tissue injuries type III B Gustilo Anderson associated with an old distal radioulnar joint dislocation. Surgical treatment consisted of antibiotics combined, tetanus prophylaxis, debridement, fasciotomy, open reduction and external fixation, decompression of the median nerve and Z-plasty of cutaneous defect. 3 months postoperatively results were satisfactory, the patient resumed activity level before the accident.
Keywords: RADIO-CARPAL DISLOCATION, OPEN, WRIST, SURGERY.

Radiocarpal dislocation is a rare dislocation with an incidence of only 0.2% (1, 2). It is generally found in young men as a result of high energy trauma, being associated with a high comorbidity and poor functional outcomes (3). According to Moneim (1) and Dumontier (2) radio-carpal dislocations are divided into pure radiocarpal dislocations (type I) and fracture-dislocation or associated to other lesions (type II) (1, 2). It has been published in the literature small series of cases in which there was no consensus about optimum method of treatment (2, 4-8). Some author prefer open reduction and fixation (2, 5) other cast immobilization (8), both reporting good results.

CASE REPORT

A 32-years-old right-handed male, was admitted in the emergency department after an accidental fall from 4 meters. He presented a "dinner fork" deformity of left wrist, a volar III B Gustillo Anderson open fracture, heavy contaminated, and functional impotence.

Local examination revealed paresthesia in the median nerve territory and absent radial pulse with low Doppler signal.

Radiological examination showed a radiocarpal dislocation, Dumontier type I, Moneim I and a chronic dorsal distal ra-

dioulnar joint dislocation. CT scan confirmed the diagnoses and revealed a minor dorsal distal radius comminution.

From the patient's personal history we found out that seven years ago suffered a fracture of the left radius, orthopedic treated.

There was an unsuccessful attempt of reduction in the emergency department under intravenous sedation. The wrist was immobilized on a splint and tetanus and antibiotic prophylaxis in dual therapy (third generation cephalosporin and aminoglycoside) was initiated.

In the operating room under general anesthesia was performed debridement and irrigation of the wound, anterior fasciotomy of the left forearm, exploration of the radial and ulnar neurovascular bundle, decompression and external neurolysis of the median nerve by opening the carpal tunnel, reduction under fluoroscopic control and external fixation. Cutaneous defect of the forearm was covered by Z-plasty.

Fig. 1. Initial clinical aspect

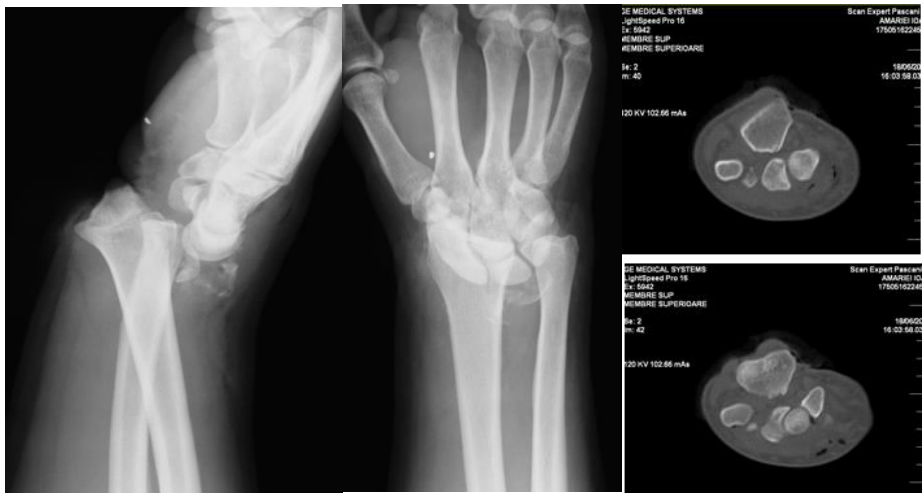

Fig. 2. Initial X-rays. AP view and lateral view

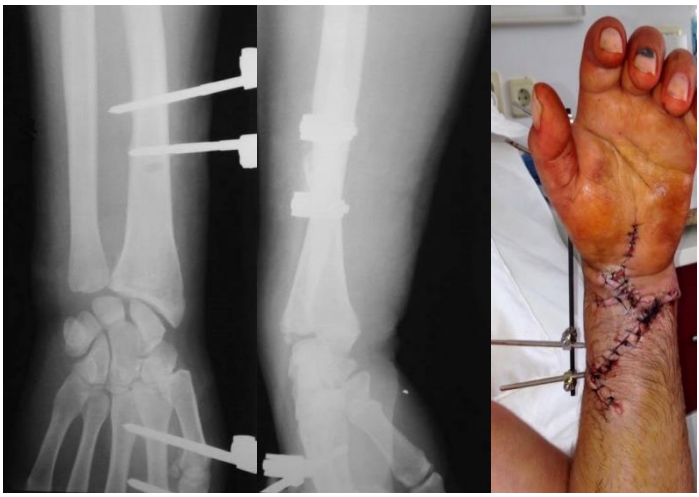

Fig. 3. Cross-sectional CT views

Fig. 1. Immediate postoperative radiographic and clinical aspect

Postoperative evolution was favorable, peripheral pulse was normal and paresthesia in the median territory ceased.

Unfortunately distal radio-ulnar dislocation could not be reduced, opting for functional treatment. Physical therapy was initiated first day postoperatively.

10 days after the intervention the patient was discharged with instructions for clinical and radiological follow-up after 2, 4, 8 and 12 weeks.

Radiological control of 4 weeks identified a dorsal radio-carpal subluxation. The patient refused surgical reintervention.

External fixator was suppressed at 8 weeks postoperatively and a functional recovery program was initiated.

Fig. 2. Clinical results after 12 months

At 3 months, postoperatively the patient had a palmar flexion of 47°, 45° extension, the Disabilities score of the Arm, Shoulder and Hand (DASH) was 28.3 (range 0-100), and the Mayo Wrist Score 60, which is considered a satisfactory result.

DISCUSSION

Starting with the first radiocarpal dislocation reported in the literature by

Varadompun N. in 1926 (8) and continuing with the series of cases reported by Dunn (7), Moneim M.S. (1) and Dumontier C. (2), the treatment of these clinical entities did not reached consensus.

These dislocations are extremely rare, the most common being type II (9). The diagnosis is best achieved by standard radiography (9), but we opiniate for CT scans which brings valuable information on the degree of comminution of the radial styloid process or dorsal/ volar rim of the radius.

Frequently dorsal radiocarpal dislocations are commonly associated with compression of the median nerve that requires decompression (10).

In type I dislocation, treatment is dictated by the possibility of achieving articular congruity and by stability of the reduction. It is imperative to check under fluoroscopy the radiocarpal stability in dynamics in flexion-extension and inclinations to establish the indication for surgical treatment (9).

Stable reductions can be treated orthopedic (8) while unstable reductions require pining (2, 10) or external fixation (2, 11) associated with open (11) or arthroscopic ligamentous repair (6).

CONCLUSIONS

Regardless urgent reduction and efficient contention, radiocarpal dislocations are serious clinical entities with unpredictable prognosis. Due to peculiarities of our patient anatomy, perfect reduction was impossible. Despite radiologic imperfect outcome, patient`s functional outcome was satisfactory. Radiocarpal dislocations require further investigation to establish optimal treatment method connection between treatment and outcome.

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THE CONTRIBUTION OF ALEXITHYMIA TO BURNOUT IN FORENSIC PHYSICIANS

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BURNOUT AND ALEXITHYMIA AMONG FORENSIC PHYSICIANS (Abstract): **The aim** of the study was to identify the relationship between the level of burnout subdomains and alexithymia among forensic physicians working in forensic institutions in Romania. **Material and methods**: A number of 37 forensic physicians were included in the survey. Burnout and alexithymia were measured by using the *Maslach Burnout Inventory* and *Toronto Alexithymia Scale.* The obtained data were processed using the *SPSS 17.00* statistical software. **Results**: The subjects obtained an average of 43.27±3.71, which corresponds to a low level of alexithymia. For burnout scores, we have obtained M=14.97±13.13 for emotional exhaustion, M=7.91±6.87 for depersonalization and M=33.18±10.59 for personal accomplishment (low-level for emotional exhaustion and medium-level burnout for the other two factors). Among the socio-demographic variables, only the age correlated positively with the burnout factor personal accomplishment. Positive correlations were identified between burnout factors and TAS-20. Comparative analysis results are important for the presence of insomnia, depression, teaching activity and looking for professional support after critical events. **Conclusions**: Scores for forensic physicians are low-level for emotional exhaustion and medium-level for two subdomains and low-level for alexithymia. Factors revealed by the comparative analysis are important to adjust professional activity and to find strategies to cope with stressful professional events. **Keywords:** FORENSIC PHYSICIANS, EMOTIONAL EXHAUSTION, BURNOUT, ALEXITHYMIA.

The burnout syndrome was originally described in relation to the healthcare profession or educators, professions that usually provide care. Burnout is characterized by a high level of emotional exhaustion, a high level of depersonalization and a low level of personal accomplishment. The most important factors related to burnout are the individual ones like personality traits such as neuroticism, extraversion, depression, conscientiousness, alexithymia or type A behavior or socio-demographic and environmental factors such as age, gender, length of experience, working conditions, number of work hours, multiple tasks in a short period, insufficient payment and reward, relationship with colleagues and superiors, or conflicting values (1).

Burnout was considered endemic in the last decades among healthcare profession-

als (2) and it was proved to be associated with a low level of professional performance and with difficult relationships with colleagues and patients. Some studies targeting the different medical specialties identified among doctors suffering from burnout a higher risk of suicidal thoughts, engagement in risky behaviors like tobacco or alcohol, and an increasing number of medical errors.

In a study of Gleichgerrcht and Decety they proved that physicians who have difficulty regulating their negative arousal and describing and identifying emotions are more prone to emotional exhaustion, detachment, and a low sense of personal accomplishment (3). On the other hand, the ability to be aware of emotions and the tendency to help others is in strong correlation with the sense of compassion. Identifying emotions is important in dealing with occupational stress (4, 5). This brings consistent help in clinical practice, where the doctor-patient relationship is important and represents the basis of a trustworthy and more appropriate medical evaluation (3).

Professionals facing critical events are more exposed to high levels of burnout and these scores may result in later psychological distress.

The aim of the study is to identify the relationship between burnout levels for each separate domain and the score for alexithymia among forensic physicians. We wanted to find a correlation between the association of the capacity to identify and describe emotions and levels of emotional exhaustion, depersonalization and personal achievement. As a secondary goal, socio-demographic characteristics and the self-evaluated presence of depression, insomnia and the impact of critical events was established. Less information is available about professional activity, burnout or related factors among forensic physicians in Romania.

MATERIAL AND METHODS

A group of 37 forensic physicians working in 11 different forensic institutions from Romania answered two questionnaires. The research was approved by the Legal Medicine Institute of Iaşi. The questionnaires were self-administered and socio-demographic data were recorded on a separate sheet. The participants were informed about the goal of the study, data privacy and dissemination of results and informed consent was obtained before filling in the questionnaires.

The burnout syndrome was evaluated using the *Maslach Burnout Inventory (MBI)*. The MBI is a 22-items questionnaire used for measuring 3 domains: emotional exhaustion (describing the feeling of being exhausted and drained by one's work, fatigued at the very idea of work, chronic fatigue, sleeping and physical troubles), depersonalization (describing emotional coldness and impersonal reactions to the beneficiaries of one's work – leading to cynicism and negative attitudes with regard to patients or colleagues, feelings of guilt, avoidance of social contacts and withdrawal into oneself), and personal achievement (describing feelings of competence and accomplishment in one's work with other people) (8). The burnout score represents evaluation at three levels: for emotional exhaustion: low (17 or lower), medium (18-29) and high level (over 30) and Cronbach's alpha coefficient was 0.85; for depersonalization, a score under 5 means a low level, a score of 6 - 11 is medium and one over 12 is high and we obtained a Cronbach's alpha of 0.69. For the personal

achievement domain, a total of 33 points or fewer characterizes a high level of burnout, one between and including 34-39 defines a medium level of burnout and a total greater than 40 shows low-level with Cronbach's alpha coefficient 0.82.

To evaluate alexithymia, we have used the *Toronto Alexithymia Scale (TAS)* to assess difficulties with emotional processing and emotional awareness. Forensic physicians rated personal statements on a Likert– scale of 1 (strongly disagree) to 5 (strongly disagree). The scores of the 20 items represent three domains: difficulty of describing feelings, difficulty of identifying feelings, and externally oriented thinking. The third scale evaluates the tendency of individuals to focus their attention externally. It is in fact a cognitive style that avoids introspective thought. A general alexithymia score is calculated as the sum of these three subscales and the final scores provide the level of alexithymia: subjects not having alexithymia (scores equal to or less than 51), subjects having borderline alexithymia (scores of 52 to 60), and subjects having scores equal to or greater than 61 are characterized by alexithymia.

Socio-demographic data were also registered, including: age, gender, department, number of children, length of experience, the self-evaluated presence of depression and insomnia, the use of pills to cope with stress or to evaluate which critical events impressed them the most during their professional work.

RESULTS AND DISCUSSION

Descriptive analysis

The study included 37 forensic physicians: 17 males, (45.95%) and 20 females (54.05%), from 11 forensic medicine institutions in 11 counties, with a mean age of 39.13±11.78 (with a minimum of 25 years and a maximum of 64 years). Considering the professional level, 17 (45.95%) are residents, 3 (8.11%) are specialists physicians and 17 (45.95%) are senior consultants. Regarding the length of experience as a forensic doctor, M=10.61±10.65 years of work experience was recorded, with a minimum of 1 year and a maximum of 32 years. The number of work hours per week was 33.51±9.56 and the mean number of medical shifts per month was 2.94±2.1. Almost ¼ (N=9, 24.32%) of the subjects have academic teaching activity.

Seven subjects (18.92%) were in a relationship, 21 (56.76%) were married, 4 (10.81%) were divorced and 5 (13.51%) were single; 17 subjects have declared that their partner has the same profession (47.22%).

The data collected also focused on recording the subjects' number of children. Over half of them (N=19, 51.35%) did not have children, 10 (27.03%) had one child and 8 (21.62%) of the surveyed forensic doctors had 2 children.

Regarding the alexithymia scale TAS-20, the cut-off scoring is the following: less than or equal to 51 (low-level alexithymia or no alexithymia), greater than or equal to 61 (high-level alexithymia) and 52-60 is possible alexithymia. Our participants obtained an average of 43.27 ± 3.71, which corresponds to low-level alexithymia.

The MBI is comprised of three factors: emotional exhaustion - our subjects obtained an average of 14.97±13.13 in this factor, which corresponds to a low level of burnout, depersonalization-our subjects obtained an average of 7.91±6.87 in this factor, which corresponds to a medium level of burnout and personal accomplishment - our subjects obtained an average of

33.18±10.59 in this factor, which corresponds to a medium level of burnout.

Correlational analysis

To make the correlational analysis and to know which tests we should use in our statistical analysis, we first tested the normality of our data distribution. For this, we used the *Kolmogorov-Smirnov* (K-S) test for all the variables we investigated. For the total score of the *Toronto Alexithymia Scale* we obtained K-S z=0.120, p=0.195 and because p > 0, we conclude that for this variable our scores are normally distributed. For the three factors of TAS-20: difficulty describing feelings K-S z=0.137, p=0.076; difficulty identifying feelings K-S z=0.156, p=0.024 and externally oriented thinking K-S z=0.120, p=0.196. We can see that for almost all the factors of the TAS-20, the score distributions are normal, except for the difficulty identifying feelings, because p<0.05. For the factors of the MBI we obtained the following results: emotional exhaustion K-S z=0.168, p=0.010; depersonalization K-S z=0.195, p=0.001 and personal accomplishment K-S z=0.175, p=0.006 and we can say that the scores are not normally distributed, since for all the factors p<0.05. For the other socio-demographic variables that we considered in this correlational analysis we obtained the following results: age - K-S z=0.160, p=0.018; number of children - K-S z=0.402, p<0.001; years of experience - K-S z=0.263, p< 0.001; hours per week - K-S z=0.300, p<0.001; shifts per month - K-S z=0.222, p<0.001. Since almost none of these variables are distributed normally, especially the burnout factors, to perform the correlational analysis, we used Spearman correlations, a non-parametric test. The results between subdomains of burnout and the other variables in our study are presented in Table I.

TABLE I

Correlation analysis burnout domains - TAS–20 and other socio-demographic variables

	Variables	Emotional Exhaustion	Depersonalization	Personal Accomplishment
TAS-20	Difficulty identifying feelings	**R= .400*, p = .014**	**R = .374*, p = .023**	**R = -.399*, p = .014**
	Difficulty describing feelings	R = .288, p = .083	R = .126, p = .459	R = -.238, p = .157
	Externally oriented thinking	R = .193, p = .253	**R = .339*, p = .040**	R = -.087, p = .610
	Total score in alexithymia	R = .317, p = .056	**R = .342*, p = .038**	R = -.323, p = .051
Variables	Age	R = .130, p = .443	R = -.016, p = .926	**R = .324*, p = .050**
	Number of children	R = -.055, p = .746	R = -.052, p = .758	R = .283, p = .090
	Experience in years	R = .045, p = .796	R = -.225, p = .187	R = .293, p = .082
	Work hours/week	R = .317, p = .064	R = -.105, p = .547	R = .168, p = .336
	Shifts/month	R = -.032, p = .859	R = -.074, p = .684	R = .274, p = .123

No correlation was identified between the following variables: number of children, years of work experience, the number of hours per week or the number of shifts per month and the factors of burnout: emotional exhaustion, depersonalization and personal accomplishment. Among the socio-demographic variables, only the age correlated positively with the burnout factor personal accomplishment. On the other hand, we can see that positive correlations are highlighted between TAS-20's factor

difficulty identifying feelings and the burnout factors: emotional exhaustion and depersonalization while the factor difficulty identifying feelings correlates negatively with personal accomplishment. Also, both the TAS-20's externally oriented thinking factor and the total score in alexithymia correlate positively with the burnout factor depersonalization. Despite this, the TAS-20 factor difficulty describing feelings did not correlate with any of the burnout factors.

Comparative analysis

To identify statistically significant differences, we used the t-test for independent samples even though the scores for most of the variables we analyzed were not distributed normally. There are a lot of authors that suggest that Independent Sample T-Tests are sufficiently robust and can be used even if not all the variables that we analyze are distributed normally. After statistical processing, no statistically significant differences were found between subjects per their gender regarding the burnout factors: emotional exhaustion, depersonalization and personal accomplishment or regarding the TAS-20 factors or the total score of TAS-20.

A recent study focusing on burnout in medical residents suggests that significant differences between male and female subjects have been identified. Gender seems to play an important role when it is about protective factors against burnout: male residents mention social support from colleagues more often, while female residents consider more often that home resources are protective factors against emotional exhaustion (6). Our lot was a small one and comparative analysis was difficult to perform considering these criteria.

Statistical differences have not been observed between the burnout factors (emotional exhaustion, depersonalization, personal accomplishment) in cases when the participant's partner works in the medical field, when the victims of the cases they work on are children, whether these children have been abused sexually or physically, whether women have been abused sexually, whether they have faced aggressive prisoners' suicides, whether they talk to a family member or whether they talk to nobody when they are affected by an event at work or whether they suffer from chronic diseases.

Regarding teaching activity (at universities or forensic institutions), there are significant differences in emotional exhaustion ($t(35) = 2.08$, $p = 0.045$), in that those with no teaching activity show lower values ($M = 12.53$) than those who teach ($M = 22.55$), the ones with teaching activity showing a higher level of emotional exhaustion. There are also significant differences in personal accomplishment ($t(35) = 4.77$, $p < 0.001$); those who do not teach have obtained lower values ($M = 30.39$) than those who teach ($M = 41.88$), the ones with teaching activity showing a higher level of personal accomplishment.

Regarding whether the participants have witnessed a critical event or not in the last five years, there are significant differences in emotional exhaustion ($t(35) = 2.96$, $p = 0.006$), in that those who have not witnessed any critical events show lower values ($M = 8.60$) than those who have ($M = 19.31$), the latter showing a higher level of emotional exhaustion.

When it comes to the cases our participants have worked on, there are significant differences in personal accomplishment, concerning some of the things that affect them the most, for example, decomposing

bodies (t (35) =4.51, p=0.001); the participants who have worked on this kind of cases (M=42.00) show higher levels of personal accomplishment than those who haven't (M=32.68).

When they were affected by an event at work, the participants who talked to one of their colleagues obtained significant differences in personal accomplishment (t (35) =2.51, p=0.017); those who talked to one of their colleagues obtained higher results in personal accomplishment (M=36.76) than those who did not (M=28.50). Also, there were significant differences in personal accomplishment if the participants talked to a specialist (psychologist, psychotherapist, social worker, officer) (t (34) =4.45, p<0.001); those who talked to a specialist (M=41.50) showed higher levels of personal accomplishment than those who did not (M=33.11).

When we consider if the participants suffer from insomnia, we obtain significant differences in emotional exhaustion (t (35) =2.74, p=0.019), those who suffer from insomnia (M=26.00) obtaining higher scores in emotional exhaustion than those who do not suffer from insomnia (M=10.88).

When we consider if the participants suffer from depression, we also obtain significant differences in emotional exhaustion (t (35) =4.93, p<0.001), those who suffer from depression (M=33.83) obtaining higher scores in emotional exhaustion than those who do not suffer from depression (M=11.32).

Regarding the drugs the participants take to relieve stress, we have obtained significant differences in emotional exhaustion (t (35) =3.15, p=0.003), the participants who take drugs to relieve stress (M=30.40) obtaining higher scores in emotional exhaustion than those who do not (M=12.56).

We have obtained significant differences in emotional exhaustion, when it comes to whether the participants think the specialty they chose has changed their vision of life (t (35) =3.41, p=0.002), the participants who think that the specialty they chose has changed their vision of life (M=17.85) have obtained higher scores than those who do not think the same (M=7.20).

All the variables presented above were considered as grouping variables when we wanted to determine if there were any significant statistical differences concerning the total scores at TAS-20, but none of the differences were statistically significant.

Regression analysis

The correlational study emphasized the influence of some variables on others (for example, the statistically significant connection between some of the alexithymia factors and some burnout factors). To identify the most effective model for estimating the burnout factors, multiple linear regression was used by the hierarchical method and the following prediction models were defined for emotional exhaustion, depersonalization and personal accomplishment. The results are presented in Table II.

For the depersonalization factor, none of the three predictive models proved to be significant.

Of the three models where emotional exhaustion was the criterion, only the first one was found to be significant. This consists of the difficulty identifying feelings factor and it explains 11% of the variance of emotional exhaustion. Also, the alexithymia factor difficulty identifying feelings has a significant influence on emotional exhaustion

(p=0.026) explaining most of the variance of the dependent variable (b=0.852, beta=0.367), its effect on the criterion being positive. So, based on these results, we appreciate that the more present this alexithymia factor (difficulty identifying feelings) is in a person's structure, the higher the emotional exhaustion scores will be.

TABLE II
Regression analysis burnout domains – TAS-20

	Models	Test	Emotional Exhaustion	Depersonalization	Personal Accomplishment
Step 1	Difficulty identifying feelings	R^2 adjusted	0.110	0.000	0.131
		ΔR^2	0.134	0.028	0.155
		F	**5.437***	0.995	**6.409***
Step 2	Difficulty identifying feelings, difficulty describing feelings	R^2 adjusted	0.087	-0.018	0.105
		ΔR^2	0.003	0.011	0.000
		F	2.720	0.676	3.119
Step 3	Difficulty identifying feelings, difficulty describing feelings, externally oriented thinking	R^2 adjusted	0.060	0.026	0.088
		ΔR^2	0.000	0.068	0.009
		F	1.762	1.314	2.153

Of the three models where personal accomplishment was the criterion, only the first one was found to be significant as well. This consists of the difficulty identifying feelings factor and it explains 13.1% of the variance of personal accomplishment. Also, the alexithymia factor difficulty identifying feelings has a significant influence on personal accomplishment (p=0.016) explaining most of the variance of the dependent variable (b=-0.737, beta=-0.393), its effect on the criterion being negative. This way, based on these results, we appreciate that the more present this alexithymia factor (difficulty identifying feelings) is in a person's structure, the lower the personal accomplishment scores will be. Our results confirm a recent study proving that only a single aspect of the alexithymia construct serves as a possibly independent predisposing factor for specific burnout dimensions (7).

From the results obtained we can see that the age correlated positively with personal accomplishment, this meaning that the older our participants get, the more personal accomplishments they will obtain. Also, significant correlations were highlighted between alexithymia factor difficulty identifying feelings and all the burnout factors: emotional exhaustion, depersonalization and personal accomplishment, this meaning that the more difficulties our participants will have in identifying feelings, the more feelings of emotional exhaustion and depersonalization they will experience and the fewer problems they will have in identifying feelings, the more accomplished they will feel personally. At the same time, alexithymia factor externally oriented thinking and the total score in alexithymia correlated positively with the burnout factor depersonalization, which means that the more alexithymia they will experience or the more they will have an externally oriented thinking style, the more they will experience feelings of depersonalization.

Regarding teaching activity, there are significant differences in emotional ex-

haustion; the participants with no teaching activity feel less emotionally exhausted than those who teach, but the ones who teach show a higher level of personal accomplishment than those who do not.

Also, the participants who have witnessed a critical event in the last five years show higher levels of emotional exhaustion than those who haven't witnessed such an event.

When it comes to the cases our participants have worked on, there are significant differences in personal accomplishment regarding some of the things that affect the participants the most, for example, decomposing bodies; those who have worked on this kind of cases show higher levels of personal accomplishment than those who haven't.

When they were affected by an event at work, the participants who talked to one of their colleagues or to a specialist (psychologist, psychotherapist, social worker, officer) obtained significant differences in personal accomplishment, those who talked to one of these categories obtained higher results in personal accomplishment than those who did not.

When we consider if the participants suffer from insomnia, depression or whether they take drugs to relieve stress, we also obtain significant differences in emotional exhaustion, those who suffer from insomnia or depression or who take drugs to relieve stress obtaining higher scores in emotional exhaustion than those who do not.

We have obtained significant differences in emotional exhaustion when it comes to whether the participants think that the specialty they chose has changed their vision of life; the participants who think that the specialty they chose has changed their vision of life have obtained higher scores than those do not think the same.

Additionally, the linear regression results showed that alexithymia factor difficulty describing feelings has a positive effect on the criterion emotional exhaustion, so we can appreciate that the more present this alexithymia factor (difficulty identifying feelings) is in a person's structure, the higher the emotional exhaustion scores will be. At the same time, the alexithymia factor difficulty identifying feelings also has a significant influence on personal accomplishment, its effect on the criterion being negative; this way, we can appreciate that the more present this alexithymia factor (difficulty identifying feelings) is in a person's structure, the lower the personal accomplishment scores will be.

One of the strengths of the present study is because less literature is available regarding forensic physicians. The limitation of the study is represented by the small number of subjects included in the research.

CONCLUSIONS

Scores for forensic physicians are low-level for emotional exhaustion and medium-level for two subdomains and low-level for alexithymia. Factors revealed by the comparative analysis are important to adjust professional activity and to find strategies to cope with stressful professional events.

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NEWS

INFLUENCE OF APPLICATION OF CHLORHEXIDINE GEL AND CURCUMIN GEL AS AN ADJUNCT TO SCALING AND ROOT PLANING

Periodontitis describes a group of related diseases resulting in the destruction of the tissues that support the tooth. The most common destructive disease of the periodontal attachment apparatus is initiated by oral microflora. Scaling and root planning remains the "gold standard" treatment for periodontal diseases against which other treatments are compared. However, after nonsurgical therapy, several deep periodontal pockets may persist and in such cases, the treatment consists of surgical procedures. Since the systemic antibiotics therapy has various disadvantages such as the development of resistant bacteria and also requires higher dosage to attain required gingival crevicular fluid concentration at the target site, this led to use of local drug delivery system. The aim of a study realized by a group of researchers was to evaluate the efficacy of subgingival application of chlorhexidine gel and curcumin gel as an adjunct to SRP in the treatment of mild to moderate (4–6 mm) periodontal pockets. The study was conducted on thirty chronic periodontitis patients who were divided into two groups as control and experimental groups using a split-mouth design. After scaling and root planning, chlorhexidine gel was applied in control and curcumin gel in experimental groups. The plaque index, gingival index, sulcus bleeding index, probing pocket depth were recorded at baseline and subsequently after 1 month and 45 days. The results revealed that both chlorhexidine gel and curcumin gel have an effect on mild to moderate periodontal pockets in chronic periodontitis patients, but greater reduction was observed in the experimental group than the control group. It can be concluded that both control and experimental gel can be used as an adjunct to scaling and root planning, but the curcumin gel was more effective than the chlorhexidine gel in the treatment of mild to moderate periodontal pockets with a significant reduction in the indices scores when compared to the baseline values. (Hugar SS, Patil S, Metgud R, Nanjwade B, Hugar SM. Influence of application of chlorhexidine gel and curcumin gel as an adjunct to scaling and root planning: A interventional study. *J Nat Sci Biol Med.* 2016 Jul-Dec; 7(2):149-154).

Irina Grădinaru

LOCALIZED FIBROUS TUMOR OF THE PLEURA UN UNUSUAL CAUSE OF SEVERE HYPOGLYCAEMIA. CASE REPORT

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LOCALIZED FIBROUS TUMOR OF THE PLEURA UN UNUSUAL CAUSE OF SEVERE HYPOGLYCAEMIA. CASE REPORT (Abstract): Localized fibrous tumors of the pleura are rare. If there are symptomatic, the symptoms are based on hormone production or size. Complete resection offers the best results. However, recurrence is reported in a significant number of patients. Usually no oncologic treatment is necessary. **Keywords**: SOLITARY TUMOR OF THE PLEURA, HYPOGLYCAEMIA, THORACIC SURGERY.

Localized fibrous tumors of the pleura are rare and originate from primitive mesenchymal cells sub mesothelial tissue (1). These tumors usually arise from visceral pleura (in 80% of the cases). Benign and malignant forms have been defined by histological criteria. Surgery is curative in most cases. These tumors may locally recur despite complete excision and benign histology (1). We present a case of a giant fibrous tumor of pleura completely resected.

CASE REPORT

A 63-year-old man presented to us with progressive breathlessness and severe hypoglycemia. At the time of hypoglycemic crisis, convulsions and syncope were observed. On the chest radiography we observed a complete opacification of left hemithorax (fig. 1). A CT scan of the thorax showed a large mass occupying the entire left hemithorax with a completely collapsed underlying left lung, descending aorta and other mediastinal structures strongly, thereby shifting them to the right side (fig. 2).

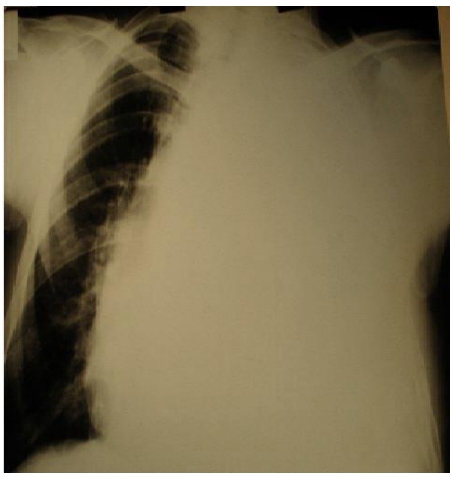

Fig. 1. Radiography with a complete opacification of left hemithorax**.**

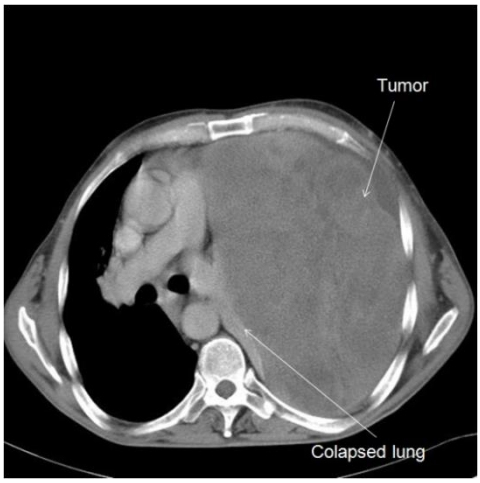

Fig. 2. Chest computed tomography scan showing a huge mass filling the entire left hemithorax.

We completely resected the mass by left postero-lateral thoracotomy (fig. 3).

During the surgery we discovered a huge encapsulated mass arising from visceral pleura.

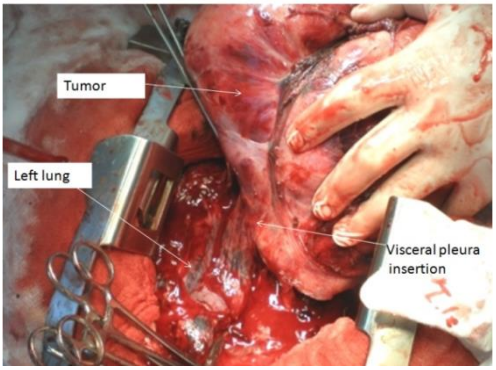

Fig. 3. Intra-operative view of the tumor.

The resected tumor was measuring 34cm in its largest dimension and weigh 3.800 g. After resection the left lung has fully re expanded. Postoperative, the glycaemia profile was normalized. Histopathological features were consistent with a solitary fibrous tumor. After an interdisciplinary discussion, no postoperative adjuvant therapy was necessary**.**

DISCUSSION

There are two types of primary pleural tumors: diffuse and solitary (1, 2). Diffuse variety is the malignant mesothelioma. Localized fibrous tumors of the pleura are relatively rare (600 cases having been reported previously (3)) and usually asymptomatic.

Cardillo et al found that 87% of solitary fibrous tumors in their series originated in the visceral pleura and only 13% in the parietal pleura (4).

In our case the symptoms were based on size and hormone production. The hypoglycemia, as in our case, is seen in 2% to 4% of cases, due to production of insulin-like growthfactor-2 by the tumor (1, 2, 5), and is more common in women. 80% of the localized tumors of the pleura are benign (1, 4).

England (6) described three histopathologic characteristics associated with malignancy: hyper cellularity, crowding and overlapping of nuclei, high mitotic activity > 4 mitoses/10 high-power fields. Using these criteria, 12% to 33% of solitary fibrous tumors of pleura could be considered as malignant.

A CT scan cannot differentiate between benign and malignant fibrous tumors of the pleura. For the most authors, tomographic guided biopsies are usually unhelpful in differentiating between benign and malignant pleural tumors with an accuracy rate less than 50 % (1, 4). Some authors consider that whole-body FDG PET is a noninvasive technique to readily and accurately differentiate benign from malignant masses, as the uptake in malignant mesothelioma would be very high (7).

Usually, the surgical intervention is re-

quired for both diagnosis and treatment. Complete resection offers the best results. The probability of recurrence is greater with malignant disease, but complete excision offers the best possibility of cure even in malignant forms. *Video assisted Thoracoscopic Surgery* (VATS) may be attempted for appropriate tumors, but conversion to open technique should be employed if margin clearance cannot be done with VATS. Some authors recommend VATS for lesions less than 5cm (2). In our case the tumor was not eligible for a minimal invasive treatment. Previously reports shows that safe surgery is possible in such patients despite poor a low forced expiratory volume (FEV1) (8). For this reason, we didn't explore the preoperative respiratory function for our patient.

In some cases, preoperative percutaneous embolization has been reported to be useful in reducing perioperative blood loss (9).

The overall operative mortality ranges from1.5% to 12% because of hemodynamic changes associated with decompressing the mediastinal structures (2). England and associates concur that the patients with incomplete excision will all die of their disease in less of five years (2).

Radiotherapy and chemotherapy have been undertaken for locally recurrent or inoperable malignant tumors but the long-term results are debatable (2).

CONCLUSIONS

The association of large pulmonary mass and hypoglycemia should highlight the possible diagnosis of localized fibrous tumor of the pleura. Surgery is the main treatment which should be attempt even in recurrent or very large tumors (10).

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PANCREATIC CANCER CT IMAGING: COMPARISON WITH SURGERY

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PANCREATIC CANCER CT IMAGING: COMPARISON WITH SURGERY (Abstract): **Aim:** To establish the best protocol for pancreatic computer tomography and criteria for staging (mainly for vascular invasion). **Material and methods**: Our research included 49 consecutive patients with pancreatic cancer examined at the Iasi "Sf. Spiridon" Hospital between January and December 2014 with a Siemens 16 Emotion CT unit. CT protocol included no enhanced CT and pancreatic phase of the superior abdomen, portal venous phase of the abdomen and pelvis. **Results and discussion**: The study patients were stratified into 5 age groups and the most frequently affected by pancreatic cancer were the patients aged 60 to 79 years. For T staging the extension in the per pancreatic fat tissue, into surrounding organs (5 patients had extension in other organs) and vessels was evaluated. We determined the degree of contact between the tumor and the artery, thrombosis and deformity of the veins and we have found 8 resettable lesions, 28 tumors in stage T3 and 13 pancreatic cancers in stage T4. Thirty-three patients had lymphadenopathies and 31 of them had distant metastases. **Conclusions**: Our study proved that computed tomography is a good method of examination for pancreatic cancer when the right imaging protocol is used; during the pancreatic phase the arteries and the tumor are well depicted, liver metastases are best evaluated during the portal venous phase. The best criterion for arterial invasion is tumor contiguity with more than half of vessel circumference, and for vein invasion deformity or thrombosis. Comparison with surgical staging was o good backup for the radiologist and depicted several differences with imaging staging, more often understating than over staging. **Keywords**: PANCREATIC CANCER, VASCULAR INVASION, COMPUTED TOMOGRAPHY

The incidence rates for exocrine pancreatic cancer worldwide equal its mortality rates (5%) and the main risk factors are diabetes mellitus, chronic pancreatitis, biliary stones, and smoking (1,2). The most frequent pancreatic malignancy is ductal adenocarcinoma which has nonspecific symptoms and blood tests, so imaging methods are very important for the diagnosis. Computed tomography (CT) is the method of choice for pretherapeutic evaluation of pancreatic malignant tumors (evaluates with maximum accuracy the tumoral extension into surrounding organs, vessels, lymph node involvement, distant metastasis) and follow-up after treatment (3,4). CT is widely available and is a validated tool for the diagnosis of pancreatic malignan-

cies because it depicts the tumor mass (with maximum lesion-to pancreatic parenchyma attenuation difference during the pancreatic phase), evaluates very well the arterial and venous invasion and detects hepatic metastases (5, 6). CT is widely available and is a validated tool for the diagnosis of pancreatic malignancies because depicts the tumor, evaluates very well the invasion in arteries and veins and detects hepatic metastases (5, 6). Computed tomography depicts in case of pancreatic cancer a poorly defined, isodense mass on non-enhanced CT which becomes hypo attenuating after contrast medium injection; the maximal contrast between the tumor and pancreatic parenchyma appears in fact in the pancreatic phase (between the arterial and portal venous phase) when the pancreas fully enhances and the tumor remains hypodense (7).

MATERIAL AND METHODS

Our research included 49 consecutive patients with pancreatic cancer examined at the Iasi "Sf. Spiridon" Hospital between January and December 2014 with a Siemens 16 Emotion CT unit; 36 (73.46 %) patients were surgically treated. The patients were examined in the morning, after digestive preparation (ingestion of 500 ml of 2% dilution of iodinated contrast medium). The imaging protocol included:

- non-enhanced CT of the superior abdomen,
- injection of 1-1.5 ml/kg, 370 – 400 mg I/ml nonionic hypoosmolar iodinated contrast medium, 3-3.5 ml per second;
- pancreatic phase of the superior abdomen – 20-30 seconds after the beginning of contrast medium injection;
- portal venous phase of the abdomen and pelvic regions – 70 - 80 seconds after the beginning of contrast medium injection;
- equilibrium phase of the hepatic region – when we have to characterize liver lesions.

We have studied the per pancreatic (including the vascular extension) and distant extension of the tumor, vascular extension of each tumor and compared it with the surgical protocol. The results were processed and interpreted by statistical methods (tables, figures, p, trend).

RESULTS AND DISCUSSION

Age-group distribution. The patients were stratified into 5 age groups: 30-39 years (1 patient, 2.04 %), 50-59 (7; 14.28%), 60-69 (22; 44.89%), 70-79 (15; 30.61%) and over 80 (4; 8.16%).

Gender distribution. The gender differences in prevalence were significant ($p<0.05$), 31 (63.26 %) of patients being males and 18 (36.73 %) females.

Computed tomography depicts in case of pancreatic cancer a poorly defined, isodense mass on non-enhanced CT which becomes hypo attenuating after contrast medium injection; the maximum tumor-to pancreatic parenchyma contrast occurs in fact during the pancreatic phase (between the arterial and portal venous phase) when the pancreas fully enhances and the tumor remains hypodense (7).

Criteria for resectability of pancreatic cancer are: lack of distant metastasis, no invasion of superior mesenteric and celiac arteries, and limited invasion of superior mesenteric vein to allow vein reconstruction (8, 9). In all cases of pancreatic cancer, according to the data in the literature, computed tomography depicted a poorly defined, isodense mass on non-enhanced CT, which became hypo attenuating after con-

trast medium injection (with the maximum contrast between the tumor and pancreatic parenchyma in the pancreatic phase) (7). For T staging we evaluated the extension into the per pancreatic fat tissue (present in 10 cases – 20.40%), surrounding organs (5 – 10.20 % patients had extension into other organs, such as duodenum, jejunum, stomach) and vessels, but in fact vascular invasion of the superior mesenteric artery and vein and of the celiac artery is essential. We have found 8 (16.32 %) resectable lesions (T1 and T2 tumors), 28 (57.14%) tumors in stage T3 (locally advanced but potentially resectable) and 13 (26.53 %) pancreatic cancers in stage T4 (unresectable tumors).

All vessels around the pancreas were examined in 3 planes (axial sections, sagittal and coronal reconstructions) and the degree of contact between the tumor and the vessel, stenosis, thrombosis and deformity was determined. The criteria used for assessing the vascular extension were the degree of contact between the tumor and the artery and for veins we added deformity and thrombosis. Tumoral invasion was found in 74 vessels: 40 (49.38 %) arteries (5–6.17 % celiac artery, 8–9.87 % hepatic artery, 8– 9.87 % superior mesenteric artery, 19–23.45 splenic artery) and in 34 (41.97 %) veins (22–27.16 % in the splenic vein and 12–14.81 % in superior mesenteric vein). Of the tumors with vascular extension only 13 (26.53 %) were not resectable (the ones with invasion of the celiac artery and superior mesenteric artery) and 28 were locally advanced (venous extension to one or more than one vein) (fig. 1).

In vascular invasion cases we found: contiguity of tumor to vessel on 75-100% of the circumference in 20 cases (24.69 %), 50-75% in 9 (11.11 %) cases, and 25-50% in 16 (19.75 %) cases, venous thrombosis in 18 (22.22 %) and venous deformity in 11 (13.58 %) cases. Tumor-to-vessel contiguity of less than 25% was found in 7 (8.64 %) cases and was not considered predictive for vascular invasion. Comparing with surgery we had only 3 (3.70%) cases of under staged vascular invasion and the best correlation between CT imaging and surgical reports for vascular invasion was contiguity of tumor to vessel on more than 50% of the circumference.

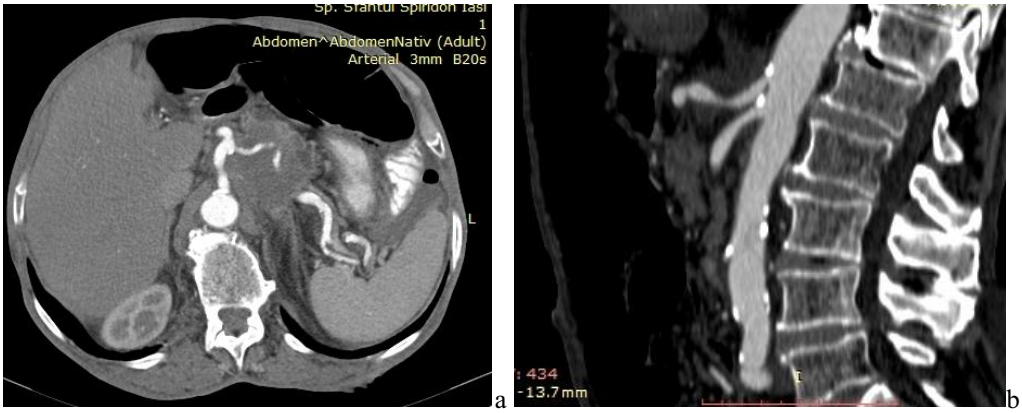

Fig. 1. Abdominal CT, pancreatic phase a – axial sections, b - sagittal reformates: celiac artery invasion

Regional lymphadenopathies were considered present when lymph nodes measured more than 10 mm in axial diameter, but if the lymph nodes were round or had an important contrast medium uptake, an axial diameter of 5-6 mm was considered pathological (fig. 2). We found 33 (67.34 %) patients with lymph node enlargement; when comparing CT with surgical findings, 3 (6.12 %) patients were under staged and 2 (4.08%) patients over staged.

Liver metastases are usually hypodense nodular lesions on non-enhanced and contrast enhanced CT and in our study we found 25 (51.02%) patients with typical pattern of liver metastasis. Comparing the CT diagnosis with pathological findings we found 3 more cases with secondary hepatic lesions, under staging being due to the fact that CT missed the isodense or small lesions (less than 10 mm in diameter). Peritoneal carcionomatosis was found in 6 cases (ascites, soft tissue infiltration of the omentum and nodular peritoneal thickening of the), compared with 10 cases at surgery (small lesions were not visible at CT) (fig. 3).

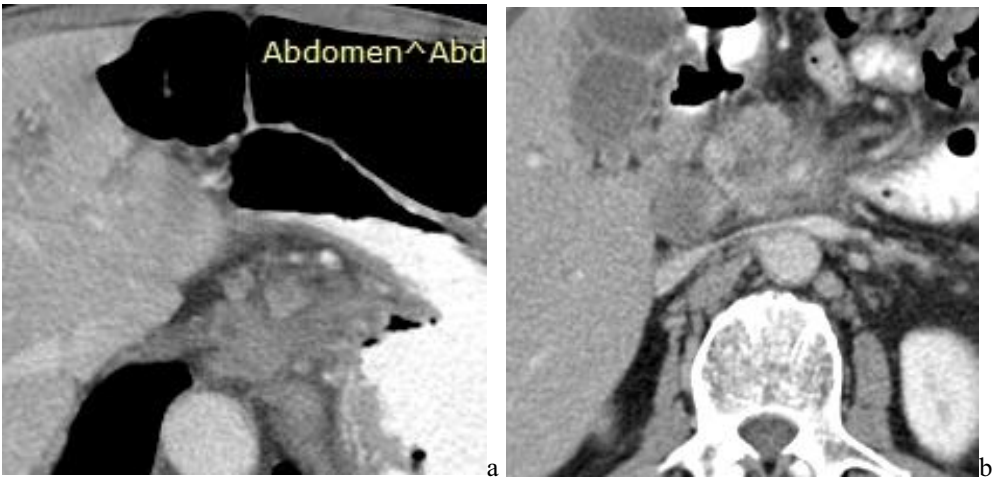

Fig. 2. Abdominal CT, portal venous phase a, b - lymphadenopathies

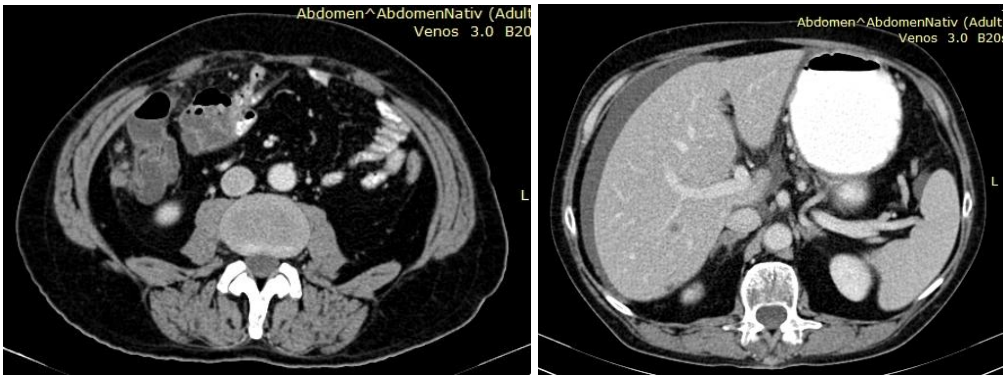

Fig. 3. Abdominal CT, portal venous phase – a - peritoneal carcionimatosis, b – hepatic metastases

Comparing CT imaging of pancreatic cancer extension with surgical evaluation, more cases of under staging than over staging were found (tab. I).

TABLE I

Pancreatic cancer extension - comparison between computed tomography and surgery

CT versus surgery	No.	%
Vascular invasion over staged	0	0 %
Vascular invasion under staged	3	3.70
Lymph nodes over staged	2	4.08
Lymph nodes under staged	3	6.12
Hepatic metastases over staged	0	0 %
Hepatic metastases under staged	3	6.12
Peritoneal carcionomatosis over staged	0	0 %
Peritoneal carcionomatosis under staged	4	8.16

CONCLUSIONS

Our study demonstrates that computed tomography is a good method for examination of pancreatic cancer when the right imaging protocol is used; the arteries and the tumor are well depicted during the pancreatic phase and liver metastases are best evaluated during the in portal venous phase. The best criterion for arterial invasion is tumor contiguity to more than half of vessel perimeter, and for vein invasion deformity or thrombosis. Comparison with surgical staging was o good backup for the radiologist and depicted several differences with imaging staging, more often under staging than over staging.

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DILEMMAS IN THE ATTITUDE TOWARDS SUICIDE

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DILEMMAS IN THE ATTITUDE TOWARDS SUICIDE (Abstract): Although apparently easy to define, the suicidal act or attempt raises complex and difficult problems due to the multitude of conditions and situations that can lead to it. In all cases the suicide's definition has always centred on the intention of one person to deliberately cause his or her death in an active manner. Defining suicide has been consecutively the temptation of philosophers, sociologists, theologians, psychologists and psychiatrists. From an epistemological point of view the suicide is an open concept without precise borders, yet not incoherent. Scientists have constantly tried to establish evaluation criteria of suicidal acts but these are variable. One can even assume that there is an infinity of combinations of characteristics that would legitimize the label of suicide, although none of them can be particularized in any way. Not even death itself represents a necessary condition for the evaluation of an act as suicide. **Keywords:** suicidal act, open concept, death by suicide.

Various attempts to define the suicide have been made in time by philosophers, sociologists, theologians, psychologists and psychiatrists. Sociologists, following the descriptive conclusions of E. Durkheim, regard suicide as the conscious assumption of a deadly risk (1). Certain psychiatrists, followers of Karl Menninger, are in striking opposition, believing that suicidal attitude is beyond mental integrity and consciousness. They state that any self-aggressive behaviour can be considered suicidal, and any lethal result of such behaviour can be qualified as suicide. In his attempt to come as closely as possible to an accurate definition of suicide Tom Beauchamp established three evaluation criteria for any suicidal act: the individual intentionally causes his/her death, there is no coercion in this respect from any other individuals and death is caused by circumstances especially created for the suicidal act purpose (2). Regardless of the accuracy of these criteria used in the identification and evaluation of the suicidal act, such a complex phenomenon cannot be comprised in a single succinct definition. It can occur in very different circumstances and can be performed through various methods. Motivation is often vague, mysterious and incomprehensible for both specialists and people who are close to the person who commits suicide.

From an epistemological perspective suicide appears as an open concept, without precise borders, without lacking coherence. There have been some attempts to establish evaluation criteria for a certain act in order to classify it as suicide, but these are variable. One could legitimately assert that there

is an infinity of combinations of characteristics that would justify labelling an action as suicide, yet they cannot be particularized, as not even death itself represents a necessary condition for evaluating an action as suicide (3).

Consequently, although it is apparently easy to define the suicide or suicide attempt, it still raises plenty of complex and difficult issues, due to the multitude of conditions and situations it can occur in. In both reductionist approaches and more liberal perspectives the definition of suicide focused on the intention of that individual to deliberately cause his/her death, in an active manner (4). The ones in favour of the reductionist approach were more radical and emphasized the self-destructive intention, establishing at the same time the purpose and the obvious motivation of the intention and the action. There is a very short distance from defining suicide in such a way to becoming judgemental; judgement requires pros and cons, leading to the paradox that the evaluation of the motivation is taken into account more than the existence of a desire to die. Many times the intention can be caused by the desire to change a certain state of things, which actually distances this act from suicide proper. The examples of people who commit suicide in order to end the pain in terminal illnesses such as cancer or of those who want to avoid betrayal during wartime are quite illustrative for the complexity of the issue. In these circumstances, suicide cannot be defined without a complex analysis of its dimensions as intention, deliberate act and consequences.

PHILOSOPHICAL AND MORAL CONSIDERATIONS

For thousands of years, religious traditions have imposed a very peremptory attitude on societies towards suicide. In India, the Upanishads tradition recognizes the suicidal act as a decent, sometimes heroic way of dying, yet only by drowning, fire or starvation. Later on, Hinduism and Jainism accepted and allowed passive self-destruction through starvation and asceticism. This form of passive self-destruction dedicated to certain deities is still opposed to certain active suicide methods, actually prohibited by the Hindi religion. In time, Buddhism adopted a very clear attitude towards any form of self-destruction. Escaping from a world of suffering was not allowed by means of suicide, the alternative being a change of attitude towards the self and the world. In the history of the Far East, two self-destructive modalities, in other words two motivations of the suicidal act were allowed, namely honour and revenge.

In ancient and medieval China, a man's suicide was disapproved because the man's duty was to support his family. Women did not have the same duty, on the contrary, they were often forced to defend their honour by suicide. As well as this, in both India and China, the cremation of the wife on a funeral pyre, together with the deceased husband was a common practiced, that persisted for many years. As long as the wife expressed her consent, this practice could be regarded as an act of suicide, accepted and even imposed by the society.

The European medieval religious tradition adopted an exceptional attitude according to which death is preferable to the betrayal of God. Thus, imposed apostasy represented the cause of many admitted suicides in both the Christian and the Judaic religions. The Islam, another monotheist religion, forbids suicide on the grounds of

God's supreme right upon any life. Consequently, the Islamic tradition forbids, through its Prophet's words, included in the Sunnah, the burial of the one whose death is caused by suicide, the Koran stating that "no soul is allowed to die unless God allows it at a certain moment".

The idea of gods' sovereignty upon human life is also to be found in the Greek philosopher's writings. The personal authority on someone's own life is limited by the priority of gods' sovereignty, suicide being regarded thus as an usurpation of divine power (5).

The same arguments were brought forward in medieval Christianity. Thomas Aquinas claimed that suicide represented an offense against one's own person, against the society and especially against God, since man belonged to his creator, who had offered him the gift of life. This concept of gift of God represented for many contemporary theologians the basic argument in their opposing the suicidal act. This opposition has not always been unanimously embraced. Thus, David Hume, in his essay On Suicide, claims that "God does not immediately appear in any activity, yet He governs everything by those general and immutable laws that have been established from the very beginning". Consequently, suicide may be part of the pre-established destiny of every human being, being regarded any other death cause (6).

We should nevertheless point out the differences between religious viewpoints and those based on moral grounds. Starting from various theories of morals, the Stoics or the Romantics of the 19th century displayed an attitude opposed to that of the church, looking for socially rooted arguments. This way, the attitude towards suicide was less radical during the 20th century. Even Theology raised the question whether suicide represents an act of self-sacrifice or self-suppression. The sinful dimension of suicide regarded as a crime against one's self is gradually lost; especially when related to medical issues, suicide becomes a more complex problem that transcends theological, philosophical or sociological areas (7).

The media mentions suicide cases very often all over the world. One argument against those who commit suicide is most of the times obvious and it seems to be shared by most people. The suicidal act is judged as cowardice in front of the hardships of life. Landsberg's objection is that "Hannibal's or Seneca's ways of dying could hardly be regarded as acts of cowardice". He also adds that "there are certainly more people who do not commit suicide because they are too coward to do it than people who commit suicide because they are coward". This does neither imply that Landsberg views suicide as act of heroism or that je justifies suicide for a noble purpose. He simply shows that the suicidal act is not merely a cowardly action, as most often labelled (8).

For Cioran, the absurdity of life is what causes a feeling of repulsion towards it. Moreover, he considers that the pursuit of truth is a battle in vain, as death is the only certainty of life. Starting from these premises, Cioran develops, in an almost Schopenhauerian manner, an ethics of renouncement. Unlike Schopenhauer, where the tragedy is more profound, as will is present in absolutely everything, influencing both the phenomenological and the intelligible worlds, Cioran still hopes that life is the only refuge. From this perspective, he states: "There are no arguments for living. On the heights of despair, the pas-

sion for the absurd is the only thing that can still throw a demonic light on chaos". It becomes evident, that under the dominance of the absurd, life loses its meaning. In this cases, suicide remains, for existentialist thinkers, a valid solution. The issue is no longer considered from a moral perspective, as Cioran himself states: "here we must think beyond normative conventions, as the issues addresses the human condition directly" (9).

CLINICAL FORMS OF SUICIDE

The suicide is probably the most dramatic and challenging clinical situation a psychiatrist is confronted with. It does not only represent a functional problem that could be addressed by means of therapeutic techniques, but also an existential issue, in both the proper and the philosophical meanings of the term (10, 11, 12). It is not about getting to lead a better life but about life being worth living or not. The fact that the starting point in the treatment of a potentially suicidal patient is seen in such different terms by the therapist and the patient qualifies the suicide as an extremely difficult case, since the psychiatrist duty goes beyond helping his/her patient by technical means. More precisely, the former is supposed to persuade the patient to alter his/her most profound beliefs and attitudes concerning life.

From a clinical viewpoint, the suicidal phenomenon displays numerous individual variations, yet some common traits have been identified (13):

- the purpose envisaged by suicide is the need to find a solution
- the target to be achieved is suspending the state of consciousness, stopping the flow of consciousness
- the triggering stimulus is uncontrollable psychical pain
- frustrate the common stressor in suicide is represented by frustrated psychological needs
- the person who commits suicide experiences an overwhelming feeling of helplessness and despair
- the inner attitude leading to autolytic actions is ambivalence, that is experiencing two contrary tendencies: of self-destruction and of planning a rescue
- the cognitive status of suicidal people is constriction, a major narrowing of the cognitive field and of the capacity to produce response alternatives
- the common action in suicide is escaping, avoidance to confront certain situations perceived as stressful (13, 14)

G. Ionescu enumerates the following forms of suicide: the idea of suicide, the suicide attempt, dissimulated suicide, suicidal drive and chronic suicide (15).

- **The idea of suicide** is not necessarily pathological in nature as it can also appear in the larger area of normality. It is probably exacerbated in crisis situations, when the individual cannot access a response method that s/he considers satisfactory.
- **The suicide attempt** is a failed suicidal act. As far as their truthfulness is concerned, suicide attempts could be placed on a bipolar axis, having as extremes full authenticity and provability.
- **Dissimulated suicide** often surprises due to the preoccupation of the subject for the feelings and wellbeing of the people around

him/her, especially those of the family members. The subject appeals to dissimulation in order to make sure s/he does not impose too heavy a moral and social burden on the loved ones, who are most often likely to experience self-culpability feelings.

- **Suicidal drive** explains the suicidal act as an expression of an irresistible, impossible to repress tendency of the individual to end his/her own life. Like any drive, the suicidal drive is transient so that the individual, once saved, is surprised by his own gesture.
- **Chronic suicide** includes the following situations:
  - risk behaviours taken to extremes to aberrant behaviours
  - refusal for absolutely necessary surgical interventions
  - refusal of absolutely necessary medical treatment
  - continuing to take drugs or drink alcohol in cases when the physical, psychic and professional degradation of the person is obvious

As opposed to various other types of psychological disorders, the suicidal state is not one that could gradually improve or worsen, but it represents a radical event that occurs at one given moment, is totally unpredictable and often irreversible.

ETHICAL ASPECTS

For the psychiatrist the ethics of suicide centres firstly upon the moral justification and the limits of an intervention policy. Thomas Szasz is known for his vehement attack against the traditional psychiatric approach of suicide, claiming that "accomplished suicide is generally the expression of an individual desire for greater autonomy and particulary for self-control upon one's own death". He considers that intervention in the suicidal behaviour is always inappropriate, actually diminishing the individual's freedom and reflecting the coercive patronizing attitude of the therapist (16).

Suicide faces thus the psychiatrist with a dilemma from both a professional and a human point of view. The therapist is challenged by the need to choose between saving a person's life, as stated in the Hippocratic Oath, and his duty to respect a personal decision, according to the ethical principle of individual autonomy (17). The critical moment extends to the point where the psychiatrist's right to interfere becomes the direct consequence of establishing an individual's ability or inability to decide upon his/her own life. Regardless of all these dilemmas, suicide prevention remains a desiderate and a reality at the same time, being regulated by national programmes adopted even by the most liberal countries as far as an attitude towards individual freedom is concerned (18).

The utilitarianism and other philosophical theories are not able to provide all necessary arguments for evaluating the value of life, as, from a moral viewpoint, life should be respected for its intrinsic value and consequently there might appear divergences of views between the psychiatrist and his/her potentially suicidal patient. The psychiatrist justifies his/her intervention pleading for the value of life, while the patient can quote arguments related to the quality of his/her life, disregarding the value of life in itself. The difficult task of the psychiatrist is reflected in his/her attitude that should replace the paternalist

approach with persuasion. He/she should make the patient perceive the new meanings his/her life might gain, allowing him/her at the same time to be completely free in making a decision.

CONCLUSIONS

All rational arguments in favour of suicide, such as avoiding pain or dishonour, actually plead in favour of giving up a certain quality of life, without actually inquiring whether death is better than life. The images of both death itself and life in its intrinsic dimension are equally difficult to perceive. Even psychiatric pathology can offer countless examples of delirium-hallucinating states related to experiences and identities that occur after death. Regarded from a life's value perspective, suicide cannot have a specifically moral representation, as morality implies a series of rules and principles that are the very grounds of life. Suicide ends up thus representing a decision beyond the sphere of moral arguments and other people's judgements. Consequently, the reason for which suicide should be prevented starts from the value of life in itself, and the medical intervention in the suicidal act is justified by the very belief in the value of life.

Suicide prevention is unfortunately still a mere palliative method, all positive results having just an individual character, while suicide rates remain, except few variations, quite constant. Suicide remains thus a ubiquitous, universal and perennial phenomenon, whose social impact and resonance cannot be neglected.

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PREDICTORS OF SUICIDE RISK IN INCARCERATED MALE OFFENDERS: THE ROLE OF PERSONALITY DISORDERS

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PREDICTORS OF SUICIDE RISK IN INCARCERATED MALE OFFENDERS: THE ROLE OF PERSONALITY DISORDERS (Abstract). **Aim:** This paper summarizes the results of a cross-sectional study aimed at exploring the role of personality disorders in predicting the suicide risk among males imprisoned for various offenses. **Material and methods:** The data set used in analyses was based on the responses of 124 males imprisoned for various violent or non-violent offenses (M_{age} = 32.39 years, SD = 8.43 years). Male offenders were interviewed using a form for demographic data and information on suicidal history. Also, they completed the Suicide Behaviors Questionnaire-Revised, as part of a broader protocol used in an ongoing study focused on the psychosocial and pharmacological aspects relevant to the diagnosis and management of personality disorders among inmates. **Results:** At the time of data collection, forty-three respondents (34.7%) were diagnosed with at least one personality disorder. Data were analyzed using hierarchical multiple linear regression. The frequency of suicide attempts during the periods of freedom and during incarceration were significant predictors of suicide risk. Moreover, the diagnosis of personality disorder brought a significant addition to the power of final regression model, explaining almost 8% of the variance in suicide risk among the inmates. **Conclusions:** The findings highlight the importance of implementing prevention programs in correctional institutions, with screening and monitoring of inmates diagnosed with severe mental disorders as an important component. **Keywords:** Male offenders, personality disorders, suicide risk, predictors.

Research data in forensic psychiatry suggest that a considerable proportion of males and females incarcerated in correctional institutions suffer from a chronic and pervasive mental disorder (1). Of the clinical entities encountered among prisoners, personality disorders are prevalent. According to a meta-analysis of about 23000 prisoners from 12 countries in Europe, USA and Australia, 65% of men in correctional institutions and 42% of incarcerated women had a diagnosis of personality disorder (2). On the other hand, it is recognized that people with personality disorders are at high risk for suicidal behaviors, which represent a stress factor for correctional officers.

Personality disorders (e.g., antisocial, borderline, paranoid, schizotypal, etc.) are fairly common among both male and female inmates (2, 3, 4). The general pattern of dysfunctional symptoms includes cognitions, affective symptoms, reactions and behaviors that deviate markedly from the expectation of the individual's culture and are associated with significant impairments

in professional and interpersonal functioning (5). This pattern is pervasive, stable over time, consistent across a variety of situations and contexts of everyday life and is not due to the direct effects of substance abuse or any other general medical condition. Compared with other clinical entities (depression excluded), personality disorders are associated with a higher risk of suicide (6, 7, 8, 9). On the other hand, all over the world, inmates are a population particularly vulnerable to suicide spectrum behaviors, as confirmed by epidemiological data (10, 11, 12). Personality disorders are one of the risk factors for suicidal behaviors among prisoners (11, 13, 14, 15). Most studies focused on the role played by mental disorders (personality spectrum disorders included) in attempted or completed suicide by inmates were conducted in US and UK. Data were also reported for other countries, such as Austria (16) and the Netherlands (17). This paper summarized the results of a study conducted in Romania on a sample of 124 inmates.

MATERIAL AND METHODS

The data set used in quantitative analyses was based on the responses of 124 male incarcerated for various offenses in two correctional facilities from Botosani and Iasi. Recruitment of female inmates was not possible as both are male correctional institutions. The first of these correctional institutions accommodates pre-trial detainees and inmates in a semi-open confinement. The correctional facility from Iasi has three units (open, closed or maximum security) and accommodates male inmates serving long-term sentences. Inmates on remand in custody or inmates in quarantine were excluded from the study. The interviewed inmates were aged 18 to 56 years (M_{age} = 32.39 years, SD = 8.43 years). Sixty-five (52.4%) were non-recidivists, the remaining being recidivists with two (N = 25; 20.2%), three (N = 21; 16.9%) or more than three prior convictions (N = 13; 10.5%). Depending on the nature of the crime they committed, inmates were divided into two subgroups: a) inmates who had committed violent crimes (e.g., attempted murder, qualified murder, robbery accompanied by murder, rape with/without sequestration, rape and robbery, attempted rape, human trafficking, etc., N = 44) and b) inmates who had committed non-violent crimes (e.g., complicity to theft and deception, driving without a license, illegally carrying a weapons, illegal crossing of border, etc., N = 80).

Inmates were interviewed individually using a form for obtaining demographic and legal data and information on suicidal history. Also, they completed the SBQ-R (18), as part of a broader protocol used in an ongoing study focused on the psychosocial and pharmacological aspects which are relevant to the diagnosis and management of personality disorders among inmates. At the time of interviews, according to medical records forty-three of the respondents (34.7%) had a diagnosis of at least one personality disorder (codes F60-F61 based on ICD-10/19). Distribution of prisoners by diagnosed personality disorders was as follows: paranoid personality disorder (N = 1; code F60.0), antisocial personality disorder (N = 18; code F60.2), emotional unstable (N = 4; code F60.30) or borderline personality disorder (N = 14; code F60.31), other personality disorders (N = 3; code F60.8) and mixed personality disorder (N = 3; code F61.0). Inmates who had enduring personality change not attributable to brain damage or disease were excluded from the

study. Most study inmates presented various other current organic diseases or antecedents.

Suicide risk was assessed with SBQ-R. This instrument is known through its simplicity and parsimoniality and allows clinical psychologists or psychiatrists who provide assistance to patients with various mental disorders and/or suicide history to obtain valuable information. The reduced number of items and their simple wording recommend the SBQ-R as a screening tool that is useful for identifying the suicide risk in individuals of different ages and health conditions. The SBQ-R is a four-item self-report scale that measures distinct aspects of suicidal behavior: lifetime suicidal ideation and/or behavior (item 1), the frequency of suicidal ideation in the past year (item 2), the communication of suicidal thoughts to others (item 3), and the likelihood of attempting suicide in the future (item 4). Participants are asked to check the number behind the statement that best applies to them. Each statement is provided with five, six or seven available responses. Each item is scored on a 0 to 6 scale and the total score is obtained by summing the scores for the four items (possible range: 3-18). Higher scores indicate higher levels of risk for suicidal behavior. Data of an exploratory factor analysis (by principal components extraction with *Varimax* rotation and eigenvalue > 1.00) carried out on the entire sample of inmates yielded a one-latent factor that accounted for 72.8% of the total variance in item scores. The standardized values of item loadings ranged from 0.71 to 0.93. In our study, internal consistency was satisfactory: 0.83 - total sample, 0.80 – inmates with personality disorders, 0.77 – inmates without personality disorders.

To determine the role of a diagnosis of personality disorder in predicting suicide risk among male imprisoned for various offenses we used hierarchical multiple linear regression. In the successive steps of the analysis we entered as independent variables only those variables for which preliminary analyses yielded significant relationships with SBQ-R score (i.e., the dependent variable). The diagnosis of personality disorder (PD) has been introduced as a dummy-type independent variable (1 = present, 0 = absent/reference category). A similar strategy has been used to recode the number of convictions (non-recidivists = 1, recidivists = 0/reference category) and type of criminal offense (violent offenses = 1, non-violent offenses = 0/reference category). For all analyses, the critical level of statistical significance was set at $p < 0.05$ (two-tailed).

RESULTS and discussion

Significant differences between inmates diagnosed with PD and those without PD (Table I) were obtained in terms of age ($\chi^2 = 15.11$, $df = 3$, $p < 0.01$), education level ($\chi^2 = 10.81$; $df = 3$, $p < 0.05$), the number of convictions ($\chi^2 = 4.38$; $df = 1$; $p < 0.05$) and type of criminal offense ($\chi^2 = 7.06$; $df = 1$; $p < 0.01$). Most inmates with PD were aged 26 to 35 years (66.7%) and had 5 to 8 years of formal education (58.1%). The group of inmates without PD was more heterogeneous in terms of age. On the other hand, in this group most inmates had 9 to 12 years of formal education (54.3%), followed by inmates with 5 to 8 years of education (33.3%). Over sixty percent of inmates diagnosed with PD (compared to only 40.7% of those without PD) were recidivists, and 51.2% of the inmates with PD (compared to only 27.2% of those without PD) committed violent crimes.

TABLE I
Cross-tabulation between demographic/forensic characteristics and psychiatric condition

Variables	Prisoners with PD (N = 43)	Prisoners without PD (N = 81)	χ^2
Age			
18-25 years (N = 33)	5 (11.9%)	28 (34.6%)	15.11 **
26-35 years (N = 53)	28 (66.7%)	25 (30.9%)	
36-45 years (N = 25)	6 (14.3%)	19 (23.5%)	
46-60 years (N = 12)	3 (7.1%)	9 (11.1%)	
Education			
Without/≤ 4 years (N = 13)	6 (14.0%)	7 (8.6%)	10.81 *
5-8 years (N = 52)	25 (58.1%)	27 (33.3%)	
9-12 years (N = 56)	12 (27.9%)	44 (54.3%)	
University (N = 3)	-	3 (3.7%)	
No. of convictions			
Non-recidivists (N = 65)	17 (39.5%)	48 (59.3%)	4.38 *
Recidivists (N = 59)	26 (60.5%)	33 (40.7%)	
Type of criminal offense			
Non-violent offenses (N = 80)	21 (48.8%)	59 (72.8%)	7.06 *
Violent offenses (N = 44)	22 (51.2%)	22 (27.2%)	

Note: * $p < 0.05$; ** $p < 0.01$

Regardless of the legal status and psychiatric condition, inmates with a history of attempted suicide during periods of freedom (N = 21) committed one to ten attempts (M = 2.80; SD = 2.67), all cases with parasuicidal behaviors being excluded. Also, inmates with a history of attempted suicide in periods of detention including the current one (N = 18) had attempted suicide one to six times (M = 2.16; SD = 1.24). On the other hand, compared to inmates without PD, those diagnosed with mental disorders had committed, on average, more suicide attempts both when out of prison ($M_{\text{inmates with PD}} = 1.23$; $SD_{\text{inmates with PD}} = 2.35$; $M_{\text{inmates without PD}} = 0.07$; $SD_{\text{inmates without PD}} = 0.34$; $t = 3.20$; $df = 122$; $p < 0.01$; $d = 0.83$), and when in prison ($M_{\text{inmates with PD}} = 0.83$; $SD_{\text{inmates with PD}} = 1.34$; $M_{\text{inmates without PD}} = 0.03$; $SD_{\text{inmates without PD}} = 0.24$; $t = 3.87$; $df = 122$; $p < 0.001$; $d = 1.00$). For these differences the effect sizes were larger. Moreover, inmates with PD had significantly higher score on SBQ-R ($M_{\text{inmates with PD}} = 9.09$; $SD_{\text{inmates with PD}} = 5.49$; $M_{\text{inmates without PD}} = 3.72$; $SD_{\text{inmates without PD}} = 1.78$; $t = 8.04$; $df = 122$; $p < 0.001$), with a very large effect size ($d = 1.39$).

Compared with non-recidivists (N = 65), the recidivists (N = 59) had a higher suicide risk based on SBQ-R score. Suicide risk was also higher among inmates who committed violent offenses ($M_{\text{non-recidivists}} = 4.83$; $SD_{\text{non-recidivists}} = 3.58$; $M_{\text{recidivists}} = 6.42$; $SD_{\text{recidivists}} = 4.96$; $t = -2.06$; $df = 122$; $p < 0.05$). Although for the previous comparisons the effect sizes were

modest (d = 0.37 şi d = 0.38), the number of convictions and type of crimes were entered as independent variables in the regression analysis.

Table II summarizes the results of the hierarchical multiple linear regression analyses. Along with the constant, the number of convictions, type of offense, number of suicide attempts committed in periods of freedom and history of suicide attempts in periods of detention (model 01) explained approximately 50% of variance in SBQ-R score, the resulting regression model being statistically significant (F = 28.98; $df_{regression}$ = 4; $df_{residual}$ = 119; p < 0.001). However, the positive predictors of suicide risk were only the number of suicide attempts in periods of freedom (β = 0.27; p < 0.01) and the frequency of suicide attempts in periods of detention (β = 0.48; p < 0.001). The first of these independent variables explained only 5.42% of the variance in suicide risk, while the second variable had a larger contribution (15.76%).

TABLE II

Results of hierarchical multiple linear regression

Model	Independent variables	B	SE	β	r^2_{sp}
01	**No. of convictions**	0.56	0.59	0.06	-
	Type of offense	1.10	0.60	0.12	-
	No. of suicide attempts (freedom)	0.79	0.22	0.27 **	0.0542
	No. of suicide attempts (detention)	2.33	0.38	0.48 ***	0.1576
*R = 0.70; F = 28.98 ***; R^2 = 0.493*					
02	**No. of convictions**	0.17	0.55	0.02	-
	Type of offense	0.46	0.57	0.05	-
	No. of suicide attempts (freedom)	0.60	0.20	0.21 **	0.0309
	No. of suicide attempts (detention)	1.89	0.36	0.38 ***	0.0967
	Diagnosis of personality disorder	3.00	0.64	0.32 ***	0.0795
*R = 0.75; F = 31.67 ***; R^2 = 0.573; Δ R^2 = 0.080 ******					

Note: The constant has been included into all models. ** p < 0.01; *** p < 0.001

In a second step of analysis, the independent variable related to the presence of a personality disorder diagnosis was entered, thus resulting a statistically significant model (F = 31.67, $df_{regression}$ = 5, $df_{residual}$ = 118, p < 0.001). Along with the constant, the independent variables entered into the model accounted for 57.3% of variance in SBQ-R score. When the presence of a personality disorder was entered as an independent variable, the explanatory power of the final regression model improved by 8% (ΔR^2 = 0.080), this increase being statistically significant (F_{change} = 20.00; df_1 = 1; df_2 = 118; p < 0.001). The number of suicide attempts committed in periods of freedom and the attempts committed during detention remained positive predictors of suicide risk (β = 0.21 and p < 0.01, β = 0.38 and p < 0.001), explaining 3.09% and 9.67% respectively of the variance in the dependent variable. Also, the presence of a personality disorder was a positive predictor of suicide risk (β = 0.32; p < .001), explaining about 8% of the variance in the score on SBQ-R.

The aim of this study was to explore the role of personality disorders diagnosed

among inmates in predicting suicide risk. The criterion considered in the strategy for quantitative analysis of raw data was inmates' vulnerability to engage in (new) suicidal behaviors. This variable was captured using SBQ-R (18). A higher frequency of prior suicide attempts and the presence of a clinically diagnosed personality disorder were associated with a higher suicide risk. The presence of a personality disorder in inmates explained about 8% of the variance in SBQ-R score distribution. This result is consistent with data reported in a case-control study conducted in Austria by Fruehwald and his colleagues (16) who in a sample of 252 inmates (almost all male) have identified the presence of a psychiatric diagnosis as an important predictor of suicide completion (OR = 17.4; $p < 0.001$). Suicide risk is even higher when associated with crisis episodes that are specific to personality disorders, especially with the severe ones (e.g. borderline or schizotypal). During such episodes, suicide is often either demonstrative or manipulative (20), or impulsive (6, 20) and does not necessarily imply a wish to die. However, besides the suicide risk associated with episodes of crisis, patients diagnosed with personality disorders may present chronic suicidal behavior (22) that is often lethal, especially when the medical condition of such patients does not improve. Moreover, by its implications for psychological and social functioning of human individuals in general, deprivation of liberty, especially life or long-term prison sentences, can complicate the symptoms of personality disorders through major crises that inmates face with. Often, these crises occurs within the first days of incarceration when the risk for suicidal behaviors is higher (14) and the committed acts are associated with feelings of helplessness and despair. The risk of death following an attempted suicide increases significantly in inmates serving life sentences (23).

Also, in our final regression model the number of suicide attempts committed by inmates while out of prison and the frequency of suicide attempts committed in periods of detention explained approximately 3.1% and 10%, respectively of the variance in suicide risk. These results are consistent with those reported by other studies (16, 17, 23). The difference in the explanatory power of these two variables can be explained by the different life circumstances in time of freedom and imprisonment. Thus, when the released inmates with known personality disorders are trying to readjust after serving a sentence of imprisonment, suicide acts that may have a sporadic and conjectural or chronic character tend to be associated with the stressful life events which cause impairments in patients with personality disorders, existential crises, psychotic episodes specific to some personality disorders (e.g. borderline) or pharmacological treatment failure/lack of psychological support. Conversely, the periods of detention imply some differences in terms of risk factors. For example, in addition to the above-mentioned factors, the physical and administrative prison environment (e.g. custodial regime, organization of detention cells, lack of socialization activities, supervision of large numbers of inmates, low vigilance of prison guards, etc.) and psychological factors (e.g., frustration related to legal sentence, shock of incarceration, need to adapt to an unknown environment and varying emotional pressures or conflicts with other inmates or administrative staff) can help create conditions favorable to the development of sui-

cidal behaviors.

This study has some limitations. Thus, the sample of incarcerated offenders with a diagnosis of personality disorder was relatively small, and consisted solely of men. Although the interview protocol contained much more questions on the family of origin climate, history of socialization, education, current marital status, personal and family medical history, risk behaviors and traumatic life experiences that we considered as being relevant for understanding the antecedents of mental disorders diagnosed in some of the participants in this study, due to the relatively small sample size the multivariate data are not presented in this paper. Moreover, due to administrative limitations related to time allocated for interviews visits, the clinical status of each participant was based solely on medical records and general clinical findings during interviews. Over three quarters of inmates with personality disorders also had other mental disorders (in various combinations), but their disproportionate distribution made grouping for statistically relevant comparisons impossible. Also, some of offenders in the control group had other mental disorders (e.g. epilepsy, mood disorder, schizophrenia, etc.) which were not included in multivariate analysis. In a future case-control study we aim at collecting data from a larger and more demographically and clinically heterogeneous sample of incarcerated offenders with mental disorders and to compare it both with a sample of offenders without a previous history of or current psychiatric problems and with a nonclinically matched sample based on community population. In addition, we plan to compare the suicide risk associated with personality disorders in male and female offending with the risk associated with other mental disorders recognized as having suicide spectrum complications, such as depressive and bipolar disorder or schizophrenia (24, 25).

CONCLUSIONS

Suicide spectrum behaviors are a common phenomenon in the general prison population. Risk factors include some demographic variables, personality vulnerabilities, history of suicide, and severe mental disorder (personality disorders included). Life adversities and certain characteristics of prison environment act as precipitating factors. These factual data highlight the importance of implementing prevention programs in correctional institutions, with screening and monitoring of inmates diagnosed with severe mental disorders as an important component. The care of inmates diagnosed with personality disorders who are at high suicide risk requires a multifaceted approach, including administrative measures, rigorous drug treatment plan and psychotherapeutic interventions. The therapeutic approach must concentrate on enhancing emotional functioning, reducing impulsive behaviors and identifying effective coping strategies to deal with factors of psychological stress underlying the suicidal behavior.

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THE MAST CELL: FROM THE OLD STRUCTURE TO THE NEW FUNCTIONS

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THE MAST CELL: FROM THE OLD STRUCTURE TO THE NEW FUNCTIONS (Abstract): Initially viewed as the central pawn in the pathologic chain of allergic reactions, the mast cell, although described microscopically over a century ago, remains an enigma. The richness of substances preformed and deposited within the cytoplasmic granules characteristic for this cell or newly synthesized after the activation of membrane receptors triggered a peculiar interest for the clarification of the intimate relationship established by the mast cell with the antigens or with the other immune cells, within the connective extracellular matrix. Nowadays, the origin of mast cell is established and its behavior is explained in accordance with the nature of the molecules stored within the cytoplasm. However, the mast cell keep displaying new abilities in other pathological conditions, justifying the major interest linked to the position and role performed in supporting the development and progression of tumor cells, especially through the involvement in tumor neoangiogenesis. This review focus on the complex functions of the mast cell, highlighting its pathogenic potential based on the activation process and mediators' biological properties. **Keywords**: MAST CELL, MEDIATORS, CARCINOGENESIS, ANGIOGENESIS.

Discovered over a century ago by Paul Ehrlich (1), the mast cells were associated for a long time only with the pathological events responsible for the initial stages of allergic reactions. Located predominantly in the perivascular territories, they are difficult to identify within the complex cell population that inhabits the loose connective tissue. At first, Ehrlich believed them to be involved in the nutritive support of the connective tissue where they are dispersed, due to the contents of their cytoplasmic granules. After 5 decades from their description the presence of high quantities of heparin was noticed in the granules of mast cells, but only at the middle of the past century the link between these cells and the type I hypersensitivity reactions (anaphylaxis) was pointed out (2, 3).

The origin of this cell raised numerous controversies explaining the variability in mast cell classification among the cell types present in the connective matrix. The intimate relationship between mast cells and the connective tissue led to the initial idea that mast cell derive from undifferentiated mesenchymal cells (4). Later, results of numerous studies suggested a link between mast cells precursors and cells of the immune system differentiated in the bone

marrow. Taking into account the structural and functional similarities between the basophiles and the mast cells, a theory of a common bi-potent precursor in the marrow (both for the mast cell and basophile line) was proposed, being deemed as valid for a long period of time (5-7). It is worth mentioning that complementary data revealed indirectly the presence of mast cell precursors in the liver and yolk sac cells, but in the absence of specific markers their existence was until recently impossible to prove (8, 9). Only two years ago, advanced immunologic methods were able to demonstrate the origin of mast cells in the aorta-gonad-mesonephros region (10) – area where the hematopoiesis is also initiated during human embryogenesis (11). This evidence not only complements the knowledge on mast cells origin, but also opens new perspectives for understanding the behavior of these cells, whose morphological aspect could be considered as insignificant.

Unlike other hematopoietic cells which differentiate and mature in the hematogeneous bone marrow, after which they enter the blood stream, the precursors of mast cells migrate early through the blood in the connective tissues of the body, where the maturation process is completed (12). They survive in the connective tissue up to 12 weeks, being capable of mitotic activity induced by specific activation factors (13, 14).

Mast cells are dispersed throughout the body, being located mainly in the skin, respiratory and digestive tract (15, 16), either in subepithelial areas (superficial dermis or *lamina propria*), either in the more profound loose connective tissue where they are concentrated mostly perivascular or around the nerves, follicles, and muscle fibers (16). The mature mast cells are unable to migrate, the dispersion ability in other connective areas being specific only to mast cell precursors (17). The intimate mechanism of preferential recruitment of mast cells in certain territories of the human body is still incompletely elucidated, the main impediments being those related to the small number of mast cell precursors and to the difficulty of their precise identification in the bone marrow and connective tissue (18-21).

MAST CELL: STRUCTURE AND ULTRASTRUCTURE

Mast cells are medium size cells (approximately 20-30 μm), oval in shape, with a spherical nucleus located in the center or slightly eccentrically, and a cytoplasm with a granular contents which cannot be easily observed in standard coloration. After fixation with glutaraldehyde and stain with toluidine blue, the cytoplasmic granules of the mast cells, rich in heparin, can be revealed, as they color metachromatically in purple red (16). In electron microscopy, the cytoplasm of the mast cell, with an irregular appearance consequent to the emergence of short and uneven microvilli, contains, besides the abundance of granules, a rudimentary RER, numerous mitochondria and well developed Golgi (16).

MAST CELL GRANULES

The complex functions of mast cells are mirrored in their ability to synthesize and release a series of biologically active molecules. Once activated, they can quickly discharge the preformed mediators stored in the cytoplasmic granules, but also synthesize several new mediators based on the membranous lipids (16, 22). Among the preformed mediators we can count histamines, serotonin, β-glucuronidase, chy-

mase, and tryptase, together with cytokines and growth factors such as TNF-α, IL-4, IL-15 or TGF-β and VEGF, while the neoformed/neosynthesized mediators comprise prostaglandin D2, E2, and leukotrienes B4 and C4, elaborated on the bases of membranous lipids, but also other molecules such as IL-10, IL-12, IL-5 or GM-CSF, PDGF, TGF-β, VEGF, and nitric oxide (Table I). Based on their contents, the matured mast cells are currently classified in two categories: tryptase/chymase-positive mast cells (MCs_{TC}), which store in the cytoplasmic granules tryptase, chymase, and carboxypeptidases, and tryptase-positive mast cells (MCs_T), which store only tryptase (23, 24). MC_T are mainly distributed in the *lamina propria* of the digestive and respiratory mucosa, while MC_{TC} are mostly present in the dermis of the skin (24). Recently, a third type of mast cells was described, observed in patients with asthma and eosinophilic esophagitis, which store tryptase and carboxipeptidase A3, but not chymase (25, 26). The differentiation of mast cell types involves also the receptors on their surface, the expression of C5aR – the receptor for the C5a component of the complement system being recorded in cutaneous and pulmonary MC_{TC}, while lacking in MC_T from the respiratory mucosa (27).

TABLE I

The main pre-formed and neo-formed mediators secreted by the mast cell

(adapted from 24)

Cytokine Family	Cytokines
Pre-formed mediators	
Biogenic Amines	Histamine, Serotonin (5-HT), Dopamine
Lysosomal Enzymes	β-hexosaminidase, β-glucuronidase, β-D-galactosidase, Arylsulphatase A,Cathepsins C, B, L, D, and E
Proteases	Chymase, Tryptase,Carboxypeptidase A, Cathepsin G, Granzyme B, Matrix metalloproteinases
Proteoglycans	Serglycin (Heparin and Chondroitin sulphate)
Cytokines	TNF-α, IL-4, IL-15
Chemokines	RANTES (CCL5), eotaxin, IL-8, MCP-1
Growth Factors	TGF-β, bFGF-2, VEGF
Others	Eosinophil Major Basic Protein (MBP)
Neo-formed and neo-synthesized mediators	
Phospolipid Metabolites	Prostaglandin D2, E2, Leukotrienes B4, C4, and Platelet Activating Factor
Cytokines	IL-33, IL-10, IL-12, IL-17, IL-5, IL-13, IL-1, IL-2, IL-3, IL-4, IL-6, IL-8, IL-9, IL-16, Type I and Type II IFN, TNF-α
Growth Factors	SCF, GM-CSF, β-FGF, NGF, PDGF, TGF-β, VEGF
Reactive Oxygen Species	Nitric Oxide

MAST CELLS ACTIVATION

The activation of mast cells is induced by several intrinsic or extrinsic factors, able to stimulate their surface receptors. Among these, the most investigated and deciphered activation pathway for the mast cells is the one mediated by Fc-RI high affinity receptors, which acts in allergic states (28). Fc-RI is a heterotetramer of the immunoglobulins superfamily, consisting

of $\alpha\beta\gamma_2$ subunits. Subunit α has an extracellular domain able to recognize the Fc segment of IgE, while subunits β and γ are intracytoplasmic (22). After the stimulation of Fc-RI, a cascade of successive protein phosphorylations is initiated in the cell, followed by the synthesis of inositol triphosphate and diacylglycerol, which will induce an inflow of intracytoplasmic calcium and the activation of protein kinase C (PKC), all of these finalized with the release of the granules contents, and the activation of neomediators synthesis (29, 30).

In the late phases of mast cells activation serines and threonine kinases, belonging to PKC and MAPK families, intervene decisively. The inflow of intracytoplasmic calcium will produce the stimulation of PI3K, followed by the activation of phospholipase D, PLA2 and of the isoforms β and - of calcium-dependent PKC (29), the latter regulating the production of Fos and Jun factors and indirectly the fabrication of cytokines (31). Also, the molecule ERK1/2 intercedes in the regulation of PLA2 activation and eicosanoids production by Fc-RI mechanism (32), through the MAPKs, PKC, and PI3K pathways, and the enrollment of the intracellular calcium (33).

However, the mast cells activation is not strictly limited to the Fc-RI pathway. The recognition of antigens is possible also through the intervention of other mast cells receptors such as TLRs1-7 and 9, nod-like receptors (NLRs) and retinoic acid-inducible gene 1 (RIG-1)-like receptors (RLRs). The stimulation of TLR is followed by the association of the adaptor protein MyD88 to its ligand, MyD88 adaptor-like/Toll interleukin-1 receptor (MAL/TIRAP), which will determine the activation of a specific kinase – IKB kinase (IKK), able to promote the transcription of the genes responsible for cytokine synthesis (34). On the other hand, the activation of mast cells stimulating factors is counterbalanced by other membranous receptors, whose stimulation modulates the synthesis and release of specific mediators (35), such as: IgG multimeric receptors (FcγR) (36), c-Kit receptor (CD117) (37), C-C Chemokine Receptor type 1 (CCR1), corticotropin-releasing hormone receptor (CRHR), and the beta-adrenoceptor (30).

MAST CELL FUNCTIONS

Through their origin and location, the mast cell is currently viewed as an immune cell, which mediates not only nonspecific defense processes, but also several other complex biologic events, such as tissue repair, angiogenesis or carcinogenesis (38).

Mast cells and tissue repair

Through the synthesis of numerous growth factors, such as PDGF, VEGF, and FGF-2, the mast cells play an important role in the maintenance of tissue integrity (39), for they are involved in all stages of tissue repair. Even from the early phases of inflammation, through the synthesis and release of platelet activating factor (PAF) associated with leukotrienes and IL-1 and IL-8 they determine platelet activation. These reactions are balanced simultaneously by the fibrinolytic effect of heparin and tryptase, also produced by the mast cells (40). The mast cells involvement continues with the production of factors that promote the migration of immune cells in the damaged territories, and the proliferation of endothelial cells or fibroblasts (41). In the late phases of tissue repair, other released proteolytic factors mediate the control of newly synthesized extracellular matrix deposits and, in association with IL-1, IL-4 and IL-6, induce the stimulation of the

myofibroblasts, which ensure local tissue remodeling (42).

Mast cells and nervous tissue

A possible relationship between mast cells and the nervous tissue is suggested by the preferential location of these cells in the vicinity of nerve ends. Moreover, the existence of a synapse-like communication between the mast cells and neurons by several molecules (i.e. N-cadherin or synaptic cell adhesion molecule (SynCAM)) is proved (43). Supplementary evidences sustains that at intestinal level the interaction between mast cells – nervous structures regulates the ion transport, vascular permeability, glandular secretion and contraction of smooth muscle, with result in local homeostasis maintenance (44).

Mast cell and allergic reactions

Classically, the mast cell is acknowledged as the main effector of the pathogenic mechanism involved in the allergic reaction, including the severe form – anaphylactic shock, through its numerous IgE – type receptors. The first contact of the immune system with an allergen induces, by antigen-presenting cells, the stimulation of T helper 2 lymphocytes which determine the activation of B lymphocytes followed by IgE synthesis (45). Therefore, mast cells have a twofold role in the allergic reaction, due to the ability to process and present the antigens in MHCI or MHCII context and to the well-represented expression of IgE – type surface receptors.

In the early phases of allergic reaction (very well studied in asthma and its allergic form), the binding of the allergen to the Fc-RI of the mast cells brutally induces the discharge of their preformed mediators, but also the stimulation of neoformed mediators synthesis responsible for the entire assembly of specific conditions (erythema, edema, increase of vascular permeability, contraction of smooth muscles and mucus hypersecretion) (46, 47).

In the late stage of allergic reaction the mast cell intervention is restricted to favoring the recruitment of other immune cells (eosinophils, basophils and T lymphocytes). Finally, in the chronic stage, mast cells act against the persistent inflammation supporting the tissue remodeling and development of local fibrosis (47).

Mast cells and immune tolerance

The idea of mast cell's intervention in the manifestation of immune tolerance dates back a decade ago (48). Towards this end, most of the researches centered on the role of these cells in the cutaneous territory, where it was noticed that the mast cells exert numerous local influences through the pre- or neoformed mediators, while concomitantly are able to modulate the immune response (24, 49).

Through the secretion of mainly IL-10 and TGF-β, already known as factors with immunosuppressive potential, the mast cells control the amplitude of the local inflammatory reaction, including the control of Fc-RI autoexpression, involved in mast cell degranulation (50). TGF-β together with IL-2 significantly influences the development and differentiation of antigen-specific regulatory T cells – T^{reg} (expressing $CD4^+$, $CD25^+$ and $FoxP3^+$) after primary lymphopoiesis in the thymus. This subtype of lymphocytes is capable of inducing the suppression of proliferation of effector T lymphocytes (51). It seems the mast cell – T^{reg} interaction is one of the defining elements in the appearance of local immunotolerance in atopic dermatitis or heart transplant (48, 52). Also, the mast cells proteases, by their specific activity, enable the occurrence of antigen immunotolerance through the decrease

of leukocyte recruitment, mediated by cytokines or direct antigen cleavage, in inflammatory foci (53).

Mast cells and angiogenesis

The angiogenesis process, defined as development and growth of preexistent or new formed blood vessels, can appear not only in physiological conditions similar to those occurring during embryogenesis, but in pathologic circumstances (as chronic inflammation or carcinogenesis) as well. Histamine and heparin were among the first factors with proven angiogenic effect, for they stimulate the formation of new vascular structures on experimental research performed on laboratory animals (54).

Nowadays, mast cell's intervention in angiogenesis is documented by the secretion angiopoietin-1, FGF-2, VEGF, IL-8, TGF-β, TNF-α, tryptase and chymase (55) – angiogenic factors that intervene in various stages of angiogenesis through the control of migration and proliferation of endothelial cells, synthesis of extracellular matrix components or mobilization of pericytes (24).

Mast cells and cardiovascular disease

Cardiac mast cells seem to play a role in the pathology of coronary heart diseases characterized by the damage of the vascular wall structure with results in the reduction of the cardiac muscle cells blood supply. Experimental research showed the presence of mast cells in the adventitia of coronary arteries during their spasms (56) or in the vicinity of atherosclerotic plaques (57). The released chymases are able to activate the conversion enzyme and the secondary synthesis of angiotensin II, a powerful coronary vasoconstrictor, or to induce proteolytic cleavages of the high-density lipoprotein cholesterol, thus favoring the flow of lipids towards the macrophages present in the vascular wall and the development of atheroma plaques (58).

Mast cells and carcinogenesis

The relationship mast cell – inflammation – cancer currently raises a major interest, proven by the numerous researches aiming to decipher the nature of the mast cell's involvement in the control of carcinogenesis and tumor progression. Recent reports in this field start from the classic histologic study, which revealed the concentration of mast cells around tumor areas (24, 59). In the tumor microenvironment, the local recruitment of the mast cells is achieved under the direct influence of several factors synthesized by the tumor cells, such as SCF (60). The mast cells activity assumes the release of pro-inflammatory, immunosuppressive or angiogenic mediators, or of mitogenic factors (61, 62). The mast cell histamine is capable of stimulating the tumor proliferation rate through the interaction with H1 histamine receptors which induce the inhibition of anti-tumoral activity of the immune cells, an effect secondary modulated also by other mast cell mediators – such as IL-10 or TNF-α (59).

However, the pro-angiogenic effect is the fundamental event where the mast cell intercedes in the support of tumor progression, for it is coordinated through a multitude of factors (such as angiopoetin-1, TNF, FGF-2, VEGF and IL-18) released from the mast cells granules, which stimulate the endothelial and vascular proliferation (63, 64).

Current research also revealed the possibility of mast cell involvement in the early stages of tumor growth through the activity of the secreted proteases such as MMP-2 and MMP-9, an effect which would later be actually completed by the modulator angiogenic role exerted through vascular stimulat-

ing factors (65). It seems that these effects are counterbalanced by the activity of other mast cells factors (TNF-α, IL-1, and IL-6), which inhibit tumor growth through a pro-apoptotic mechanism, such as it was recorded in malignant melanoma (64). Consequently, the complex interrelations established in the tumor microenvironment between the mast cells and tumor cells are still only partially elucidated. Nevertheless, the results of most studies support the multifaceted intervention of the mast cells in the peritumoral immune inflammatory profile, with a dominant effect on the development of neoangiogenesis, resulting in the local or distant invasion of malignant cells (62, 66, 67).

FINAL REMARKS

Although known for over one hundred years, the mast cell is currently viewed as a multifunctional immune cell, for it mediates several physiological processes such as tissue growth and repair, but also pathologic, such as allergic reactions, inflammation and, recently, tumor angiogenesis, tumor development and progression. Distributed evenly in the human body, with perivascular, perimuscular and periglandular concentration in the connective territories located at the interface with the exterior environment, the mast cell acts as a "guardian of local immune processes", as it controls the activity amplitude of other immune cells. The mast cells presence in the tumor microenvironment opens today a new perspective regarding the influence in carcinogenesis, through the possible therapeutic control of the neoangiogenesis.

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NEWS

ASPIRIN USE AND COLORECTAL CANCER

Colorectal cancer (CRC) is the second most frequent type of cancer in Europe, with higher incidence for men after 50 years of age. Aspirin has anticancer effects. The aspirin use reduced the colorectal adenomas risk and the distant metastasis for patients with CRC. A recent meta-analysis summarized the results of eleven studies and showed that aspirin therapy after CRC diagnosis improved survival particular among patients with positive prostaglandin endoperoxide synthase 2 (PTGS2) expression and mutated PIK3CA tumors. PTGS2 is an enzyme involved in prostaglandin synthesis which promote inflammation and cell proliferation. Phosphatidylinositol 3-kinase (PI3K) has an important role in carcinogenesis. Also, the meta-analysis highlighted no association between aspirin use before diagnosis and CRC mortality. Future research is needed in order to establish the optimal doses and duration of aspirin adjuvant therapy in CRC (Peiwei Li; Han Wu; Honghe Zhang; Yu Shi; Jinming Xu; Yao Ye; Dajing Xia; Jun Yang; Jianting Cai; Yihua Wu. Aspirin use after diagnosis but not pre-diagnosis improves established colorectal cancer survival. *Gut* 2015;64(9):1419-1425).

Cătălina Luncă

RELATIONSHIP BETWEEN THE PROANGIOGENIC ROLE OF EG-VEGF, CLINICOPATHOLOGICAL CHARACTERISTICS AND SURVIVAL IN TUMORAL OVARY

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RELATIONSHIP BETWEEN THE PROANGIOGENIC ROLE OF EG-VEGF, CLINICO-PATHOLOGICAL CHARACTERISTICS AND SURVIVAL IN TUMORAL OVARY (Abstract) **Aim:** To prove the presence of EG-VEGF in tumor ovary and to analyze its involvement in the ovarian carcinogenesis, as promoter of angiogenesis, in relationship with the clinicopathological prognostic factors and survival. **Methods:** The study group comprises tumor tissue specimens from 50 cases of surgically treated ovarian cancer that were immunohistochemically investigated. A scoring system based on the percentage of positive cells and the intensity of staining was applied for the semiquantitative assessment of EG-VEGF, as negative or positive. Statistics involved χ^2 test, and Kaplan-Meier and log-rank test. **Results:** EG-VEGF was positive in 35 cases (70%) and negative in 15 cases (30%). Our data confirmed the predominance of EG-VEGF positivity in the serous subtype as compared to endometrioid and clear cell subtypes, and its absence in mucinous subtype. Moreover, we demonstrated that EG-VEGF is overexpressed mainly in high-grade ovarian carcinomas (type II) than in low-grade ones. Significant differences were registered between the EG-VEGF positive or negative expression and tumor stage and histological subtypes, respectively. Survival analysis showed no differences in patient's survival and EG-VEGF positive and negative cases. **Conclusions:** The analysis of EG-VEGF expression in ovarian tumors points out the relationship between the enhanced potential for tumor angiogenesis and the tumor aggressivity. **Keywords**: OVARIAN CANCER, EG-VEGF, TUMOR ANGIOGENESIS, IMMUNOHISTOCHEMISTRY.

Although the existence of organ-specific angiogenic factors was hypothesized in the early '80s (1), the presence of such a factor has been later confirmed (2). Its specificity results from the expression characteristics, concretely by its identification and isolation in endocrine organs, as: testis, adrenal gland, ovary, and placenta (2). Consequently, it has been named endocrine gland - derived vascular endothelial growth factor (EG-VEGF). EG-VEGF action is restricted to endothelial cells be-

longing to the steroidal endocrine organs, without known potential on other types of endothelial cells. EG-VEGF has a complex role as regulator of the angiogenic processes including cell proliferation, migration, and survival, pseudovascular and spheroidal organization, permeability and paracellular transport (3).

Besides the above mentioned organs, a low expression of EG-VEGF has been noticed in brain, colon, skeletal muscle, small intestine, spleen, thymus, uterus, and liver, whereas it is absent in non-lactating breast and skin (2). The EG-VEGF overexpression is reported in colorectal (4), pancreatic (5) and ovarian carcinoma (6-9).

The ovarian site offers a generous model for angiogenesis research, both in physiological status characterized by a cyclic evolution and in pathological conditions that includes a large heterogeneity of morphological changes. However, data concerning the EG-VEGF involvement in the ovarian carcinogenesis are limited.

Within this context, our study aimed to analyze the relationship between the EG-VEGF ovarian expression and clinicopathological prognostic factors and survival.

MATERIAL AND METHODS

The study group consisted of 50 cases with epithelial ovarian carcinoma, aged 41 to 77 years, diagnosed in "Cuza Voda" Obstetrics and Gynecology University Hospital from Iasi, Romania, during 2009-2012 intervals.

According to tumor stage, 15 cases (30%) have been classified as stage I, 12 cases (24%) as stage II, and 23 cases (46%) as stage III. Five cases (10%) have been graded as G1, 21 cases (42%) as G2, and 24 cases (48%) as G3, respectively. Epithelial ovarian carcinomas have been included in the following histological subtypes: 33 cases (66%) as serous, 10 cases (20%) as endometrioid, 4 cases (8%) as mucinous, and 3 cases (6%) as clear cell subtype; 14 cases (28%) have been classified as type I category (low-grade) and 36 cases (72%) as type II category (high-grade).

Serial sections have been cut at 4 μm thickness from the tumor specimens, in order to perform routine histological technique, followed by immunohistochemical (IHC) staining with anti-EG-VEGF antibody (Santa Cruz, Biotechnology Inc., Santa Cruz, USA) using the automated system Dako Autostainer Plus (Dako Cytomation, Glostrup, Denmark). A semiquantitative method has been used (8) to quantify the results, each slide receiving a score for the percent of immunoreactive tumoral cells and a score for the tumor tissue staining intensity from the same microscopic field. The sum of the two scores determined the final score, ranging between 0 and 6. A final score less than 2 has been considered negative, while ≥3 score has been considered as positive for the evaluated marker.

Statistical analysis has been performed using the commercially available GraphPad Prism software. We have applied χ^2 test for correlation with clinicopathological characteristics, and Kaplan-Meier curves and log-rank test for survival analysis.

RESULTS

Our study had shown a positive expression of EG-VEGF in 35 cases (70%) and a negative expression in 15 cases (30%). The distribution of EG-VEGF expression within tumor stages has shown the following pattern: 9 cases were positive and 6 cases were negative in stage I, 10 cases were positive and 2 cases were negative in stage II, and 7 cases were positive and 16 cases

were negative in stage III. The correlation of EG-VEGF expression with the tumor grade has been the following: 2 positive cases and 3 negative cases in G1, 14 positive cases and 7 negative cases in G2, and 19 positive cases and 5 negative cases in G3. Expression of EG-VEGF has been more common among the serous subtype (fig. 1), represented by 26 cases followed by endometrioid subtypes, represented by 6 positive cases (fig. 2), and clear cell subtype – 3 cases (fig. 3). EG-VEGF has been undetected in 15 cases (7 case of serous, 4 cases of endometrioid, and 4 cases of mucinous type, respectively). Regarding the tumor type, EG-VEGF expression has been overexpressed in 28 cases of type II, comparative to 7 cases of type I tumors.

The statistical analysis had revealed significant differences between the EG-VEGF expression (positive versus negative) and tumor stage (p=0.009) and histological subtypes (p=0.017), respectively (tab. I). No differences in patient's survival and EG-VEGF positive and negative cases had been registered (p=0.218) (fig. 4).

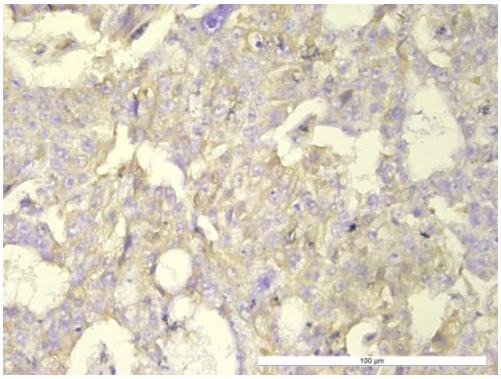

Fig. 1. EG-VEGF positive reaction in serous ovarian carcinoma (IHC, anti-EG-VEGF, x 40).

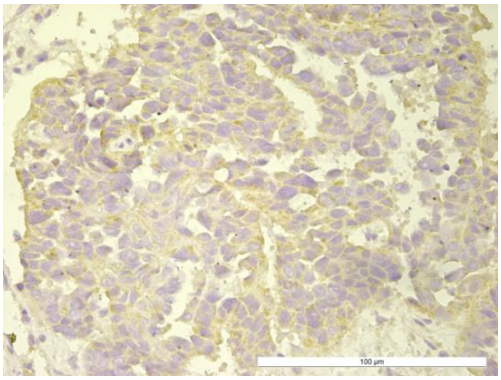

Fig. 2. EG-VEGF positive reaction in endometrioid ovarian carcinoma (IHC, anti-EG-VEGF, x 40).

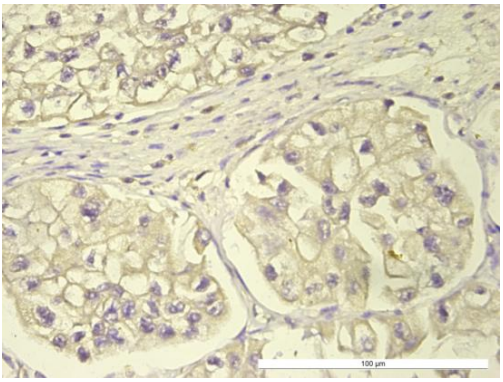

Fig. 3. EG-VEGF positive reaction in clear cell carcinoma (IHC, anti-EG-VEGF, x 40).

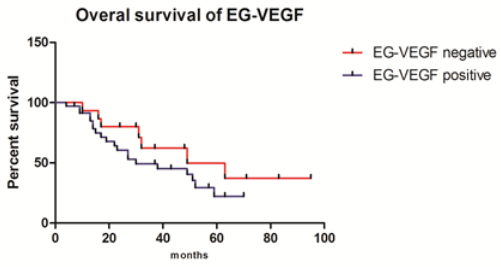

Fig. 4. Kaplan-Meier curves showing no differences in overall survival (p=0.2186).

TABLE I.
EG-VEGF expression in ovarian epithelial carcinoma

Clinicopathological characteristics	Cases		EG-VEGF positive cases		EG-VEGF negative cases		p value
	#	%	#	%	#	%	
Tumor stage							
I	15	30	9	60	6	40	0.009
II	12	24	10	83.33	2	16.67	
III	23	46	7	30.43	16	69.57	
Tumor grade							
1	5	10	2	40	3	60	0,200
2	21	42	14	66.67	7	33.33	
3	24	48	19	79.17	5	20.83	
Histological subtype							
Low-grade serous carcinoma	4	8	3	75	1	25	0.017
Low-grade endometriod carcinoma	3	6	1	33.33	2	66.67	
Mucinous carcinoma	4	8	0	0	4	100	
Clear cell carcinoma	3	6	3	100	0	0	
High-grade serous carcinoma	29	58	23	79.31	6	20.69	
High-grade endometrioid carcinoma	7	14	5	71.43	2	28.57	
Tumor Type							
Type I	14	28	7	50	7	50	0.054
Type II	36	72	28	77.78	8	22.22	

DISCUSSION

The first data concerning EG-VEGF involvement in the angiogenic processes in steroidal organs have been published 3 decades ago (2). EG-VEGF belongs to a class of proteins that includes Bv8 (7, 10). Although EG-VEGF accomplishes comparable functions as VEGF, its structure is completely different (10).

As a difference from its physiological functions, the EG-VEGF role in tumor environment is to induce endothelial cells proliferation and fenestration in tumor vascular capillaries, by different growth factors activation, as a consequence of hypoxia and action of proinflammatory cytokines (4). The binding of EG-VEGF to its specific receptors (PKR1 and PKR2) determines angiogenic effects (mediated by PKR1) and cellular permeability (mediated by PKR2) (7, 10). As a consequence, EG-VEGF/EG-VEGF-R system represents a paracrine system, the ligand being produced by non-endothelial cells, while the receptors are selectively expressed in vascular endothelium present in the steroidal tissues (7, 10). These facts justify its specificity for the endocrine organs characterized by steroid hormones secretion (8).

Few data concerning EG-VEGF in ovarian carcinoma have been reported in the mainstream publications (6, 8, 9) and its involvement in regulating tumoral cells growth and survival is yet unclear.

As reference in the research in this field, the study conducted by Zhang et al. (6) had analyzed EG-VEGF and VEGF comparative expression at mRNA level (using real-time quantitative RT-PCR) in 50 cases of human ovarian tumors, suggesting that EG-VEGF is not directly expressed by ovarian tumor cells, but more likely it is the result of leukocyte inflammatory activity or of stromal cell intervention. The authors have demonstrated EG-VEGF mARN higher expression in benign and borderline ovarian tumors, and also in tumor stage I (early stage) compared with stage III. VEGF expression had been opposite to that of EG-VEGF, VEGF exhibiting a predominant expression in advanced cases, compared to early stages. Moreover, the investigation of the inflammatory extracellular matrix role related to EG-VEGF expression had identified a higher EG-VEGF mRNA in peritumoral stroma compared to tumoral one; reported to inflammatory peritumoral cells (lymphocytes CD3, CD19, CD45, and NK), EG-VEGF had been overexpressed in CD3 lymphocytes supporting the inflammation role in EG-VEGF liberation.

Later on, an attempt to determine the potential prognostic value of EG-VEGF in ovarian carcinoma had been accomplished by Bălu et el. (9) who analyzed EG-VEGF expression in 30 patients with ovarian tumors, aiming the correlation of EG-VEGF expression related to classical parameters. The obtained results have shown statistically significant differences between EG-VEGF and tumor stage, tumor grade and histological subtypes.

Therefore we consider our research is worthwhile in supplementing data on the EG-VEGF characterization in relationship with the behavior of ovarian tumors. The novelty of our study, based on our previous interest on the role of this molecule in ovarian carcinogenesis (11), is sustained not only through the correlation with the clinicopathological characteristics, but also by those with patients' survival – to the best of our knowledge not yet reported in the literature.

In accordance with the published papers (6, 9), our data revealed statistically significant differences related to tumor stage and histological subtypes. The EG-VEGF decline in advanced stages of ovarian carcinoma may be attributed to a negative action of the steroidal stroma on tumor growth. This observation opens perspectives for considering EG-VEGF as a therapeutic agent in early phases of ovarian carcinogenesis. Within the framework of the histological heterogeneity of ovarian carcinoma, we confirmed the predominance of EG-VEGF positivity in the serous subtype as compared to endometrioid and clear cell subtypes, and its absence in mucinous subtype. Moreover, we demonstrated that EG-VEGF is overexpressed mainly in high-grade ovarian carcinomas (type II) than in low-grade ones. These statements can be interpreted as evidences for a direct relationship between the enhanced potential for tumor angiogenesis and the tumor aggressivity.

The EG-VEGF prognostic role for survival was not proved, but this negative result may be attributed to the relatively reduced number of cases included in the study – a more extensive research being necessary to clarify this issue.

CONCLUSIONS

The analysis of EG-VEGF expression in ovarian tumors, founded on the peculiar

morphology of endocrine organs characterized by steroid hormones secretion, offers complementary data for a better understanding of the angiogenesis role in carcinogenic mechanisms.

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EMPHYSEMATOUS CYSTITIS: AN UNREPORTED COMPLICATION AFTER PANCREATICODUODENECTOMY

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EMPHYSEMATOUS CYSTITIS: AN UNREPORTED COMPLICATION AFTER PANCREATICODUODENECTOMY (Abstract): Pancreaticoduodenectomy was associated with an increased morbidity, and septic complications are the primary cause of death in these patients. However, severe sepsis, caused by a postoperative urinary tract infection, is uncommon. It is presented the case of a 72 years old man, with a pylorus-preserving pancreaticoduodenectomy for an ampullary adenocarcinoma. Postoperatively, the patient developed a chyle leak, and a severe urinary tract infection (i.e., emphysematous cystitis), with septic shock. The diagnosis, management and outcome are discussed. In conclusion, emphysematous cystitis is a potentially life-threatening complication, which may occur due to the postoperative immunodepression after pancreaticoduodenectomy. **Keywords**: PANCREATECTOMY, COMPLICATION, CHYLE LEAK, IMMUNODEPRESSION, EMPHYSEMATOUS CYSTITIS.

Emphysematous cystitis is an acute infection, characterized by the presence of the gas into the walls of the urinary bladder (1). Pancreaticoduodenectomy is a complicated surgical procedure, which is associated with increased morbidity (2). Infectious complications after pancreatectomies are encountered in around 30% of the patients (3). Septic complications represent the primary cause of death after pancreaticoduodenectomy, and in most of the cases are consequences of a postoperative pancreatic fistula (3). Urinary tract infections after pancreatico-duodenectomy are encountered in 7% of the patients, but extremely rarely may lead to septic complications (3).

CASE REPORT

A 72 years old man, with no significant medical history, underwent a pylorus-preserving pancreaticoduodenectomy, with standard lymph nodes dissection(2), for an ampullary tumor. The pathology report showed an ampullary adenocarcinoma, pT1, pN0 (17 lymph nodes retrieved). The postoperative outcome was complicated with a chyle leak, which occurred two days (postoperative day 4) after starting the enteral feeding on the naso-jejunal tube, which was intraoperatively mounted. The triglycerides level in the abdominal drainage fluid was 253 mg/ dL, with normal levels for amylase and lipase. Postoperatively, the patient had spontaneous normal

glycaemia levels. Total parenteral nutrition was initiated, along with fasting and octreotide administration, with apparently favorable outcome (decrease of the daily drainage from 7 liters/day to 1 liter/day). On postoperative day 36 fevers appeared, along with chills.

Laboratory tests showed increased leucocytes count (21,000/ mm^3); no other abnormalities were observed, including the serum urea and creatinine levels. The computed tomography identified the presence of gas within the wall of the urinary bladder (fig. 1, white arrow). On notice, the patient did not present any urinary symptoms or signs, and he had a daily urinary output volume within the normal ranges. A urinary catheter was inserted, and broad-spectrum antibiotics and antifungal treatment were started. The evolution was rapidly progressive to septic shock, multiple organ dysfunctions, and death within 48 h after diagnosis. Hem cultures were positive for *Candida*.

Fig. 1. An axial computed tomography showing the aspects of emphysematous cystitis (presence of the gas within urinary bladder walls – white arrows).

DISCUSSION

A chyle leak is defined as a milky white fluid enriched in triglycerides (>110 mg/ dL) (4), and it is considered a rare complication after pancreaticoduodenectomy, with a reported prevalence ranging from 1.8% to 16% (4-10).

The cause of a chyle leak after pancreatectomy is a lymphatic disruption (4, 9). Manipulation of the para-aortic region (8), retroperitoneal invasion (8), the number of harvested lymph nodes (≥ 18) (6) or an extended lymph nodes dissection (5), vascular resection (6), portal vein/ superior mesenteric vein thrombosis (5), chronic pancreatitis (10) and early postoperative feeding (7, 8) are considered independent risk factors for development of a chyle leak after pancreaticoduodenectomy. Furthermore, a chyle leak is also more frequently encountered after pancreaticoduodenectomy for a malignant pathology (8), in males (6), in patients with lymph nodes metastases(5;8), early T stages tumor (≤ T2) (7), advanced ages (≥ 66 years) (7), soft pancreas texture (7), and without postoperative pancreatic fistulae (7).

Although an early enteral nutrition was associated with high risk for chyle leak in some studies (7, 8), it is worth to mention

that the early enteral nutrition reduces the postoperative infectious complications after pancreaticoduodenectomies (11). Nevertheless, some studies did not identify an early enteral intake as a risk factor for a chyle leak development after pancreatectomies (9).

Postoperative enteral nutrition brings an intake of fats, and fatty meals increase the flow in the lymphatics (4). This is the reason a postoperative chyle leak can be clinically diagnosed after the enteral nutrition is restored. A drain output volume > 335 ml on postoperative day four after pancreatectomies was correlated with a high suspicion for a chyle leak diagnosis in some studies, particularly in the absence of a postoperative pancreatic fistula(7). A grading system for chyle leak after pancreatectomies was proposed in a study(10). The data from the literature have shown that the occurrence of a chyle leak after pancreatectomy is usually observed in the postoperative day 5- 8 (4-7).

In the reported case, male gender, advanced age, soft pancreas texture, a malignant early stage tumor (i.e., T1) and early enteral nutrition might be speculated as risk factors for postoperative chyle leak.

A persistent chyle leak may potentially induce malnutrition, immunodeficiency and sepsis because of the depletion for immunoglobulin, lymphocytes and long-chain triglycerides (6, 8); it is also associated with increased mortality (6). Peritonitis or other forms of sepsis were observed in about 20% of patients with pancreatectomies and a postoperative chyle leak or chylous ascites (6).

Total parenteral nutrition, octreotide (a somatostatin analogue) and medium-chain triglycerides are recommended for the treatment of a chyle leak, and most of the patients have a favorable outcome (5, 8). An intraoperative milk test was suggested to identify a lymphatic leakage after pancreatectomies (4).

Emphysematous cystitis is a severe, life-threatening complication that occurs mainly in diabetic or immunodepressed patients (1). Escherichia coli represent the most frequent causative bacteria for emphysematous cystitis, while *Candida* remains an uncommon finding (1).

In the reported case, the infectious complication was most likely due to the persistent high output volume chyle leak, with substantial malnutrition and immunodeficiency, that couldn't be compensated by the parenteral nutrition.

The clinical symptoms of emphysematous cystitis are not particular, and the imaging investigations are the most important tool for diagnosis (12). Although a plain abdominal radiograph may diagnose the presence of the gas within the urinary bladder walls, computed tomography is considered the gold standard for the imaging diagnosis in patients with emphysematous cystitis, because it may exclude other causes of presence of the gas into the bladder, such as colo-vesical fistulae etc (12). Moreover, computed tomography may also detect the associated pyelonephritis (12).

In the reported case, there were no clinical or bioumoral signs to suggest a urinary disease. Furthermore, the diagnosis was established at computer tomography examination. An uncommon finding for the reported case of emphysematous cystitis was the *Candida* etiology.

The mortality rate in patients with emphysematous cystitis is 7% (1, 13), but may increase to over 50% in cases with associated pyelonephritis (1). Only an early diagnosis and an immediate aggressive treatment may save the patient from a fatal

outcome (1, 13).

In the reported case, although the diagnosis of emphysematous cystitis appears to be made in the early setting, however, the evolution was rapidly progressive to a fatal outcome. This unfavorable evolution might be explained by the *Candida* etiology in a severely immunodepressed patient.

In conclusion, emphysematous cystitis is an uncommon, but severe complication that may occur due to the postoperative immunodepression.

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HIGHLIGHTING CONSIDERATIONS ABOUT PREDISPOSING FACTORS IN THE DYNAMICS OF ETHANOLIC DEPENDENCE SYNDROME

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HIGHLIGHTING CONSIDERATIONS ABOUT PREDISPOSING FACTORS IN THE DYNAMICS OF ETHANOLIC DEPENDENCE SYNDROME (Abstract): **Aims:** This research aims to highlight the involvement of behavioral disorders as predisposing vulnerable factors in the pathology of patients with ethane deficiency, compared to healthy subjects. **Material and methods:** For 120 patients included in the study a statistical and mathematical representation of the factors of personality and temperament was made: impulsivity, socialization, social desirability, somatic anxiety, social-cognitive anxiety and muscle tension, in relation to the dynamics of the ethanolic challenge syndrome. **Results:** The "Karolinska Scales of Personality - KPS" was used to evaluate the representation. For the statistical analysis was used *SPSS version 17.0* and for the charts and tables was used Microsoft Excel. **Conclusions:** Blood tests were performed (OGT, PGT, TG-gamma) to determine the degree of severity and the predisposition to develop subsequent ethanolic complications. **Keywords**: PREDISPOSING FACTORS, ETHANOL DEPENDENCE SYNDROME, STATISTICAL AND MATHEMATICAL REPRESENTATION.

The term to alcoholism was first medical developed by Magnus Huss (1849) and considered by the WHO (1967) "the fourth public health problem" as a set of physical and mental disorders caused by the consumption of alcoholic beverages" (1) with somatic and psychological effects and serious consequences on work capacity and interpersonal relationships.

Chronic alcoholism and psychiatric complications represent 5.5% of the psychiatric pathology in the country's population and have major consequences, causing: temporary inability to work, accidents at work and significant financial costs for medical care (2).

According to Sher and Trull, (3) in the sense of Deshaies and in developmental theories and vulnerability to the onset of alcoholism (4), personality factors, especially those of temperament, are classified as vulnerability and risk factors.

MATERIAL AND METHODS

The study included a group of 60 subjects selected by age, gender, social status, professional training and domicile, between 30 and 65 years of age, assisted in the Department of Occupational Medicine of the Arad County Emergency Hospital in 2016

and 2017, diagnosed with alcoholic addiction syndrome.

In comparison, the study uses 60 homogeneous control batch consisting of healthy subjects as a first batch and another batch, identical, but without alcoholic addiction syndrome over a two-year period.

The "Karolinska Scales of Personality - KSP" questionnaire (6), which contains 135 items in 15 scales, grouped in 5 categories, was used to evaluate the representation. Item capitalization was done on the Likert scale with 4 anchors (1-4) (7).

For the statistical analysis we used *SPSS version 17.0* and for the charts and tables we used *Microsoft Excel*.

Blood test were performed (OGT, PGT, TG-gamma) to determine the degree of severity and the predisposition to develop subsequent ethanolic complications.

RESULTS

The main idea of this study was to establish is there are any significant differences between our two groups. For this we run a Wilcoxon sum - rank test to compare and quantify the responses given by our patients in all 135 questions. We obtained that in most of our questions 132 (97.7 %) out of 135 there are extremely significant differences ($p < 0.001$). The only three exceptions are question number 25, 36 and 69, here there are no significant differences between our groups ($p > 0.05$). In each case the healthy sample had significantly better results (tab. I, II, fig. 1, 2):

TABLE I

Comparative analysis between pathologies diagnosed in the study groups

Pathological disorders	Healthy group (N1=60) (%)	Diseased group (N2=60) (%)
Depression	15%	90%
Somatic anxiety	22%	93%
Impulsivity	27%	60%
Psychopathic personality	5%	74%
Avoiding monotony	41%	87%
Nervousness and suffering	19%	89%
Muscular tension	8%	76%
Social cognitive anxiety	21%	67%
Psychic anxiety	12%	81%
Psychic asthenia	4%	56%
Inhibiting aggressiveness	82%	24%

TABLE II

The distribution of level of OGT, PGT and TG-gamma in 120 subjects

OGT-less than 50mg./dL = I		OGT over 50mg./dL = 2	
No	42	No	78
%	35	%	65
PGT-less than 50mg./dL = I		PGT over 50mg./dL = 2	
No	26	No	94
%	22	%	78
TG-gamma = I		TG-gamma = 2	
No	36	No	84
%	30	%	70

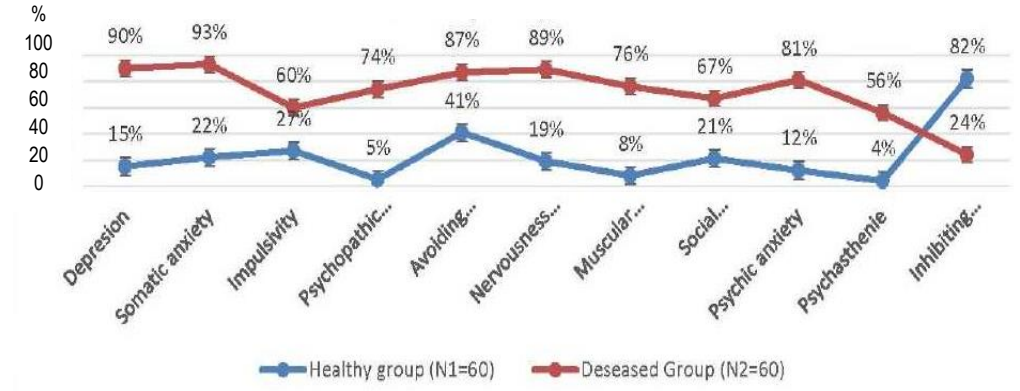

Fig. 1. Predisposing factors

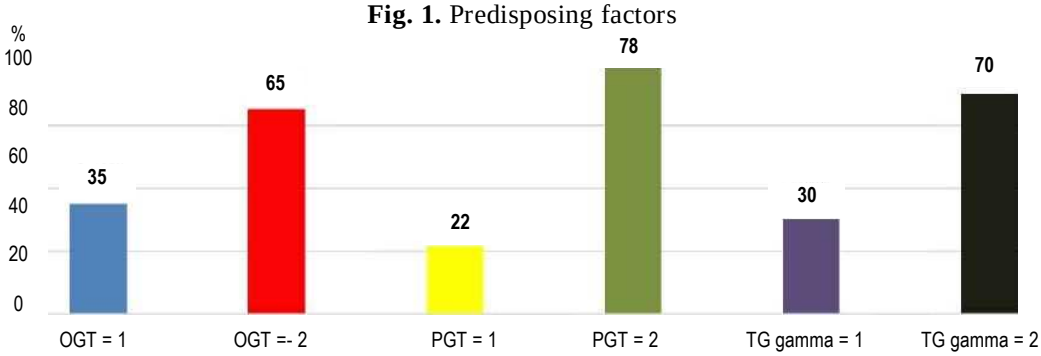

Fig. 2. The distribution of the subjects according to their medical tests

DISCUSSION

Psychopathic personality of patients with ethanolic dependence is recognized with the healthy group through a significant difference (1, 2, 3). Many study highlights the high percentage of factors involved in the occurrence of ethanolic addiction syndrome: low work potential, low skills, low power of attention and concentration, increased impulsivity, low socialization, nervous and muscular tension, social-cognitive anxiety, indirect aggression and irritability (8, 9, 10).

Alcoholism is multifactorial disorder both environmental and biologic factors contribute (10, 11).

Because many drinkers occasionally imbibe to excess, temporary alcohol-related pathology is common in nonalcoholic.

The period of heaviest drinking is usually in late teens to the late twenties. This is also a time of high risk for temporary alcohol-related social, occupational or driving difficulties.

The phenomena are often isolated events or self-limited, but when repeated problems in multiple life areas develop, the person is likely to meet criteria for alcohol abuse or dependence (12, 13).

In the absence of alcohol dependence, an individual can be given a diagnosis of alcohol abuse if he or she demonstrates repetitive problems with alcohol in any one of four life areas: an inability to fulfill major obligations, use in hazardous situa-

tions such as driving, legal problems, or use despite social or interpersonal difficulties (14, 15, 16).

CONCLUSIONS

In our study was demonstrated like the subjects selected for the study show an increased level of neuroticism, a lower degree of socialization, and a tendency of increased impulsivity in interpersonal relationships, compared to those subjects in the healthy group.

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AORTOCORONARY BYPASS GRAFT PATENCY ASSESSED BY CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY – UNICENTRIC STUDY

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AORTOCORONARY BYPASS GRAFT PATENCY ASSESSED BY CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY – UNICENTRIC STUDY (Abstract): Coronary artery bypass graft (CABG) surgery is used to revascularize ischemic myocardium affected by coronary artery obstruction. Conventional coronary angiography (CCA) is an invasive method used for graft evaluation. Nowadays, in post CABG patients grafts can be assessed noninvasively by using coronary computed tomography angiography (CCTA). **Aim**: To determine the late patency rate in coronary bypass grafts using CCTA. **Methods**: To assess graft patency, sixty post CABG patients (51/60 males and 9/60 females) were evaluated using dual source computed tomography (DSCT) angiography. All patients underwent a primary isolated CABG surgery between January 2000 and December 2004, performed by a single surgeon, at the Department of Cardiovascular Surgery, "Prof. Dr. George I. M. Georgescu" Institute for Cardiovascular Diseases, Iaşi, Romania. The grafts were classified as patent, occluded and stenotic (stenosis >50%). **Results**: The mean age of patients was 68.05±7.59 years and the mean time after graft implantation was 129.53±16.74 months. The number of assessed grafts was 200, including 146 saphenous vein grafts (SVG) and 54 in situ left internal mammary artery (LIMA) grafts. A total number of 105/146 (71.92%) venous grafts and 52/54 (96.3%) arterial grafts were patent. **Conclusion**: Nowadays, computed tomography angiography is a modern noninvasive imaging modality used to evaluate the aortocoronary graft patency in early and late postoperative settings. In our study, the late saphenous graft patency rate was superior to that found in earlier angiographic studies. **Keywords:** CORONARY COMPUTED TOMOGRAPHY ANGIOGRAPHY, CORONARY ARTERY BYPASS GRAFT, SAPHENOUS VEIN GRAFT, LEFT INTERNAL MAMMARY ARTERY GRAFT

Coronary artery bypass graft (CABG) surgery represents the standard of care in the treatment of advanced coronary artery disease in order to restore myocardial perfusion. Triple vessel disease, one or two vessel disease involving the proximal left anterior descending artery (LAD), left main artery stenosis and left ventricular failure are the main clinical indications for CABG (1). The long-term prognosis after myocardial revascularization depends on bypass graft patency and progression of native coronary artery disease (2). Conventional coronary angiography (CCA) is the gold

standard in evaluating the patency of CABG, but it is a two-dimensional projection method that allows evaluation of the vessel lumen, not of the vessel walls, and is characterized by important limitations (invasiveness, high cost, need for hospitalization, low patient compliance). The development of electrocardiographically (ECG) gated multidetector computed tomography angiography has allowed accurate visualization of graft patency noninvasively, to detect or exclude significant stenosis in bypass grafts (3). The superior temporal resolution of dual source computed tomography (DSCT) has substantially improved coronary imaging. Weustink *et al*., using DSCT angiography, reported a diagnostic accuracy of 100% in the evaluation of graft stenosis on a segment basis. Coronary computed tomography angiography (CCTA) is now used more widely in postoperative settings and in preoperative planning for repeat CABG surgery (2). While asymptomatic CABG patients are not routinely followed by CCA, DSCT represents an opportunity for graft follow up in such as patients, as well as in symptomatic post CABG patients. The aim of this study was to assess graft patency noninvasively using DSCT.

MATERIAL AND METHODS

We performed a single center study that included 60 post-CABG patients, 51 (85%) males and 9 (15%) females, who underwent a primary isolated CABG surgery in the interval January 2000 - December 2004, performed by a single experienced surgeon, at the Department of Cardiovascular Surgery, "Prof. Dr. George I. M. Georgescu" Institute for Cardiovascular Diseases, Iași. All patients underwent CTCA after a written informed consent was obtained. Exclusion criteria were allergy to iodine contrast agents, renal failure and inability to hold breath. The number and types of grafts and the native recipient vessels were extracted from each patient surgical record. An 18G cannula was placed in an anticubital vein for the administration of the iodinated contrast. All patients received nitroglycerin spray 0.8 mg within 5 minutes of scan acquisition.

All CCTA examinations were performed on a second-generation 128-slice DSCT scanner (Somatom Definition Flash; Siemens Healthcare, Forchheim, Germany). Scanning parameters were 100 or 120 kV tube voltage, tube current modulation with Care Dose 4D (reference 320mAs), 128 x 0.6 mm collimation, gantry rotation time 280ms. Peak arterial enhancement time was determined by the bolus tracking method. The contrast was injected by a power injector at 6 ml/sec, followed by 50ml saline flush. Images were reconstructed with a slice thickness of 0.75 mm, using a soft-tissue convolution kernel (B26f) and a sharp-tissue convolution kernel (B46) for vessel wall calcifications, to compensate blooming artifacts. Three-dimensional volume-rendered reconstructions were used to obtain general information about the status and anatomy of the grafts and native coronary arteries. Axial slices were examined for the presence of significant narrowing by determining the presence of ≥50% reduction in luminal diameter and vessel occlusion. The analysis was assisted by curved multiplanar reconstructions of all vessels.

The patients mean age was 68.05 years (range 48 to 82 years), and the mean interval between CABG surgery and CTA was 129.08±16.64 months. Mean body mass index (BMI) was 28.3±3.76 (range 20.53 to 40.57) kg/m^2. Arterial hypertension was the most common risk factor followed by dyslipidemia and diabetes mellitus (tab. I).

TABLE I
Patient characteristics

Variable		
Continuous	**Mean**	**SD**
Age, years	68.05	7.59
Follow-up interval, months	129.53	16.74
BMI, kg/m^2	28.27	3.88
Total cholesterol	187.28	45.88
HDL	48.93	13.06
LDL	107.33	40.82
Triglycerides	157.71	81.83
Category	**N**	**%**
Ever smoked	26	43.33
Male gender	51	85.00
Family history CAD	18	30.00
Dyslipidemia	51	85.00
Diabetes mellitus	26	43.33
Hypertension	56	93.33

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; CAD, coronary artery disease.

RESULTS

The maximum number of grafts per patient was 5 (one patient) and the minimum number was one (three patients), with a mean number of 3.33 grafts per patient. Six patients had only saphenous vein grafts (SVGs); three of them had only one venous graft on the right coronary artery (RCA), one patient had two venous grafts (on RCA and diagonal arteries), and two patients had three venous grafts (on RCA, diagonal and obtuse marginal arteries).

The grafts were classified as patent if there was continuous lumen visualization at origin, in the body and at its distal anastomosis with native recipient vessels; occluded when only their stumps were seen; stenotic if there was ≥50% diameter narrowing.

The total number of assessed grafts was 200, including 146 SVGs and 54 arterial grafts of in situ LIMA. A total of 105/146 (71.92%) SVGs and 52/54 (96.3%) LIMA grafts were patent; 41 venous grafts and 2 arterial grafts were occluded. The patients with a single SVG/RCA graft were two males (both SVG/RCA were patent) and one female (occluded SVG/RCA).

Thirty-one of the 60 patients (51.67%) had all grafts patent, 17/60 (28.33%) patients had just one occluded graft, 10/60 (16.67%) patients had two occluded grafts, whereas only two patients had three occluded grafts (43/200 occluded grafts and 157/200 patent grafts).

The third segment of RCA was grafted in 40 patients (20% females and 80% males), while posterior descending artery (PDA) was grafted in 20 patients (5% females and 95% males); all patients were right dominant (fig. 1). Left anterior descending (LAD) artery was the most grafted vessel (54 grafts, 7 in females and 47 in males), followed by diagonal branch, 45 grafts in 42 patients, 7 females and 38 males (3 males

with two grafted diagonal branches). Obtuse marginal branch (OM) was grafted in 40 patients (10% females, 90% males) with 41 grafts, one male with two grafted obtuse marginal branches (fig. 2).

A number of 54 patients had a LIMA graft to LAD. LIMA graft was not developed in 2 patients.

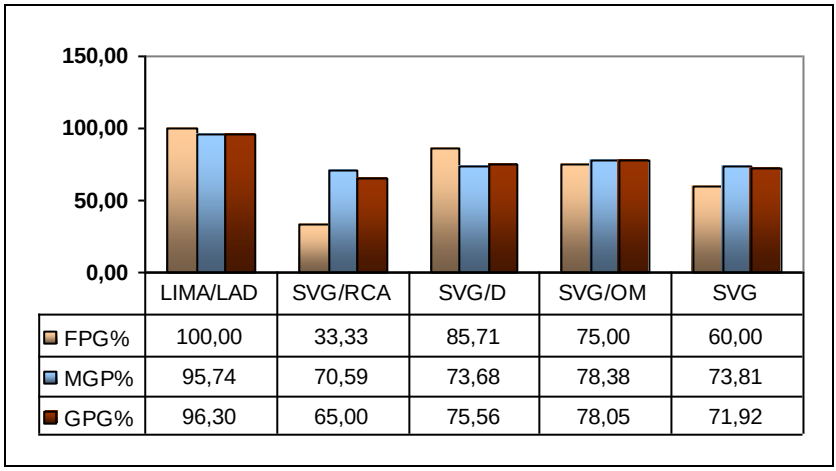
Fig. 1. Patient M.V. 71-years-old, 12 years after CABG surgery (2001-2013), has 4 grafts (SVG/PDA, LIMA/LAD, SVG/D1, SVG/OM1), all of them permeable

Fig. 2. Patient P.V. 72-years-old, 13 years after CABG surgery (2000-2013), has 5 grafts (SVG/RCA, LIMA/LAD, SVG/D1, SVG/D2, SVG/OM), all of them permeable, except SVG/RCA

The diagonal branch was the second most grafted artery. 45 diagonal branches were grafted in 42 patients, of which 34 (75.56%) patent and 11 (24.44%) occluded venous grafts were visualized. There was one stenotic (stenosis of ≥50%) graft to the diagonal branch.

OM was grafted in 40 patients. There were 41 venous grafts to OM, 32 (78.05%) patent and 9 (21.95%) occluded. Four patent SVGs to OM were stenotic grafts.

On right coronary artery, there were 60 venous grafts, of which 39 were patent (65%), with a worse patency in females. In males, 36/51 SVGs to the RCA (70.59%) were patent, while in females 3 of 9 (33.33%) SVGs to RCA were patent. According to their distal anastomoses, 40 venous grafts anastomosed distally to RCA, proximal to the *crux cordis* (8 females and 32 males), while 20 SVGs had distal anastomoses to PDA (one female and 19 males). Patent grafts to RCA proximal to the crux had been found in 25/40 (62.5%) patients and 15/40 (37.5%) patients had SVGs to RCA occluded, with a much worse outcome for females 6/8 (75% occluded) than males 9/32 (28.12% occluded). Two patients had stenotic graft to RCA (≥50% stenosis). 14/20 (70%) grafts anastomosed distally to the crux, to PDA, were patent, while 6/20 (30%) were occluded, with a better outcome for female (100%) than for male (68.42%) (fig. 3).



	LIMA/LAD	SVG/RCA	SVG/D	SVG/OM	SVG
FPG%	100,00	33,33	85,71	75,00	60,00
MGP%	95,74	70,59	73,68	78,38	73,81
GPG%	96,30	65,00	75,56	78,05	71,92

Fig. 3. Distribution of graft patency rate by distal anastomoses and sex (FPG, graft patency in females; MPG, graft patency in males; GPG, general graft patency)

The overall patency rates for arterial LIMA and venous grafts in this patient population were 96.3% and 71.92% respectively. There were 67.12% (98/146) saphenous venous good quality grafts, without any stenosis at origin, in the body or at their insertion with native vessel (7 SVGs were stenotic grafts).

DISCUSSION

Conventional coronary angiography (CCA) is the standard investigation for the evaluation of native coronary arteries and bypass grafts in post CABG patients (4). CCA is an invasive procedure, which has a significant risk in post CABG patient, associated with stroke, cardiac arrhythmia,

contrast allergy, nephrotoxicity, high cost, and radiation exposure (5). CCTA is a noninvasive method used for the assessment of coronary arteries and bypass grafts, which has been improved with the advent of DSCT scanners and has shown a high degree of accuracy for the detection of significantly lesions (6). Nonselective injection of contrast on CCTA abolishes such serious risks as arrhythmia, graft dissection, myocardial infarction or embolic events as compared to the selective injection of contrast agents in bypass graft on CCA. The current CCTA technology is able to evaluate a higher number of grafts as compared to the earlier CCTA technology, with a sensitivity of 100% for detecting significant lesions and a NPV of 100% (7). High sensitivity and high NPV indicate that negative CCTA results exclude significant graft lesions and the need for diagnostic CCA. Moreover, CCTA graft imaging can be done prior to planned CCA for graft visualization or in redo CABG for preoperative planning (8).

In contrast to arterial grafts, SVGs usually appear larger on CCTA images and fewer streak artifacts from surgical clips are seen. The proximal anastomosis of a graft is typically better visualized than its distal part on CCTA. Distal anastomoses that are not well visualized are considered patent when contrast is present within the graft conduit lumen.

All right-sided grafts in this study were SVGs, whereas left-sided grafts were represented by in situ LIMA graft on LAD and SVGs on diagonal branches or obtuse marginal arteries.

The advantages of LIMA graft conduit include decreased postoperatively mortality and improved cardiac event-free survival rates. The LIMA grafts are more resistant to atherosclerosis and have a long-term patency rates superior to those of SVGs (9). The wall of the arterial conduit is better suited to a high-pressure system and high flow velocity after CABG surgery. LIMA is used most often to revascularize the LAD or its branch territory. In situ LIMA grafts are commonly used to target the LAD (10). Sequential distal grafting is sometimes performed proximal side-to-side anastomosis to diagonal branches and distal end-to-side anastomosis to LAD or both to LAD (11). In our study, all LIMA were in situ grafts, with end-to-side anastomosis to LAD, without sequential anastomosis performed. On CTA, the LIMA graft is usually seen as a vessel along the left anterior mediastinum and may be difficult to visualize distally because of its size and artifacts. Long-term patency rate of the LIMA grafts, over 90% at 10 years after the surgery, was demonstrated by several studies.

Using SVGs for coronary arteries revascularization offers many advantages such as availability, accessibility, ease of harvest, resistance to spasm, but SVG occlusion in the later stages, due to plaque formation in the bed of fibrointimal hyperplasia and development of atherosclerosis, represent the main limitations. SVGs patency rate have increased due to continued improvements in surgical techniques, combined with antiplatelet or anticoagulant agents and lipid lowering drug therapy. The use of no-touch techniques to haverst the saphenous vein (SV) has been reported to significantly improve long term graft patency. Souza *et al.* (12) reported that the harvesting of SV with surrounding tissue provides high short- and long-term patency rates comparable to the LIMA (angiography at 8.5 years showed a patency rate for the conventional group of 76% and 90% for the no-touch group). Hata *et al.* (13) reported a SVG patency rate of 64.7% at 8 years.

Campbell *et al*. reported 66.7% patency rate of SVG at 7.3 years, while Naveed *et al*. (14) reported 66.1% venous grafts as patent at a mean follow up of 8.01 years.

CONCLUSIONS

In our study, patency rate for SVGs at 129 month was 71.92%, which is higher than in the previous reports. The secondary prevention methods, statins, antiplatelet agents, lipid lowering therapy, and lifestyle factor modification, contributed to improvement of SVG patency rate thus, venous graft occlusion had developed at a slower rate than found in earlier angiographic studies. While the association of arterial hypertension with the other risk factors related to the metabolic syndrome in elderly patients makes them more prone to the development of cardiovascular diseases (15, 16), further studies are required to determine whether a more aggressive pharmacological therapy could favorably modulate patency in venous conduits.

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NEWS

CYTOLOGICAL ANALYSIS OF THE PERIODONTAL POCKET IN PATIENTS WITH AGGRESSIVE PERIODONTITIS AND CHRONIC PERIODONTITIS

Oral exfoliative cytology includes the study and interpretation of the features cells exfoliated from the oral mucosa. The aim of a study realized by a group of argentinian researchers was to analyze cytological changes in the periodontal pocket of patients with different clinical stages of aggressive periodontitis and chronic periodontitis. Patients, aged between 24-54 years, of whom 41 were diagnosed with aggressive periodontitis, 40 with chronic periodontitis, sub-classified as mild, moderate and severe periodontitis, and 40 healthy individuals who were the control group. For the cytological study were taken samples of the epithelium of the periodontal pocket. Superficial and intermediate cell values were significantly greater in patients with aggressive periodontitis than in patients with chronic periodontitis or the control group. Histiocyte number was higher in patients with chronic periodontitis than in those with aggressive periodontitis, and differed significantly in both types of periodontitis compared to the control group. There were significant differences in polymorphonuclear neutrophil leukocytes when both types of periodontitis were compared to the control group. Microbial flora was statistically higher in patients with chronic periodontitis, and there were differences between patients with periodontitis and the control group. The cytological study demonstrated that patients with aggressive periodontitis had greater tissue damage, shown by the increase in intermediate and superficial cells of the epithelium of the periodontal pocket compared to the group of healthy subjects and to a lesser extent, to patients with chronic periodontitis. Only superficial cells made it possible to differentiate the sub-stages of the disease (Cecilia EC, Myriam AK, María EL. Cytological analysis of the periodontal pocket in patients with aggressive periodontitis and chronic periodontitis. *Contemp Clin Dent*, 2014; 5 (4) : 495-500).

Irina Grădinaru

THE HIV-POSITIVE PATIENT IN INTENSIVE CARE – PSYCHOLOGICAL PROFILE

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THE HIV-POSITIVE PATIENT IN INTENSIVE CARE – PSYCHOLOGICAL PROFILE (ABSTRACT). **Aim:** This paper aims to outline the profile of HIV-positive patients in intensive care, in terms of psycho-emotional and vital parameters. **Material and methods:** We evaluated the HIV-positive patients that required intensive care (IC), from January 2011 to December 2013, in the HIV/AIDS Regional of the „Sf. Parascheva" Infectious Diseases Clinical Hospital Iaşi. **Results:** From January 2011 to December 2013, the HIV/AIDS Regional Centre in Iaşi recorded 2649 hospitalizations, of which 0.67 % (18 cases) required intensive medical care. Of these 10 were males and 8 females, aged between 0 and 65 years with a median of 24 years. There were 29 deaths (1.09 % of all hospitalizations), 11 of which in intensive therapy (38 % of all deaths) - 7 men and 4 women. CD4 counts in persons requiring IC care were between 1 and 112/mm^3, and most naïve patients who died were late-presenters. The main diseases diagnosed were pulmonary tuberculosis and pneumocystosis, the main cause of death being multiple organ failure. The duration of hospitalization ranged between 4.5 and 30 days. Treatment success rate was correlated with the CD4 and biological status: liver and renal failure, respiratory failure, meningeal coma, hypoproteinemia, diselectrolitemia. From a psychological perspective, patients that arrived in the intensive care showed a history of non-compliance and non-adherence, a personality structure often marked by a lack of respect for them, indifference or ignorance regarding the factors that generate well-being. **Conclusions:** HIV-positive patients in the position of requiring intensive care showed a marked immunological collapse due to abandonment of therapy or late detection. **Keywords**: HIV/AIDS, INTENSIVE CARE, ADHERENCE, ANTIRETROVIRAL THERAPY, PSYCHOLOGICAL PROFILE

The HIV infection is currently considered a worldwide pandemic. Some of the main problems concerning HIV patients are epidemiological, regarding the means of transmission, confections with hepatitis B, C and D, HIV outspread in vulnerable populations (intra-venous drug abusers), viral resistance to antiretroviral therapy (ART), choosing the most adequate ART treatment. At the same time, the medical world is confronting with numerous cases of HIV patients in extreme health conditions, which require intensive care assistance (1).

A patient's survival essentially depends on the characteristics of the infectant HIV clade (resistance to ART), on establishing an optimal therapeutic regimen and also on the patient's co morbidities (renal, cardiac,

diabetes) (2).

Given this general context, we consider the multidisciplinary collaboration between doctors to be essential in order to prevent the adverse events and also to keep track of drug interactions.

In Romania, the "pediatric cohort", represented by the young people born in 1986-1989 and infected in the hospital, is still the main part of all HIV infected people. However, we have observed in the past years a shift in patients' morbidity, from the adolescent age to adults and older.

From a psychological point of view, young people living with HIV/AIDS experience a series of particular emotional states: "therapeutic exhaustment" from having grown up with multiple ART regimens, abandonment of therapy, denying HIV status. This has an impact on the overall health status, making patients vulnerable to opportunistic infections that have an increased effect on the weakened immune system (3, 4).

Another situation to be considered is that of patients living with HIV/AIDS that are unaware of their diagnosis, slow-progressers (with a slow increase in viral load and a slow decrease in immunity throughout a long period of time) and late-presenters (HIV-infected patients with a very low immunity, CD4 cell count under 200/mm^3) (5). They represent the majority of HIV patients assisted in a medical intensive care (IC) unit.

This paper aims to describe the clinical-biological and psycho-emotional profile of the HIV patient requiring intensive care assistance.

MATERIAL AND METHODS

We realized a retrospective study for a period of 36 months, from January 2011 to December 2013, evaluating HIV positive patients in intensive care. In the mentioned time span, the Infectious Diseases Hospital Iasi reported 28.773 admissions, of which 2649 were HIV infected patients. Of these, 18 HIV positive patients required intensive care (0.67%) (fig. 1).

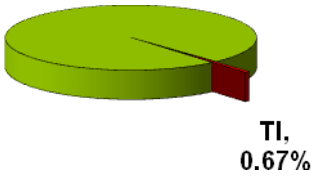

Fig 1. HIV patients admitted to ICU in 36 months

We evaluated the viro-imunological parameters at admission, and the clinical and biological status, highlighting organ deficits and failure. We also studied the psychological reports written at admission and at discharge, for patients whose biological status allowed psychological evaluation.

The psychological evaluation consisted mainly in clinical semi-structured interviews that explored the patients' overall emotional status and their emotions towards the HIV diagnosis.

The demographics revealed 10 male patients and 8 female, with a median age of 32.4 (range 24-65 years).

From the total of 18 cases (100%) of HIV in intensive care, 61% (7 male, 4 female), setting the survival rate 39% (fig 2).

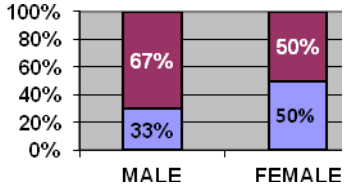

Fig 2. Gender distribution, and survival rate

RESULTS

In our study, we found that patients were admitted to the intensive care unit (ICU) with the following associated pathology, which revealed the HIV-infection:

- pneumocystosis and Kaposi sarcoma – one case;
- generalized tuberculosis and ongoing pregnancy – one case;
- ovary tumor – one case;
- HIV myelitis and encephalopathy – one case;
- cryptococosic meningitis – 2 cases;
- respiratory failure caused by a pneumocystis jirovecii infection – 5 cases;
- generalized tuberculosis with pleural-pulmonary, ganglionary, meningean and gastro-intestinal spread – 7 cases.

From an immunological point of view, CD4 cell count varied between 1 and 112/mm^3. Patients were admitted with severe immunosuppression, mainly with respiratory failure due to pulmonary pneumocystosis or pulmonary tuberculosis.

The number of days of hospital admission varied between 4.5 and 32 days in the ICU.

The initial psychological interview was conducted for 16 of the 18 patients in ICU. In 2 cases the severely deteriorated health status did not allow communication. The 7 survivors were evaluated at discharge from ICU and also monitored long term.

The psychological interview was semi-structured; questions regarded general life aspects, the moment of diagnosis and the progression of the HIV disease, coping with antiretroviral therapy, motivations for taking or refusing the ART, family, personal life, projects and life goals.

After analyzing the interviews, we observed two directions in HIV patients.

One category of patients was represented by those that had "given up" on life, deciding to stop taking the ART in order to end life. This form of "suicide" by not taking medication reveals a profoundly melancholic emotional profile, characterized by moderate to severe depression, a sense of being overwhelmed by life and all that society expects from the individual (financially, marital, maternally, educationally). Patients described a wish to "shut down", to stop existing, and often times to punish those around them (family, friends, and community) for their attitude towards them (rejection, stigma, work exploitation, indifference, and hate).

Another category was represented by the survivors – the patients that found motivation for living and for creating a close to normal existence. This motivation came from different sources – from wanting to take care of their family and children, from getting the support they needed from their parents or their life partner, from wanting to function normally in society, as successful individuals.

Long term psychological monitoring for these survivors revealed an increase in adherence to ART and a change in lifestyle. The motivation found in the ICU acted as psychological support for re-evaluating their life status and for taking the necessary actions to prevent further medical complications.

DISCUSSION

The number of cases in our study – 18 (0.67%) out of 2,649 – could be considered a small sample, when compared to the total number of HIV positive admitted patients. However, the author's motivation for evaluating the psychological status of the HIV patient in ICU was driven by the complexity of the cases, the medical and human costs, and especially the profound psychological implications of this pathology. The

survival rate of almost 40% encourages long term management and is a solid platform for clinical therapy and for the activity of the infectious diseases doctor and the IC specialist.

The HIV positive patient goes through a series of emotional changes, after the diagnosis. These changes are similar to those described in mourning: first there is denial, followed by anger, negotiation and finally acceptance. This acceptance of the positive status leads to a natural behavior of adherence to ART. However, some of the patient remain trapped in one of these states, and don't go through all the emotions necessary in order to reach acceptance. In this case they lack the ability or the intrinsic desire to take medication (6).

The Romanian "pediatric cohort" represents a particularly vulnerable population, due to the fact that it is made up of young adults that have spent their childhood and adolescence taking pills and fighting different co morbidities (7, 8, 9).

Adherence in this population is an ongoing problem due to both the long term therapy that causes "therapeutic exhaustion" and to lack of social support to go through all the emotional stages described above.

From a psychological point of view, practice has revealed two opposite attitudes:

1. denial of the disease and all the aspects concerning it, when meeting a new partner, a new group of friends, or in new social contexts; wanting to be like everybody else by NOT taking pills;

2. acceptance of the disease and coping with every-day social challenging situations, having a functional family, healthy children, a workplace etc.; wanting to be like everybody else WHILE taking pills.

Unfortunately, the first category of patients is the most common in ICU, being admitted with different organ failures and opportunistic infections that require intensive care assistance.

For slow-progressers and adult naive patients, the psychological impact is greatly increased by having to cope with a highly stigmatizing diagnosis while in ICU. They find themselves in the position to either give up or find strength and self-motivation to overcome the physical illness and the psychological trauma. Family support is essential in establishing a "safety net" for the patient, in his attempt to overcome the disease.

Also of crucial importance is the psychological support that is offered throughout hospitalization. The relationship that the patient builds with the psychologist is the stepping stone for future adherence to ART (10, 11).

CONCLUSIONS

In our research, we realized that, in order to fully address the needs of the HIV positive patient, the infectious diseases doctor, along with the ICU specialist must collaborate with the psychologist. This allows a multidisciplinary perspective on the patient, and professional support from all sides. It is of great value to identify the patient's psychological profile and his motivation, thus increasing life expectancy through a high level of ART adherence.

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NEWS

ANTIRETROVIRAL THERAPY AND ACQUIRED RIFAMPICIN RESISTANCE

According World Health Organization (WHO), in 2013, 13% of new cases of tuberculosis (TB) were HIV positive. Antiretroviral therapy (ART) reduced the risk of TB morbidity and mortality among patients HIV+ (1). A group of researchers from the National Institute for Research in Tuberculosis India have evaluated the impact of ART on acquired rifampicin resistance (ARR) in HIV-TB coinfection. The study group included 574 new pulmonary TB cases with and without HIV (246 TB+HIV-, 212 TB+HIV+ART-, 116 TB+HIV+ART+), receiving a TB treatment regimen which comprised thrice-weekly rifampicin for 6 months. The results showed that the relative risk for ARR was 21.1 (95%CI 2.6-184), $p<0.001$ for patients TB+HIV+ART- and 8.2 (95%CI 0.6-104), $p=0.07$ for patients TB+HIV+ART+, compared to patients TB+HIV-. ART reduced the relative risk for ARR among the patients TB+HIV+ by threefold (2).This study sustain the WHO recommendation for ART initiation in all cases HIV+TB+, irrespective of CD4 cell level (1) (World Health Organization. Global tuberculosis report 2013. www.who.int/tb/publications/global_report/en/ ; Narendran G, Menon PA, Venkatesan P et al. Acquired Rifampicin Resistance in Thrice-Weekly TB Therapy: Impact of HIV and Antiretroviral Therapy. *Clin Infect Dis*. 2014. doi: 10.1093/cid/ciu674).

Cătălina Luncă

RESEARCH ON PLASMA AND SALIVA LEVELS OF SOME BIVALENT CATIONS IN PATIENTS WITH CHRONIC PERIODONTITIS (SALIVARY CATIONS IN CHRONIC PERIODONTITIS)

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RESEARCH ON PLASMA AND SALIVA LEVELS OF SOME BIVALENT CATIONS IN PATIENTS WITH CHRONIC PERIODONTITIS (Abstract): The purpose of this study was to determine whether chronic periodontitis can stand behind modifications in the salivary and blood concentration of some bivalent cations (Calcium, Magnesium, Zinc and Copper). For this purpose, we formed a group of 30 adult patients with clinically onset chronic periodontitis, and another one of 30 healthy patients as control. Both groups were free from acute oral pathology and general illnesses. The groups were divided again according to the habit of smoking. Total saliva samples were obtained as "first time in the morning", then weighed and processed. Cations were read on Atomic Absorption Spectrophotometer and by Ion Chromatography (Magnesium). The same patients were required to undergo laboratory blood tests for Calcium, Magnesium and Zinc. Data obtained was normalised, then statistically interpreted using two-tailed heteroscedastic *t-Student* tests. Our data confirmed the existence of a connection between salivary calcium, magnesium, zinc and copper, and of blood magnesium, and chronic periodontitis. Salivary calcium and magnesium are affected by smoking.
Keywords: CHRONIC PERIODONTITIS, CALCIUM, MAGNESIUM, ZINC, COPPER.

The meaning of the words "chronic periodontitis" was argued upon for decades. It took a great deal of systematic work (1) to put together all definitions and mechanisms that come under it.

Chronic periodontitis is caused by plaque (2), but disease progression depends on an individual's susceptibility (3, 4, (5). While many factors have been cited as influencing the progression of the disease, there appears to be a fundamental association with the nature of the host response. Therefore, chronic periodontitis is a chronic inflammatory condition that recognizes the influence of local and general factors in its evolution. Identification of the histological characteristics of the development of chronic periodontitis has also influenced treatment philosophies and how clinicians treat individual patients affected by chronic periodontitis.

Bivalent cations take many involvements in acute and chronic inflammatory pathology. The studied cations (calcium, magnesium, zinc and copper) and many others play complex roles in the human organism. They

are found both intracellular and extracellular and are crucial for many normal processes within the normal physiology. From this point of view, the tissues of the oral environment have the unique particularity to be in contact with both blood and saliva. Therefore, bivalent cations' concentration in blood and saliva may have important effects on physiological processes but also on pathogenic mechanisms in the oral environment. Salivary concentrations of bivalent cations vary in certain conditions of the oral cavity and of the head and neck (such as malignant tumours) (6, 7, 8).

There are several conditions (glossopyrosis and oropyrosis) where salivary magnesium is constantly low (9).

Variations in the concentration of salivary cations are sometimes consistent with and some other times different than the serum concentrations of the same cations, under different pathological conditions.

The development of gingivitis and subsequently of the chronic periodontitis lesion has been classically described as progressing through a series of stages, i.e. the initial, early, established and advanced lesions (10). These stages are not always discernible as distinct entities in their own right. The "initial lesion" of chronic periodontitis is a subclinical entity occurring within the first four days of plaque accumulation. The characteristic immune response to bacterial enzymes and metabolic end products is observed as a result of complement activation of the alternative pathway. There is also a release of tumor necrosis factor-a. However, the presence of an organized plaque biofilm induces the neutrophils to release their lysosomal agents, in an act of "abortive phagocytosis". The action of these extremely active agents exacerbates local tissue damage; however, the lesion is not clinically discernible, and only occupies 5–10% of the surrounding connective tissues (10). A number of other factors have been described as being involved in the pathogenesis of chronic periodontitis (11, 12, 13, 14, 15).

The aim of this study was to assess the differences in some bivalent cation concentration (calcium, magnesium, copper and zinc) in the blood and saliva of patients in their initial status (with and without chronic periodontitis).

PATIENTS AND METHODS

The research was aimed at adult patients of both sexes.

The first lot consisted of adult subjects of both sexes, non-smokers and free from any pathological conditions of the oral cavity. The second lot consisted of adult subjects of both sexes, smokers and free from acute pathological conditions of the oral cavity. The third lot consisted of adult subjects of both sexes, non-smokers and with onset chronic periodontitis. The fourth lot consisted of adult subjects of both sexes, smokers and with onset chronic periodontitis. The demographic data of the four lots are described in Table I.

TABLE I

Demographic structure of the lots

Parameter	Lot I Control non-smokers	Lot II Control smokers	Lot III Periodontitis non-smokers	Lot IV Periodontitis smokers
No. of persons	9	21	12	18
Age & Sex				
Age	29±5.66	38±8.29	34.33±8.6	31.75±8.93
Sex distribution M/F	7/2	18/3	11/1	11/7

Lots I and II were built up of adults of both sexes, without acute oral pathology, and who did not feature general illnesses.

Inclusion criteria: Patients included in the study lots needed to:

- be of age;
- feature chronic periodontitis;
- be fully compliant to a treatment scheme.

Exclusion criteria:

- Patients with massive metallic dental restorations, that would hinder proper readings for copper and zinc;
- Patients that would not comply with assuming a proper and constant hygiene technique (brushing, flossing);
- Patients that were afterwards called on duty in other places (therefore, impossible to follow);
- Patients with drug-induced gingivitis or with other forms of periodontitis;
- Patients with chronic ethylism;
- Pregnant and breast-feeding patients;
- Patients with systemic illnesses such as diabetes mellitus, renal insufficiency, liver cirrhosis, malabsorption syndromes;
- Patients who used food supplements that contained bivalent cations;
- Patients with any kind of psychosis.
- Patients who used drugs that could bias cation levels (such as diuretics, cardiac tonics).

The diagnosis criteria for chronic periodontitis were: true loss of gingival-dental attachment; existence of periodontal pockets deeper than 3 mm; radiological loss of interdental septa (horizontal bone loss) or of lamina dura (vertical bone loss); all these for at least six dental-periodontal units per arch (16).

In what smoking was concerned, the lots were divided as follows:

- The first lot consisted of 21 periodontitis-free persons, non-smokers.
- The second lot consisted of 9 periodontitis-free persons, of which 6 smoked less than 10 cigarettes a day, and 3 smoked more than 10 cigarettes a day.
- The third lot consisted of 12 persons with periodontitis who didn't smoke at all.
- The fourth lot consisted of 18 persons with periodontitis, of which 10 smoked more than 10 cigarettes a day, and 8 smoked less than 10 cigarettes a day.

Patients were first subjected to an initial clinical examination and a thorough anamnesis.

The patients were required to have their Orthopantomogram (OPG) taken, or to produce a recent one if available. The x-rays were eventually obtained for each patient clinically diagnosed with chronic periodontitis.

Periodontal probing followed. We used a first-generation calibrated periodontal probe, of the 'O'-type by the University of Michigan, with Williams's markings. Each tooth (meaning a tooth that is not so damaged as to prevent measuring) was subject to six measurements, three on the buccal side and three on the oral side. After performing such measurements, and combining them to the chart head, patients received a clear and final diagnosis.

The structure of oral and general pathology, as well as that of hygiene status and scale deposits may be found in table II.

Saliva sampling

Patients were then instructed to produce a saliva sample in a sterile sealed single-use polyethylene syringe. They would have to do this the morning of the next appointment, just after waking up, before any hy-

giene or intake (eating or drinking) (17). After collecting the saliva sample, patients were asked to bring it to the practice as they would come to the appointment.

The patients were also required to perform a routine blood test, or to bring in any recent tests, if available; the tests included total blood calcium, magnesium and zinc, blood cell count and distribution, PCV, Hb and several other parameters. The blood samples and tests were performed at patient chosen locations.

The saliva samples were tare-weighed in ceramic and platinum crucibles on an analytical scale (*Balanţa*, Sibiu, Romania), then oven-desiccated at 400ºC for 24 hours. The crucibles were identified with numbers written with heat-resistant marker. The residue was taken up with 5 mL nitric acid 5%, then flushed and diluted with distilled water up to 50 mL (working volume for the atomic absorption spectrophotometer (AAS)). The solution obtained was filtered (using filter paper) into sterile dry polypropylene containers. Containers were labeled according to the original sample ID number and then the concentrations of cations were read on an AAS in the Faculty of Pharmacy. For magnesium, the determinations were performed by the Analytical Chemistry department of the Chemistry Faculty in the "Al. I. Cuza" University Iaşi.

Statistic interpretation of the data series was performed using a *t-Student* test, two-tailed and of the heteroscedastic type.

TABLE II

Oral and general pathology, hygiene status and scale deposits

Parameter	Lot I Control non-smokers	Lot II Control smokers	Lot III Periodontitis non-smokers	Lot IV Periodontitis smokers
Oral pathology				
New caries	1	3	2	11
Improper filling	3	4	7	14
Fillings with caries	1	1	1	6
Gangrenes	0	0	1	0
Root remains	1	2	5	13
General pathology				
Chronic inflammations	0	0	0	0
Diabetes mellitus	0	0	0	0
Neoplasia	0	0	0	0
Chronic medication	0	0	0	0
Oral hygiene (OHI)				
2.33 – 3	0	1	1	12
1.33 – 2	4	6	1	10
0 – 1	5	14	0	5
Scale deposits	4	8	12	16

As far as dental treatments were concerned, for the third and fourth lot, we found the following situation: Patients were found with new onset dental caries (13 in the periodontitis lots, 4 in the control lots), with improper fillings (21 in the periodontitis

lots, 7 in the control lots), with caries under fillings (7 in the periodontitis lots, 2 in the control lots), with gangrenes (6 in the periodontitis lots), root remains (18 in the periodontitis lots, 3 in the control lots). In the periodontitis lots, 26 patients featured more than one crown (4 ceramic, 11 metal – acrylic, 3 metal – diacrylic and 7 metallic), while in the control lots we only found 15 people with crowns or bridgework (5 ceramic, 3 metal–acrylic, and 7 metallic). Cervical margins of crowns were thoroughly checked, and found inappropriate in 24 patients of the periodontitis lots and 3 patients in the control lots (tab. II).

The study was performed according to the EU norms for clinical studies, and after the issue of the endorsement of the Research Ethics Committee of the University of Medicine and Pharmacy "Grigore T. Popa", Iaşi.

RESULTS

In saliva samples, there were several significant differences. We tried to allocate such differences to known or alleged phenomena in chronic periodontitis.

Salivary calcium was lower in non-smoker patients with periodontitis. That is, in control groups salivary calcium had an average of 84.24±0.87 mg/L (lot I) / 86.88±3.11 mg/L (lot II), while in the periodontitis lots, salivary calcium had an average of 53.37±6.57 mg/L (lot III) / 56.12±8.45 mg/L (lot IV), $p<0.05$ for periodontitis, $p<0.05$ for periodontitis and smoking (fig. 1).

Blood calcium did not feature any significant differences ($p=0.5894$) (Fig. 1).

Contrary, salivary magnesium increased in patients with periodontitis. The control group featured an average of $0.81 \pm 8.5 \times 10^{-3}$ mg/L salivary magnesium, while in periodontal patients, it reached 1.07 ± 0.18 mg/L, $p<0.05$ (fig. 2).

Blood magnesium decreased in patients with periodontitis. We found in the control group an average blood magnesium of 2.05 ± 0.08 mg/L, compared to an average of 1.87 ± 0.12 mg/L in patients with periodontitis, $p<0.05$ (fig. 2).

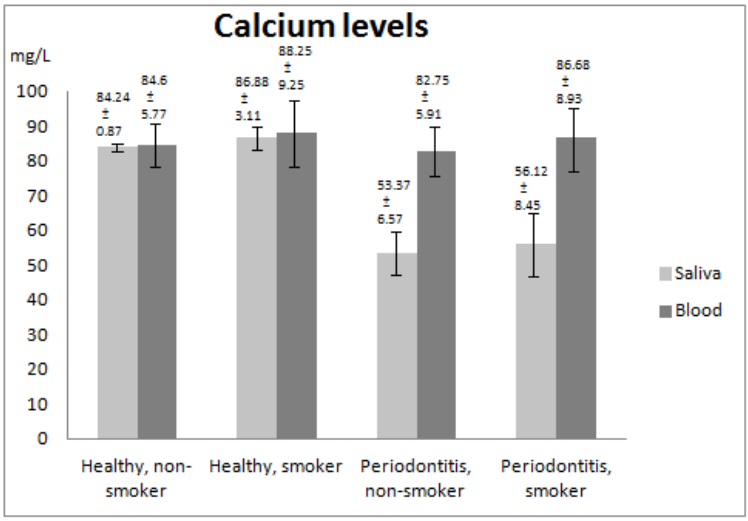

Fig. 1. Differences in calcium levels

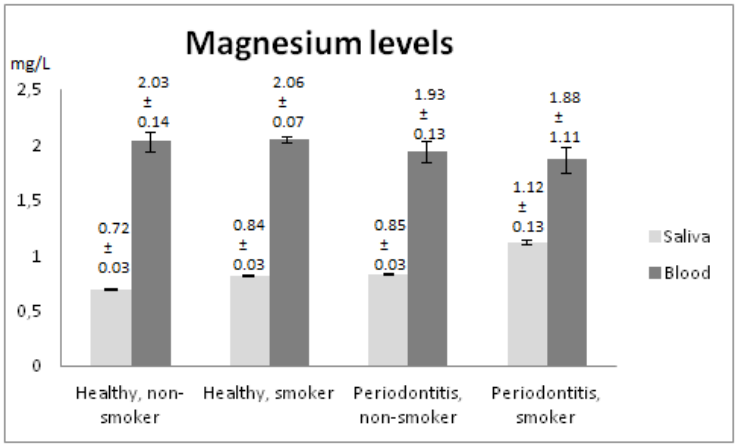

Fig. 2. Differences in magnesium levels

Smoking severely affected the salivary magnesium level in patients with periodontitis. Lot III had 0.82±0.03 mg/L, while lot IV had 1.12±0.13 mg/L, $p<0.05$. Salivary magnesium was also affected by smoking in healthy patients, lot I having 0.72±0.03 mg/L and lot II having 0.84±0.02 mg/L, but the statistical significance was not enough to state a clear difference, $p=0.05$.

Salivary copper increased in periodontitis (healthy average of 0.83 ± 0.18 mg/L, compared to 1.2 ± 0.2 mg/L in the periodontal patients), but this was not so evident ($p<0.05$) (fig. 3). Smoking had a positive effect on salivary copper in healthy subjects (lot I having a "basal" 0.66±0.01 mg/L while lot II had 0.92±0.16 mg/L, $p<0.05$) as well as in those with chronic periodontitis (lot III had 0.99±0.21 mg/L while lot IV had 1.18±0.15 mg/L, $p<0.05$).

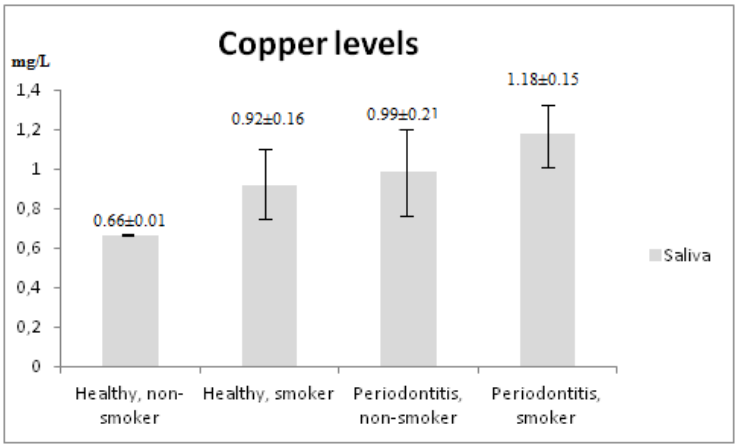

Fig. 3. Differences in salivary copper levels

Salivary zinc was not that accurately distributed among patients with periodontitis (lot III with 0.37 ± 0.01 mg/L, and lot IV with 0.21 ± 0.12 mg/L), but was defi-

nitely higher in healthy subjects (lot I with 0.48 ± 0.05 mg/L and lot II with 0.53 ± 0.18 mg/L). The difference was statistically significant ($p<0.05$) (fig.4).

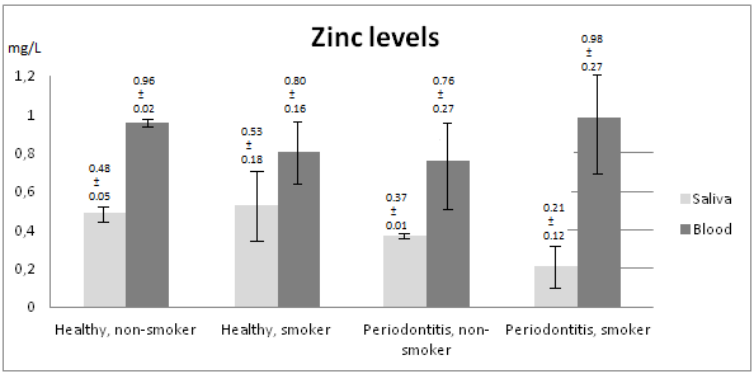

Fig. 4. Differences in zinc levels

DISCUSSION

Bivalent cations have various involvements in acute and chronic inflammatory mechanisms. Extracellular calcium triggers NLRP3 inflammasome which enables inflammatory monocyte and macrophage to release high levels of interleukin 1β (18). Some elements of the molecular mechanism of inflammation depend on the calcium release from intracellular structures, or on the entrance of calcium ions from the extracellular area into the cells (19). Human macrophages' activity is calcium-dependant (20).

In periodontitis, Kuraner (21) identified an increase in serum calcium and a diminishment of plasma zinc. In parotid saliva, calcium had a lower concentration than in normal subjects.

Lowering of salivary calcium may be charged to the deposition of scale that occurs by precipitation of soluble calcium from the saliva, therefore decreasing calcium concentration. This mechanism could also explain the relative uniformity of figures in the working lot ($\sigma=7.65$, that is 14.06%) compared to the control lot ($\sigma=34.3$, that is 29.7%), probably by reaching a saturation threshold of the precipitation phenomenon.

Magnesium is an essential bivalent metal for the activity of over 300 enzymes. It features an anti-oxidant activity since it reduces the forming of peroxide radicals. It is involved in the modulation of vascular permeability, and in the endothelial function. Magnesium reduces the synthesis of cytokines (22). Administration of magnesium suppressed some inflammatory responses in various tissues (23).

In rats with experimental magnesium deficiency, an inflammatory syndrome was discovered, characterized by the increase of the release of inflammatory cytokines, and by leukocyte activation. The synthesis of acute phase proteins increases (24).

The relative uniformity of the salivary magnesium values in the control lot is probably caused by a strict tuning of salivary secretion in periodontitis.

In our research the serum level of calcium and magnesium does not feature significant differences in patients with chronic periodontitis (either smoker or non-smoker)

compared to the control lots.

The salivary concentration of calcium is significantly lower though in patients with periodontitis. Smoking did not affect, either in the periodontitis lot or in the control lot, the level of salivary calcium.

The salivary concentration of magnesium is significantly higher in the periodontitis lot compared to the control lot, and higher in the smokers with periodontitis than in the non-smokers, also with periodontitis. Thus, in the case where the salivary Magnesium / salivary Calcium ratio is obviously higher in patients with this periodontal condition, magnesium is suspected to reduce the bacterial LPS-induced inflammation (25), and an increased salivary magnesium level could contribute to the put-off of the periodontal inflammation, mostly caused by bacteria. Respectively, a reduced level of magnesium increases phagocytosis and the yield of reactive oxygen species in the neutrophils (26).

An increased magnesium level could diminish apoptosis and also sustain tissue regeneration (27). A higher salivary magnesium level could have a diminishing action on the destruction of some pro-inflammatory cytokines such as TNF-α and IL-6 (22). Magnesium deficiency supports the development of inflammatory conditions (28).

The increase of copper is not very obvious, and it can be charged to other causes, such as contamination from present or prior bronze bridgework, and smoking (Figure 4).

Zinc is an essential element for the activity of the immune system. In case of zinc deficiency, the oxidative stress exacerbates (29). Administration of zinc diminishes the oxidative stress hence inhibiting the development of certain inflammatory processes (30). The ratio of copper / zinc concentrations is important for the intensity of the oxidative stress. Thus, an increase of the copper / zinc ratio is correlated to an increase of the level of the oxidative stress (31). There is no assertion of what an increased zinc level could mean. Probably it is due to a very host-dependent immune mechanism involving salivary IgA and complement, as a reaction to the presence and multiplication of periodontal bacteria.

The salivary level of copper is significantly increased in patients with chronic periodontitis, compared to their respective control lot. The accentuated decrease in the salivary zinc level in patients with periodontitis has an alleged connection with smoking, since in the control lot there is no significant difference in salivary zinc concentration between smokers and non-smokers.

The existing data on the variation of salivary and blood concentrations of the above-mentioned bivalent cations in relation to chronic periodontitis are still contradictory. By analyzing the salivary concentrations of calcium in patients with periodontitis (both chronic and aggressive periodontitis), adult smoker and non-smoker, Kiss (32) found that the level of salivary calcium in smokers is significantly higher than in non-smoker periodontitis patients (57.76 ± 18.8 mg/L *vs.* 44,6 ± 7.8 mg/L). The higher calcium level was associated to an increased bone loss and probing pocket depth.

By analyzing salivary calcium concentration in patients with periodontitis, smokers and non-smokers, Sutei (33) found no significant differences.

Kolte and his team analyzed in 2012 the saliva in smokers compared to non-smokers and found that total proteins, calcium, magnesium, and zinc are significantly reduced in smokers *vs.* non-smokers (34).

The same diminishment of calcium and magnesium concentration in the smokers' saliva was also found by Zuabi et al (35).

CONCLUSIONS

In our research we were unable to depict significant differences in salivary calcium from patients with chronic periodontitis, that is in smokers *vs.* non-smokers, but in both cases these concentrations were significantly lower than the concentrations in the saliva of healthy subjects, both smokers and non-smokers, but in both situations these concentrations were significantly lower than those in the saliva of periodontally healthy patients, either smoker or non-smoker.

In our study, the level of salivary magnesium in smokers with chronic periodontitis is significantly higher compared to both healthy smokers and non-smokers with chronic periodontitis.

The heterogeneity of the data in the literature is rather difficult to explain. Our results show a clear increase of the salivary copper / zinc ratio and the diminishment of salivary and blood zinc in patients with chronic periodontitis, compared to the healthy ones. We reckon that the lower zinc level may cause a slower and weaker immune activity, thus supporting the development of bacteria involved in the pathogenity of chronic periodontitis.

Thus, testing the salivary concentrations of bivalent cations may be useful for the assessment of patients with periodontitis, smokers or non-smokers.

ACKNOWLEDGEMENTS

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NEWS

RECEPTOR-MEDIATED RECOGNITION OF MYCOBACTERIAL PATHOGENS

Mycobacterium is a genus of Actinobacteria that includes more than 50 different species, ranging in virulence from the non-pathogenic *M. smegmatis* to the causative agent of tuberculosis (TB) in humans, *M. tuberculosis.*Infection with mycobacteria most commonly occurs through inhalation or ingestion of bacilli. The bacilli are phagocytosed by host macrophages and dendritic cells (DCs) at the site of infection, for example by alveolar macrophages in *M. tuberculosis*-infected lungs or by intestinal macrophages in animals infected with *M. avium* subsp. *paratuberculosis* (MAP). Mycobacteria have evolved a range of mechanisms to circumvent phagosome maturation, preventing lysosomal degradation, and are therefore able to both survive and replicate inside the host phagosome. In order for mycobacteria to be phagocytosed by macrophages and DCs, the pathogen is first recognized by pattern recognition receptors (PRRs) on the host cell. This is achieved through the recognition of highly conserved molecular structures found on the surface of pathogens, often critical for microbial survival, termed *pathogen associated molecular patterns* (PAMPs) Several families of PRRs exist, all of which are capable of recognizing a different repertoire of PAMPs; including plasma membrane-bound and intracellular Tolllike receptors (TLRs) and cytosolic NOD-like receptors (NLRs) and RIG-I like receptors (RLRs). Future studies targeting different combinations of receptors may lead to a better appreciation of the complex interactions between phagocyte and mycobacterium. Ultimately, increasing our understanding of innate immune recognition of mycobacterial pathogens will aid in our abilty to treat and prevent mycobacterial diseases. (Kate E. Killick, Clíona Ní Cheallaigh, Cliona O'Farrelly, et al. Receptor-mediated recognition of mycobacterial pathogens. *Cellular Microbiology* (2013) 15(9): 1484–1495)

Doina Butcovan

CLOSTRIDIUM DIFFICILE EPIDEMIC OUTBREAK IN AN ONCOLOGY UNIT

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CLOSTRIDIUM DIFFICILE EPIDEMIC OUTBREAK IN AN ONCOLOGY UNIT (Abstract): **Aims:** Contributions to the knowledge of some peculiarities of *C. difficile* involvement in human pathology, nosocomial infections (NI) included. **Material and methods**: This clinico-epidemiological and laboratory study included 14 patients admitted to the Medical Clinic of the Iasi Regional Cancer Institute with the diagnosis *of C. difficile* NI based on detection of toxins A and B in stool samples. The data were assessed and interpreted using an algorithm which revealed the peculiarities of *C. difficile* NI outbreak occurrence and evolution. **Results:** Of the 14 cases included in the study, 5 (35.8 %) had community-onset infections and 9 (64.3 %) oncology unit-onset infections. The average hospital stay was 22 days. Immunocompromised condition and the use of antibiotics in the ciprofloxacin, colistin and cefotaxime group for 4 - 10 days were the main risk factors. **Conclusions**: This epidemic *C. difficile* NI outbreak which affected immunocompromised patients with high susceptibility to infection, common finding in oncology care was brought under control by active preventive measures associated with concomitant antimicrobial therapy effective in *C. difficile* infections. **Keywords**: *CLOSTRIDIUM DIFFICILE*, NOSOCOMIAL INFECTION, ONCOLOGY, EPIDEMIC OUTBREAK.

Similar to other species of the 200 described, *Clostridium difficile* can induce invasive phenomena and toxigenic processes through the mediation of some histotoxins (enterotoxins, haemolysins, proteases) that destroy the soft tissues, especially in immunocompromised patients treated with antibiotics who may develop colitis, sometimes severe, and invasive infections, such as bacteraemia (1, 2).

Although the role of clostridia in human pathology was reported before 1870, in the era of antibiotics *C. difficile* is still an important causative agent of human diseases, and the most common cause of nosocomial infections (IN), producing toxin-mediated diarrhea, pseudo membranous enterocolitis and toxic mega colon (3, 4, 5).

Recent clinico-epidemiological observations have demonstrated the involvement of highly virulent *C. difficile* strains in the occurrence of epidemic outbreaks in various health care units, requiring effective diagnostic, treatment and prevention inteventions (6, 7).

The possible involvement of *C. difficile*

in intestinal infections has been suggested ever since 1935, but, in the absence of accessible methods of investigation, these infections were thought to be caused by *Staphylococcus aureus*, recognized as the main causative agent of NI at the onset of antibiotic era (6, 7, 8).

The identification in 1978 of *Clostridium difficile* as a Gram positive sporulated bacillus allowed its inclusion among the etiologic agents of antibiotic-associated diarrhea, proving its widely emerging tendency with various clinico-epidemiological manifestations (9, 10, 11).

The spread and aggressiveness of *C. difficile* strains may be favored by: the use of antibiotics (even in a single dose) and cytostatics, digestive tract surgery, severe chronic diseases, administration of proton pump inhibitors, immunosuppression from various causes; frequent hospitalizations, age over 65 years (12, 13, 14).

The risk of *C. difficile* infections is maintained by the healthy carriers in the general population, with a prevalence of 2 - 5%, with the possible involvement of other risk groups including children, women after delivery, and tissue and organ recipients, stem cells included.

MATERIAL AND METHODS

The clinico-epidemiological study was conducted in the interval January-October, 2013, using the data in the observation sheets and laboratory results.

The study group included 14 patients with a mean age of 56.4 years, admitted to the Medical Clinic of the Regional Cancer Institute for *C. difficile* NI diagnosed by the presence of toxins A and B in stool samples.

The data were assessed and interpreted by using an algorithm which revealed the peculiarities of *C. difficile* NI outbreak occurrence and evolution (fig. 1).

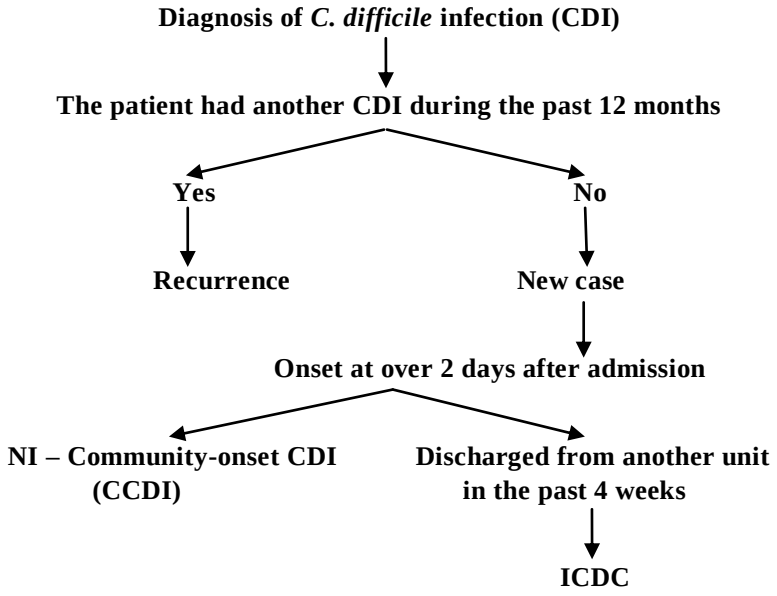

Fig. 1 The scheme of the structural algorithm for *C. difficile* infection outbreak

RESULTS

Community-onset C. difficile NI (CCDI). The assessments, based on the clinico-epidemiologic algorithm, showed that of the 14 cases included in the study, 5 (35.8%) were considered community-onset NI. The 5 patients were aged between 30 and 72 years, 4 were females and 4 from urban areas, with onset on average 72 hours before admission, multiple previous hospitalizations, no surgeries, and clinical symptoms started on average 2 days after admission (tab. I).

Clinical symptoms occurred 3 to 9 days after prior hospitalization (mean: 2 - 6.4 days) with fever state, abdominal pain and accelerated intestinal transit (3-20 stools/ day).

TABLE I

Some characteristics of the patients with community-onset *C. difficile* infection and clinical onset during hospital stay

No. case	Age	Sex	Area	Diagnosis upon admission	Co morbidities	Antibiotic therapy	
						Type	No. days
1.	72	M	urban	Acute myeloid leukemia Pseudo membranous colitis	Renal failure Heart failure Viral hepatitis C	Cefotaxime Ciprinol	7
2.	61	F	urban	Multiple myeloma Secondary immune deficit	Respiratory failure Cardiac failure	Ciprinol	8
3.	52	F	rural	Myeloproliferative syndrome	Myocardial infarction Breast cancer	Cefotaxime Ciprinol	7
4.	39	F	urban	Myeloproliferative syndrome Prolonged febrile syndrome	No	Augmentin	7
5.	30	F	urban	Diffuse non-Hodgkin's lymphoma	No	Vancomycin Metronidazole	3

Despite cancer-induced immunosuppression, of the 5 patients with CCDI only 1 case (20%) presented a severe evolution.

Oncology unit-onset C. difficile NI: 9 cases (64.3%), mean age of 60.5 years, onset 9- 35 days (average 22 days) after admission, characterized by abdominal pain (50%) accelerated intestinal transit (> 5 stools/day) (100%), fever (> 38.5 °C) (66.6%), and a mild clinical course (77.7 %).

In these patients, the clinico-epidemiological investigation showed that the risk factors involved in the occurrence of CDNI were the use of antibiotics in the ciprofloxacin, colistin, cefotaxime group for 4-10 days in 4 cases (44, 4%); cefotax-

ime and a dose of cytostatics in 2 patients (22.2%), chemotherapy and radiotherapy alone in 3 patients (33.3%).

The most commonly recorded co morbidities were: hepatitis C virus, arterial hypertension and anemia (tab. II).

TABLE II

Some characteristics of the patients with oncology unit -onset *C. difficile* infection

No. case	Age	Sex	Area	Diagnosis upon admission	Co morbidities	Surgery	Antibiotic therapy	
							Type	No. days
1.	67	F	urban	Malignant biliary tract tumor	Anemia Malnutrition AHT	yes	no	no
2.	68	F	urban	Lymphoid leukemia	AHT	no	Vancomycin	3
3.	67	M	urban	Malignant rectal tumor	Viral hepatitis C	yes	Cefuroxime	3
4.	70	M	urban	Malignant rectal tumor	AHT	yes	Cefotaxime	3
5.	76	F	urban	Malignant sigmoid colon tumor	Viral hepatitis C	yes	no	no
6.	30	F	urban	Diffuse non-Hodgkin's lymphoma	-	no	Vancomyicn Metronidazole	3
7.	73	F	rural	Diffuse non-Hodgkin's lymphoma	Anemia Osteoporosis AHT	no	Vancomycin Metronidazole	3
8	78	F	urban	Malignant ovarian tumor	-	yes	Cefotaxime Cytostatics	3
9.	67	M	urban	Malignant rectal tumor	AHT Nephropathy	yes	Cefotaxime	3

DISCUSSION

C. difficile - associated diseases cause polymorphic clinical manifestations dominated by diarrhea of varying severity and possible severe course in the case of pseudo membranous enterocolitis or toxic mega colon.

Both isolated cases, as well as those involved in nosocomial outbreaks have as characteristic feature the association of antibiotic and immunocompromised status.

The use of the classes of antibiotics, including penicillins, cephalosporins, macrolides, aminoglycosides, and lincosamides, can be associated with the induction of human *C. difficile* infections.

The onset of *C. difficile* infection is associated with the use of antibiotics that disrupt the equilibrium of intestinal flora. The virulence of the pathogenic agent strains is dependent, in principle, on the production of two enteric cytotoxins (Ted A and Ted B), and the outbreaks of *C. difficile* infection, particularly those with

severe symptoms, are related to the presence of ribotype O_{27}/NAP_1 (1, 2, 5, 6, 9).

The clinico-epidemiological diagnosis may benefit from laboratory investigations, which include detection of toxins in the stool samples of patients with suspected *C. difficile* infection.

The use of culture media for the isolation of this pathogen is not recommended as approximately 2-5% of the general adult population may be intestinal carriers.

Cell cultures are exposed to stool filtrate for 24-48 and a specific cytopathic effect is observed followed by a confirmation test with neutralizing antibodies, the results being available in 48-72 hours.

Rapid and highly specific results for both *C. difficile* toxins can be obtained using some variants of the immunoenzymatic test (12, 13, 14).

C. difficile selective agar is a medium that allows the isolation of *C. difficile* strains from stool samples, and colony characteristics allow the differentiation from other clostridia possible causes of antibiotic-associated diarrhea.

As to the cultures grown on blood agar media the colonies can be easily identified by the odor due to the production of cresol 12, 13, 16).

Roberts et al. (17) and Byrne et al. (18) analyzing the results of clinico-epidemiological researches on the incidence of *C. difficile* infections in various geographical areas have concluded that due to the emerging trend, the medical and socioeconomic implications these infections are a major population health issue.

Redelings et al. (11) reported that in the USA, in 2005-2006, hospitalizations for *C. difficile* infections increased by 117% with a corresponding increase in hospital costs and death compared to a decade earlier. Wysowski (15) showed that in the USA *C. difficile*-related mortality rate increased by 35%, from 5.7 per million population in 1999 to 23.7 per million population in 2004, the same being true for England.

Since 1990, the first-line therapy of non-severe *C. difficile* infections is metronidazole while vancomycin is recommended in severe infections, given the fact that there is a relatively low level of persisting spores at risk to germinate after reducing the concentration of antibiotics (5, 6).

Onderdonk and Garrett (5), and Vonberg (10) found that the spread of *C. difficile NI* can be controlled by consistently applied common hygiene-sanitary and epidemiological measures, directed to patients, suspects, health care workers and ambient environment. It is envisaged that *C. difficile* spores can live for long periods on surfaces, objects, food, and can be spread through aerosols especially in rural hospital without being destroyed by the usual methods of decontamination, which are effective only for the strains in vegetative form.

CONCLUSIONS

The nosocomial infection outbreak presented in this study although occurring in a group of immunocompromised patients and thus high susceptibility to infection, common situation in an oncology unit was brought under control by active preventive measures including daily screening, patient isolation, disinfection chemicals, including sporicidal agents, and the standard precautions of the health care professionals and support staff. Concomitantly, the antimicrobial therapy effective in *Clostridium difficile* infections was initiated, monitored by laboratory tests and customized in patients with certain co morbidities.

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STATISTICAL STUDY ON THE INCIDENCE OF ALLERGIC DISEASES TREATED WITH DESLORATADINE AND LEVOCETIRIZINE AT "ATOPIA" ALLERGOLOGY MEDICAL CENTER, IAŞI, ROMANIA

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STATISTICAL STUDY ON THE INCIDENCE OF ALLERGIC DISEASES TREATED WITH DESLORATADINE AND LEVOCETIRIZINE AT "ATOPIA" ALLERGOLOGY MEDICAL CENTER, IAŞI, ROMANIA (Abstract): Allergies and allergic manifestations are a major health problem in our country and around the world. Epidemiological data have reported an increased incidence of allergic diseases up to 10-30% over the past decades. **Aim:** To evaluate the incidence of the most common allergic diseases treated with desloratadine or levocetirizine, second-generation antihistamines. **Material and methods:** For diagnosis and assessment of treatment with the two antihistamines the following tests were performed: allergy skin testing, immunoglobulin E blood levels, evaluation of target organ for allergic reaction (lung function tests, nasal and conjunctival examination, assessment of bronchial inflammation). **Results:** We conducted a retrospective study that included 365 patients (64% women and 36% men) diagnosed with different types of allergy in the *"Atopia" Allergology Medical Center* at Iaşi during the interval August 2012 – August 2013. Patients were aged 2 to 90 years (mean age group 35.98 years; 39.55 years for women, and 29.74 years for men). **Conclusions:** The majority of patients were from urban areas (82%). The most common allergic diseases in the study group were: urticaria (21%), allergic rhinoconjunctivitis (14%), allergic bronchitis (14%) and allergic asthma (12%). **Keywords:** DESLORATADINE, LEVOCETIRIZINE, URTICARIA, ALLERGIC ASTHMA, RHINOCONJUNCTIVITIS

According to the latest statistics, today, one of four people is affected by an allergic disease (1). Allergy is the immune response to common elements in the environment, which usually do not cause any reaction. An allergic reaction is triggered by substances called allergens and they are frequently represented by pollens, molds, dust mites, pet dander, specific foods, and chemicals (isocyanates, anhydrides, formaldehyde, some metals - chromium, nickel) (2).

Hereditary predisposition is a major risk factor; if one parent is allergic, the estimated risk of the child to be allergic is 48% and increases to 70% if both parents are allergic.

A growing number of studies have shown that allergies are becoming more complex and persistent, patients being now sensitized to more and more trigger factors, facing increasingly severe and persistent

symptoms.

Despite great progress achieved in the last 10 years in the research of treatment and causes regarding allergic disorders, there are still many issues to be solved towards achieving the objectives of more effective therapies, even a possible prevention (3, 4).

Desloratadine and levocetirizine, the most commonly prescribed antihistamines in the world nowadays, have a better efficiency and safety compared with first-generation antihistamines and a long latency period.

Desloratadine and levocetirizine are very efficient in the treatment of allergic diseases, including seasonal allergies, such as perennial allergic rhinitis, urticaria, and allergic conjunctivitis.

Although they are among the most frequently prescribed drugs, second-generation antihistamines are not without side effects. The most important potential side effects include effects on the nervous and cardiovascular system and drug interactions (5).

MATERIAL AND METHODS

We conducted a clinical and pharmacotoxicological retrospective study on a series of 365 patients diagnosed and treated at the Iasi "Atopia" Allergology Medical Center during a one-year interval (2012-2013). The diagnosis of allergic disorder was made on history and some allergy tests: allergy skin testing, immunoglobulin E blood levels, lung function tests, nasal and conjunctival examination, assessment of bronchial inflammation.

Patients diagnosed with allergic disorders received either desloratadine or levocetirizine alone, or association of these two along with other drugs such as antileukotriene agents, steroids (inhaled or topical), immunomodulatory agents.

Of the study patients, 64% were females and 36% males (fig. 1). Patients came from urban areas (82%) and rural areas (18%) (fig. 2). Patients were aged between 2 years and 90 years; the average age was 35.98 years (39.55 years for women, and for men 29.74 years) (fig. 3).

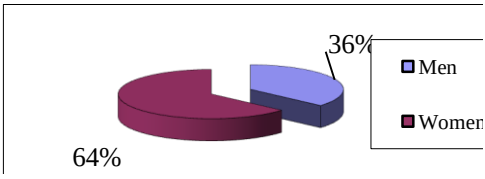

Fig. 1. Gender distribution of patients

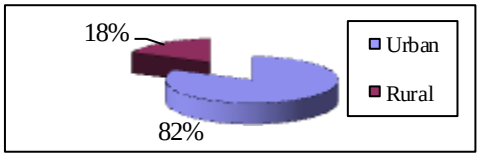

Fig. 2. Environmental distribution of patients

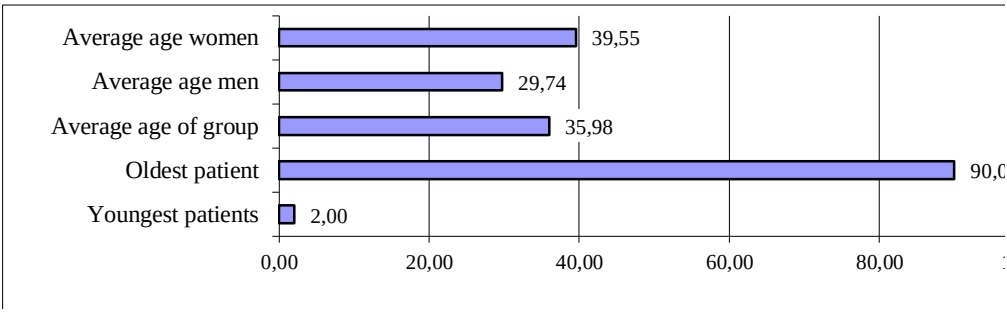

Fig. 3. Age distribution of study patients

From allergy observation sheets we collected data regarding the incidence of allergic clinical manifestations of patients treated with desloratadine or levocetirizine, and data on side effects caused by these drugs to the study patients.

Data were statistically analyzed using *MS Excel* software, interpreted, and corroborated with references in the literature.

RESULTS AND DISCUSSION

Distribution of allergic diseases in the studied group is presented in figure 4.

We mention that each patient presented one to three allergic diseases and the total number of diseases was 756, corresponding to an average of 2.07 diseases / patient.

The most common allergic diseases in the study group were: urticaria, allergic bronchial asthma, allergic bronchitis, allergic rhino conjunctivitis and other allergic manifestations, such as: allergic rhinitis, allergic dermatitis, angioedema, allergic conjunctivitis and allergic laryngitis.

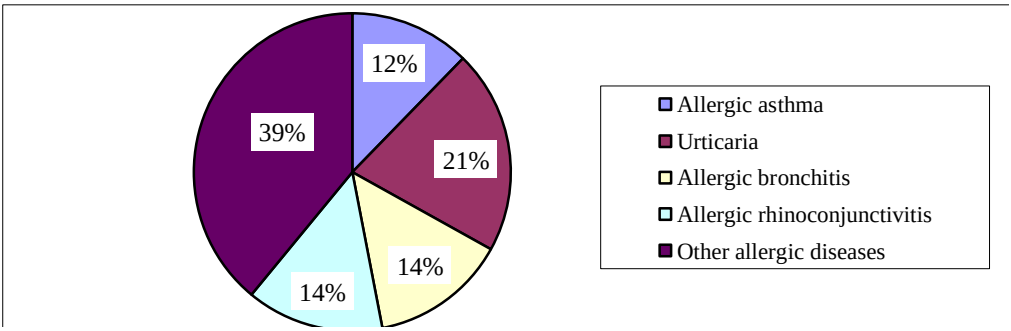

Fig. 4. Distribution of allergic diseases in the study group

In table I and figure 5 are shown the demographic data of patients (gender, average age and environment), as well as the incidence of major diseases.

Analysis of allergic diseases in the studied series showed predominance of urticaria (67.52%) and allergic rhino conjunctivitis (66.04%) in women, and allergic asthma (41.94%) and allergic bronchitis (35.24%) in male.

TABLE I

The structure of the study group

Allergic diseases	Gender		Average age (years)	Environment		Total diagnoses
	M (%)	F (%)		R (%)	U (%)	
Allergic asthma	41.94	58.06	35.34	17.20	82.80	93
Urticaria	32.48	67.52	36.80	19.75	80.25	157
Allergic bronchitis	35.24	64.76	36.22	22.86	77.14	105
Allergic rhino conjunctivitis	33.96	66.04	34.40	18.87	81.13	106
Other allergic manifestations	36.61	63.39	34.67	18.64	81.36	295

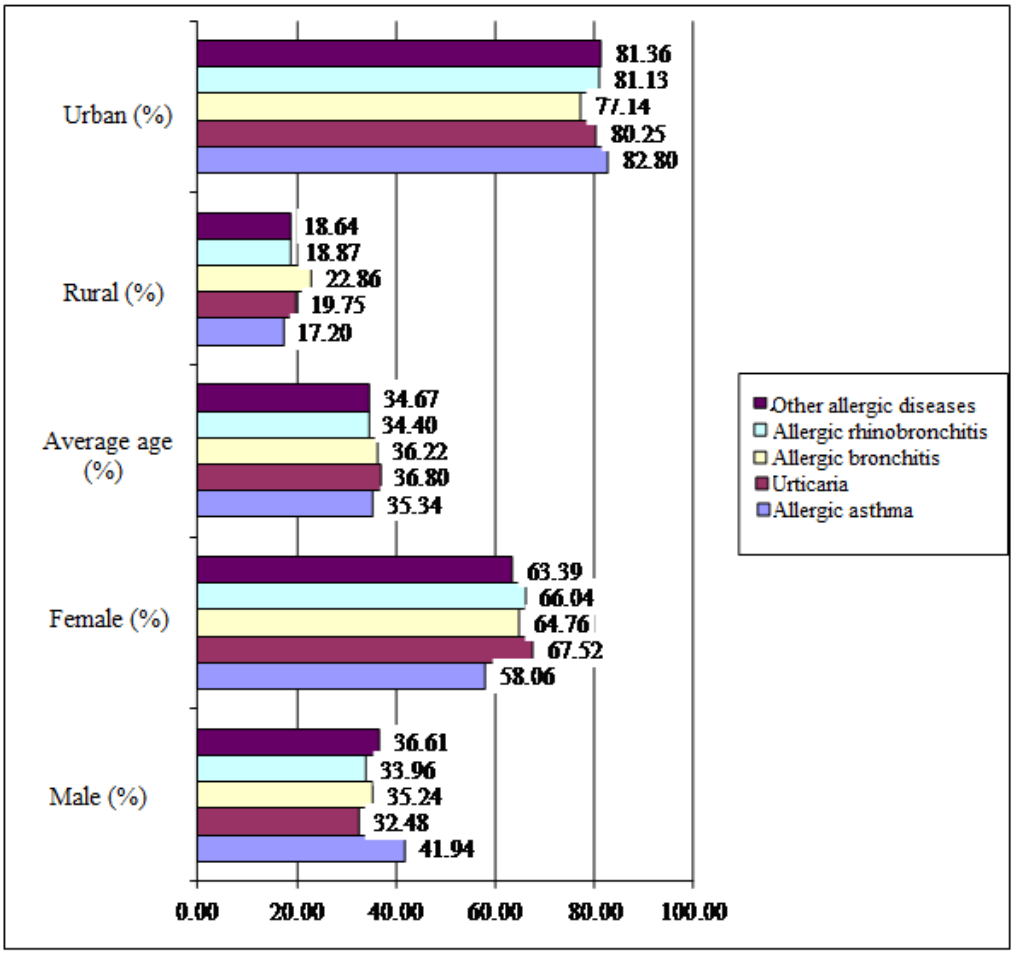

Fig. 5. Demographic data of the groups of allergic patients included in the study

The study group included a two-year-old patient with urticaria by sensitization to anti-inflammatory syrup (ibuprofen), and a 90-year-old patient with chronic urticaria by sensitization to antibiotics.

Table II presents the number of atopic cases and cases with predominant allergic diseases in the study group, respiratory and cutaneous type. Figures 6 and 7 show that 8% of the cases were atopic and 41% had skin allergies, this type of allergy being the most common in the atopic patients. Associated pathologies were present in 21.4% of the patients.

Therefore, 8% of the patients had hereditary predisposition to develop allergy, a much lower percentage than in the literature (6, 7).

The most common allergens involved in sensitization of the patients, responsible for producing allergic manifestations, were: mites, house dust, pollen, mold, animal dander, food (especially fish); these allergenic agents can be found in similar studies (7). Overall, the prevalence of sensitization to indoor allergens was increased in patients

with cutaneous manifestations, while the prevalence of sensitization to outdoor allergens was linked to respiratory manifestations. The presence of concomitant polysensitization to multiple allergens was frequent in patients with cutaneous disorders. Also, patients who simultaneously show sensitization to more than one allergen manifest a greater allergic poly symptomatology than those with mono sensitization.

TABLE II

Atopy, allergic diseases and associated pathology of patients

Indicator	Number of cases	Percentage distribution (%)
Family history		
Cases with no atopy	8	2
Cases with atopy	29	8
Undefined cases	328	90
Total	**365**	**100**
Allergic diseases		
Allergic asthma	86	24
Urticaria	150	41
Other allergic diseases	129	35
Total	**365**	**100**
Associated pathology		
Cases with associated pathology	78	21.4
Cases with no associated pathology	287	78.6
Total	**365**	**100**

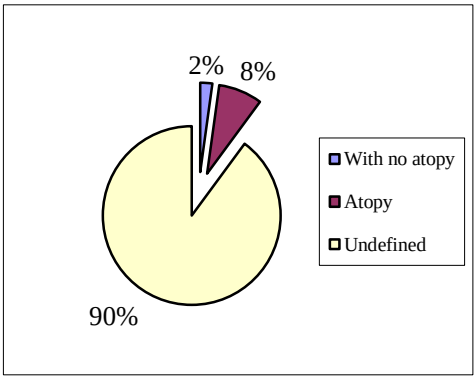

Fig. 6. Distribution of cases according to atopic structure

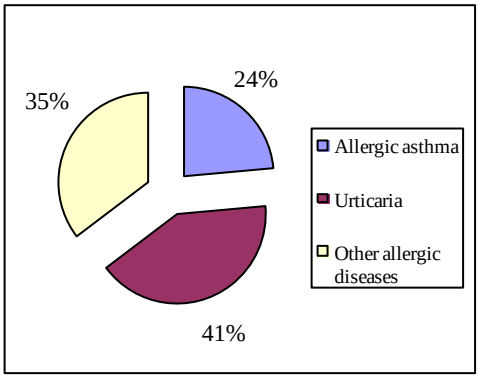

Fig. 7. Incidence of allergic diseases

We studied the incidence of the most significant side effects noted in specialized studies (8, 9).

Of all patients (365) treated with desloratadine or levocetirizine (5 mg/day), 2.74% experienced side effects (xerostomia, headache, fatigue, drowsiness, exhaustion and myalgias). At 61 years-old female patient, from an urban area, diagnosed with bronchitis and allergic rhino conjunctivitis, treated with desloratadine (5mg/day), the following side effects were recorded: asthenia, headache, myalgias.

Of the two study substances levocetirizine has greater potency, as confirmed by *in vivo* studies, in humans.

Levocetirizine may cause drowsiness in sensitive individuals.

Although desloratadine is less efficient, it has the advantage of a longer half life. Its most important side effect is drowsiness.

Desloratadine has a safety profile similar to levocetirizine, does not cross the blood-brain barrier, and has a very low sedation rate, which gives increased safety and tolerability (10). According to the literature, desloratadine and levocetirizine are "non-sedating" antihistamines, but the best would be to describe them as having minimal sedative effects, at therapeutic doses (11, 12).

The efficacy and safety of second-generation antihistamines, supported in the literature, is also confirmed by our study by the diminution of allergic symptoms and decreased incidence of treatment-emergent adverse events in our study patients.

CONCLUSIONS

We conducted a statistical study (August 2012 - August 2013) on the incidence of allergic diseases in 365 patients treated with desloratadine or levocetirizine in the *"Atopia" Allergology Medical Center* at Iaşi. We followed the adverse drug reactions during treatment.

Data were collected from allergy observation sheets and statistical analysis was performed using *MS Excel* software.

In the study group (365 patients), the incidence of allergic diseases was: urticaria (21%), allergic bronchitis (14%), allergic rhino conjunctivitis (14%), allergic asthma (12%), and other allergic manifestations (19%).

Of all the patients treated with desloratadine or levocetirizine (5 mg/day), 2.74% experienced side effects: xerostomia, headache, fatigue, drowsiness, and muscle aches.

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NEWS

CAESAREAN SECTION INCREASED RISK OF ALLERGIES

Intestinal microbiota stimulates the development and maturation of the immune system early in life. At birth the child's gut is colonized by bacteria from the mother's vaginal microbiota, included *Bacteroidetes* phylum which is linked to protection against allergies. The caesarean section (CS) reduces microbial exposure and the immune system reacts excessively against innocuous antigens in its surrounding. Using DNA sequencing and computer analysis methods Jakobsson and colleges showed that infants born through CS were less colonized with the *Bacteroidetes* phylum, and have lower blood levels of the Th1-associated chemokines for inhibit allergic immune responses. The researchers concluded that CS induced a "lower total microbial diversity, delayed colonization of the Bacteroidetes phylum and reduced Th1 responses". These results explain the higher frequency of allergies in babies born by cesarean section than in those born vaginally (Jakobsson HE, Abrahamsson TR, Jenmalm MC, Harris K, Quince C, Jernberg C et al. Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by Caesarean section. *Gut*, 2013; DOI: 10.1136/gutjnl-2012-303249).

Cătălina Luca

ASSESSMENT OF NUTRITIONAL HABITS ON A GROUP OF PUPILS FROM A COUNTRYSIDE ELEMENTARY SCHOOL IN ROMANIA

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ASSESSMENT OF NUTRITIONAL HABITS ON A GROUP OF PUPILS FROM A COUNTRYSIDE ELEMENTARY SCHOOL IN ROMANIA (Abstract): Food is a major contributor to healthy children and youth. The study of this factor must be complex, considering the dietary habits of the population. **Material and methods**: 65 pupils from rural area were surveyed weekly about eating chicken, fish, fruits, bread and cereal by-products. A special attention was given to daily consumption of fruits (to those who recognize this contribution). **Results and discussion**: chicken appears in menus mostly 2-3 times a week (40.0%) or 4-7 times (27.7%). Fish is particularly present in menus one time (47.6%) or 2-3 times (26.2%). Fruits are provided in 75.4% cases 4-7 times a week. The dominant consumption of bread is of 4-7 times (90.8%) and of cereal by-products is of 2-3 times (58.5%) or even of 4-7 times (27.7%) a week. Self body weight is considered to be appropriate by 63.1% teenagers and in 23.1% cases they considered their body weight is too high. **Conclusions**: The implementation of national dietary programs can change the nutritional habits of children if they are based on a real assessment, achieved only with the help of surveys conducted by questionnaire method. **Keywords**: CONSUMPTION FREQUENCY QUESTIONNAIRE, INTAKE OF CHICKEN, FISH, FRUITS, BREAD, CEREAL BY-PRODUCTS.

Food is an external factor that plays an essential role in maintaining the health of children and youth. Currently, there are many issues related to diet, due to the current difficult economic situation in most families, especially in those from rural areas. Economic problems are difficult in rural areas where the income is assured only by household items. Products obtained must assure the family needs and also designated for sale, to obtain funds used to ensure future crops. In this context the study of rural youth nutrition is very important (1, 2).

The study is particularly important, as today there are more questions about national dietary programs, intended to change the nutritional habits of the population. For example it is ongoing a program that gives fruit to the pupils in the school. We must know the exact situation because providing fruits to a population that already consumes fruits is a useless action.

MATERIAL AND METHODS

The investigation was conducted on a

group of 65 pupils who live in rural areas and learn at the School grades I-VIII of Băceşti countryside area, Vaslui County. Sex distribution of cases is relatively balanced, as assessed 36 boys (55.4%) and 29 girls (44.6%). To these young people was applied frequency questionnaire of food consumption. (3.4) is a weekly frequency questionnaire contributions (the fruit is estimated daily consumption) questionnaire was completed by pupils in the study group. We insist on weekly consumption of chicken, fish and seafood, fruits, bread and cereal by-products.

Statistical processing of results was done with Pearson Chi-Square test.

RESULTS AND DISCUSSION

The study was conducted on a sample of 65 pupils in eighth grade, aged between 13 and 14 years. We insisted on the frequency of eating chicken, fish, fruits, bread and cereal by-products in their menus.

1. Chicken meat is specific to rural area as baby grows quickly, so the presence of this meat in meals is not a problem. We must insist on this point because buying food in rural areas may be disrupted by lack of money. Population eats mostly products obtained in their household. Dominant contribution is 2-3 times per week (40.0%), followed by 4-7 times (27.7%). 18.5% of all pupils consume chicken meat only one time a week, while other 13.8% pupils do not consume at all (Fig. 1).

The situation is serious in 30% of families where the chicken is missing or is a very rare presence. Hygiene specialist and GP should know these issues to try to remedy the situation.

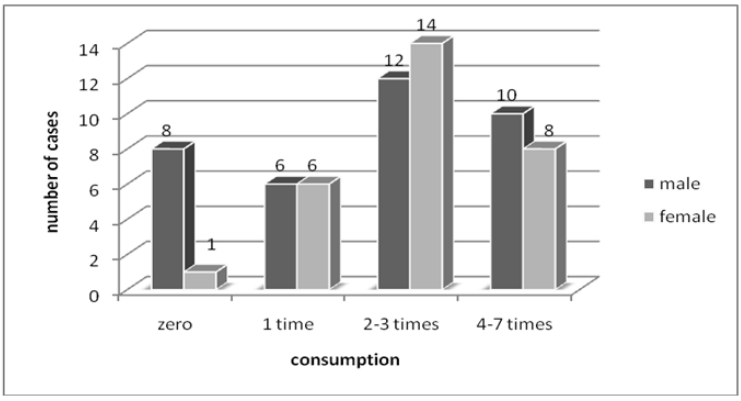

Fig. 1. Weekly intake of chicken meat at studied pupils

It is important to make assessments also on gender. At males, there are 22.0% pupils who do not eat chicken, while at females only 2.7%. Dominant contribution is 2-3 times both in boys (33.4%) and girls (48.8%). Pearson Chi-Square shows statistically significant differences ($\chi^2 = 5.126$, df = 3, p = 0.163) that directs to a traditional chicken diet.

These aspects are very important because any attempt to change the diet is likely to fail, if we start from the reality represented by a given population anchoring in tradition. Advices from television like "drink to your health daily…" have little chance of success.

2. Fish consumption is not a great option for our questioned pupils. It is not consumed especially in rural areas, where is not purchased. Throughout the group, the dominant contribution is one time (47.6%) or 2-3 times (26.2%). There are only 3.1% of families consuming fish 4-7 times, and 23.1% of families who do not eat at all (Fig. 2).

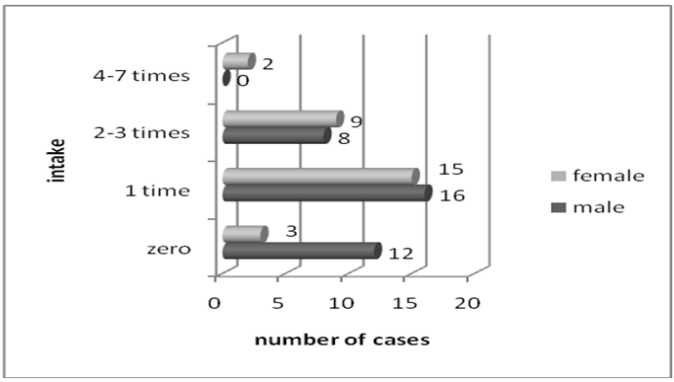

Fig. 2. The frequency of fish in the meals of studied pupils

At present menus the boys eat fish 2-3 times in 44.7% cases and 51.7% girls. In these situations there are no statistically significant differences ($\chi^2 = 6.816$, df = 3, p = 0.078).

3. Fruits are consumed fresh to give to the body an adequate intake of vitamin C. You should pay particular attention to the results due to the insistence of national programs to achieve this. A population consuming fruits in large quantities does not require supplementation by such programs. People who do not need supplements can consume, but they should be carefully provided.

We have found that 75.4% from all consumers eat fruits 4-7 times per week and other 23.07% consume fruits only 2-3 times (Fig. 3).

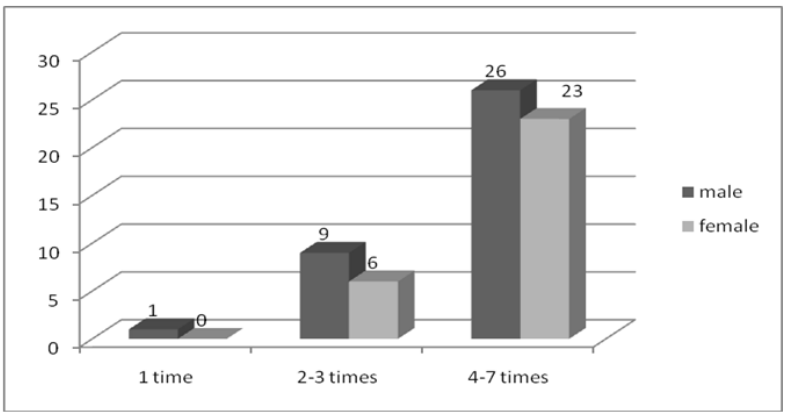

Fig. 3. The frequency of fruits in the meals of studied pupils

Basically surveyed pupils consume sufficient fruits; the intake was mostly 4-7 times a week. To this group is not necessary to make a further contribution. Food corrections are necessary, but not to fruits. To give fruits to these children is a futile gesture that has no educational value. Being tired of fruits that are offered, pupils become irritated, without a positive result.

In fact, you expect to get positive results from a large population which is consuming fruits. National programs should be oriented to feature food consumption of the population and not the opinion of one or another who does not know the reality and does not want to know.

On gender, the study is less important because boys are eating 4-7 times a week appear in 72.3% cases and in 76.3% cases for girls. The statistically significant differences obtained between genders ($\chi^2 = 1.042$, df= 3, p = 0.594) are logical and expected.

Fruits consumption is normal in rural areas where in every yard there are at least 2-3 trees. Keeping them for winter is not a problem as apples, pears, quinces are less perishable. Providing to these children fruits is a serious tactical mistake.

We must study here daily frequency of fruit consumed where 40 young people (61.5%) stated the presence of fresh fruit in daily menus (tab. I).

TABLE I

Daily intake of fruits

Daily consumption	One time	Two times	Three times	Four times	Many times
Male	0	5	5	2	8
Female	3	3	6	1	7
Total number	3	8	11	3	15
Percentage (%)	7.6	20.1	27.6	7.1	37.6

A fruit presence in the menu 1-2 times a day is not a problem. 7.6% pupils observe a contribution of one time and 20.1% observe an input of 2 times. If you eat fruits every day, one or two fruits are sufficient to provide the necessary nutrients. From an intake to 3 times per day and higher start too high values. We remark that 7.1% pupils are eating fruits 4 times a day and 37.6% who eat them several times a day. Practically 40% of surveyed pupils are eating fruits daily.

Fruits are needed by the body only in recommended quantities of rational consumption norms. They are low in protein and fat, have significant amounts of low molecular weight carbohydrates, but are important sources of vitamins and minerals. Their caloric intake is less than 100 kcal per 100 g, so it is very small (5, 6).

Providing these products in an excessive amount does not have a beneficial effect on consumers' health. Mass media programs provide only partial information on the role of vegetables and fruit in the diet without disadvantages offered and insisting on excessive consumption. Professionals must properly inform consumers about the importance of each product, with its nutritional value. Any such exaggeration is related to fruits has a negative impact over population. We do not need national programs offering fruits to a population which already consume them in large quantities.

Money allocated could be spent more effectively if it is done an accurate and extensive food survey.

4. Bread and cereal by-products. A balanced diet must include animal and vegetable products. Bread and cereal by-products are an important source of vegetable proteins and carbohydrates, giving the body an increased calorie intake. On the entire group, the dominant bread consumption is of 4-7 times (90.8%). There are 1.5% responses of absent or one time consumption. Otherwise bread is consumed very frequently. It is a traditional consumption for our population, especially for the wheat one which is widely grown in Moldavia (Fig. 4).

Of course it is also important the distribution of responses according to the pupils' gender. The consumption of 4-7 times appears in 91.6% cases at males, and in 89,7% cases at females, the calculated differences being statistically insignificant (χ^2 = 2.101, df = 3, p = 0.552). On the study group the consumption of bread doesn't raise special problems, being well represented.

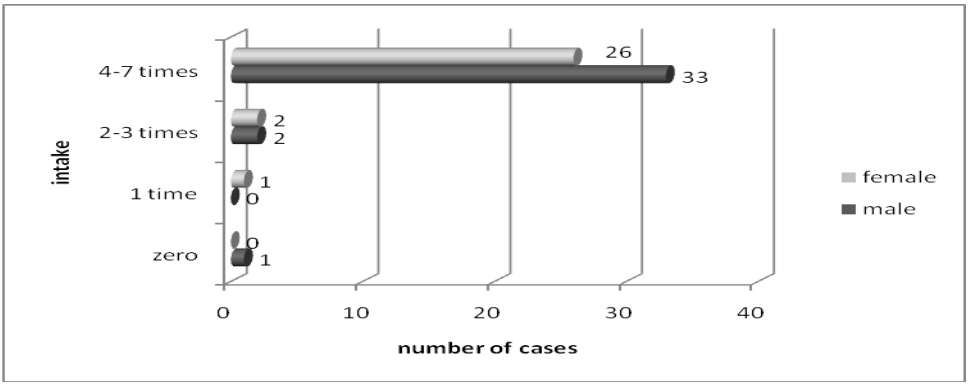

Fig. 4. Presence of bread in the questioned pupils' menus

The consumption of cereal by-products is a new issue, with no relation to tradition. The elders didn't usually consume rice, pasta or croup. These products have evolved simultaneously with the industrialization of nourishment. The dominant consumption of cereal by-products is of 2-3 times (58.5%) or even of 4-7 times (27.7%). There are also 10.8% responses of one time and even 3.1% negative responses. The small or absent consumption is not a major problem because this is food that provides mostly a quantitative intake, and not a qualitative one. The balanced intake of 2-3 times a week appears both at boys and girls, Pearson Chi-Square showing no statistically significant differences (χ^2 = 1.086, df = 3, p = 0.780). Even with these new products introduced in the menus the nourishment appears similar.

5. Assessment of body weight

To approach this aspect we will first discuss children's view regarding the role of nourishment in maintaining their health. Proper nourishment helps maintaining health – it is an assertion sustained by 81.5% pupils. There draw attention a percentage of 14.5% of negative responses of some pupils who can't make the connection between balanced nourishment and ensuring a normal growth and development of the body.

Body weight is closely connected to the quantity and quality of food given to teen-

agers. Their opinion about their own body weight is very important because it can change some food habits. Children who think they are too fat can start worrying about this aspect and try to lose weight (Fig. 5).

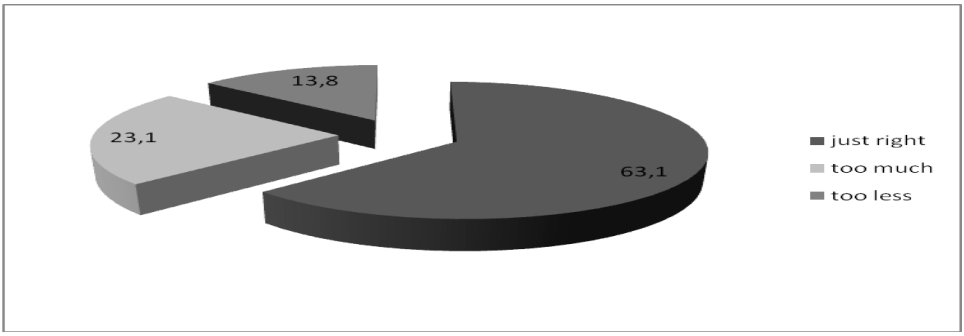

Fig. 5. Pupils' opinion about their own body weight

On the entire group, appear only 63.1% responses of just right weight, which is quite low for 13-14 years old pupils. Too much weight is stated in 23.1% cases which should be objectified by weighing.

On gender, too much weight is stated by 22.7% boys and 24.1% girls, and too less by 8.3% boys and 20.7% girls. The calculated differences are statistically insignificant ($\chi^2 = 2.281$, df =2, $p>0.05$) and reveals a similar perception of self body weight.

The study is very important because it provides an overview of eating habits of pupils in this group and allows a close observation of the mistakes that appear. The noticed mistakes are not those stated on TV, related to an insufficient fruit intake. Food changing programs must be carefully made, after a thorough study about the actual eating habits of the target population. On a population so rooted in traditions as the one in the study group, any attempt of forcing consumption in a certain direction has little chance of success.

CONCLUSIONS

Chicken is present especially 2-3 times (40.0%) in the menus, followed by 4-7 times in consumer cases (27.7%) and also there are cases of absence (13.8%).

Fish is a food with a great nutritional value, but is not widely used in our country. In most of the cases (47.6%) it is on the table only one time a week or 2-3 times (26.2%) a week.

Fruits appear to be consumed in this group by 75.4% of pupils for 4-7 times a week, and by 23.07%, 2-3 times a week. Particularly should be noted the existence of non-response to zero and only 1.5% of responses for one time.

The dominant consumption of bread is of 4-7 times (90.8%) and of cereal by-products is of 2-3 times (58.5%) or even of 4-7 times (27.7%) a week.

Self body weight is considered to be appropriate by 63.1% teenagers. There are 23.1% cases in which body weight is considered too high, but these teenagers should be weighted to get the objectivity of the statement.

Any national dietary program geared towards changing nutritional habits of a population group should be based on the actual assessment of targeted populations. Otherwise the results are less encouraging.

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NEWS

POLYSPECIES BIOFILM FORMATION ON IMPLANT SURFACES WITH DIFFERENT SURFACE CHARACTERISTICS

Rough implant, while being beneficial for initial bone formation and osseointegration, have been generally considered to enhance initial adhesion and the subsequent colonization of oral bacteria. The aim of this study was to investigate the microbial adherence and colonization of a polyspecies biofilm on 7 differently processed titanium surfaces. Six-species biofilms were formed anaerobically on 5-mm-diameter sterilized, saliva-preconditioned titanium discs. Material surfaces used were either machined, stained, acid-etched or sandblasted/acid-etched. Samples of the latter two materials were also provided in a chemically modified form, with increased wettability characteristics. Surface roughness and contact angles of all materials were determined. The discs were then incubated anaerobically for up to 16.5 h. Initial microbial adherence was evaluated after 20 min incubation and further colonization after 2, 4, 8, and 16.5 h using non-selective and selective culture techniques. Results at different time points were compared using ANOVA and Scheffé post hoc analysis. The mean differences in microorganisms colonizing after the first 20 min were in a very narrow range (4.5 to 4.8 log CFU). At up to 16.5 h, the modified sandblasted/acid-etched surface exhibited the highest values for colonization (6.9±0.2 log CFU, $p<0.05$) but increasing growth was observed on all test surfaces over time. Discrepancies among bacterial strains on the differently crafted titanium surfaces were very similar to those described for total log CFU. Fusobacterium nucleatum was below the detection limit on all surfaces after 4 h. Within the limitations of this in vitro study, surface roughness had a moderate influence on biofilm formation, while wettability did not seem to influence biofilm formation under the experimental conditions described. The modified sandblasted/acid-etched surface showed the highest trend for bacterial colonization. From a clinical point of view related to hygienic requirements, the choice of implant surface characteristics may be salient to the long-term health of any implant placed [Schmidlin PR, Müller P, Attin T, Wieland M, Hofer D, Guggenheim B. Polyspecies biofilm formation on implant surfaces with different surface characteristics. (*J Appl Oral Sci* 2013; 21(1): 48-55).

Gianina Iovan

THE SEROPREVALENCE OF HEPATITIS C VIRUS INFECTION AMONG BLOOD DONORS FROM THE NORTH WEST REGION OF ROMANIA

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THE SEROPREVALENCE OF HEPATITIS C VIRUS INFECTION AMONG BLOOD DONORS IN THE NORTH WEST REGION OF ROMANIA (Abstract): The infection with hepatitis C virus (HCV) known also as a blood-borne infection can be life-threatening by delayed consequences of persistent infection. This study aimed to estimate the hepatitis C infection prevalence, its epidemiological mode of manifestation and its recent evolution among blood donors from North West region of Romania. We made a chronological study using the HCV seropositive confirmed results to serological blood donors screening, provided by two Blood Transfusion Centres, chosen for their quality of provided data: Cluj and Satu Mare. The statistics were descriptive and the time series modelling used the polynomial regression. In both counties, about three fourth of donors were male and in Satu Mare, female donors (29.2%; $p<0.001$) have been significantly more represented than in Cluj (24.6%). Hepatitis C virus seroprevalence was significantly ($p<0.01$) higher in Satu Mare ($279.8/10^5$ donors, CI: $241.7\text{-}317.9/10^5$ donors) than in Cluj ($212.6/10^5$ donors, CI: $187.4\text{-}237.8/10^5$ donors). The mode of manifestation was endemic in different patterns, one with wave and a stable one with a particular high value of $1.091.1/10^5$ female donors (CI: $674\text{-}1508.2/10^5$ donors) seroprevalence, in 2008, in Satu Mare. The variation of the prevalence value was higher for women and particularly (coefficient of variation: 91.9%) for those from Satu Mare. The hepatitis C virus seroprevalence among blood donors reflected a stable, endemic manifestation with particular variation and high transmission in female populational subgroups. **Keywords**: HEPATITIS C VIRUS, BLOOD DONORS SEROPREVALENCE, TIME SERIES.

Hepatitis C virus (HCV) infection is a serious public health concern because of the high risk for persistent infection (75-85% of infections) and delayed consequences as chronic hepatitis, cirrhosis and hepatocellular carcinoma (1, 2).

In the epidemiology of hepatitis C infection there are many differences between countries (even within the same country) related to risk factors, health care practices and socioeconomic issues. In the countries of Eastern Europe and also in Romania, there were many changes in population condition of life, habits, movements, socioeconomic situation and as well in the reforms of healthcare system. These factors

may significantly influence the populational manifestation of hepatitis C and in consequence, the selection of donors.

In developed countries, blood transfusion or plasma-derived products used to be the main route of HCV transmission, but after the systematic selection and screening of donors, the residual risk of transfusion-transmitted HCV, now is very low (3, 4).

In Romania, EU Directives and WHO recommendations for tissues and cells donations were adopted in the legal documents and are applied, except blood donors, which receive compensation (three days free time, tickets for bus and restaurant) for each donation. Blood donation and transfusion safety are centralized, in the Ministry of Health coordination and administration trough the National Institute of Blood Transfusion (NIBT). Across the country, there is one Blood Transfusion Centres (BTC) in each county, which use the same regulation and the quality assessment, particularly for screening of the transfusion transmissible infections (5). The donors are selected from the population aged between 18 and 62 years, documented as being free of chronic diseases, disabilities, acute infections or the belonging to some risk groups, in order to select individuals with low probability of the presence of HCV infection. Since 1995, the routine serological screening for HCV infection has been introduced in all Romanian BTCs.

To our knowledge, the HCV seroprevalence among blood donors in Romania has not been previously studied. Only few papers related to patients or specific high or low risk groups for the HCV infection, in some Romanian areas, have been published (6, 7, 8).

The aim of this study was to estimate the HCV infection prevalence, the mode of epidemiological manifestation and its recent evolution among blood donor population of the North West region of Romania. This study is part of a larger objective that we have established in our activity, which aims to identify and use objective sources of data in order to accurately describe the epidemiology of C hepatitis in our region.

MATERIAL AND METHODS

To estimate the HCV infection prevalence and its populational manifestation among blood donors we have made a chronological (longitudinal ecologic) study, including the confirmed positive results to the routine serological screening tests, for HCV antibodies. A confirmed positive result was considered the donor with positive results to all serological tests made in the local laboratory of BTC and in the National references laboratory for transfusion transmitted virus (NRLTTV) of the NIBT. The usual serological screening consisted in two simultaneous enzyme-linked immunosorbent assays (ELISA) tests, made in local laboratory of BTC and for the positive results, other different two ELISA tests and a Western Blot test, made at NRLTTV. For the indeterminate results, all tests were repeated from a new blood sample, at 3-6 weeks interval from the first determination.

Between departmental BTCs of the North West region of Romania, we have chosen two collaborative centres in our academic training: Cluj and Satu Mare, known for a good quality of provided data. Data source were the laboratory registers of the two selected BTCs, where were recorded the results of tests made in both BTC

laboratory and NRLTTV. Were collected summed anonymous data about the year of donation, gender and positive confirmed screening results for the presence of HCV antibodies. Data validation was made by the person in charge with the management of the laboratory register.

Collected data were recorded in an Excel worksheet, Office for Windows 2007 and the statistics were performed in the same software. The descriptive statistics was made by using proportions, confidence intervals, z test for statistical significance (comparison of proportions), the chi-square significance test for trend, the variance coefficients and the modelling for chronological analysis used the polynomial regression (9).

RESULTS

The period for which were found confirmed and valid results, for both Cluj and Satu Mare counties, was 2003 to 2010. In the 8 years period, in Cluj BTC were laboratory screened for HCV antibodies, 128,398 persons, with a yearly average of 16,050 persons and a variation from 15,177 to 17,365 donors (tab. I). In Satu Mare BTC were screened 73,969 persons, among which in average were about 9,264 person per year, and a variation from 7,487 to 10,808 persons in each year. All screened donors were aged between 18 and 62 years, the legal age for blood donation.

In both counties, about three fourths (75.4% for Cluj, 70.8% for Satu Mare) of donors were male (tab. I). For the entire period, the proportion of women donors was significantly higher ($p<0.001$) in Satu Mare County (29.2%) than in Cluj County (24.6%) and for each year it was the same situation, except the year 2008 and 2009.

From 2003 to 2010, among Cluj County donors, 273 ($212.6/10^5$ donors, confidence intervals CI: $187.4\text{-}237.8/10^5$ donors) were found positives to HCV antibodies and for Satu Mare County donors, 207 ($279.8/10^5$ donors, CI: $241.7\text{-}317.9/10^5$ donors) were positives, the prevalence being significantly ($p<0.01$) different in the two geographical areas.

In Cluj County, according to gender, the frequency of seropositive female donors, varied between the highest value of $375.7/10^5$ donors (CI: $192\text{-}559.4/10^5$ donors) in 2004 to the lowest of $72.5/10^5$ donors (CI: $9.5\text{-}154.5/10^5$ donors) in 2010. determining a range of $303.2/10^5$ donors, but without a significant (chi test for trend -0.2857) decreasing trend in evolution (tab. III). For male, the minimum value was $103.9/10^5$ donors (CI: $45.1\text{-}162.6/10^5$ donors) in 2009. the maximal of $331.1/10^5$ donors (CI: $227.4\text{-}434.8/10^5$ donors) in 2007, the range was $227.1/10^5$ donors and with a insignificant (chi test for trend -0.6667) decreasing in time. For entire Cluj County donors, the trend of evolution in the time interval was stable with insignificant (chi test for trend -0.5952) variation of seroprevalence between the highest level of $360.6/10^5$ donors (CI: $266.3\text{-}454.9/10^5$ donors) in 2007 and the lowest of $108.5/10^5$ donors (CI: $56.9\text{-}160.1/10^5$ donors) in 2009. There were no statistically significant differences between male versus female, yearly seroprevalence rates, except in 2003, when was a significant ($p<0.002$) higher prevalence among males.

In Satu Mare area, the seroprevalence in female donors had variations between $130.8/10^5$ donors (CI: $2.7\text{-}258.9/10^5$ donors) in 2005 and a particularly very high value of $1.091.1/10^5$ donors (CI: 674-

1508.2/10^5 donors) in 2008, but without a significant (chi test for trend 0.71429) trend evolution (tab. IV). In male donors of the same county, the seroprevalence had variation with the highest value of 343.5/10^5 donors (CI: 206.3-480.7/10^5 donors) in 2008, the lowest of 87.2/10^5 donors (CI: 22.6-151.8/10^5 donors) in 2009, but without significant (chi test for trend -0.1667) decline in the trend evolution. The range of the prevalence rate was of 960.3/10^5 donors, for female and of 256.3/10^5 donors, for male donors. Differences statistically significant ($p<0.05$) of the specific seroprevalence by gender was in 2005 with higher level in males and a particularly higher value for females in 2008 ($p<0.01$) and in 2009.

TABLE I

The number and the distribution by gender of blood donors serologically screened for hepatitis C virus antibody, in Cluj and Satu Mare Blood Transfusion Centre, in the 2003-2010 periods.

Years	Donors from Cluj County			Donors from Satu Mare County			p value[£]
	All	Female (%)*	Male (%)*	All	Female (%)*	Male (%)*	
2003	15,177	3,425 (22.6)	11,752 (77.4)	7.487	2,495 (33.3)	4,992 (66.6)	<0.01
2004	17,365	4,259 (24.5)	13,106 (75.5)	8.055	2,685 (33.3)	5,370 (66.6)	<0.01
2005	16,491	4,064 (24.6)	12,427 (75.4)	9.174	3,058 (33.3)	6,116 (66.6)	<0.01
2006	16,248	3,879 (23.9)	12,369 (76.1)	9.425	3,141 (33.3)	6,284 (66.6)	<0.01
2007	15,530	3,751 (24.2)	11,779 (75.8)	9,250	2,545 (27.5)	6,705 (72.5)	<0.01
2008	15,598	3,998 (25.6)	11,600 (74.4)	9,370	2,383 (25.4)	6,987 (74.6)	NS
2009	15,663	4,110 (26.2)	11,553 (73.8)	10,400	2,373 (22.8)	8,027 (77.2)	<0.01
2010	16,326	4,140 (25.4)	12,186 (74.6)	10,808	2,902 (26.8)	7,906 (73.2)	<0.05
Total	128,398	31,626 (24.6)	96,772 (75.4)	73.969	21,582 (29.2)	52,387 (70.8)	<0.01

*percents from all donors;

[£]p value for the comparison between Cluj and Satu Mare, through gender proportions;

NS - no significance.

TABLE II

Number of subjects with confirmed positive results for the presence of the hepatitis C virus antibodies, the prevalence and confidence intervals for seropositive blood donors from Cluj Blood Transfusion Centre, for the 2003-2010 period.

Years	Number of confirmed HCV seropositive persons			Seroprevalence for 100.000 donors		
	Female	Male	Total	Female	Male	Total
2003	3	32	35	87.6 [11.5-186.7]	272.3 [178.1-366.5]*	230.6 [154.3-306.9]
2004	16	40	56	375.7 [192-559.4]	305.2 [210.7-399.6] £	322.5 [238.2-406.8]
2005	7	25	32	172.2 [44.7-299.7]	201.2 [122.4-280] £	194 [126.8-261.2]
2006	7	19	26	180.5 [46.9-314.1]	153.6 [84.6-222.6] £	160 [98.5-221.5]
2007	17	39	56	239.9 [83.3-396.5]	331.1 [227.4-434.8] £	360.6 [266.3-454.9]
2008	9	23	32	225.1 [78.2-372]	198.3 [117.3-279.3] £	205.2 [134.2-276.2]
2009	5	12	17	121.7 [15.1-228.3]	103.9 [45.1-162.6] £	108.5 [56.9-160.1]
2010	3	16	19	72.5 [9.5-154.5]	131.3 [67-195.6] £	116.4 [64.1-168.7]
Total	67	206	273	211.9 [161.2-262.6]	212.9 [183.9-241.9] £	212.6 [187.4-237.8]
Test for trend	-	-	-	-0.2857 NS	-0.6667 NS	-0.5952 NS

*Female versus male seropositivity rate, statistical significant for a $p<0.05$;

£ Female versus male seropositivity rate, not significant;

NS - no significance.

TABLE III

Number of subjects with confirmed positive results for the presence of the hepatitis C virus antibodies, the prevalence and confidence intervals for seropositive blood donors from Satu Mare Blood Transfusion Centre, for the 2003-2010 period.

Years	Number of confirmed HCV seropositive persons			Seroprevalence for 100.000 donors		
	Females	Males	Total	Females	Males	Total
2003	4	11	15	160.3 [3.3-317.3]	220.4 [90.3-350.5] £	200.3 [99-301.6]
2004	6	16	22	223.5 [44.9-402.1]	297.9 [152.1-443.7] £	273.1 [159.1-387.1]
2005	4	18	22	130.8 [2.7-258.9]	294.3 [158.5-430.1]*	239.8 [139.7-339.9]
2006	6	21	27	191 [38.3-343.7]	334.8 [192-477.6] £	286.5 [178.6-394.4]
2007	5	23	28	196.5 [24.4-368.5]	343 [203.1-482.9] £	302.7 [190.7-414.7]
2008	26	24	50	1091.1 [674-1508.2]	343.5 [206.3-480.7]*	533.6 [386.1-681.1]
2009	9	7	16	379.2 [131.9-626.5]	87.2 [22.6-151.8]*	153.8 [78.5-229.1]
2010	11	16	27	379 [155.4-602.6]	202.4 [103.3-301.5] £	249.8 [155.7-343.9]
Total	71	136	207	328.9 [252.5-405.3]	259.6 [216-303.2] £	279.8 [241.7-317.9]
Test for trend	-	-	-	0.71429 NS	-0.1667 NS	0.11905 NS

*Female versus male seropositivity rate, statistical significant for a $p<0.05$;
£ Female versus male seropositivity rate, not significant;
NS – no significance.

The graphical representation of HCV antibody prevalence among blood donors reveals different patterns of evolution in the two counties: some wavy evolution for Cluj and more stable, but with an accidental transmission for 2008 In Satu Mare (fig. 1).

The models of polynomial regression denote a time stability of the endemic manifestation, without significant variation in time. For Cluj, in the graphical model there are (for both male and female donors) two peaks at 3 years interval, reflecting a probable high HCV transmission in certain particular population, but for both gender the modelling of the time-series show a stable evolution over time (fig. 2).

In Satu Mare for the first 5 years, the graphic curves, for both genders are in slowly increase and then they are divergent, with a high peak for female in 2008 and a sharp decline for male's curve, in 2009 (fig. 3).

The modelling of time-series show for men a slight decrease of prevalence in time (the coefficient of determination: 51.3%) and for female a stable evolution in time.

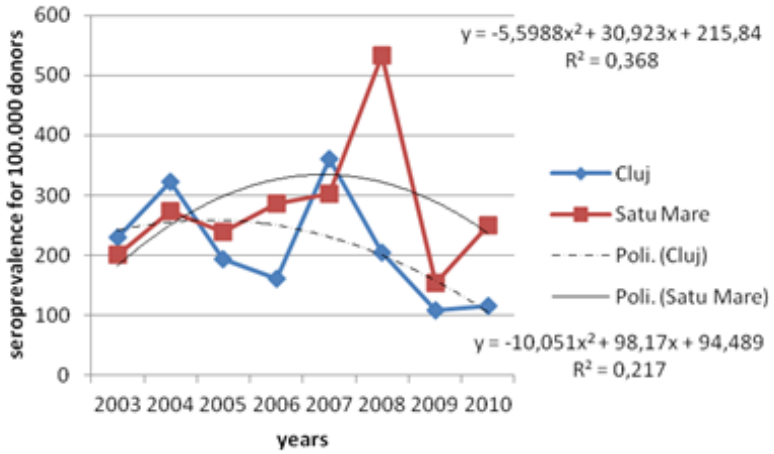

Fig. 1. The annual prevalence of seropositive persons for hepatitis C virus antibody, among blood donors from Cluj and Satu Mare County, in the 2003-2010 period and the time series modelling by polynomial regression.

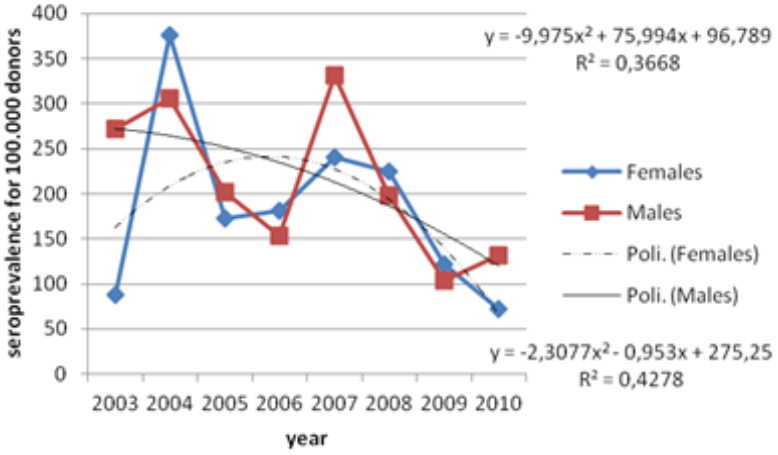

Fig. 2.The annual prevalence of seropositive persons for hepatitis C virus antibody, among males and females blood donors from Cluj County, in the 2003-2010 periods and the time series modelling by polynomial regression.

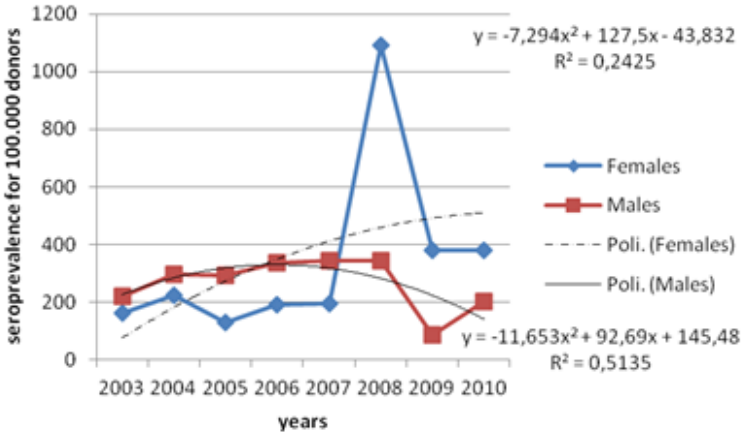

Fig. 3. The annual prevalence of seropositive persons for hepatitis C virus antibody, among males and females blood donors from Satu Mare County, in the 2003-2010 periods and the time series modelling by polynomial regression.

TABLE IV

The coefficients of variation for the hepatitis C virus antibody prevalence, among Cluj and Satu Mare blood donors, in the 2003 – 2010 period.

County	Female (%)	Male (%)	Total (%)
Cluj	53.2	39.2	42.7
Satu Mare	91.9	33.9	40.4

The variability of seroprevalence rates, evaluated through the coefficient of variation was higher in Cluj (42.7%) than in Satu Mare (40.4%) and among the female blood donors, both in Cluj (53.2%) but mainly in Satu Mare for whom the coefficient (91.9%) was the highest (tab. IV).

DISCUSSION

Keeping the risk of HCV contamination for blood products as low as possible is an important issue of patient security. In this sense, improvements in the selection of blood donors depend on the knowledge about infection prevalence in general population and detailed epidemiological data, particularly about transmission.

The present study provides some epidemiological aspects based on high quality and exhaustive data, for two counties of the North West region of the country, using the same standardized and centralized selection method for blood donors.

In the region the different attitude of people towards blood donation (difference by gender), may reflect the educational campaigns for blood donation, sometimes made under the pressure of the urgent practical needs for blood products. On the other side the behaviour to donate blood, may be associate with some personal characteristics and may represent persons with high HCV infection prevalence (risk groups). These aspects show the importance and the needs for implementation and improvement of programs for educate and prepare people to donate.

In comparison with European countries, the observed blood donors HCV seroprevalence in the presented study, is higher than in blood donors from developed countries

($0.35/10^5$ in France, $1.5/10^5$ in Switzerland), but similar with other or bordering countries like Turkey ($380/10^5$ donors), Hungary ($426/10^5$ donors) or Kosovo ($300/10^5$ donors) (10. 16). However, comparisons with other studies have to be carefully discussed, because discrepancies may be explained by the study periods, the included populations, and by the performances of serological tests used in the study (10, 11, 12, 14, 16). In addition, the HCV seropositivity varies between new and repeated donors ($41.07/10^5$ for new donors, against $1.55/10^5$ for repeated donation in United Kingdom; $97.4/10^5$ respectively $1.5/10^5$ in Germany), which were not distinguished in our study (13. 15). Besides the methodological reasons mentioned above, this difference observed with similar tests, could be explained by the higher prevalence of HCV infection in Romania, level never measured on representative sample, only estimated or evaluated in some risk groups (6. 7. 8). In addition, the effectiveness of low risk blood donor's selection in Romania has not been assessed. These differences are barriers for achieving a homogeneous blood safety in Europe: they therefore warrant investigation and maybe other specific preventive and/or control measures.

In the presented study were found in some years, significant differences in HCV seroprevalence distribution by gender and an accidentally high rate in 2008 for female blood donors from Satu Mare County. This higher prevalence in women, in our study, could reflect a similar phenomenon as in the general population, or could be the result from a less effective screening in female donors. Recent results on different population subgroups in south-western Romania, are in favour of the first hypothesis (men 3.4% versus women 5.5%; p = 0.002) (8). This higher prevalence in women, if confirmed by other studies, should be related to an obstetrical transmission, among other assumptions (16). Differences between genders were founded in other published study and in those from developed country, the higher prevalence in young men were associated with intravenous drug use (10, 16, 17, 18). There are also some studies made in development countries, who founded an important impact of medical invasive practice for the increasing prevalence of HCV infection in community and especially for elevated values in women (16. 19).

In evolution the stable endemic manifestation of HCV infection in the blood donors population of the two analysed county of north west region, even with apparently high transmission in some subgroups of population are contrary to the constantly decrease in HCV seroprevalence for blood donors, observed in other studies (12. 13. 14. 15). These issues should encourage the study of HCV infection epidemiology in the region and stimulate the evaluations for improvements of the blood donors' selection methods.

In this study the comparisons seems to be reliable because were used homogenous data from two large donor populations selected with the same methods. In addition were included only confirmed HCV positive subjects registered in the same manner. Under these circumstances, the results are worrying and should lead to further investigations. The weaknesses of the study are the short analyzed period of only eight years, not enough to assess the long evolution in time and the number of only two BTC included affecting the representativeness for the North West region of country.

CONCLUSIONS

In the studied blood donor's populations of the North West region of the country, the populational manifestation was endemic and stable in time. The pattern of populational manifestation was different, reflecting on one side a stable transmission and of the other part a transmission in wave and a particular very high accidental among female candidate blood donors. For the analysed period the highest variability denoting heterogeneity of the prevalence was among female blood donors. The individual differences on the attitude toward blood donation may favour the selection of persons at increased risk for the HCV infection. These aspects should lead to further investigation for identifying the particular risk factors for transmission in the regional population and then to improving the donor's selection.

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NEWS

NEW PROPOSALS ON CLASSIFICATION OF LUNG CARCINOMA

The World Health Organization (WHO; Geneva, Switzerland), in conjunction with the International Association for the Study of Lung Cancer (Aurora, Colorado), published its last official classification in 2004, and this book has served well as a benchmark for classification of lung tumors since then. Since 2004, lung cancer pathology has seen an enormous growth in data on the molecular pathology of lung cancer and the way that influences both the development of the disease and how it should be treated. These advances have had major repercussions for pathologists dealing with lung cancer, not only on our core morphologic diagnoses but also on the need for molecular data to inform therapeutic decisions. All the updates culminated in proposals for a revised classification of adenocarcinoma, driven by William D. Travis et al and sponsored by the International Association for the Study of Lung Cancer, the European Respiratory Society, and the American Thoracic Society. In Muller-Hermelink HK opinion, there is both a clinical need and an academic desire to integrate molecular data into the classification of lung cancer. There is already good evidence that doing so enhances the clinical relevance of the classification, makes it more biologically meaningful, and, in some circumstances, makes it more accurate. Fundamentally, the classification remains a morphologic one, which may, when appropriate, be enhanced or qualified by IHC and/or molecular pathologic findings. There is no need or indication to replace the morphologic classification with a molecular classification, even if such a thing existed. In today's lung cancer clinic, neither the morphologic nor the molecular approach, in isolation, serves patients adequately; in combination, they provide significant improvements in the way patients with lung cancer can be treated. (Kerr KM. Classification of Lung Cancer: Proposals for Change? *Arch Pathol Lab Med*. 2012; 136: 1190–1193)

Doina Butcovan

TRUTH OR LIE – SOME ETHICAL DILEMMAS IN THE COMMUNICATION OF A SEVERE DIAGNOSIS

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TRUTH OR LIE – SOME ETHICAL DILEMMAS IN THE COMMUNICATION OF A SEVERE DIAGNOSIS (Abstract): Informing the patient about his disease is a very important issue in the medical practice, thus communication becomes imperative in healthcare services. Communicating the diagnosis of a pathology that doesn't bear any imminent risk for life is a common procedure for physicians. However, the situation changes drastically in the case of a severe diagnosis or a hopeless prognosis, with terminally ill patients. **Aim:** In this article the authors proceed to a critical analysis of the dilemma of communication or non-communication of a severe diagnosis. **Material and Methods:** The problem of communicating a severe diagnosis is described from a philosophical perspective (in terms of three fundamental ethical theories: Aristotle's theory of virtue, Kant's ethical theory and Bentham and Mill's moral theories). At the same time, certain physiological and medical approaches are presented. **Results and conclusions:** In order to avoid any communication conflict, especially considering the particular situation of the seriously ill patient in the context of the contemporary medical activities, the authors propose the debating of two opposing approaches: "the sacred lie principle" and "the justified medical truth" in the physician - patient relation. **Keywords:** SEVERE DIAGNOSIS COMMUNICATION, MEDICAL ETHICS, INFORMED CONSENT.

Communication is a personal ability that cannot be separated from the human being. It is the process used by individuals to exchange information, using a common and adequate system of signs, symbols and actions that allow both the adjustment of own behavior and the understanding of other people's behavior (1).

In the physician – patient relation the communication is essential, and its necessity for the communication between the patient and the physician / medical team is widely recognized and proved by researches. Due to its high importance in the medical activity and to the fact that the ability to communicate is not innate, but learned, the study of this topic is more and more frequently included in the medical curricula (2). This approach allows the future physicians to develop themselves globally, combining the professional technical aspects with the communication and rational interpersonal skills that form a "measurable and changeable set of behavior features that can further evolve" (3).

Over the years medicine has considera-

bly increased its complexity and intricacy, imposing over-specialization, through which the areas of expertise of the medical specializations were narrowed and deepened in order to improve performance. The doctor-patient relationship, in turn, has met a number of important changes, becoming much more complex, involving more professionals. On the other hand, the time dedicated to medical consultations has decreased considerably, raising the gap between patient and physician. The commercial character that society imposed on the doctor-patient relationship has also contributed to this, turning the doctor into a service provider under the risk of malpractice accusation in case of breaking the contractual relationship established between him and his patient.

Thus modern medicine has moved the doctor away from his patient, reducing the interpersonal interaction and communication between them, while the technical aspects of the medical profession have become increasingly conspicuous. The emphasis on the scientific and technical side of the modern medical profession in the higher education system has greatly contributed to this state.

Considering the increasing pace of industrialization and bitter preoccupation to heal at any price, placing in the forefront the disease to be treated rather than the patient as a human being, in the recent years, the adequate and effective communication has become increasingly important. There are a certain number of researches proving the importance and the role of social and psychological aspects in the evolution of pathology. The 2004 report of the Institute of Medicine (IOM) in the United States shows that about half of the causes of mortality and morbidity in this country are related to social and behavioral factors which led to the conclusion that the medical standards should incorporate psychological and social knowledge and communication skills in order to create an efficient therapeutic relationship between the physician and his patient, ensuring patient compliance and improving treatment outcomes (4).

Parallel to the technical progress of medicine, doctor-patient relationship has undergone a major transformation by moving from paternalistic model when the doctor makes all the decisions concerning the treatment, towards the relationship of partnership, which promotes patient autonomy, in which doctor and patient work together to identify the best therapeutic solutions. In this type of relationship, the physician invests medical expertise, considering the "best interests" of the patient from the perspective of the given medical data. The patients, in turn, invest their own set of values and life principles as a filter for all the information received. Such doctor-patient relationship, which aims to develop a medical decision by articulating the medical information provided by the physician to the patient's life principles, can be functional only by means of establishing efficient communication between the two parts (5).

THE INFORMED CONSENT OF THE PATIENT

The patient’s informed consent has become a reality of the Romanian clinical activity in the last decade. Although it is often regarded as a formal procedure of the patient signing a "document" (in fact, its meaning remains unclear to the patient), which authorizes the necessary medical intervention, according to the national and

international regulations; however, the informed consent tends to overcome the excessive formalization by practically displaying its moral, legal and social advantages (4).

The moral benefits may be limited to the patient's awareness of his/her own moral value and the right to be treated respectfully by the doctor, as well as the doctor`s moral satisfaction, gained by refraining from unconscious tendencies to dominate, encouraged by his advantageous professional position over the patient's vulnerability and dependence.

The legal advantage of the informed consent, if properly conducted, becomes relevant when used as a means of protection in case of legal action initiated by the patient against the physician.

The social benefit derives from the fact that getting an informed consent can be considered an education process with informative features, since a certain sequence of the medical science development is revealed to the subject that enhances his knowledge, and at the same time requires him to take the responsibility for his own actions within the therapeutic program, which consolidates the core of personal autonomy. This advantage of the informed consent takes higher importance especially for countries that overcame totalitarian political regimes that inhibited both the individual autonomy and the cultural pattern or collective spirit. The doctors assuming the role of "educator" of the patient supposes an implicit acknowledgment of the ideals of the human rights consigned within the Declaration of Human Rights (1947), but also their contribution to the maintaining and strengthening of these relatively recent acquisitions of the human civilization.

CONTEMPORARY CIRCUMSTANCES

Besides the advantages detailed above, informing the patient remains a highly debated problem, both in the academic and clinical terms, when it's a matter of a life-threatening diagnosis. In Western countries, the practice of direct revealing of the lethal diagnosis is consistently implemented since the second half of the twentieth century. This practice is based on the principle of patients' autonomy, under which he has the right and full power of deliberation – decision concerning his own life regardless of the existential context, justified by clinical need of the patient`s compliance to the therapy (6).

In the Romanian clinical environment, in spite of the globalization trends to create common standards of activity in various fields, including that of medical practice, a denial of adequate or complete information about a patient's diagnosis persists, especially a serious one and it occurs without there being the patient's prior refusal to be informed, as required by national and international legal practice (for example, the Oviedo Declaration of the European Council in 1996, on the right of "not knowing" stipulates that the information may be waived only if the patient wishes not to be informed).

Sometimes the patient reveals certain ambivalence toward "truth", an uncertain desire to know his diagnosis, which requires the specification of the information that the patient really wants to receive. The patient is frequently not able to understand at once what he is informed about, including the full spectrum of consequences of his disease. At each stage of the disease, we can observe apparent reactions of escape, of denial or avoidance, which should

not be taken as a resignation, but reflect the patient's resentment and his way to react, which must be accepted and respected. The interweaving of the patient's responsibility regarding his decision with the support provided by the physician enables the efficient collaboration between the two and excludes the possible conflicts between the "principle of the sacred lie" and "the legitimate medical truth".

In literature there are frequent descriptions of disputes different opinions whether a serious diagnosis should be communicated or not, ranging from total rejection and assessing it as inhuman and accepting it as fundamental right of the patient (7, 8), the doctor often facing the dilemma of informing the patient or not of the serious or terminal stage of the illness.

On one hand, in Romania the legislation forces the physician (under the potential threat of punishment) to inform the patient about his medical condition and the patient has the right to file a complaint of malpractice in case the doctor breaks his right to information. On the other hand, non-disclosure of bad news to patient is based on an attempt to protect the patient, under paternalist approach of the doctor-patient relationship, the doctor being authoritarian by competence, and fully responsible in the relation with the patient, whilst the patient is dependent (subject), and fully confident of the good intentions of the physician towards him. In this type of relationship the physician decides either to inform the patient about a lethal diagnosis or not, with the condition that such decision is taken in accordance with the promotion of the patient's benefit. Since the general, most often accepted perception is that the negative information and especially the one about the state of incurability, produces negative effects (consequences), the doctor, desiring to protect and not to damage the patient, tends to avoid the communication of the diagnosis.

PHILOSOPHICAL PERSPECTIVES

The philosophical valences regarding the dilemma of whether to inform patients suffering from a serious illness will be analyzed considering three fundamental ethical perspectives: the teleological perspective (Aristotle's theory of virtue), the deontological perspective (Kant's ethical theory) and the utilitarian one (Bentham and Mill's moral theories).

Aristotle (9) developed an ethical theory of self-realization according to which we, as human beings, hold potential moral virtues that can and are updated only through practice. Only by proceeding correctly do we become righteous or only by offering do we become generous. But we can, for example, offer too little and then we are considered stingy, or we give too much and then can be considered wasteful. In this context, Aristotle defined virtue as a middle state between two vices, one that is born of excess and the other of deficiency. Thus, generosity is the middle way between of avarice (deficiency), and waste (excess) contrarily, courage is born by avoiding cowardice (deficiency) and at the same time fearfulness (excess), and modesty is the middle way between shyness and naughtiness. In order to live morally and virtuously we must find the midpoint between two extremes. In other words, a life lived virtuously is that the moderate way. If we want to be sober we must consider the "golden middle" of our own perspective, in other words, we must know our own measure.

According to the Aristotelian view, the concept of virtue cannot be applied to all

types of activity as for some behavioral acts there is no middle way. For instance, we can eat and drink moderately (to some extent), but we cannot kill, steal or lie moderately, as these actions themselves are labeled as immoral. Under these circumstances, if the doctor wants to be moral, he must be honest at any time, even towards a patient facing a terminal or serious diagnosis. The moral act is done not under the influence of pressing/ stressful circumstances, sometimes rather despite them, but always reasonably. At the same time, according to the Aristotelian view, a virtuous act cannot be committed accidentally, but only as the manifestation of sustainable moral traits of character. Returning to informing the terminal patient, in Aristotelian terms, the appropriate question is not telling the truth or not, but how and when that truth must be told. The answer to this issue, in terms of his theory, is that the truth must be told in a moderate (cautious) way that could mean "the right words in a proper case" avoiding over-information on the one hand and refusal to inform him on the other.

The utilitarian's theory changes perspective in approaching morality: from virtue and self-development towards the considerations about society and law. Morality is not just a personal matter devoted solely to self-realization (self-becoming), as Aristotle sees it, but it also refers to the common good and public order. Establishing a foundation of moral criticism is an indispensable process of building a better society by means of improving the legislative base. From the utilitarian approach of the two significant representatives (Jeremy Bentham and John Stuart Mill), legislation is the crucial element in achieving individual and general social welfare (10).

Both utilitarian philosophers promote the principle of "maximum happiness for the maximum number of people" as the moral basis of an adequate legislation; according to this principle, an action can be judged as morally right if its consequences are as good as possible for a number of people as large as possible. If legislation promotes such actions and consequences, they are considered appropriate.

Returning to the problem of communicating the diagnosis, we can assume that from the utilitarian perspective, the relationship physician – patient must be guided by laws, codes and social conventions based on the principle of the best consequences for the largest number of individuals. Let us assume that the doctor lies to the patient about the diagnosis, with the good intention of not harming them. As a result, the patient will experience a state of satisfaction/ happiness gradually subdued by the persistence or worsening of the illness's symptoms and ultimately replaced by a deep sense of disappointment that can dangerously affect the patient's life and, in a bad way, the physician's life. Therefore, not disclosing the truth cannot be a rule of conduct as it does not maximize the happiness or subdue the pain of a maximum number of individuals. By contrast, utilitarian analysis of consequence shows a new rule of conduct for medical doctors: "the medic must tell the truth to his patients".

From the Kantian perspective, the priority in ethical evaluation moves from the consequences towards the action itself. According to Kant, each time we act voluntarily, we are guided by certain rules, of which, we may or may not be aware at the time of the action, but which we acknowledge, when we have to justify our behavior to others or to ourselves. In order to distinguish the moral behavior norms

from the immoral ones, Kant formulated the categorical imperative, the most well-known formula: *Act only according to that maxim whereby you can, at the same time, will that it should become a universal law* (11). According to this formula, the maxim is moral if, without contradiction, it is required to be a rule of conduct for everyone, without exception. For example the maxima "*Lie in order to get what you want*" cannot be moral for no one, even the most experienced liar, would accept it (or like it) as a universal rule of behavior. If a person chooses to lie in some cases, they should want to be victimized by a liar, crook or cheat. Presumably, nobody wants to be deceived, so no rational thinker, according to Kant, would accept such maxima.

As for the topic of this paper, from the Kantian perspective, in order to stay a moral person, the doctor, like any other human being, must tell the truth under any circumstances. This *must*, coming not from external constraints, but from the holy will (each man's duty) to follow their own moral law (or in other words from the categorical imperative of human beings by virtue of freedom, personal autonomy), is by definition (due to universality) the moral law of all people.

From all of the above, we find out that all three ethical approaches reach the same result by different means, namely: non-disclosure of adequate information about their medical condition to the terminal patient is, theoretically, morally unjustified. It should be noted, however, that within these theoretical reflections, slightly modified by empirical necessity, a moderate opinion may be added, according to which the refusal to provide information is still unacceptable; however, it is morally accepted to limit information in order to safeguard the patient's interest, complying with the requirement of prior critical analysis of the condition, the nature of pathology, the expected evolution and patient's personality. As stated, this view is possible, provided that it is not the case of some limitation of *content* but merely of an apparent one, with a background in the spirit of Aristotelian, utilitarian and Kantian ethics.

MEDICAL PERSPECTIVES

Despite the perspective offered by the philosophical approach, many practitioners argue that providing full and detailed information to the patient with serious diagnosis can lead to the worsening of their state, inducing possible state of depression, anxiety, apathy etc. These assumptions are often rooted in practice (12). On the other hand, communicating serious diagnosis or bad prognosis is one of the most difficult aspects of the doctor-patient communication which, unfortunately, is insufficiently taught during the training of future specialists in our country.

The way of communication and informing the patient is extremely important. How the patient perceives, internalizes and reacts to the information received, strongly depends on the form of communication. Even if the statement is a seriously bad diagnosis, the physician should not try to minimize the importance of information, but tell the truth in an empathic manner, with the aim of actively involving the patient in their recovery. The minimization of the importance of the disease risks to suggest the patient that there is no need to worry or get deeper involved in the therapeutic process, which will lead to a poor therapeutic compliance. Contrarily, the disclosure of reality must be accompanied

by instilling confidence in the physician as well as hope for the better (13).

The act of information in medicine should be individualized while the way of communication must vary from case to case. In order to avoid the negative effects of information, the doctor should get more information about the patient, especially on his values and cultural context from which he comes before disclosing a diagnosis of serious illness or a poor prognosis. A patient may need additional information which requires that the relationship between the doctor and the patient be based on trust and hope. After a longer contact with the patient, the doctor may find important data on the patient`s character and their emotional status that allows him to choose the correct way of providing information about the disease and its prognosis.

In the communication process, patience is essential. The patient's satisfaction is higher when the duration of the meeting with his doctor is in line with his expectations, especially when the doctor proves to be a real communication partner. Unfortunately, it was found that doctors would listen, on average, just 23 seconds before stopping the patient from the story of his complaints and turn the discussion into another direction. Allocating enough time for discussion allows the patient to better understand the information that must be provided in accessible terms, according to his degree of education and age (14).

There are several studies showing that effective communication in medicine has positive therapeutic effects on short and long term, both at the psychic and somatic level (15).

On short term, good communication creates a state of satisfaction with the interaction with the physician and the medical care received, and promotes adherence to the prescribed treatment and recommendation.

In the long run, good communication is likely to improve the patient's health condition and quality of life, proved by the resolution of symptoms, especially pain relief and improvement of physiological parameters such as blood pressure and blood sugar level. In addition, it leads to anxiety reduction that has positive effects on the health of the patient.

The efficiency of the communication between doctor and patient is reflected in meeting its goals, among which the most important is to create a therapeutic relationship. Only throughout efficient communication can a doctor withdraw useful information from the patient, based on which the physician can make the best decision together with the patient and his family and launch the therapeutic procedure. In this sense the patient will adopt the appropriate behavior related to his disease and treatment. In order to comply with the medical prescriptions, the potential benefits and risks, the patient must understand and accept the medical diagnosis (16).

Taking into account the results of several studies on the importance and beneficial role of communication in medicine, WHO insisted in recent years on the need to promote the ethical values within medical communication, stating that "information is a basic right for all as health is a priority for the whole world" (3).

An important aspect of the effective communication in medicine is reducing the risk of malpractice claims. An example of this is provided by the Netherlands, a country where about 40% of health care services are provided by family doctors, who go along patients for long periods and estab-

lish with them and their families long term relationships based on trust; this makes patients less likely to act legally against their doctors even if they are mistaken. This last fact is based on the premise that physicians have the best intentions in relation to their patients (18).

PSYCHOLOGICAL PERSPECTIVES

In the communication process the doctor and the patient should be placed on equal positions, a condition necessary for providing a partnership, focused on patient autonomy (17).

In his interaction with the patient, the physician should adopt a cognitive empathetic approach that might allow the doctor to get closer to the patient and gain his confidence. It is necessary for the doctor to avoid excessive emotional involvement because it could affect the diagnosis and therapeutic behavior. The "compassionate detachment" helps the doctor to better understand emotions, inner experiences and the patient's perspective on the disease; it is beneficial for the latter as he does not need pity from his doctor, but understanding and support. The studies show that the empathic communication between the doctor and his patient determines the satisfaction of the latter, the adherence to the treatment and perception of his interaction with the physician as being of good quality. Other studies indicate a lower risk of malpractice claims and obvious improvement of health status of the patient if a relationship is based on empathy (19).

In the process of communication the physician must be prepared for any reaction from the patient, especially when communicating a diagnosis of seriously bad or incurable illness. The patients' reactions to bad news may be very different and can be placed on a range from stoically facing reality to hysterical manifestation, crying, anger or denial. No matter where he is placed in this continuum, the patient has the right express his emotions in reaction to the diagnosis and prognosis of their disease, and the doctor must allow and even encourage this (14).

Some oncology professionals suggest some ways of dealing with patients with no hope for cure (20). It is proposed to avoid communication of the diagnosis to those patients that never suspected the pathology and are in a good state for the moment. The information can be cautiously suggested to those with a strong character who value truth more than tragic subjectivity, as well as to those who already feel the sad diagnosis and refused a treatment or intervention. In this perspective, the physician's careful deliberation based on psycho-medical criteria becomes essential, also taking into account the socio-professional and family environment of the incurable patient.

THE ROLE OF THE FAMILY IN THE DOCTOR - TERMINALLY ILL PATIENTS COMMUNICATION

In establishing an ethical and collaborative relationship between doctor and terminal patient it is also important to highlight two aspects: vulnerability and limited autonomy of the dying patient and the importance of relationships and family roles.

The current Romanian legislation, connected to Western values and ethical principles, promotes individual patient information and making decisions based on his autonomy. Nevertheless, in some cases these patterns of behavior cannot be achieved in the interaction with some patients. The medical practice and legislation

in many European countries, including ours , provide the possibility that – in case of patients with diminished or absent capacity of decision – the closest kin should take part in the medical decision according to what the patient would want in that situation (21).

Often, the patients' family members want to get as much information as possible about their relatives in serious condition. At times, they might be the ones to hold the most information about the patients. This knowledge could entail greater clinical approach of the latter (22).

We must also consider the situations when the patients, although accompanied by family members, do not feel comfortable being examined in their presence. It can be seen as a violation of their dignity or privacy. Such conflict situations are not rare and an inadequate solution to difficult ethical dilemmas is an additional source of stress (23-25).

Confidence is extremely important in interacting with the medical personnel, both for the patient and his relatives. The lack of information or the inadequate communication can lead to loss of confidence and limitation of the patient in his decision making ability. For the medical staff the situation becomes even more delicate when interacting with patients from particular ethnic groups with cultural values different from those of the majority of population. Some studies on communication between medical staff and patients from certain ethnic groups (Chinese and Asian population in the U.S.) showed that the latter may have less trust in physicians (26, 27) or have a distant attitude towards health care workers.

Such studies show that familiarizing the medical personnel with the behaviors and beliefs of the patients is essential for a good relationship and communication, allowing the identification and resolution of potential conflicts of values (28-31).

CONCLUSIONS

Along with the progress of medical science, communication is nowadays thought to be essential in establishing a therapeutic relationship between doctor / healthcare professional and patient. However, communicating the serious diagnosis and prognosis remains a real challenge for the physician, who is often "caught" inside the dilemma of the "principle of sacred lie" and the "legitimate medical truth".

Thus, the solution of this difficult issue of disclosing the serious diagnosis and prognosis is supported both by theoretical, philosophical arguments, and by the results of studies and clinical practice. All these arguments support and promote disclosure of the diagnosis to the patient, no matter how serious, emphasizing the great importance of the way to communicate and the doctor's ability to morally support the patient, instilling them the confidence they need.

The necessity and importance of communication in medicine result from the essence of the medical profession itself which comprises of a blend between the Asclepian tradition promoting the importance of technical knowledge and critical current practice and the Samaritan tradition that promotes the doctor's compassion towards his patients the good relationship between physician and patient outlining the humanistic character of medicine.

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NEWS

TRANSARTERIAL CHEMOEMBOLIZATION FOR HEPATOCELLULAR CARCINOMA

Transarterial chemoembolization (TACE) is used as treatment for hepatocellular carcinoma. Cytolysis, caused by necrosis of tumour or of non-tumoral parenchyma, may occur days after the procedure and can influence tumour response and liver function. A study by Marquez *et al*. evaluated the impact of cytolysis on tumour response, hepatobiliary complications and survival of patients with liver disease who underwent treatments for hepatocellular carcinoma. The researchers analyzed the associations between cytolysis and radiologic tumor response, as well as adverse hepatobiliary events. Also, they compared the survival rate at 18 months after chemoembolization between the groups with and without cytolysis. The study found that cytolysis was associated with a favourable radiological response, but had no impact on the occurrence of short-term adverse hepatobiliary events or survival at 18 months. **(**Marquez V, Sylvestre MP, Wartelle-Bladou C, Bouchard L, Perrault P, Grégoire P, Pomier-Layrargues G. Impact of cytolysis following transarterial chemoembolization for hepatocellular carcinoma. *J Gastrointest Oncol*. 2013;4(1):45-52. doi: 10.3978/j.issn.2078-6891. 2012.061).

Teodora Vremera

STRATEGY OF SURGICAL TREATMENT OF GIANT PRESACRAL TUMOR - A CASE REPORT

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STRATEGY OF SURGICAL TREATMENT OF GIANT PRESACRAL TUMOR - A CASE REPORT (Abstract): Presacral tumors are uncommon lesions that can be difficult to diagnose because of their nonspecific presenting signs and symptoms. Surgery is the mainstay of treatment as it establishes the diagnosis and prevents the adverse consequences associated with malignant degeneration and secondary bacterial infection. Large, highly vascularised pelvic tumors may pose intraoperative difficulties as bleeding and intraoperative tumor perforation. Cross-sectional imaging is essential in evaluating these lesions to determine the optimal surgical approach and the extent of resection. We emphasize a multidisciplinary expert individualized approach. We report a case of a presacral giant gastrointestinal tumor initially considered as unresectable but further on successfully managed by preoperative vascular embolization followed by resection via abdomino-perineal approach. **Keywords**: PRESACRAL TUMOR, ANGIOEMBOLIZATION, RESECTION, PRESACRAL SPACE.

Presacral or retrorectal tumors represent a spectrum of heterogeneous lesions ranging from simple benign cysts to complex malignant masses invading surrounding pelvic structures. The incidence of presacral tumors in the general population is not known, as the majority of reports on these lesions are from tertiary referral centers and thus do not represent the true incidence of these tumors (1).

Despite their infrequent occurrence, a basic comprehension of the etiology, presentation, diagnostic evaluation and management of these lesions is important as incorrect diagnosis or inappropriate management can result in significant morbidity and adverse outcome (1).The only large series not from a referral center was published almost 30 years ago by Uhlig and Johnson and found an average incidence of two presacral tumors per year in the metropolitan Portland area (2).

The benefit of percutaneous or laparoscopic biopsies in the presacral region has not been presented thoroughly in the literature since this region known to have unique anatomic and oncologic properties (3). The standard treatment for the most giant presacral tumors remains the complete surgical resection, with the aim to obtain negative microscopic margins over the organ origin. However, because of the anatomic site and

tumor size, complete resection is either not feasible or possible only through extensive aggressive procedures with expected major functional morbidity (4).

Angioembolization can be used for many types of malignant tumors to achieve good surgical results. It may be employed either preoperative or palliative to alleviate symptoms and reduce further dissemination as well as increasing the response to other treatment modalities by occlusion of the feeding vessels in order to cut off the growing of the tumor using different substances (5).

CASE REPORT

A 38 years old man, with no previous medical history, was admitted for a palpable right abdomino-pelvic mass, lower abdominal pain with difficulty in voiding and passing motion for two weeks associated with history of chronic constipation on laxatives and poor appetite. There was no history of fever, vomiting, hematemesis, melena and hematuria. No neurological symptoms were recorded. A weight loss of 5 kg was recorded in 6 months. The patient was initially diagnosed in his country two years ago when only an excision biopsy was performed via laparotomy because of the large volume and high vascularisation of tumor. The pathological examination showed a perihistiocytoma. Thus, chemotherapy was advised but not done. Abdominal examination revealed a soft, firm palpable well defined tender mass at the right lower quadrant extending to the pelvis. Hernia orifices and genitalia were free as well as renal angles. A digital rectal examination revealed a retro rectal mass invading the posterior wall of the rectum and normal mucosa. Colonoscopy was normal.

Computed tomography (CT) showed large well defined mass in the pelvis located at the presacral area, measuring 15 x 10 x 20 cm in size, with calcification and much dilated tortuous venous drainage channels; erosion and osteolytic destruction of coccyx and S5 were present. The tumor extended to the left ischiorectal fossa pushing the rectum right antero-laterally and the urinary bladder superiorly (fig. 1). CT scan of the thorax was normal.

Fig. 1. CT finding of a huge mass filling the whole pelvis with infiltration of sacral bone

Laparoscopic incision biopsy was performed. Histopathology revealed hyper cellular spindle cell tumor and no mitotic activity. The case was discussed in multidisciplinary meeting and the consensus was for angioembolization followed by surgical resection. Angiographic evaluation and super selective embolization for right internal iliac feeding branches was performed (fig. 2).

Fig. 2. Angio-CT showing complex vascular network of the tumor

Three weeks post-embolization the patient underwent complete surgical excision of the mass through abdomino-perineal approach, the tumor was resected en bloc including rectum, sigmoid and oscocygum and a permanent colostomy was performed. The mass weighted 1.5 kg and measured 18 x 11x 9.5 cm (fig. 3).

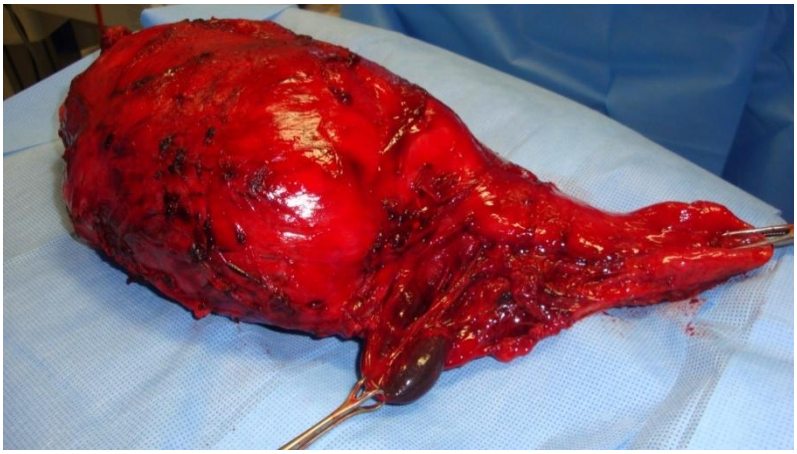

Fig. 3. Specimen of resected mass with intact mesorectal surrounding fascia

Intraoperatively, the hypervascular tumor was dissected successfully and carefully from all adjacent structures - urinary bladder, both ureters, as well as iliac vessels and sigmoid colon. Metastatic nodules were noted only on cecum and one ileal loop and were wedge resected as frozen sections turned positive. The postoperative recovery was, except stress incontinence grade II, uneventful and he was discharged in good general condition. Glivec (Imatinib) therapy has been started because of the metastasis to the cecum and small bowel as well as the giant size of the tumor.

Histopathology revealed extra-gastrointestinal stromal tumor (EGIST)**,** mixed type**,** predominant spindle cells type with 20% necrosis of the tumor. The tumor was highly cellular with high grade histological; margins were positive for the tumor, mitotic rate 11/50 HPF and lymphovascular invasion. The pathology TNM staging – pTNM was recorded as T4 N0 M1.

DISCUSSION

Extensive pelvic surgery in oncologic patients is highly demanding because of the irregular and complex shape of the bony pelvis, numerous muscles attachments and the proximity of major blood vessels, nerves and visceral organs. Surgeon's ability with the extent and involvement of vital structures determine the resectability, keeping in mind that inadequate clearance results in local recurrence. Retrosacral space is a virtual space also called retrorectal space and retrorectal tumors are rarely encountered in surgical practice. Irrespective of their origin they cause similar symptoms and due to their location in presacral space. Although in 26-50% of cases are asymptomatic, the majority of patients present with vague symptoms reflecting the compression, displacement or invasion of pelvic structures, viscera and nerves (4), and occasionally with perirectal pain, mass sensation, constipation, painless rectal bleeding, change in caliber of stool, or urinary frequency (6).

Generally, malignant or inflammatory tumors present with pain (7). Differential diagnosis of retro-rectal tumors can be narrowed using a combination of diagnostic tools. Thorough history and comprehensive clinical assessment including per rectal examination are essential as retro rectal tumors are palpable as extrinsic masses, and they are usually soft and compressible (7). These features may lead to misdiagnosis unless the examiner is careful and suspicious in his examination (2). In case of rectal bleeding, colonoscopy can rule out any mucosal pathology, on the other hand, barium enema can detect extrinsic mass but it will not add additional information (7).CT or MRI scan are the optimal imaging modality to delineate the margin, location and the extent of the tumor as well as possible invasion and displacement of adjacent structures, which is vital for decision making regarding surgical approach, also will add more information regarding the metastases as well, when dealing with malignant tumors (8, 9). Radiological evidence of sacral erosion is the only reliable indicator of malignant pathology as in reported case (10).

Angiography is advisable for highly vascularized tumor to evaluate the relation of the major blood vessels to and around the tumor to define the road map for the resection, in the same time it will illustrate the vascularisation of the tumor which can be tackled by selective embolization in order to decrease the tumor size by blocking the feeding branches. This multidisciplinary approach markedly decreased blood

loss and improved visualization to help in achieve complete surgical resection and resolution of clinical symptoms (11, 12).

Complete surgical resection is the main-line of treatment for all presacral tumors even if they are thought to be benign (4).The approach is dictated by the size, site, and the extent of the tumor also the tissue planes between neighboring structures, which mandate a more aggressive approach if it's malignant tumor. The surgeons need to think three dimensionally in approaching such a huge tumor and in many patients both abdominal and pelvic dissection are required. Moreover, positioning of the patient plays an essential role for total excision. The presacral space may be approached anteriorly (abdominal), posteriorly (transsacral / transcoccygeal), combined anterior-posterior, an anterior approach is performed in a tumor with lowest extension above the fourth level of the sacral element, while the posterior approach preferred for small tumors which doesn't extend above the level of the fourth sacral elements(13). Third approach is the combination between two methods for large tumor beginning under the fourth level of the sacral element and extending above it and it is selected for high grade malignant disease involving adjacent organs (13). These different kinds of approaches should offer a wide abdominal and pelvic exposure in order to achieve good hemostasis as the surgeon may encounter considerable difficulties in controlling presacral bleeding. Overall, 45%-50% of presacral tumors are malignant or have areas of malignant changes in them (7). The consistency may vary and solid tumors are more prone to be malignant than cystic lesions (60% *vs.* 10 %) (14).

Successful surgical dissection demands optimal exposure, skill in dealing with intestinal adhesions, meticulous hemostasis that is maintained throughout the procedure, a thorough the knowledge of anatomy, a plan of attack the tumors at difficult anatomic sites and experience in reconstruction is needed. Undoubtedly, the best results will be obtained by high volume centers with special interest in this form of surgery.

CONCLUSIONS

Surgical approach with different technique of dissection following the path of the least resistance is a wise policy in working round large tumors. Tumor angioembolization may precede surgery for selected patients with large, highly vascularized tumors.

It is certainly a team work involving a panel of expert colorectal surgeons, interventional radiologists and medical oncologist with significant clinical experience.

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NEWS

NEW ANTIBIOTIC IN TREATING EXTENSIVELY DRUG-RESISTANT TUBERCULOSIS

Extensively drug-resistant tuberculosis (XDR-TB) is a form of tuberculosis that is resistant to at least rifampicin and isoniazid as well as to any member of the quinolone family and at least one of the following second-line anti-TB injectable drugs: kanamycin, capreomycin, or amikacin. Treatment requires extensive chemotherapy for up to two years and can cause serious side-effects. A significant number of patients with XDR-TB develop resistance to treatment. A group of Korean researchers conducted a clinical trial on the use of linezolid in the treatment of pulmonary XDR-TB (sputum-culture–positive after six months of treatment). The researchers found that 87% of patients enrolled in the study had no positive cultures after 6 months of treatment with linezolid. In the study, 82% of patients experienced clinically significant adverse reactions, resolved quickly after briefly stopping the drug or using the lower dose. The study concluded that linezolid is a therapeutic option in XDR-TB but dose adjustment is necessary to reduce adverse effects in long-term treatment (Myungsun Lee, Jongseok Lee, Matthew W. Carroll et al. Linezolid for Treatment of Chronic Extensively Drug-Resistant Tuberculosis**.** *N Engl J Med*, 2012; 367:1508-1518).

Cătălina Luncă

SEVERE INVASIVE LISTERIOSIS – CASE REPORT

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SEVERE INVASIVE LISTERIOSIS - CASE REPORT (Abstract): Listeriosis is a rare food borne infection which, in the invasive form, presents as bloodstream infection, central nervous system infection, materno-fetal infection, or focal infection. Certain immunosuppressive conditions have been identified as risk factors for severe invasive disease. The invasive forms of listeriosis are associated with a high case fatality rate. We present the case of a 62 year old male with an unremarkable medical history admitted to the Iasi Infectious Diseases Hospital for fever, headache, ataxia, and diplopia. Physical examination revealed high temperature, confusion, relative bradycardia, and signs of meningeal irritation. Laboratory test showed leukocytosis with neutrophilia, pathological CSF findings (high WBC count with predominance of neutrophils, low glucose and high protein levels), increased liver enzymes (ALAT, ASAT, AP, γGT), and important renal impairment (normal levels at presentation). No abnormalities at chest x-ray, cranial CT and abdominal ultrasound. CSF and blood cultures were positive for *Listeria monocytogenes*. Under antibiotics (ampicillin and ciprofloxacin), the course was marked by respiratory failure requiring mechanical ventilation, coma, hypotension, tachycardia, and death 12 days after admission. The particularity of this case consists in the association of the two classical forms of invasive listeriosis, meningitis and bacteriemia, with a focal infection, acute hepatitis, and a course marked by multiple organ dysfunction syndromes and exitus in a previously apparently healthy individual. **Key words**: LISTERIOSIS, MENINGITIS, BACTERIEMIA, MULTIPLE ORGAN DYSFUNCTION SYNDROME.

Listeriosis is a rare but potentially serious infection caused by *Listeria monocytogenes*. This agent has been recognized as a human pathogen for over 80 years. Epidemiological investigations during the past 30 years have shown that epidemic or sporadic listeriosis is mainly caused by consumption of contaminated food (1). Food borne listeriosis can occur in large or small outbreaks or as sporadic cases; however, the predominant form of disease is probably that of single sporadic and unrelated cases (2). The widespread distribution of *Listeria monocytogenes* provides numerous potential ways by which the disease may be transmitted to humans, although it is now generally accepted that the consumption of contaminated food is the main route of transmission (2). The infection has three major clinical presentations: bloodstream infection, CNS infection, materno-fetal listeriosis) (3).

Listeriosis is diagnosed by positive culture from a normally sterile site (clinical specimens such as blood, cerebrospinal fluid (CSF) (1) When *Listeria* causes disease, it is usually severe with a high case fatality rate of 20-50% (4, 5).

CASE REPORT

In February 2012, a 62-year-old man presented to the Infectious Diseases Hospital for fever, chills, headache, vomiting, and cervical pain, symptoms that occurred four days earlier, associating after two days, ataxia and diplopia. His medical history was unremarkable except for hearing loss requiring auditory prosthesis. Physical examination revealed high temperature (39.5°C), confusion, relative bradycardia (74/min), signs of meningeal irritation, frequent vomiting, and diplopia.

On admission, laboratory findings were: white blood cell (WBC) count 10,850 per mm^3 (88.7% neutrophils), hemoglobin level 12.2 g%, erythrocyte sedimentation rate 115 mm/h, urea 45 mg%, creatinine 1.39 mg%, alanine aminotransferase (SGPT) 37 UI/l, alkaline phosphatase (AP) 192 UI/l, gamma glutamyl transferase (γGT) 69 UI/l. CSF exam revealed WBC count 714 per mm^3 (78% neutrophils), glucose level 0.20 g/l (blood level 1.25 g/l), protein level 4.62 g/l, chloride level 6.60 g/l. Chest radiography revealed no pulmonary abnormalities, cranial computed tomography showed no features suggestive for brain abscess, normal abdominal ultrasound. HIV test was negative. Cultures from blood and CSF were performed and empirical treatment with ampicillin associated with pathogenic therapy was initiated. Two days later, blood and CSF cultures showed a heavy growth of *Listeria monocytogenes*. An epidemiological history including food consumption was taken, and consumption of raw milk on a regular basis was found.

On day 4, the course was marked by a continuous alteration of mental status, leading to coma and requiring specific management in intensive care unit. Ampicillin was associated with ciprofloxacin. Biochemical exams revealed elevated liver enzymes and acute renal failure. The dynamic of laboratory parameters is detailed in the tab. I.

TABLE I

Evolution of laboratory parameters

Parameter	Day 1	Day 4	Day 5	Day 7	Day 8	Day 9	Day 11
WBC/mm^3	10 850	9680	-	9610	-	26 980	40 670
(Neutrophils %)	88,7	78,9		83,4		82,9	94
Urea mg%	45	179	206	201	116	-	191
Creatinine mg%	1,39	5,50	7,27	4,99	2,38	-	3,81
ALAT UI/l	37	130	84	53	49	-	-
AP UI/l	192	340	270	-	239	-	-
γGT UI/l	69	221	165	-	-	-	-
CSF WBC/mm^3	714					960	
CSF glucose g/l	0,20					0,30	
CSF protein g/l	4,62					9,31	

On day 6, the patient developed respiratory failure requiring mechanical ventilation. On day 8, fever occurred again and ventilator-associated pneumonia was sus-

pected. Therefore, new blood and tracheal aspirate cultures were performed and an antiinfectious therapy with vancomycin, colistin and caspofungin replaced the initial antibiotic association. Despite adequate vasopressive therapy the patient presented persistent hypotension and died 12 days after admission.

DISCUSSION

Listeria is widespread in the environment. It can be found in soil, vegetation and animals. Considering the high exposure rate, the pathogenicity of this organism must be low. Listeriosis is a rare disease, with an annual incidence in most countries of <100,000 inhabitants (3, 5). Whereas much has now been learned about epidemic listeriosis, little is known about sporadic listeriosis which, in fact, represents the majority of cases (6).

Listeria is a facultative intracellular, Gram-positive, motile rod which causes both sporadic disease and outbreaks of food borne infection in humans. The genus *Listeria* comprises six species (*L. monocytogenes, L. ivanovii, L. innocua, L. welshimeri, L. seeligeri* and *L. grayi*). Almost all cases of human listeriosis are due to *Listeria monocytogenes* (2). This organism occurs ubiquitously in nature and, therefore, it is not possible to eliminate it totally from raw produce or ready-to-eat products prepared without a bacterial inactivation step (5). Consequently, humans are exposed regularly to *Listeria* (3). The main route of transmission is believed to be through consumption of contaminated food. *Listeria monocytogenes* is not a spore-producing bacterium and does not have unusual characteristics which allow survival in conditions commonly used in food processing (2). The bacterium is particularly successful in causing food-borne disease because it survives food-processing technologies and, unlike many pathogens, can continue to multiply slowly at low temperatures (7). Dietary risk factors (such as unpasteurized milk or other dairy products) for sporadic listeriosis have been assessed through case-control studies (1). The peak of human listeriosis occurs during late summer and autumn; the reasons for this being unknown (2).

The incubation period between consumption of contaminated foods and onset of clinical listeriosis is extremely variable and ranges from the first day to over 90 days (2). It is not known whether the differences in incubation period after oral ingestion are dose or strain dependent, or perhaps reflect unknown differences in host susceptibility (2). Certain conditions have been identified as risk-factors for severe invasive listeriosis, including the extremes of age, malignancies, diabetes mellitus, alcoholism, liver disease and other immunosuppressive diseases and treatments (3). The major host defense against listeriosis is cell-mediated immunity and, therefore, individuals with T-cell dysfunction seem to be particularly at risk for contracting the infection (8). Listeriosis also occurs in previously apparently healthy individuals without any of the above risk factors (2). Serious systemic listeriosis in previously healthy individuals has been reported, but is rare (9).

Listeria monocytogenes causes two forms of listeriosis: non-invasive gastrointestinal form and invasive form.

In immunocompetent individuals, non-invasive listeriosis develops as a typical febrile gastroenteritis; in immune-compromised adults, listeriosis can manifest as sepsis or meningoencephalitis. Invasive listeriosis can also be acquired by the fetus from its infected mother via the placenta (10). The onset of meningo-

encephalitis can be sudden, as in our patient, with fever, intense headache, vomiting, and signs of meningeal irritation. Rhomboencephalitis (with signs of ataxia, cranial nerve deficits), involving the brainstem, may be an unusual clinical form. *Listeria monocytogenes* can also produce a wide variety of focal infections: skin lesions, pleuropulmonary, eye or joint infection, lymphadenitis, liver impairment, brain or spleen abscess, cholecystitis, peritonitis, osteomyelitis, pericarditis or myocarditis, arteritis, necrotizing fasciitis. Focal non-meningeal infections are uncommon and very few cases affecting the liver have been reported (11). Two patterns of liver infection have been described: solitary or multiple liver abscesses and acute hepatitis with elevated liver enzymes (11). Recent observations that gastrointestinal disease and/or fever may be the only symptom in the majority of cases suggest that there may be a considerable number of undiagnosed subclinical cases of this infection (9).

Despite the high contamination rates of certain foods with *Listeria monocytogenes*, listeriosis is a relatively rare disease as compared with other common food borne illnesses, such as *Campylobacter* or *Salmonella* infection. However, because of its high case-fatality rate, listeriosis is, after salmonellosis, the second most frequent cause of food borne infection-related deaths in Europe (1). Clinicians may need to exercise more caution since the outcome involves increased morbidity and mortality.

The particularity of the reported case consists in the association of the two classical forms of invasive listeriosis, meningitis and bacteriemia, with focal infection, acute hepatitis, with a course marked by multiple organ dysfunction syndromes and death, in a previously apparently healthy individual.

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RETROSTERNAL GOITERS

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RETROSTERNAL GOITERS (Abstract): The term of retrosternal or substernal goiter means that more than 50% of thyroid gland descends in the thorax. **Material and methods:** There is a retrospective study on retrosternal and substernal goiter and its patological features among 2482 patients who underwent thyroidectomy between 2000 and 2010 in the First Surgery Clinic of Iaşi. Retrosternal goiter was identified 54 patients (2.17%). All patients were refered to surgery from the Clinic of Endocrinology. **Results**: Mean age at diagnosis was 55.3 ± 3.58 years, and most cases were found in women (83.3%). The clinical pictures of retrosternal goiter was dominated by compressive disorders. Thyroid function abormalities were identified by hormonal assays performed on Endocrinology Clinic Iasi in 15 cases (27.7%). The diagnosis of retrosternal goiter was suggested by clinical examination and confirmed by imagery: thorax X ray, ultrasonography, CT scan. The cervical approach was being safely performed. Only in 8 cases (14,8%), sternotomy was necessary. There was no mortality and morbidity was 5.5% (3 cases). The length of stay in the hospital was 4.3 days. We compared our recent data with a previous report on retrosternal and thoracic goiter treated in First Surgery Clinic of Iasi during 1950 to 1979 and published in the journal "Chirurgia" in 1981. **Conclusions**: Retrosternal goiter is a particular form of thyroid surgical pathology presentation with declining incidence. Diagnosis and treatment of retrosternal goiter involve a multidisciplinary team. The endocrinologist has an important role in diagnosis and postoperative follow-up. Surgery is the treatment of choice for substernal goiters, but there are still some controversies on surgical approach, and complication rate. The cervical approach can be safely performed in almost all cases but when required, sternotomy should be performed without hesitation. **Key words**: SUBSTERNAL GOITER, RETROSTERNAL GOITER, THYROIDECTOMY

Substernal goiter was first described by Albert von Haller in 1749, but the term was introduced in the medical literature by de Souza and Smith.

The term of retrosternal, substernal or cervicomediastinal goiter refers to goiter of which more than 50% of thyroid gland goes down into the thorax. According to the relationship with aortic arch, Huins et al. proposed a classification which indicates the type of necessary surgical approach: degree I: goiter goes down up to aortic arch (cervi-

cal approach), degree II : goiter goes beyond aortic club to the pericardium (the approach needs cutting the upper part of the sternum), degree III: goiter goes behind the rigth atrium (medial sternotomy is necessary) (2).

Different statistics give an incidence of 1-30% of thyroidecomies or 1 in 5.000 individuals (1).

True or primary intrathoracic goiters are very rare (1%), develop of ectopic thyroid tissue situated within mediastinum (mostly posterior) and receive their vessels from mediastinum.

Secondary cervicomediastinal goiters which will be discussed in this study develop by extension of the thyroid within the thorax and their vessels arise normally of superior and inferior thyroid vessels. The treatment of retrosternal goiters is a challenge for surgeon. Total thyroidectomy is the golden standard for cervicomediastinal goiters.

MATERIAL AND METHODS

From January 2000 to December 2010, 2482 patients underwent thyroidectomy in the First Surgery Clinic, University Hospital "St. Spiridon" Iassy. After reviewing the files we identified 54 patients (2,17%) with mediastinal extension of thyroid gland. A mediastinal goiter was defined as the goiter that, in the judgment of surgeon, both preoperatively and intraoperatively extends below the plane of thoracic inlet when the neck is hyperextended. Data from files: preoperative, intra and postoperative, surgical protocols and pathological findings were statistically analyzed. Theese data were compared with o previous statistics regarding cervicomediastinal goiters surgically treated in the First Surgery Clinic of Iasi during 1950-1979 and published in the Journal of Surgery (Chirurgia) in 1981 (3).

RESULTS

During the analyzed interval (2000-2010), among 2482 thyroidectomies there were identified 54 cases of cervicomediastinal goiters representing 2.17%. There was an obvious higher incidence in females (45 females (83.3%) and 9 males) with a sex ratio between females and males of 5/1. Mean age at diagnosis was 55.3±3.58 years with limits between 36 and 82 years. Mean age in women was higher than in men (56.15±1.5 vs 51.5±5.1). 90.7% of cases have been diagnosed after 40 years and 68 % after 50 years.

In the studied group there were 39 cervico-mediastinal goiters (72.2%) and 15 mediastinocervical goiters (27.8 %). There were no cases of primary or independent mediastinal goiters in this study; opposed to the group from 1950-1979 in which 3 cases were found (3).

The most important clinical symptoms of retrosternal goiter were compressive symptoms which were more important in large goiters deeply descending in the thorax. Dyspnea and cough was most frequent – 40 cases (70.07%) and more severe in aging patients. Dysphagia was seen in 8 cases (14.8 %) and dysphonia in 6 cases (11.1%). Compression on thoracic veins was seen in 4 cases (7.4%) with secondary dilated superficial veins of the neck.

TABLE I

Symptoms and signs of cervicomediastinal goiters

	2000-2011 – 54 cases	**1950-1979 – 100 cases**
Cervical mass	39 (72,2%)	79%
Dyspnea/cough	40 (74,07%)	59
Dysphagia	8 (14,8%)	16
Dysphonia	6 (11,1%)	10
Superior vena cava syndrome	4 (7,4%)	6
Hyperthyroidism	7 (12,9%)	15

Cervical mass were detected by palpation was the most cases (tab. I).

Hormonal assessment made in the Clinic of Endocrinology Iasi detected 15 patients (27.7%) with thyroid dysfunction: 7 hyperthyroid and 8 hypothyroid patients. 11 patients had associated cardiovascular abnormalities confirmed by ECG: sinus tachycardia – 8 cases, atrial fibrillation – 3 cases, ventricular extrasystoles – 2 cases.

The diagnosis of retrosternal goiters was suggested by clinical examination and confirmed by imagery. Chest X-ray identified a rounded mass with regular margins, situated in the upper part of the anterior mediastinum, in midline or paramedian positon, sometimes in slightly lateral position with trachea deviation. The upper pole of the mass was less visible being confounded with soft tissues of the base of the neck (fig. 1). In 4 cases calcifications were seen within the goiters (fig. 2). Trachea was deviated in most cases. Profile X-ray identifies the lower pole of the goiter, situated in the anterior mediastinum, anterior of the trachea and pushing the esophagus (fig. 3).

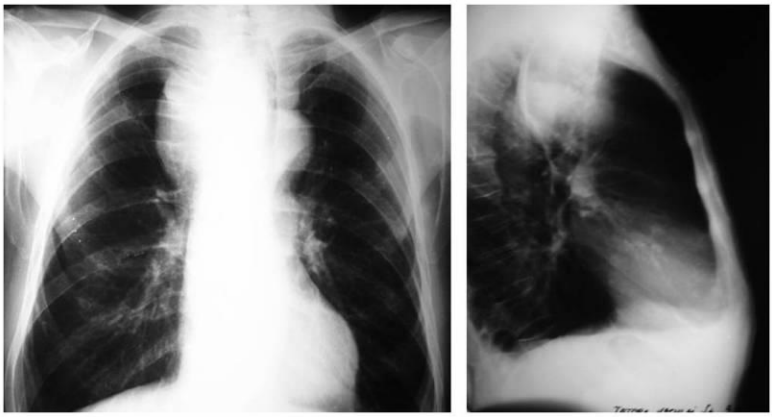

Fig. 1. Thorax X-ray postero-anterior and profile.
Enlarged superior mediastinum due to a soft tissue opacity of 10/6 cm, which deviates trachea to the left side and is situated anterior from the trachea – plonged goiter

Fig. 2. Cervical and thorax X-ray: trachea deviation to the right in the cervical and thorax part due to soft tissue opacity of 10/6 cm. The lower pole of the mass descends to 2 cm. from he o arch and has multiple calcifications within central and peripheral area – pronged goiter with calcifications'

Fig. 3. Cervical X-ray profile: well delimited in homogenous soft tissue opacity of 11/8 cm situated in the anterior cervical area, with the lower pole situated under the sterno-clavicular joint.

Ultrasound examination, done in all cases, has identified the dimensions of the thyroid, the echogenicity of the nodules, the dominant place of the gland, its vessels and its vascular reports (fig .4).

The most useful examination was computed tomography. This was done in 16 cases and gave us important information regarding vascular reports and laringeal nerve position allowing the anticipation of surgical approach and intraoperative difficulties.

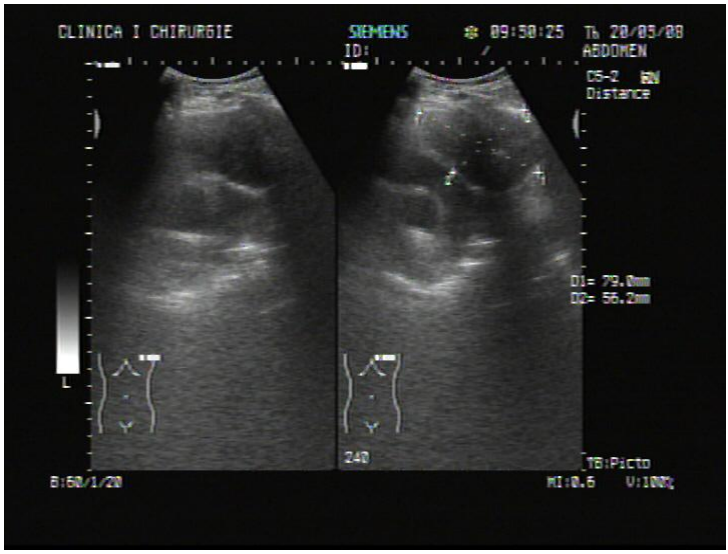

Fig. 4. Cervico-mediastinal ultrasound examination: hypoechoic, homogenous mass, well delimited, of 79/56 mm situated behind left clavicle and behind the sternum, and superior from the thoracic aorta, without Doppler signal inside: plunged aorta

The most useful examination was computed tomography. This was done in 16 cases and gave us important information regarding vascular reports and laringeal nerve position allowing the anticipation of surgical approach and intraoperative difficulties.

The 99mTechnetium scintigram was done in 44 cases and confirmed the presence of radiotracer within the thorax.

Direct laringoscopy showed preoperative laringeal nerve palsy in 3 cases (5.5%)

Surgery was done by cervical approach initially and sternotomy was necessary in 8 cases (14.8%) (fig. 5) After exposure of the gland in the neck, the blood supply of the upper pole and the middle thyroid veins were isolated and ligation was done. Parathyroid glands were identified and preserved of harm. The inferior thyroid vessels and the laryngeal nerve were identified when possible, but at times this could not be accomplished until the intrathoracic part of the gland had been delivered from the mediastinum. This was done either by digital mobilization alone or by addition of a spoon technique. Infrequently further exposure was necessary and was done by median sternotomy to allow complete mobilization. After mobilization of the intrathoracic part of the goiter the inferior thyroid vessels if not already controlled, were ligated and divided, and the goiter was completely removed (fig. 6).

The volume of retrosternal goiter was:

- large goiter (up to 200 g) - 5 cases (9,25 %);
- very large goiter (200-300g) - 40 cases (74.08%)
- giant goiter (over 300g) - 9 cases (16.67%)

Macroscopically retrosternal goiters were multinodular (76%), with a solitary nodule (15 %) and rarely diffuse (9%).

Pathological examination showed coloid goiter – 31 cases (57.4%), hyperplastic hyperfunctional goiter – 7 cases (13%), adenomatous goiter – 6 cases (11.1%) and malignant nodules in 3 cases (5.5 %).

Total thyroidectomy was performed in 41 cases (76%) and subtotal thyroidectomy in other cases.

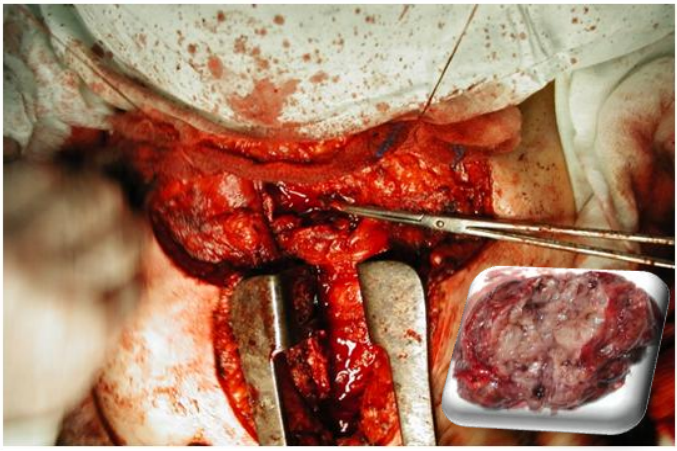

Fig. 5. Medial sternothormy for retrosternal and posterior nodular and cystic goiter. The resected goiter is showen in the lower right part of the figure.

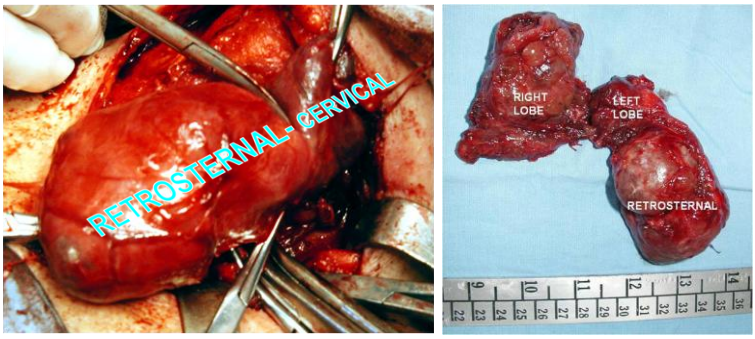

Fig. 6. Total thyroidectomy through cervical approach for retrosternal goiter (left). Resected goter (right)

There was not intraoperative mortality, Morbidity was seen in 5.5% of cases: 1 case of transitory hypoparathyroidism, a unilateral laringeal nerve palsy and a post-operative bleeding which required reintervention for haemostasis.

Average length of stay in the hospital was 4.3 day. The patients were followed from one month to 5 years in the Clinic of Endocrinology and no relapse was seen.

DISCUSSION

The current literature data use different terms such as retrosternal goiter, substernal, intrathoracic or mediastinal goiter. The term of substernal goiter is used when more than a half of thyroid gland descends in the thorax. There is no consensus regarding the plunger goiter. Moron and Shen define these goiters as goiters of which more than a half of their mass is situated under the upper thorax opening (4,5). Makaieff proposed 3 criteria to define a plonger goiter: goiter with substernal development when the neck is in operatory position, a substernal extension of minimum the width of two fingers, the need for supplementary extraction maneuvers (6). Zainine R defines plonger goiters in cases in which the lower pole of the gland is not palpable when neck is hyperextended and differentiates them from endothoracic or ectopic goiters by the fact that thyroid intrathoracic parechima is linked to the cervical part of the gland by a bridge of thyroid parenchyma (7). It is important to emphasize that large cervical goiters have an intrathoracic part in 20 % of cases. Factors that could facilitate the migration of the goiter from cervical region into the thorax may be: gravity, negative intrathoracic pressure and a potentially receptor space within the thorax. In rare cases a goiter develops “de novo”, primary within the mediastinum (intrathoracic goiter). Taking into account the reports with venous brachicephalic trunk the plonger goiters are divided prevascular and retrovascular (7). Most of substernal goiters are situated within anterior mediastinum in prevascular position as may be also in our data. Posterior goiters are seen in 10-15 % of cases (8). In our study the frequency of posterior plonger goiters was 3%.

The incidence of retrosternal goiters in our clinic decreased from 4.1% during 1950-1979 (100 cases among 2400 thyroidcctomies) to 2.1% in this study, and this may be due to a precocious detection and treatment of this particular form of goiter. All over the world iodine deficiency is the most important cause of goiter, but in areas with normal or increased iodine supply, Hashimoto’s thyroiditis is the main cause. In our cases there was noticed an increase of inci-

dence on autoimmune thyroiditis from 7 % in the previous group to 13 % in the actual group. Although there is a well known increased incidence of goiter in women (sex ratio F/M=5/1), the incidence of cervicomediastinal goiter is even higher in females in our study. Mean patients' age was 55.3 with limits between 36 and 82 years in concordance with other reported data (7). Patients between 51-60 years are particularly affected, the age being more advanced than that of patients with normally situated goiters. The fact that 90 % of female patients with retrosternal goiter have more than 40 years demonstrates that these goiters have a long silent evolution during which they first develop in the normal position and then migrate within the thorax. By comparison with our first study there was noticed an increased incidence of retrosternal goiter in aging patients. 79 % of patients studied between 1950-1979 had more than 40 year and 90.7% of patients in this study have more than 40 year.

Regarding the symptoms, only 5.5 % of our cases had no symptoms and were found by imagery techniques. Torre gives a similar percentage (5.5%) on 237 patients (9). Most patients presented with a cervical part of their goiter, and only in 3 cases the thyroid was not found at cervical level. In literature such cases may represent up to 11.8%10.

Compressive symptoms were dominant in our patients (tab. I). The incidence of compressive symptoms was similar in both groups and that demonstrates that a goiter that descends into the thorax may produce compression.

Laringeal nerve palsy was preoperatively detected in 3 cases (5.5%) with a similar incidence with other authors (6% in Vadasz report) (11). This could have the significance of a thyroid cancer (3 cases in our group).

As in other series (12) most of our patients were in an euthyroid state, and only 11% had hyperthyroidism. The prevalence of hyperthyroidism in patients with retrosternal goiter is estimated to 9.4% and 13.8 % of cases (6, 13, 14). Preoperative evaluation of thyroid function is mandatory because hyperthyroid patients must receive an appropriate preoperative treatment.

Dysphagia was seen in our 14.8% to 16% of our patients and is revealing sign for the development of retrosternal goiter on left side or within posterior mediastinum with esophageal compression (15).

The syndrome of superior vena cava was detected in 6% and 7.4% of cases (11).

The preoperative evaluation of retrosternal goiter is very important. Direct laringoscopy must identify laryngeal nerve compression (10%-11% of cases) and tracheal compression (increased from 59 % to 74 % of case). Thorax X-ray is a useful and not expensive method to discover retrosternal goiter. It shows superior mediastinal opacity with or without tracheal deviation (16). It is the most frequent sign seen in the literature (60%-90%) (7).

Ultrasound examination confirms the thyroid features of the mass which descends into the thorax. It was performed in the actual studied group and may approximate the goiter volume, the echogenicity of the nodules and their vascularity.

Cervical and thoracic computed tomography has opinion the optimal sensitivity (17) to evaluate intrathoracic extension and its place, the degree of tracheal narrowing and most of all the reports of goiter with vascular and nervous structures (18). Other authors consider magnetic resonance imaging the most precise method to detect the reports of goiter with brachiocephalic trunk, subclavian artery, aortic arch and other structures of the superior mediastinum (19). Faringoesofageal transit and tracheobronchial fibroscopy could eliminate or identify an invasion due to a thyroid cancer (6).

These evaluations are sufficient for the diagnosis of retrosternal goiter and allow the surgeon to plan the surgical approach.

The factors which decide surgical approach are: the dimensions of goiter, bilateral lesions, and the goiter in the posterior mediastinum, extension under the aortic arch, superior vena cava syndrome, and suspicion of obstruction of the trachea by a malignant lesion. Although rarely necessary, sternothomy may be indicated for giant substernal goiter or in patients with previous cervical surgery (relapse was noticed in some studies in up to 37% of cases) (20). After De Perot two factors frequently require medial sternothomy: medial ectopic goiter and suspicion of an invasive cancer (21). Rarely, medial sternothomy is required in order to mobilize a large substernal goiter which is associated with perithyroid inflammatory lesions (Hashimoto's thyroiditis), or in reinterventions for a goiter which extends below the aortic arch (22).

The removal of large substernal goiters may be done, with a few exceptions, by cervical approach with the patient in semi-Fowler position, with the neck hyperextended. A large cervical incision offers an appropriate acces. Subhyodian muscles may be cut laterally possibly the omohyoidian muscle tendon too. The medial thyroid vein is than ligated and cut in order to allow mobilization of the thyroid gland. The thyroid part situated within the thorax in than pulled up and medially with a special concern for the dissection of posterior cervical fascia outside of the thyroid gland.

In order to avoid a lesion of the superior laryngeal nerve, the branches of the superior thyroid vein are safely ligated on the surface of the thyroid gland. Because in cervicomediatinal goiter the laryngeal nerve and parathyroid glands are dificult to be identified, a subcapsule dissection is of choice. Similarly, the thyrotimic ligament must be ligated directly on the surface of the thyroid in order to avoid an injury of inferior parathyroid gland. Sometimes the parathyroid glands are situated on the anterior and lateral surface of the gland and must be safely dissected and preserved. O small margin of thyroid tissue must be preserved at the level of Zuckerkandl body, because at this level may be situated anterior, laterally or frequently posterior the laryngeal nerve.

Total thyroidectomy is recommended in all cases because a relapse may create difficult problems of surgical technique (23).

Minimal invasive thoracoscopic approach or robotic-assisted is now possible in specialized centers with very good results (24, 25). Robotic surgical techniques for removal of substernal goiter and other thyroid masses with mediastinal extension, in combination with cervical incision are also effective. Robotic-assisted techniques can complement video-assisted thoracic surgical techniques and amplify indications for minimally invasive surgery (26).

Independently of the used approach technique in the removal of large substernal goiters the personal experience of the surgeon offers a clear advantage in order to avoid morbidity and mortality associated with this surgery. Surgical management of patients with substernal goiter requires an appropriate preoperative diagnosis, a considerable experience of the surgical team, and must be performed in specialized centers (27).

Most of substernal goiters are benign: diffuse, coloid or adenomatous nodular goiters. The incidence of malignancy in these goiters may be up to 12 %28. It was of 6% in our cases. Risk factors for malignancy are: familial history of thyroid cancer, history of irradiation of the neck, cervical lymph node enlargement (grade C recommendation) (29).

Postoperatively the patient must be monitored in the ICU. Assisted ventilation overnight may be necessary in cases with

previous difficulties at intubation, large dissection of the neck, upper airway edema.

Postoperative complications are more frequent than those associated with thyroidectomy for cervical goiters: transient laryngeal nerve palsy occurs in 7 % and permanent laringeal nerve palsy occurs in 4 % of cases. The incidence of transient hypoparathyroidism is 10% and that of permanent hypoparathyroidism is 2.6 % (30). tracheomalacia is a severe but rare complication and compressive heamatoma and edema of the upper airway need intubation. Mortality associated with surgery is rare (1.1%). Surgical complications are associated with large goiters suggesting an increased caution in these patients (31). Intrathoracic goiters reaching carina tracheae have a high risk of postoperative complication and mortality (32).

No mortality was noticed in the group operated recently but in the previous group mortality was of 2%. There were 3 cases of surgical complications (5.5%), one being a bleeding which needed reintervention for haemostasis. Length of stay in the hospital after surgery decreased from 12.5 day for the first group to 4.3 in the actual group, that fact being an obvious progress.

CONCLUSIONS

Retrosternal goiters are a particular form of thyroid surgical pathology with a decreasing incidence. The diagnosis and treatment of retrosternal goiters involve o multidisciplinary team.

A retrosernal goiter is a clear indication for surgery.

It is important to clarify preoperatively the relationship between goiter and other structures of neck and mediastinum in order to plan the optimum surgical approach. The best investigation for these reports is neck and mediastinum CT.

Total thyroidectomy is the treatment of choice. When necessary, manubriothomy or sternothomy may be performed without hesitation.

The treatment of retrosternal goiters must pe performed only in specialized centers, in which surgeons' experience guarantees a minimal postoperative morbidity.

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HOW DO PARENTS EXPERIENCE THE RARE DISEASE OF THEIR CHILD?

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HOW DO PARENTS EXPERIENCE THE RARE DISEASE OF THEIR CHILD? (Abstract): **The aim of the study** is to identify the opinions of caregivers of children with rare diseases regarding some aspects of their life or those of their child. The rare diseases included are: cystic fibrosis, phenylketonuria and hypothyroidism. **Material and methods**: A number of 35 parents provided socio-demographic data and answered to a questionnaire with 19 items. Data were processed using *SPSS v. 20.0.* **Results**: 90% of parents consider that their relationship with their partner did not change after the time of diagnosis. More than 75% of parents discussed their worries about the illness with their life partner. They consider that the most affected aspects of their child's life are: relationship with friends and school activities. Most mothers self-evaluate themselves as being more involved in child's health problem compared to their partner. Parents declared that they consulted a health professional to cope with stress (32.7% of parents consulted a psychologist and 19.6% a psychiatrist). No change was identified by parents regarding the child' relationship with their siblings or teacher. **Conclusions**: Psychological support should be provided to parents having a child suffering from a rare disease. Comparative results are provided. **Keywords**: RARE DISEASE, CAREGIVER, PATIENT, CHILD.

A rare disease is defined as one affecting less than 1 in 2,000 people, and more than 6,000 diseases are identified, conforming to the EURORDIS report.

Over 30 million European citizens are affected by one rare disease (1).

A rare disease is a social challenge, with national programs providing equal access to medical services for all patients with healthcare problems and to support costs related to them.

Living with a rare disease is also a challenge for both pediatric patients and their families. Affected children experience loss of social, educational and economic opportunities and difficulties in receiving medical care in good time caused by the need for a specialized medical team or proper medical investigation, with the latter sometimes being delayed (2, 3). After a clear diagnosis, the relationship with the medical encounter is crucial, the doctor-patient

relationship influences the quality of life of a patient with a rare disease. Even if studies identified that such a patient is actively searching information and help, the difficulties and importance of providing information about a rare disease are a challenge also for the healthcare provider (4).

Most of the families are affected by the child's health condition: more financial expenses are needed to cover the specialized medical care, medical treatment, specialized equipment, and special education services (5). In addition, considering the type of disease, some families must travel to obtain a clear diagnosis, or they have to settle in a new location in order to have access to the treatment or medical equipment. Greater geographical distances between patients and physicians have been identified in a study lead by EURORDIS (6). Professional difficulties for parents are also noted, with some studies identifying a high level of unemployment among parents of children with rare diseases and a lack of social network (7, 8). A rare disease does not influence only a patient's and caregiver's life, but also the other children in the family. Some studies demonstrate that the quality of life of the sibling of a child with a rare disease is also affected (9).

Family plays a central role in assuring the quality of life of children affected by rare diseases. Around the world, support programs for parents of children with rare diseases were developed within the last decade. They help the caregiver cope with stress and train them to develop parental skills in order adjust properly to life with a rare disease. That is why factors like economic status, professional duties or other socio-demographic data like marital status, type of chronic disease, geographic area or the possibility to access medical services are also studied. The psychological and physical well-being of the career will influence the quality of life of the affected child. In a recent study in Germany (10) it was revealed that the marital status influences this level, with single parents of children with cystic fibrosis obtaining significantly lower scores for quality of life than partnered parents. The mother of children with rare disease are usually affected mentally and physically, having significant lower than average physical and mental well-being scores (7, 11, 12). Also, single mothers and full-time employment seem to increase the level of stress among mothers of children with rare diseases. A study of Dellve et al identified that fathers are more likely to need supportive programs than mothers focusing on family competence (8). That is why, a family centric support should also be considered in cases of children living with rare diseases.

Since the parent psychological and physical health is important, the present study focuses on the opinion of parents regarding the disease and on some difficulties that they must deal with: personal problems or issues related to their child's life.

MATERIAL AND METHODS

Sample

53 caregivers of children diagnosed with rare diseases were included in the research. Children are registered and supervised by medical teams in a university child hospital in north-eastern Romania. The parents were questioned while they were staying with their children in the hos-

pital. The pediatric patients were registered with cystic fibrosis, hypothyroidism or phenylketonuria.

The participants were informed about the purpose of the study, they agreed to fill in the papers after they had read and signed the inform consent. It was explained that consent could be withdrawn at any stage of the research and they were assured about the confidentiality of the data that they provided.

Instruments

The participants had to complete two documents. The first one referred to socio-demographic data: age, gender, environment, religion, marital status and educational level. The second document was designed specifically for parents having children with rare disease and included nineteen items focusing on identifying the parents' opinion regarding their child's life at home, at school, how the disease is seen by relative or friends, or how they relate the disease with family or religious factors.

Statistical procedure

The collected data were processed using *SPSS version 20.0* for Windows and the following types of statistical methods were used:

- descriptive statistics, which pursued the central tendency and dispersion indicators (the mean and standard deviation) for socio-demographic characteristics and medical data. Also, included were the frequency of answers to all nineteen items, for both male and female caregivers.
- *chi-square* tests were used to point if there are any associations between various categorical variables: the items of the questionnaire in association with socio-demographic data and the type of chronic disease.

Ethical conduct

The research was approved by the ethical committee of "Sf. Maria" University Hospital for Children, Iasi, Romania.

RESULTS

Descriptive analysis

Socio-demographic data gathered information about gender (male/female), age, environment (rural/urban), religion, ethnicity, marital status (single, in relationship and married), educational level (primary, secondary, high school and university degree or uneducated). The data is presented in first table.

Collected data also included medical information about the chronic disease and child's age and gender. A total of 63.3% were boys and 36.4% were girls. The average is M = 7.1± 6.44, ranged 1 to 22-year-old. More than half of the patients (68.8%) are aged between 1 and 6-year-old, 28.1% are aged between 6 and 12-year-old, and the rest (3.1%) are older than 12-year-old

The distribution of rare diseases of their children is the following:

- cystic fibrosis 39.6 %,
- phenylketonuria 30.2%
- hypothyroidism 30.2%.

When parents were asked about their participation in child's treatment (see tab. II, *Item* 19), all fathers declared that are not more caring about their child's disease than mothers. In the case of female subjects, 70% appreciated that they are more concerned about it.

TABLE I.
Socio-demographic data

Variables	**N (%) or M ± standard deviation**
Gender	
male	N = 20 (37.7%)
female	N = 33 (62.3%)
Age	M = 33.5 ± 8.22 (ranged 18-55 years old)
Environment	
urban	N = 26 (49.1%)
rural	N = 27 (50.9%)
Religion	
Orthodox	N = 48 (90.6%)
Pentecostals	N = 3 (5.7%)
Adventist	N = 2 (3.8%)
Marital status	
single	N = 3 (5.7%)
in relationship	N = 2 (3.8%)
married	N = 48 (90.5%)
Educational level	
primary school	N = 3 (5.7%)
secondary school	N = 8 (15.1%)
high school	N = 27 (50.9%)
university	N = 8 (15.1%)
uneducated	N = 7 (13.2%)

TABLE II
The frequency of answers for total/male/female participants

I	**Items and multiple answers**	Total (%)	Male (%)	Female (%)
1.	*I think the time given to my child is*:			
	a. less than that given to other children	3.8	5	3.1
	b. the same time as other children	48.1	50	46.9
	c. longer than the time given to other children	42.3	40	43.8
	d. I do not know how to appreciate	5.8	5	6.3
2	*I discussed the diagnosis of my child with family members*			
	a. yes	98.1	95	100
	b. no	1.9	5	0
3.	*I advised my relatives to have screening for this disease*			
	a. yes	46.2	50	43.8
	b. no	53.85	50	56.3
4.	*I think the other siblings have inappropriate manifestations of it*:			
	a. rarely	8.5	5.9	10
	b. often	2.1	0	3.3
	c. never	89.4	94.1	86.7

I	Items and multiple answers	Total (%)	Male (%)	Female (%)
5.	*It is hard for me to see my baby's disease difficulties*			
	a.yes	47.1	31.6	56.3
	b.no	49	63.2	40.6
	c.I do not know	3.9	5.3	3.1
6.	*When the members of my family learned about my child's illness, they*:			
	a. encouraged me	62.3	75	54.5
	b. helped me	34	25	39.4
	c. I noticed no change	3.8	0	6.1
7.	*When they learned about my child's illness, school teachers*:			
	a. started to isolate him	4.5	0	6.7
	b. began to favor him	0	0	0
	c. started ironing it	0	0	0
	d. began to protect him	54.5	57.1	53.3
	e. I have not noticed changes in teacher behavior	40.9	42.9	40
8.	*When they learned about the illness, the friend of my child*:			
	a. begun to protect him/her	33.3	37.5	31.6
	b. started to isolate him/her	0	0	0
	c. started ironizing it/her	3.7	0	5.3
	d. started to bypass him/her	7.4	0	10.5
	e. I have not noticed changes in the behavior of my child's friends	55.6	62.5	52.6
9.	*Regarding the illness, I blame*:			
	a. family	10.3	14.3	8
	b. child	0	0	0
	c. God	23.1	21.4	24
	d. unhealthful	61.5	64.3	60
	e. ignorance of the family	0	0	0
	f. doctors	2.6	0	4
	g. mother	2.6	0	4
	h. father	0	0	0
10.	*I've checked a second opinion regarding the illness of my child*			
	a. yes	43.1	42.1	43.8
	b. no	56.9	57.9	56.3
11.	*The relationship with my partner had changed since we learned about the diagnosis*			
	a. yes	5.8	5.3	6.1
	b. no	94.2	94.7	93.9
12.	*The relationship with my other children has changed since the diagnostic*			
	a. yes	2.2	0	3.7
	b. no	97.8	100	96.3

I	Items and multiple answers	Total (%)	Male (%)	Female (%)
13.	*When I'm upset and want to talk to someone about my child's suffering, I call on*:			
	a. the life partner	78.4	78.9	78.1
	b. friends	11.8	10.5	12.5
	c. acquaintances	0	0	0
	d. service colleagues	0	0	0
	e. grand parents	0	0	0
	f. priest	0	0	0
	g. physician	5.9	5.3	6.3
	h. other parents who have a child in the same situation	3.9	5.3	3.1
14.	*I consulted a psychologist to help me cope with this situation*			
	a. yes	32.7	21.1	39.4
	b. no	67.3	78.9	60.6
15.	*I consulted a psychiatrist to help me cope with this situation*			
	a. yes	19.6	10.5	25
	b. no	80.4	89.5	75
16.	*When I think about my child, the hardest thing to deal with is*:			
	a. fear of death	25.5	22.2	27.6
	b. fear of treatment	4.3	11.1	0
	c. fear of pain	17	11.1	20.7
	d. fear of illness	53.2	55.6	51.7
17.	*I think my most difficult child must bear*:			
	a. the pain	44	31.6	51.6
	b. treatment	24	26.3	22.6
	c. disease	32	42.1	25.8
18.	*The most affected aspect of my child's life I think is*:			
	a. school activity	40.7	37.5	42.1
	b. relationships with friends	44.4	62.5	36.8
	c. family relationships	7.4	0	10.5
	d. intimate relationships	7.4	0	10.5
19.	*I think I'm involved in treating my child's disease more than my partner is*.			
	a. yes	44.7	0	70
	b. no	55.3	100	30

Comparative analysis

To determine whether there are any associations between type of disease, child's gender, child's age, parents' gender and each item, chi square tests were used. The statistical interest is represented by the cells with positive and negative adjusted residuals within a (-2, +2) interval.

The chi square tests used to determine the association between parents' gender and environment (rural/urban) and each of our instrument's items show no significant associations.

The type of disease

The chi square tests used to determine the association between child's disease and each of our instrument's items show an association with:

- *Item 5*: $\chi^2(4) = 15.239$, $p = .004$.

Two positive adjusted residuals were identified.

- In the case of phenylketonuria, there were more participants in the category of parents who do not understand the child's difficulties with the disease than would be expected by chance;
- in the case of cystic fibrosis there were more participants in the category of parents who understand the child's difficulties with the disease than would be expected by chance.

Also, two negative adjusted residuals were identified:

- in the case of phenylketonuria there were less participants in the category of parents who understand the child's difficulties with the disease than would be expected by chance;
- in the case of cystic fibrosis there were less participants in the category of parents who did not understand the child's difficulties with the disease than would be expected by chance.

- *Item 7:* $\chi^2(4) = 15.475$, $p = .004$.

By inspecting the adjusted residuals, resulted two positive ones.

- concerning phenylketonuria, there were more participants in the category of parents who think their child's teacher started to isolate him/her than would be expected by chance,
- in the case of hypothyroidism there were more participants in the category of parents who do not see any modifications in their child's teachers' attitude than would be expected by chance.

- *Item 10*: $\chi^2(4) = 7.701$, $p = .021$.

By inspecting the cross tabulation, two positive adjusted residuals were revealed:

- in the case of phenylketonuria there were more participants in the category of parents who think their child's teacher started to isolate him/her than would be expected by chance;
- in the case of hypothyroidism there were more participants in the category of parents who do not see any modifications in their child's teachers' attitude than would be expected by chance.

- *Item 16*: $\chi^2(6) = 17.186$, $p = .009$.

In the case of this item, two positive and two negative residuals were found.

- in the case of phenylketonuria, there were less participants in the category of parents who find it difficult to deal with the fear of death than would be expected by chance and more participants in the category of parents who find it difficult to deal with the treatment than would be expected by chance.
- in the case of hypothyroidism, there were more participants in the category of parents who find it difficult to with the fear of death than would be expected by chance and less participants in the category of parents who find it difficult to deal with the fear of the disease than would be expected by chance.

- *Item 17*: $\chi^2(4) = 12.753$, $p = .013$.

In this case, one negative and two positive residuals resulted.

- in the case of phenylketonuria, there were less parents in the category that think it's difficult for their children to face the pain and more parents in the category that think it's difficult for their children to face the treatment than would be expected by chance.
- in the case of cystic fibrosis, there were more parents in the category that think it's difficult for their children to face the pain than would be expected by chance.

Child's gender

The chi square tests used to determine

the association between child's disease and each of our instrument's items show an association with the item No 17 - $\chi^2(2) = 9.557$, $p = .008$.

By inspecting the cross tabulation, one negative and one positive adjusted residual were noticed: in the case of boys, there were less parents, and, in the case of girls, there are more parents in the category that think it's difficult for their children to face the pain than would be expected by chance.

Child's age

To provide a comparative analysis considering the age of the children, three groups were considered: 0-6 years old, 6-12 years old, and over 12 years old. The chi square tests used to determine the link between child's age and each of our instrument's items show the following significant associations:

- *Item 5*: $\chi2(4) = 16.124$, $p = .003$

with two positive and two negative adjusted residuals:

- in the case of children aged between 1 and 6 there were less participants in the category of parents who understand the child's difficulties with the disease and more in the category who do not understand the child's difficulties with the disease than would be expected by chance,
- in the case of children aged between 6 and 12, there were more participants in the category of parents who understand the child's difficulties with the disease and less in the category of parents who do not understand the child's difficulties with the disease than would be expected by chance.

- *Item 12:* $\chi2(2) = 20.988$, $p = .000$ - one positive and one negative adjusted residuals:
  - in the case of children older than 12 years, there were less participants in the category of parents who do not think their relationship with their other children has changed and more in the category who believe their relationship with their other children has changed than would be expected by chance.
- *Item 13*: $\chi2(6) = 28.163$, $p = .000$ - two positive and two negative adjusted residuals:
  - in the case of children aged between 1 and 6, there were more participants in the category of parents who turn to their life partner and less in the category who turn to their friends than would be expected by chance,
  - In the case of children aged between 6 and 12, there were less participants in the category of parents who turn to their life partner and more in the category of parents who turn to their friends than would be expected by chance.
- *Item 14*: $\chi2(2) = 11.158$, $p = .004$

with two positive and two negative adjusted residuals:

- in the case of children aged between 1 and 6, there were less participants in the category of parents who turned to a psychologist and more in the category who did not turn to a psychologist than would be expected by chance,
- in the case of children aged between 6 and 12, there were more participants in the category of parents who turned to a psychologist and less in the category of parents who did not turn to a psychologist than would be expected by chance.

- *Item 15:* $\chi2(2) = 12.470$, $p = .002$ - two positive and two negative adjusted residuals:
  - in the case of children aged between 1 and 6, there were less participants in the category of parents who turned to a psychiatrist and more in the category who did not

turn to a psychiatrist than would be expected by chance,

- in the case of children aged between 6 and 12, there were more participants in the category of parents who turned to a psychiatrist and less in the category of parents who did not turn to a psychiatrist than would be expected by chance.

- *Item 16*: $\chi 2(6) = 13.672$, $p = .034$ - two positive and two negative adjusted residuals.

- in the case of children aged between 1 and 6, there were less participants in the category of parents who feared death and more in the category who feared the disease than would be expected by chance,

- in the case of children aged between 6 and 12, there were more participants in the category of parents who feared death and less in the category of parents who feared the disease than would be expected by chance.

DISCUSSION

Dealing with a rare disease is a challenge for both patients and parents, but also for the medical team. A multidisciplinary team is needed, to share decision and knowledge. Individually tailored treatments are provided, that is why the treatment is also a source of psychological distress, especially when family is vulnerable (single caregiver, unemployed or family difficulties) or the child presents some problems (behavioral problems, deficiencies, adolescence, psychological disturbances).

The present research focused on the caregiver's opinion regarding some aspects of the parent and the child's life. The results showed that mothers are more involved in child's treatment, and this aspect is revealed also by fathers who were questioned. Treatment, in case of rare disease, is extremely important, and closely-supervised diets or early identification in the changes in the patient's physical status are extremely important (13,14). This aspect has also been identified by other researchers, showing that mothers are more often hospitalized or more involved in child' treatment and diets. Mothers seems to need more psychological support and they are more often looking for a professional help.

No significant associations were identified between parents' gender and environment (rural/urban) and each of the instrument's items. In a study of Medfort *et al.* (15) it was pointed that socioeconomic factors were not associated with parental wellbeing among parents of children diagnosed with phenylketonuria. Some other studies showed that age of the child did not impact the difference of quality of life between single and partnered parents (10).

The limitations of the study. The first limitation is due to the small number of parents. This aspect is due to the small number of children registered in the university hospital where the study was conducted. The second limitation is determined by the unbalanced number of parents considering the gender, so comparative results were not possible to be revealed. Taking into consideration the limitations of the study, results should be treated with caution.

CONCLUSIONS

For both patients and families, living with a rare disease is a ongoing learning experience. Research should address the many challenges in the management of rare disease and focus on identifying factors that improve life for people with rare diseases. Patient well-being does not involve

only medical care, but also psychological support, for both patient and caregivers, especially mothers, single parents or the unemployed. Financial expenses and investments in studying rare diseases is approached differently by medical teams from different countries. This vulnerable population must be cared for carefully to reduce psychological and physical distress for those affected by this type of illness.

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TRATAMENTUL CHIRURGICAL AL TUMORILOR CUTANATE. PREZENTARE DE CAZURI

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SURGICAL TREATMENT OF SKIN TUMORS. CASE REPORT (Abstract): This is the overview of several hospitalized cases of skin tumors this year based on which we analyzed the information in the literature concerning the clinical features, the evolution and therapy of these skin neoplasms. The tumors that develop in the skin fibrous tissue sometimes on a prior lesion need to be analyzed from a multidisciplinary perspective, therefore it is essential for the surgeon, the pathologist and the oncologist to consistently cooperate and to solve all issues related to the evolution of the case, which may be at times less favorable. **Patients and method:** In what follows we will introduce two clinical cases of dermato-fibrosarcoma protuberans (Darier-Ferrand) met in our recent practice this year, accompanied by the data found in the specialized literature concerning the clinical features, evolution and therapy of these quite rare skin lesions. Also we present another two cases of skin neoplasm. **Results:** All operated patients have survived since the surgery without any indication of relapse. **Discussion:** The attention of the surgeons is focused quite rarely on such skin tumors, since the patients with such lesions are usually treated by dermatologists. This cases we met in the recent years made us analyze the specialized literature in this field and to draw different conclusions. **Conclusions:** Each skin tumor where there are doubts concerning the diagnosis must be treated with extreme care and caution. We personally prefer a larger excision of an apparently benign tumor rather than making an insufficient excision. The operated patients must be consistently monitored throughout their lives. **Key words**: SKIN TUMORS, DERMATO-FIBROSARCOMA PROTUBERANS , TUMOR RELAPSE, METASTASES.

Aceste tumori care se dezvoltă din țesutul fibros de la nivelul pielii (tumori fibrohistiocitare), uneori pe o leziune preexistentă, necesită o abordare multidisciplinară fiind necesară colaborarea coerentă dintre chirurg, anatomopatolog şi oncolog şi pentru a nu avea surprize neplăcute datorate evoluției ulterioare nefavorabile a cazului. *Dermatofibrosarcomul protuberans* (Darier-Ferrand) este o tumoră cu malignitate intermediară care recidivează frecvent şi poate metastaza dar numai după recidive repetate. Afectează de predilecție tinerii şi adulții de sex masculin. În toate cazurile de leziuni maligne rolul chirurgului devine important deoarece excizia largă a tumorii cu limite de securitate oncologică reprezintă componenta principală a protocolului terapeutic. Prezentăm două cazuri clinice de dermatofibrosarcom întâlnite în practica noastră din ultimii ani, împreună cu datele găsite în literatura de specialitate cu privire la particularitățile clinice, evolutive şi terapeutice ale acestor leziuni cutanate destul de rar întâlnite.

MATERIAL SI METODA:

Cazul 1. Pacientul S. S. de 39 de ani se prezintă la un consult de rutină afirmând că este deranjat din ce în ce mai des de către o formațiune proeminentă situată în 1/3 inferioară a gambei stângi, pretibial care la fiecare atingere mai tare de alte corpuri sângerează uşor şi chiar îl doare. Pacientul prezintă această formațiune cutanată de aproximativ 15 ani, dar despre care poate spune că a fost mai mică inițial şi că a crescut în volum în ultima perioadă de timp. La examenul obiectiv se constată pe fața antero-internă a gambei stângi, în 1/3 inferioară, pretibial o formațiune tumorală cutanată cu un mic pedicul şi un aspect de "ciupercă" cu diametrul de circa 1 cm, de culoare vineție şi aspect conopidiform la exterior.

Având acceptul pacientului se excizează limitat această formațiune cutanată, sub anestezie locală şi se trimite la examen anatomopatologic.

Macroscopic tumora are aspect de "ciupercă" lobulată, de culoare violacee, vineție, de aproximativ 1cm diametrul, de consistență crescută, iar examenul microscopic evidențiază celularitate tumorală asemănătoare fibroblastelor, cu aranjament storiform în jurul vaselor de sânge caracteristice dermatofibrosarcomului protuberans (fig. 1).

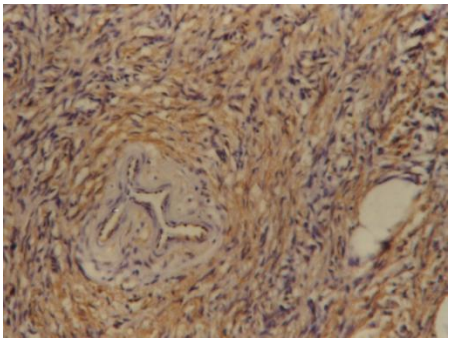

Fig. 1. Ob 20x.Col. IHC pentru CD34-pozitivitate citoplasmatică in celulele tumorale

În aceste condiții am reintervenit chirurgical practicând excizie largă cu limite de securitate oncologică în suprafață şi în profunzime în jurul cicatricei (fig. 2) de după prima intervenție chirurgicală acoperind defectul cutanat rămas printr-un lambou romboid rotativ. În acelaşi timp am mai excizat două formațiuni situate premaleolar drept pentru a fi siguri că pacientul nu mai rămâne cu alte posibile leziuni.

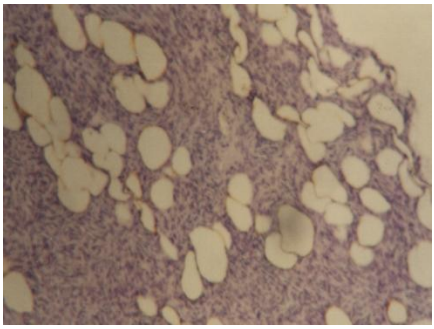

Fig. 2. Ob20x.Col.IHC pt S100-invazie în țesutul adipos

S-au efectuat testele de imunohistochimie care au evidențiat pozitivitate citoplasmatică intensă in toate celulele tumorale pentru Vimentin şi CD34 şi focală pentru Actin SM şi negativitate pentru S100, CD68,CKc,HMB45.De asemenea s-a decelat pozitivitate nucleară intensă si omogenă pentru Ki67 in 4-5% din celulele tumorale ceea ce a permis incadrarea cazului in grupa de malignitate joasă. Testele de imunohistochimie confirmând diagnosticul de dermatofibrosarcom protuberans şi prin excluderea altor forme de exemplu dermatofibromul, pacientul a fost îndrumat către medicul oncolog pentru luare în evidență şi supraveghere ulterioară. Evoluția pacientului a fost favorabilă neconsemnându-se recidivă locală şi nici un fel de metastaze la 18 luni de la intervenția chirurgicală.

Cazul.2. Pacientul G.H. de 38 de ani se prezintă la un consult chirurgical cu bilet de trimitere de la medical dermatolog pentru prezența unei formațiuni situată la nivelul trunchiului, dorsal, pe spate fiind suspicionat de la început de un melanom malign al trunchiului. S-a intervenit chirurgical practicându-se de această dată excizia cutanată în limite oncologice *per primam* .

Examenul anatomopatologic microscopic evidențiază caracteristicile unui dermatofibrosarcom protuberans (fig. 3), iar imunohistochimia confirmă diagnosticul (fig. 4).

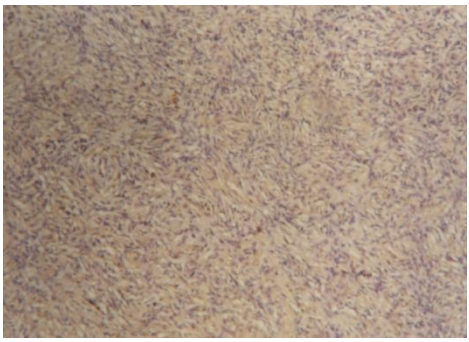

Fig. 3. Ob.20x. Col.IHC pt Vimentin-aranjament storiform

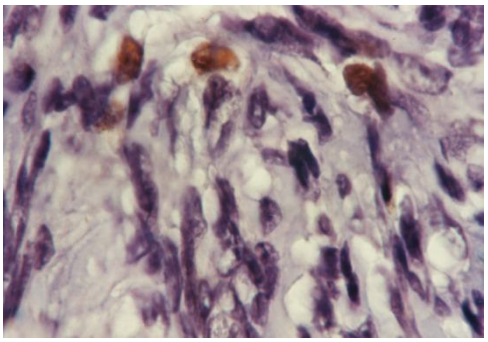

Fig. 4. Ob100x Col.IHC pt Ki67 – colorație nucleară difuză şi omogenă

Cazul 3. Pacientul Gh. S. de 71 de ani se prezintă în serviciul nostru pentru a se opera de o formațiune tumorală cutanată mare situată în regiunea temporală dreapta fiind refuzat de către serviciul de chirurgie al spitalului județean de care aparținea.

După pregătirea de rigoare datorită tarelor asociate s-a intervenit chirurgical sub anestezie locală practicându-se excizia acestei formațiuni. Examenul anatomopatologic efectuat.

Fig. 5. Tumora cutanată a pacientului Gh. S

Cazul 4. Pacientul C. B de 76 de ani se prezintă în serviciul nostru fiind deranjat de o formațiune axilară stângă mare.

După o scurtă investigare şi pregătire

s-a intervenit chirurgical sub anestezie locală şi s-a extirpat această formaţiune. La explorările efectuate s-au decelat metastaze hepatice. Examenul anatomopatologic efectuat a decelat degenerare sarcomatoasă. Pacientul a fost îndrumat către serviciul de oncologie pentru tratament complementar.

Fig. 6. Formaţiunea cutanată a pacientului C. B.

DISCUŢII

Dermatofibrosarcomul protuberans (Darier-Ferrand) este o tumoră cu malignitate intermediară care recidivează frecvent şi metastazează după recidive repetate.

Afectează de predilecţie tinerii şi adulţii de sex masculin. Clinic tumora se poate dezvolta oriunde dar apare mai frecvent pe trunchi şi pe extremităţi. Evoluţia tumorilor este marcată de numeroase recidive, metastazele fiind mai rare (apreciate la 1-2%) şi sunt localizate în plămâni sau în ganglionii limfatici regionali (1). Microscopia electronică a pus în evidenţă faptul că celulele tumorale sunt fibroblaste deoarece au un reticul endoplasmatic dezvoltat care sintetizează activ colagen. Diagnosticul acestor forme tumorale se realizează strict prin examen histopatologic, la simplul examen microscopic adăugându-se recent si criteriile imunohistochimice bazate pe o serie de markeri, şi anume: Vimentin+, S100−, CD34+(2).

Incidenţa recidivelor locale şi a metastazelor este in toate referinţele studiate mai mare la cei eronat diagnosticaţi şi deci trataţi incorect iniţial. Mortalitatea legată de evoluţia neoplaziei variază intre 0-6% la 10 ani de la prima intervenţie chirurgicală şi este semnificativ redusă (0-1%) la cei trataţi iniţial corect(3,4).

Tratamentul de elecţie este cel chirurgical si constă in excizia tumorii in limite de siguranţă oncologică, apreciate prin examenul histopatologic extemporaneu (*„frozen - section") sau* la parafină urmat, dacă este cazul, de reexcizie. Majoritatea autorilor consideră că o limită de 2 cm de la marginea macroscopică a zonei modificate este suficientă. La aceasta se asociază evidarea limfoganglionară, locoregională dacă este prezentă adenopatia. În cazul recidivelor, se fac excizii largi şi evidare limfoganglionară de principiu. În ambele situaţii, radioterapia se poate asocia postoperator (4).

În cazul tumorilor prezentate tardiv, adesea metastazate, se consideră că excizia

chirurgicală îmbunătăţeşte prognosticul vital prin încetinirea evoluţiei tumorilor secundare .Sintetizând, in final, datele din literatură, am remarcat faptul ca există mai multe puncte de consens, dar si aspecte aflate încă in dezbatere (cum ar fi eventualele criterii de clasificare).

Punctele de consens acceptate sunt utile practicienilor:

- potenţialul de malignizare a oricărei formaţiuni cutanate, in special a celor expuse la stimuli mitotici repetaţi, constituie un risc real;
- posibilitatea existenţei in evoluţia tumorii a unei perioade lungi asimptomatice sau cu semne clinice şterse, nespecifice, impune prudenţa in evaluarea tuturor leziunilor cutanate;
- frecventele erori de diagnostic, in special in cazul tumorilor suprainfectate/ulcerate pot influenta negativ evoluţia;
- excizia chirurgicală corectă reprezintă componenta de bază a tratamentului;
- tratamentul chirurgical incomplet/incorect are consecinţe cel puţin la fel de nefaste ca şi lipsa tratamentului;
- posibilitatea decelării recidivelor locale sau a metastazelor la un interval mare de timp după cura chirurgicală aparent corectă , impune urmărirea pacienţilor pe termen lung (5,6).

CONCLUZII

În faţa celui mai mic dubiu de diagnostic pentru orice formaţiune tumorală cutanată atitudinea medicală trebuie să fie prudentă. Din acest motiv trebuie să efectuăm o excizie mai largă de la bun început a unei formaţiuni benigne decât o excizie insuficientă a uneia maligne.

Examenul histopatologic al piesei este obligatoriu. Postoperator pacientul trebuie îndrumat către medicul oncolog pentru a fi luat în evidenţă.

Monitorizarea pacienţilor trebuie făcută periodic la început, apoi anual.

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OPȚIUNI DE TRATAMENT ÎN TUMORILE DE UNGHI INTERN AL OCHIULUI

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TREATMENT CHOICES IN THE TUMORS OF THE INNER ANGLE OF THE EYE (Abstract). Surgery of the tumors of the inner angle of the eye is difficult given the fact that beside tumor size, local peculiarities (concave area, thin and adhering skin, internal ligament, orifices, and tear duct), eyelids -mobile structures, vicinity and importance of visual receptor) need to be considered. **Aim:** To determine, based on data in the literature, what are the treatment choices in the various types of tumors of the inner angle of the eye. **Material and methods:** During a six-year interval (2004-2009) we diagnosed with and treated for tumors of the inner angle of the eye 135 patients. Patients age, sex, environment, causes – tumor epidemiology, and course length have been studied A thorough history, including the clinical features and progression of the tumor, previous treatments, as well as aspects related to the general pathologic picture was obtained from every patient. The treatment included tumor excision and subsequent reconstruction, the methods used for covering the defect and the type of used tissue depending on the local characteristics. The main areas of the study are: type and onset area of the tumor, tumor size and implicitly the size of postsurgical defect and the method for the reconstruction of postsurgical defect. **Results:** The reconstruction plan was individualized and included at least one alternative option depending on the size and depth of the excision. Aiming at best functional and esthetic results has lead to changes in the classical techniques which proved beneficial. **Conclusions:** The comparative analysis of these methods allowed us finding the most effective treatment of these tumors. For best results the collaboration plastic surgeon-ophthalmologist-maxillofacial surgeon-neurosurgeon was often required. We support the radical tumor removal with intraoperative pathologic confirmation. Surgical approach according to a previously established plan gives treatment safety, this paper suggesting the best type of surgery planning, thus representing a short guide to preoperative options. **Key words:** TUMOR, INNER ANGLE OF THE EYE, TUMOR REMOVAL, SURGICAL RECONSTRUCTION

Patologia tumorală a ariei interne a ochiului, în stadiile incipiente, nu prezintă dificultate pentru un chirurg, deoarece pleoapele au o structură elastică, excizia unei formațiuni cu diametrul sub 0,5 cm putînd beneficia de o sutura directă a defectului (1, 2).

În mod normal o pierdere de substanță mai mică de 1/3 din lungimea pleoapei poate fi compensată prin adaptarea ulterioară a țesuturilor. Totuşi în aceste cazuri plastiile locale cu triunghiuri încrucişate ori lambouri de rotație sau avansare se impun pentru o vindecare fără sechele cicatriciale

importante.

Formațiunile tumorale cu diametre ce impun o excizie mai mare de 1-1,5 cm obligă la folosirea unei metode de acoperire de tipul plastiei cu piele liberă, lambouri de rotație ample sau lambouri pediculate. Uneori reconstrucțiile acestei regiuni se pot practica folosind lambouri pediculate lungi sau cele liber transferate. În studiul nostru acestea au fost folosite limitat doar în cazurile în care marimea exciziei impunea o suprafață mare de acoperit (3).

Din punct de vedere al exciziei tumorii, condițiile locale nu permit ca acestea să fie largi, cu asigurarea unor limite de siguranță suficiente. De aceea considerăm că se impune utilizarea în cazul tumorilor din această regiune a unei metode care să asigure o excizie limitată a țesuturilor, cu o pierdere de substanță minimă în afara leziunii tumorale (4).

În reconstrucția postexcizională în afară de dimensiunea tumorii, se va ține seama și de particularitățile locale: tegumente aderente, caruncula și ligamentul intern, orificiile și canalul lacrimal, peoapele – structuri mobile și structura lor, vecinatatea și importanța receptorului vizual (5).

Utilizarea grefelor de piele în refacerea defectelor de substanță la nivelul jumătății interne a pleoapelor și a unghiului intern, reprezintă o metodă care oferă, în general, satisfacție în ceea ce privește calitatea țesutului și aspectul acestuia în timp.

Utilizarea lambourilor de vecinatate se impune ca o consecință a defectelor de țesuturi postexcizionale, care interesează toate structurile moi, pînă la planul osos.

Au fost inventariate în acest scop metodele propuse în literatură, utilizându-se în relație cu caracteristicile locale pre- și postexcizionale toate tipurile de lambouri de vecinatate (6).

Bazîndu-se pe circulația întimplătoare, lambourile de vecinătate pot fi translate sau rotate, acoperind astfel defectul postexcizional. Deşi cu un țesut de calitate apropiată celui a unghiului intern, aceste lambouri sunt limitate de dimensiunea pleoapei şi de menținerea funcționalității ei (7).

MATERIAL ŞI METODE

Am realizat un studiu pe un lot de 135 pacienți cu tumori ale ariei unghiului intern al ochiului, în perioada 2004 – 2009, în cadrul Clinicii de Chirurgie Plastică Reconstructivă a Spitalului Clinic de Urgențe Iaşi.

S-a luat în considerație vârsta, sexul, mediul de proveniență, caracteristicile tumorii. Am evaluat referințele din literatura de specialitate pentru metodele de tratament aplicate în relație cu particularitățile pacienților incluși în studiu (8).

Informațiile au fost acumulate prin anamneză, consemnând detaliile evolutive şi caracteristicile clinice ale tumorii, tratamentele efectuate anterior şi comorbiditățile fiecărui pacient.

Am stabilit planul operator pentru fiecare caz în parte, utilizînd excizia cea mai sigură şi completă pentru eradicarea formațiunii tumorale şi estimând modalitatea de reconstrucție postexcizie (9).

Experiența practică chirurgicală acumulată a permis utilizarea metodelor cele mai eficiente în reconstrucția defectelor tisulare postexcizionale, cu efecte funcționale şi estetice minime (10, 11).

Au fost apreciate în studiul comparativ rapoartele regionale față de elementele structurale importante (caruncură, ligament intern, canale lacrimale); tratamentul chirurgical aplicat (tipul de excizie); tipul de reconstrucție postexcizională în relație cu dimensiunile defectului de țesut rezultat;

rezultatele cicatriciale şi funcțional - estetice postoperatorii.

Direcțiile de studiu au inclus: tipul, aria de debut şi dimensiunile tumorii, extinderea defectului postexcizional şi metoda de reconstrucție a acestuia.

REZULTATE ŞI DISCUȚII

Debutul tumoral se realizează la nivelul structurilor de vecinatate, cel mai frecvent la nivelul pleoapei inferioare, de unde datorită laxității țesuturilor se produce relativ rapid o invazie tumorala locală. La aceasta contribuie mobilitatea zonei, vascularizația optimă, umiditatea şi traumatismele locale mai frecvente.

Tumorile benigne sunt semnificativ mai numeroase (71,1%) decît cele maligne, şi sunt reprezentate ca formațiuni tegumentare în stadiul incipient, necomplicat, pentru care sunt suficiente excizia şi sutura (χ^2=141,80; GL=1; p<0,001) (fig. 1).

Tumorile maligne ale unghiului intern cu 39 (28,9%) cazuri au fost în majoritate carcinoame bazocelulare cu stadiu evolutiv de la ulcerație la tumora vegetantă, cu sau fără infiltrație locală (fig. 2).

Tratamentul leziunilor necomplicate s-au realizat în majoritatea cazurilor prin excizia leziunii şi sutura tegumentară. Plastiile locale s-au impus ca urmare a dimensiunilor tumorale sau pentru un aspect cicatricial mai bun din punt de vedere estetic. Defectele postexcizionale estimate în cazul tumorilor complicate cu caractere clinice clare de malignitateau fost preponderent de dimensiuni ce depaşesc 1,5 cm diametru. Acest aspect corelat cu condițiile regionale justifică complexitatea reconstrucției (11, 12) (fig. 3).

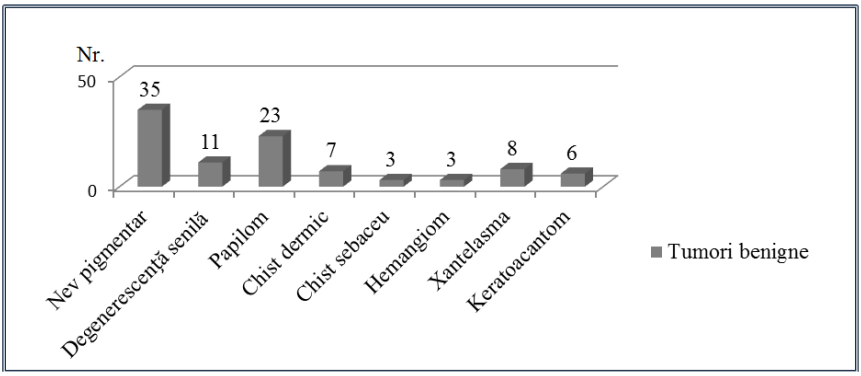

Fig. 1. Frecvența tumorilor benigne ale ariei unghiului intern la pacienții incluşi în studiu

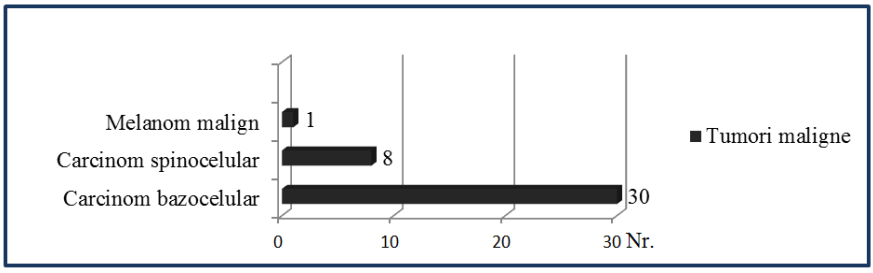

Fig. 2. Frecvența tumorilor maligne ale ariei unghiului intern la pacienții incluşi în studiu

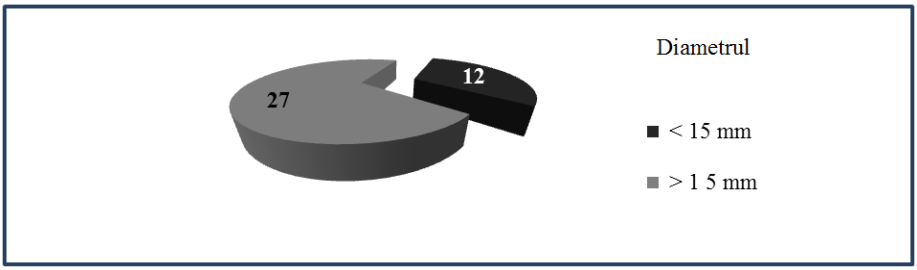

Fig. 3 Evaluarea comparativă a dimensiunilor defectelor de țesut după excizie, la pacienții incluşi în studiu

Planingul tratamentului în cazul leziunilor tumorale complicate a avut în vedere dimensiunea tumorală, corelată cu estimarea clinică a invaziei locale. În afară de aceste aspecte s-a ținut cont de capacitatea de reconstrucție şi de factorul estetic şi importanța acestuia pentru pacient (13) (tab. I).

TABELUL I

Abordul chirurgical al unghiului intern în realție cu dimensiunea tumorii

Tehnica	Marimea defectului		
	Mic (<5mm)	Mediu (5-10mm)	Mare (>10mm)
Sutură primară	X		
Sutură primară + cantotomie	X	X	
Sutură primară + cantotomie + cantoliză		X	
Sutură primară + cantotomie + cantoliză + lambou de veciatate		X	X
Grefă tarso-conjunctivală + lambou tegumentar		X	X
Grefă tarso-conjunctivală + grefă de piele		X	X
Grefă marginală compozită + grefă de piele		X	X
Lambou tarsoconjunctival + grefă de piele			X
Grefa compozită + lambou facial			X

În raport cu grupele de vârstă am utilizat grefa de piele liberă la pacienții cu vârsta de peste 65 ani, însă distribuția cazurilor în cadrul acestei perioade, nu prezintă diferențe semnificative comparativ cu practicarea lamboului de vecinătate (χ^2=7,96; GL=5; p=0,158) sau a celui pediculat (χ^2=5,46; GL=2; p=0,065) practicat numai la pacienți cu vârsta cuprinsă între 45-64 ani (fig. 4).

Reconstrucțiile postexcizionale au necesitat reinserția ligamentului intern în două cazuri, în care invazia tumorală interesa această structură, iar după retracția

lamboului refacerea unghiului intern a impus reconstrucția ligamentului intern. Proceduri de reconstrucție asociate sau practicat la un număr redus de pacienți (χ^2=48,18; GL=1; p<0,001).

Tumorile ce depășec dimensiuni de 10 mm, în contextul regiomal, pot fi asociate chiar în cazul celor benigne cu rezultate funcționale modeste (tab. II).

Planificarea reconstrucției necesită să includerea inițială a unor variante în relație cu dimensiunile şi profunzimea exciziei şi care vor fi adaptate la necesitățile operatorii.

Urmărirea unor rezultate cu caracteristici funcționale şi estetice cît mai bune a dus la modificarea unor tehnici clasice, care şi-au confirmat utilitatea în cadrul studiului (14, 15).

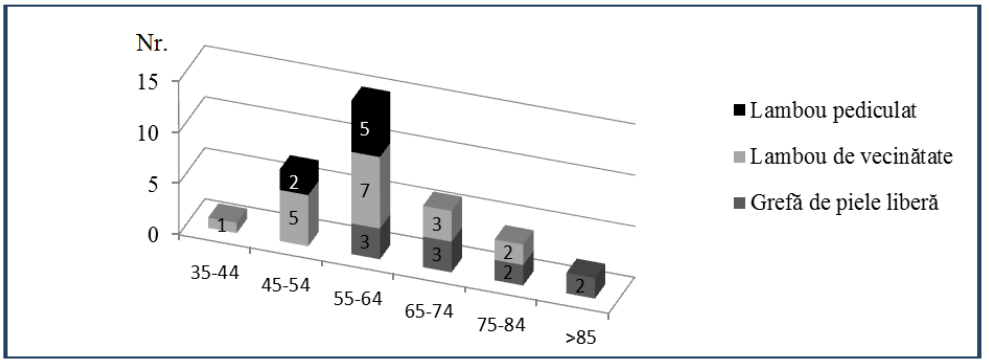

Fig. 4. Repartiția pe grupuri de vârstă a metodelor tratamentului chirurgical al tumorilor complicate

TABELUL II

Evaluări asupra rezultatelor tratamentelor chirurgicale

Tipul tumoral	Dimensiunile formațiunii (mm)	Tipul de recondtrucție	Rezultate cicatriciale	Rezultate functionale
Tumori benigne	<5	sutură	+++	+++
	5-10	plastie locală	+++	+++
	>10	plastie locală	+++	+
Epiteliom bazocelular	<5	plastie locală	+++	++
	5-10	lambou vecinătate	++	+/-
	>10	grefa de piele, lambou pediculat, alt lambou	+/-	+/-
Epiteliom spinocelular	<5	grefa de piele, lambou vecinătate	++	+
	5-10	grefa de piele, lambou pediculat	+	+/-
	>10	lambou pediculat, alt lambou	+/-	-

Dimensiunile exciziilor tumorale mai mici de 5 mm au fost abordate prin suturi directe sau plastii cu lambouri încrucişate în relație cu poziționarea acestora : localizările la distanță de carunculă – sutură directă sau localizările din preajma caruncu-lei – plastie cu lambouri.

In leziunile cu dimensiuni cuprinse între 5 - 10 mm, diametrul exciziei, a fost utilizată pentru acoperirea defectului grefa de piele (daca excizia nu interesa toate planurile până la cel osos).

Practicarea lamboului frontal a constituit a doua obțiune pentru cura reconstructivă a defectelor cu dimensiuni mai mari de 5 mm, acesta având o mobilitate mare dar şi posibilitatea ajustării în funcție de defectul postexcizional. Refacerea este grevată totuşi de grosimea tegumentului frontal (tab. III) (fig. 6).

TABELUL III

Reprezentarea schematică a rezultatelor

Marimea defectului (mm)	**Metoda**	**Nr.**	**Rezultate funcțional - estetice**
> 15	Transfer liber	0	+
	Lambouri pediculate	9	+
	Grefe de piele	26	++
<15	Lambouri de rotație	11	+++
	Lambouri triunghiulare	29	+++
	Lambouri de avansare	17	++++
	Aproximare directă	43	+++++

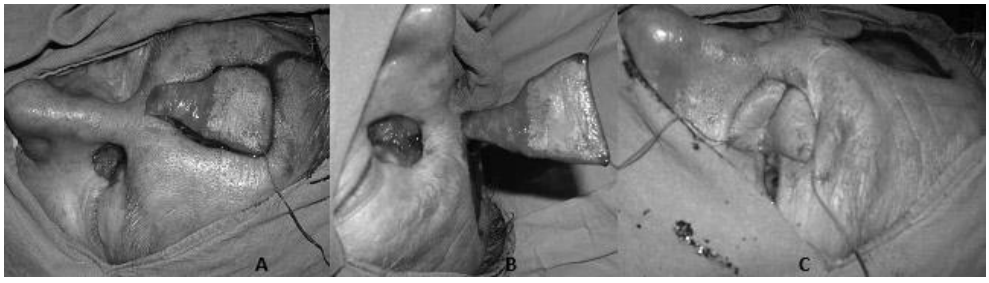

Fig. 6. Etapele operatorii cu lambou frontal dezepidermizat pentru unghiului intern

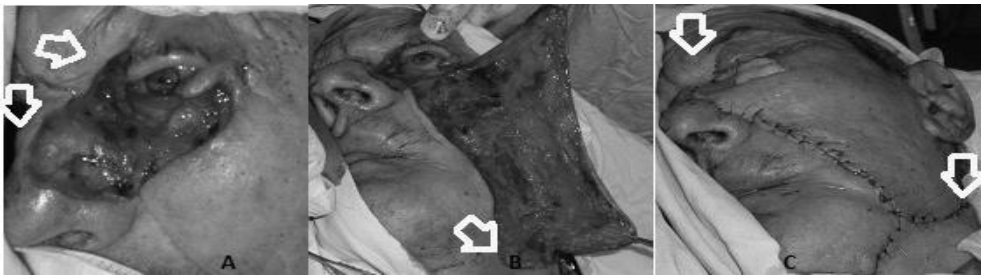

Fig. 7. Etapele operatorii cu lambou cervico-facial pentru tratamentul unui defect important al unghiului intern al ochiului

Pentru leziunile cu dimensiuni mai mari de 10 mm trebuie folosite lambouri care sa aducă un surlus de țesut, dar care să asigure şi o refacere de calitate a regiunii orbitare (fig. 7).

CONCLUZII

Analiza comparativă mai multor metode a permis aprecierea orientării spre alegerea unui tratament eficient al tumorilor de unghi intern al ochiului.

Planul operator a fost specific pentru fiecare formațiune tumorală în parte, necesitând de multe ori colaborarea între oftalmolog şi chirurgul plastician, dar şi cu chirurgul maxilo-facial sau chiar neurochirurgul.

Rezultatele cercetărilor pledează pentru abordarea formațiunii tumorale în mod radical, excizia completă a formațiunii trebuind certificată intraoperator, deoarece recidivele tumorale la acest nivel sunt urmate de cele mai multe ori de operații complexe, cu efect important asupra fizionomiei şi psihicului pacientului.

Abordarea chirurgicală după un plan prestabilit, cu variante posibile conferă siguranță în tratamentul tumorilor de la nivelul ariei unghiului intern al ochiului, iar lucrarea de față sugerează varianta optimă de planing al operației, constituindu-se ca un ghid succint de orientare preoperatorie.

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CEFALEEA DE CAUZĂ NAZALĂ – REALITATE SAU DIAGNOSTIC ERONAT ?

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SINONASAL HEADACHE – REALITY OR MIS-DIAGNOSIS? (Abstract): Facial pain can be the presenting, and sometimes the only, complaint of many disorders that originate from cranial structures. One causes of facial pain/headache is sinusitis, in both its acute and its chronic presentation. Successful management of headache is preceded by the identification of an etiology and precise diagnosis. **Material and methods**: A prospective study was carried out on a series of 71 patients treated in the ENT Department of Iasi "Sf. Spiridon" Hospital between January and august 2009 for pathology implying facial pain, headache, facial pressure, nasal obstruction, rhynoreea. The therapy was medical and surgical and pursued nasal desobstruction, sinusal drainage and ventilation. Data regarding different parameters were analyzed pre and post therapy. **Results:** After the proper treatment for each situation, the parameters analyzed improved significantly, although, in some cases the main accuses regarding the pain remained unchanged or slightly ameliorated. **Conclusions**: Depending on the clinical suspicion of the possible etiologies of facial pain and headache, the appropriate consultations should be made. For concerns of head and neck lesions or sinus-related headache, an otolaryngologist should be consulted. If a primary headache syndrome or a cranial neuralgia is of concern, the patient should be evaluated by a neurologist. Dentists and oral surgeons should be involved. In some cases, a psychiatry referral is appropriate. The surgical treatment can be effective if the pathology meets the criteria of sinonasal facial pain. **Key words**: SINUS-RELATED FACIAL PAIN, CHRONIC SINUSITIS, HEADACHE, NASAL ENDOSCOPY

Durerea este un simptom frecvent întâlnit în cadul afecţiunilor rinosinusale, datorită inervaţiei bogate senzitive şi vegetative a foselor nazale şi a anexelor acestora. Cefaleea este o durere situată deasupra liniei orbito-meatale iar o durere facială, inferior acesteia. Există numeroşi pacienţi cu cefalee sau durere facială ce consideră această suferinţă datorată unei sinuzite, diagnostic stabilit de ei-înşişi sau chiar de catre un medic (1) .

Cefaleea de origine rinosinusală se poate împarţi în două categorii:

- algiile simptomatice ale unei sinuzite, cu un diagnostic în general simplu,
- algiile fară sinuzită, mai dificil de diagnosticat, fiind considerate rezultatul unui dezechilibru trigemino-simpatic.

Cefaleea de cauză nazală suscită în continuare vii controverse. În cazul unui sindrom cefalalgic rino-sinusal, trebuie analizate cele trei mari mecanisme fiziopatolo-

gice de durere: durerea prin exces de nociceptie, durerea neurogenă şi durerea psihogenă. Recunoaşterea lor va orienta conduita terapeutică, dar nu de puţine ori, putem întâlni asocierea acestora (2).

Tegumentul jumătăţii superioare a piramidei nazale este inervat de nervii supratrohleari şi infratrohleari din ramului oftalmic al trigemenului, iar tegumentul jumătăţii inferioare de nervul infraorbitar, ram al nervului maxilar din trigemen.

Mucoasa nazală primeşte o inervaţie bogată din nervul oftalmic (V1), nervul maxilar (V2). Acesta din urmă este strâns conectat cu ganglionul neurovegetativ pterigo-palatin. Inervaţia sinusului maxilar este dependentă de ramuri ce provin din nervul maxilar: nervul mare palatin, nervul nazal, nervul infraorbitar. Celulele etmoidale anterioare sunt inervate de nervul etmoidal anterior, cele posterioare şi sfenoidul de nervul etmoidal posterior şi ramuri din sfenopalatin. Sinusul frontal primeşte o inervaţie din nervul supraorbitar, ram din oftalmic (V1).

Un stimul electric, chimic sau mecanic la nivelul mucoasei rinosinusale declanşează o durere locală şi/sau reflectată (iradiată). Zonele cele mai sensibile sunt cornetele nazale, ostiumurile sinusale, septul, canalul nazo-frontal. Mucoasa sinusală este puţin sensibilă. Cornetul inferior proiectează durerea la arcada dentară maxilară, procesul zigomatic, regiunea orbitară, cornetul mijlociu iradiază durerea spre regiunea temporo-zigomatică, ochi, frunte. Fenomenul de convergenţă a aferenţelor nociceptive trigeminale la nivelul cortexului senzitiv explică non-discriminarea senzorială între tegumentul şi mucoasa rino-sinusală (3) .

Durerea din sinuzită este binecunoscută, sinuzitele sunt acute sau cronice, limitate la un sinus sau la mai multe, infecţioase propriu-zise sau de însoţire a unui proces tumoral. Examenul clinic, endoscopic şi imagistic pun diagnosticul şi ghideaza atitudinea terapeutică. Tratamentul acestor dureri rinosinusale se suprapune cu tratamentul etiologic al afecţiunii declanşatoare, al sinuzitei. În toate cazurile, examenul endoscopic şi imagistic al nasului şi sinusurilor paranazale trebuie să elimine o patologie rinosinusală subjacentă.

Sechelele dureroase ale sinuzitelor operate - Traumatismul nervos periferic reprezintă principala cauză a sechelelor dureroase postoperatorii. Sinusul maxilar este cel mai frecvent implicat, cel mai rar sinusul frontal şi etmoidal. Durerea este de tip ”neuro-vascular” asociată unei afectari nervoase a nervului infraorbitar sau a ramurilor sale şi are un complex de manifestări vasomotorii şi secretorii foarte bogate: rinoree, lăcrimare abundentă, eritem şi edem cutanat. Algia evoluează în pusee, declanşată de variaţiile de temperatură, de stimularea mecanică a nervului. Tratamentul acestui tip de patologie dureroasă este în primul rând preventiv, în cursul intervenţiei chirurgicale iniţiale. Tratamentul curativ este dificil, având în vedere mecanismul neurogen al durerii şi constă în tratamentul cu antidepresive, antiepileptice, blocuri de ram nervos (6).

Cefaleea de ”vacum sinus” a lui Sluder (1927) - Este cauzată de obstrucţia mecanică aseptică a infundibulului frontal ducând la o hipopresiune dureroasă a sinusului frontal cu un edem secundar al mucoasei. Durerea orbito-frontală determinată de acest sinus este exacerbată de efortul de acomodare la citire. Restabilirea ventilaţiei sinusale face se dispară simptomatologia. Barotramatismul sinusului frontal este de doua ori mai frecvent decât al sinusului maxilar. Examenul imagistic confirma

hiperplazia in cadran a mucoasei sau epanşamentul intrasinusal sero-mucos sau serohematic.

Cefaleea din ageneziile sinusale (sinus frontal sau sinus maxilar) - Se caracterizează prin durere surdă, foarte rar intensă, cateodată asociată cu manifestari vasomotorii oculare. În realitate, maformaţia sinusală nu explică de una singură sindromul dureros, având în vedere frecvenţa ageneziilor sinusale asimptomatice, mai ales frontale descoperite imagistic (7).

Sindroml de ganglion pterigo-palatin al lui Sluder (1908) - În acest sindrom apare o durere paroxistică la rădăcina nasului şi periorbitar, unilaterală, iradiind catre mastoidă şi ceafă. Această durere se asociază cu manifestari vaso-secretorii: congestie nazală, strănut, lăcrimare, eritem hemifacial, senzaţie de plenitudine auriculară.

Anestezia pe cale endonazală sau infiltrarea ganglionului pterigopalatin cu xilocaina prin canalul palatin posterior pe cale transpalatină fac sa dispara simptomatologia. Acestă patologie se aseamană cu sindromul de nerv vidian al lui Vail.

Sindromul nervului nazal al lui Charlin - Acest sindrom este responsabil de dureri paroxistice la nivelul aripii nazale şi periorbitare, unilaterale asociate de manifestări vaso-secretorii: rinoree abndentă, strănut, lăcrimare, hiperemie palpebrală şi conjunctivală. Examenul clinic evidenţiază sensibilitatea ramurilor terminale ale nervului naso-ciliar. Anestezia capului cornetului mijlociu face sa dispara simptomatologia (2).

Aceste sindroame istorice ale lui Sluder şi Charlin sunt acum asimilate algiei vasculare a feţei (8).

Cefaleea "nazală" - De la începutul secolului, numeroase algii cranio-faciale fară cauză organică propriu-zisă cum sunt migrena şi cefaleea de tensiune, au fost considerate a avea o cauză nazală oligosimptomatică. Au fost incriminate deviaţiile septale, nasul ingust, hipertrofiile de cornete, sinuzitele posterioare latente etc. Multiple manevre chirurgicale cu viză antalgică au fost efectuate de rinologi intre anii 1940-1950. De aproape 30 de ani, cea mai mare parte a acestor intervenţii au fost privite cu neîncredere fiind considerate abuzive, chiar nefaste. În prezent, răspandirea examenului endoscopic, a imagisticii CT si IRM rinosinusale, progresul neurochimiei au permis reconsiderarea acestor algii aşa zise "nazale" (9,10) .

Diferite modificări anatomice ale cavităţilor rinosinusale au fost descrise: un contact mucos între cornetul nazal superior sau mijlociu şi septul nazal, o deviaţie de sept cu o creastă septală, o concha bulosa mai voluminoasă, un mucocel, dehiscenţa intrasinusală a nervului oftalmic. Aceste anomalii sunt luate în considerare dacă sunt de aceeaşi parte cu durerea craniofacială (11,12).

MATERIAL ŞI METODĂ

Am evaluat un numar de 71 de pacienţi internaţi în Clinica ORL a Spitalului "Sf. Spiridon" Iaşi în perioada ianuarie-august 2010 pentru investigaţii şi tratament privind o patologie rinosinusală însoţită de cefalee, durere cranio-facială, senzaţie de presiune facială.

La examenul clinic se evidenţiază următoarea patologie rinosinusală asociată sau factori favorizanţi (fig. 1): deviaţie sept nazal, rinită cronică hipertrofică, polipoză nazală, focare infecţioase dentare.

Dintre cele mai importante acuze remarcăm durerea spontană, senzaţia de presiune facială, obstrucţia nazală, cefaleea, rinoreea anterioară sau posterioară.

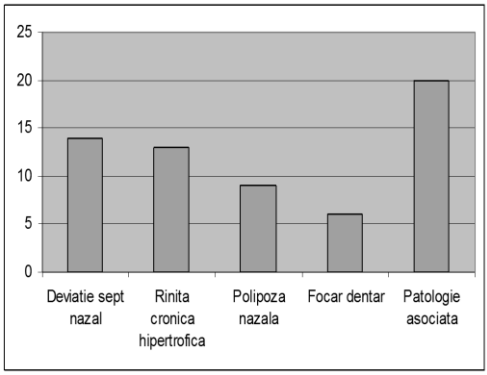

Fig. 1. Patologie asociată şi factori favorizanţi

Dintre investigaţiile imagistice, au fost disponibile radiografiile de sinusuri anterioare ale feţei, examene computer tomografice cu sau fără substanţă de contrast, investigaţii de imagistică prin rezonanţă magnetică. Pe aceste investigaţii s-au urmărit modificările anatomice şi patologice: deviaţii de sept nazal, creste septale, hipertrofii de cornete, puncte de contact osoase şi mucoase, voalarea sau opacifierea cavităţilor sinusale, prezenţa nivelului de lichid etc.

Mijloacele terapeutice au constat în tratament medicamentos, chirurgical sau combinat. Clasele de medicamente administrate au fost antibiotice, antiinflamatorii steroidiene şi nesteroidiene, antalgicele, vasoconstrictoarele nazale, corticoterapie topică nazală.

Tratamentul chirurgical a urmărit obstrucţia nazală, drenajul şi lavajul sinusurilor cu colecţii mucopurulente, cura radicală a sinusurilor afectate. Dintre intervenţii, au fost efectuate rezecţii subpericondromucoase ale septului nazal, septoplastii, mucotomii, cauterizari laser sau elecrocauterizări ale cornetelor nazale inferioare, excizia de concha bulosa, antrostomia maxilară intranazală, cura radicală a sinuzitei maxilare prin procedeul Caldwel-Luc, etmoidectomia anterioară.

În general pacienţii ce au suferit intervenţii chirurgicale au fost tamponaţi nazal pentru 24-48h sub protecţie antibiotică şi antalgice. Au revenit în clinică pe perioade de 7-10 zile pentru defibrinarea foselor nazale, panasamente, anemizari.

Vindecarea locală s-a obţinut la 14-20 zile postoperator constând în diminuarea zonelor denudate de mucoasă, dispariţia crustelor şi a secreţiilor seromucoase, dispariţia senzaţiei de obstrucţie nazală.

REZULTATE

Vom urmări în următoarele grafice evoluţia simptomatologiei comparativ pre şi postterapeutic.

Se observă diminuarea numărului de pacienţi ce acuzau durere facială precum şi scăderea severităţii acesteia (fig. 2).

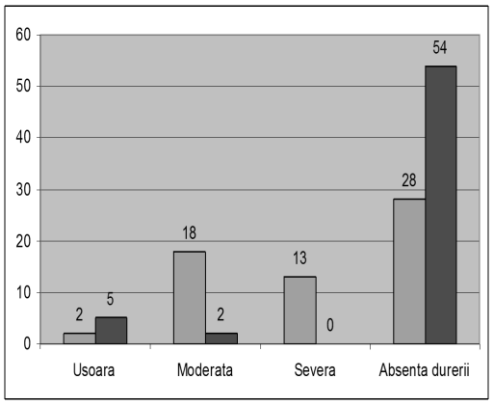

Fig. 2. Evoluţia durerii sub tratament

Senzaţia de presiune facială se diminuează semnificativ la marea majoritate a pacienţilor (fig. 3).

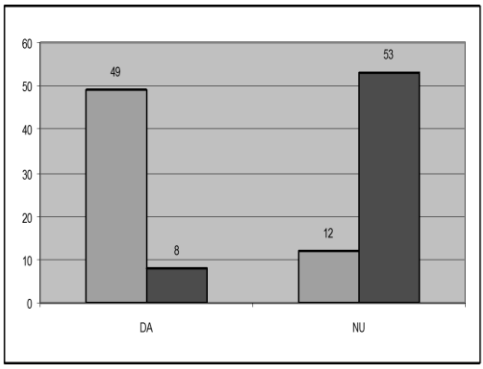

Fig. 3. Evoluţia senzaţiei de presiune facială

Postterapeutic, obstrucţia nazală a dispărut la marea majoritate a pacienţilor (fig. 4).

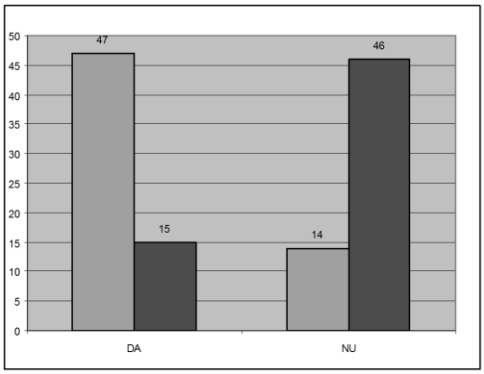

Fig. 4. Evoluţia obstrucţiei nazale sub tratament

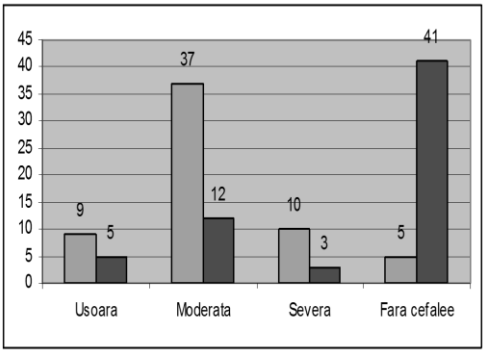

Fig. 5. Evoluţia cefaleei sub tratament

De asemenea s-a modificat semnificativ numarul pacienţilor ce sufereau de cefalee, la cei la care aceasta persistă scăzând în intensitate şi frecvenţă (fig. 5).

Un alt parametru evaluat a fost rinoreea, care în lotul studiat a fost iniţial prezentă la 65,57% din cazuri, iar postterapeutic s-a diminuat pâna la 22,95%.

DISCUŢII

Tarabichi raportează un studiu efectuat pe 82 de pacienţi operaţi pe cale endoscopică de sinuzită cronică asociată cu durere cranio-facială. Dupa un an de la operaţie, 38% din pacienţi acuzau în continuare durere deşi nu mai exista nici un tip de sinuzită; de asemenea, nu s-a putut stabili nicio corelaţie între severitatea durerii şi extensia sau localizarea afectarii rinosinusale preoperatorii. Autorii au concluzionat ca cefalalgiile non-sinusale trebuie luate în considerare la aproximativ o treime din pacienţii operaţi pentru sinuzită (4). O anamneză preoperatorie riguroasă trebuie să precizeze tipul durerii, topografia, factorii declanşatori, semnele asociate nazale, oculare, digestive. O cauză frecventă de cefalee (migrena, cefaleea de tensiune) nu exclude coexistenţa altui tip de cefalee.

În 1988, M. Stammberger a postulat că un contact mucos endonazal poate fi responsabil de cefalee prin intermediul secreţiei locale a unui neuropeptid, substanţa P. Stimularea mecanică, chimică, termică a fibrelor C ale nervului oftalmic sau a nervului maxilar determină eliberarea de diverse neuropeptide cum ar fi substanţa P, dar şi calcitonin-gene-related-peptide (CGRP) sau neurokinina A. Ramurile colaterale ale trigemenului, mai ales nervul oftalmic, ce inerveaza de asemenea meningele precum şi vasele sangvine intracraniene constituie sistemul ”trigemino-vascular” al lui Moskowitz.

Neuropeptidele algogene produse de fibrele nervoase trigeminale sunt la originea unei vasodilataţii, a unei extravazări plasmatice prcum şi a unei infiltrări plasmocitare. Acest mecanism fiziopatologic neuro-vascular este de asemenea incriminat in migrena şi algiile vasculare ale feţei (13).

Aplicarea locală sub control endoscopic a unui amestec de tetracaină 2% şi efedrină 1% pe zonele de conflict mucos pot determina ameliorarea sau dispariţia durerii. În acest caz, un tratatment chirurgical corector poate fi recomandat. Aplicarea mucoasă de capsaicină se poate efectua pentru a realiza o desensibilizare a receptorilor substanţei P şi degenerarea fibrelor C nociceptive aferente, fară modificarea sensibilitaţii mucoasei nazale. Tratamentul local cu capsaicină poate reduce durerea şi efectele vasculare ale aplicarii pe mucoasă de acid lactic sau de ser salin hiperton.

Indicaţia operatorie a fost aşadar propusă la adultul sau copilul care îndeplinea un anumit numar de criterii:

- cefaleea a fost tratată de un neurolog şi nu s-a ameliorat prin terapie medicală,
- nu există semne de sinuzită propriu-zisă decelabilă prin examen endoscopic sau imagistic,
- endoscopia şi imagistica pun în evidenţă un contact mucos de aceeaşi parte cu dureea,
- cefaleea dispare sau se ameliorează după aplicaţia topică a unui anestezic local pe zona de conflict mucos.

Intervenţia chirurgicală sub control endoscopic corectează anomalia anatomică decelată: turbinectomie sau turbinoplastie, septoplastie, etmoidectomie anterioară etc. Corticoterapia locală se poate prescrie postoperator dacă fenomenele inflamatorii asociate sunt importante (14).

Rezultatele apreciate cu ajutorul Scalei Analogice Vizuale (VAS) nu evidenţiază diferenţe semnificative pe vârste, sexe, tip de durere, vechimea acesteia, manevra chirurgicală efectuată. Dispariţia durerii sau scăderea frecvenţei de apariţie au fost înregistrate între 78 si 91% din cazuri la 12 luni.

Această ipoteză fiziopatologică a contactului mucos endonazal ce generează cefalee ramane controvesată. Un studiu recent al lui D. West a fost efectuat pe 101 pacienţi ce au fost consultaţi ORL pentru durere cranio-facială fară nici un semn obiectiv de sinuzita (endoscopic si CT). Studiul clinic a permis clasificarea durerilor faciale într-un cadru neurologic clar definit de Societatea Internaţională de Cefalee cu excepţia durerii de segment mijlociu al feţei ce aparţine cefaleei de tensiune. La majoritatea pacienţilor s-a ameliorat simptomatologia la administrarea de doze mici de Amitriptilină (10 mg/zi pe parcurs de 6 săptamâni). În caz de eşec s-a administrat Carbamazepina sau Propranolol. Nici un pacient nu a fost operat de vre-un contact mucos.[15,16]

CONCLUZII

Durerea facială şi cefaleea reprezintă unele din cele mai frecvente acuze ce aduc pacienţii în cabinetul ORL. Foarte puţini pacienţi ce se prezintă la medic cu diagnosticul iniţial de sinuzită, chiar au sinuzita.

Pacienţii ce au cu adevarat sinuzită, însoţită de semne şi simptome nazale, examen endoscopic şi imagistic concludent pot fi trataţi eficient obţinându-se o rată crescută de succes cu diminuarea pâna la dispariţie a simptomatologiei.

Datorită progresului mijloacelor de investigaţie imagistică precum şi a disponibilităţii tot mai mari a examenului endoscopic nazal, pacienţii pot fi mai corect diagnosticaţi şi se poate face o selecţie mai

corectă a cazurilor ce necesită tratament chirurgical.

Tratamentul chirurgical ar trebui să ramană ultima soluţie terapeutică după efectuarea unor consultaţii interdisciplinare oftalmologice şi neurologice. Cazurile ce vor urma un tratament chirurgical trebuie atent selecţionate pentru a întruni cât mai multe criterii ce indică faptul ca sindromul cefalalgic este de cauza rinosinusală.

Chiar şi în aceste condiţii, un procent însemnat de pacienţi trataţi chirurgical nu prezintă dispariţia simptomatologiei sau ameliorarea semnificativă a acesteia.

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EVALUAREA FUNCȚIEI VIZUALE SUB TRATAMENT CU TOPICE ANTIGLAUCOMATOASE ŞI VASODILATATOARE SISTEMICE. STUDIU CLINIC PE LOTURI DE PACIENȚI CU GLAUCOM, ASISTAȚI ÎN CLINICA A II-A OFTALMOLOGIE, SPITALUL CLINIC DE URGENȚE „PROF. DR. N. OBLU" IAŞI

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ASSESSMENT OF VISUAL FUNCTION UNDER TREATMENT WITH TOPICAL ANTI-GLAUCOMA AGENTS AND SYSTEMIC VASODILATORS. CLINICAL STUDY ON GLAUCOMA PATIENTS (Abstract)**:** The authors present some observations regarding the use of topical anti-glaucoma therapy and systemic vasodilators. **Material and methods**: The study was carried out on a series of 996 glaucoma patients, of which 54.1% with primary open-angle glaucoma, 5.7% closed-angle glaucoma, and 17.6% with normal intraocular pressure. **Results**: Intraocular pressure and visual field were assessed after the use of such therapeutic methods as carbonic anhydrase inhibitors (dorzolamide) and prostaglandin (latanoprost) and dorzolamide (cosopt) association. **Conclusions**: The favorable progression of glaucoma could be improved by using new etiopathogenic mechanisms and treatment approaches. **Key words**: OPEN-ANGLE GLAUCOMA, ANGLE-CLOSURE GLAUCOMA, RETINAL CIRCULATION, TOPICAL THERAPY

Neuropatia glaucomatoasă este un răspuns la acțiunea factorilor de risc primar, interacționând cu cei secundari, între care şi predispoziția genetică, dar nivelul presiunii intraoculare (PIO) este factorul principal în inducerea neuropatiei optice (NO). Indiferent dacă NO, la om, este urmarea patologiei scurgerii trabeculare în glaucomul primar cu unghi deschis (GPUD) sau a celor secundare (GS) ori are o bază genetică, iar la animalele vertebrate (iepure, câine, şoarece, şobolan sau maimuță) este indusă experimental, PIO crescută determină o afectare a retinei. S-a confirmat şi posibilitatea ca o PIO scăzută, să determine o progresie mai lentă a modificărilor glaucomatoase.

La pacienții cu glaucom cu tensiune normală (GTN), factorii de risc primar pot fi reprezentați de modificările hemodinamice cronice, localizate în microcirculația oftalmică. Aceşti factori lezează, în principal, celulele ganglionare retiniene (CGR) şi axonii lor localizați în zone unde vascularizația este limitată, cum ar fi retina şi capul

nervului optic (microvascularizația *laminei cribrosa*).

Localizarea patologiei microvasculare în subregiuni de la nivelul capului nervului optic explică afectarea focală a CGR şi subțierea fibrelor nervoase, inițiind neuropatia optică glaucomatoasă, cu interes în cunoaşterea modelelor pentru dezvoltarea agenților neuroprotectori în glaucom (1, 2, 3, 4).

MATERIAL ŞI METODE

Studiul efectuat în Clinica a II-a Oftalmologie, Spitalul Clinic „Prof. Dr. N. Oblu", pe un număr de 8118 pacienți, internați în perioada 2006-2010, împărțiți în 2 loturi, cel tratat pentru afecțiuni generale oftalmologice (7122 cazuri: 87,7%), celălalt format din 996 (12,3%) pacienți cu glaucom, şi subîmpărțiți în relație cu forma clinică: 539 (54,1%) cu GPUD; 225 (22,6%) cu GTN; 57 (5,7%) glaucom primar cu unghi închis (GPUI) şi 175 (17,6%) cu GS.

Pentru studiul comparativ al efectelor neuroprotectoare a diferitelor programe terapeutice utilizate, pacienții cu GPUD şi GTN au fost împărțiți în 3 loturi: unul martor, cu pacienți care nu au primit medicație neuroprotectoare, fiind tratați prin scăderea PIO; al doilea care a primit medicație topică, certificată şi de alte studii (1, 2) ca având acțiune neuroprotectoare şi al treilea care a primit medicația locală pentru scăderea PIO, asociată cu una cu acțiune generală.

Cei 764 (76,7%) pacienți cu GPUD şi GTN au fost studiați în relație cu aspectul câmpului vizual la prima vizită (pierdere incipientă, medie şi avansată), conform Ghidului European de Glaucom (1, 2), constatându-se că 268 (35%) aveau o pierdere incipientă de câmp vizual, 442 (57,8%) - moderată şi 54 (7%) - avansată.

Dintre pacienții cu afectare incipientă, 165 (61,6%) au fost împărțiți în 3 subloturi, cu câte 55 (33,3%) pacienți care au fost informați asupra tratamentului ce li se va efectua, precum şi asupra efectelor adverse şi termenelor la care se va face evaluarea.

REZULTATE ŞI DISCUȚII

1. Evaluări asupra evoluției PIO medii. Pentru a vedea efectul asupra funcției vizuale (CV) al medicației administrate am inclus în studiu numai pacienții cu pierdere glaucomatoasă incipientă, evaluați în decurs de 1 an. Câmpurile vizuale au fost analizate la 6 luni, concomitent cu înregistrarea valorilor PIO (2).

La prima prezentare, examinarea pacienților a inclus: PIO (curbă nictemerală), examenul complet al polului anterior şi al celui posterior, perimetria computerizată statică, simțul cromatic, pahimetrie, OCT). De asemenea, s-au realizat investigații privind patologia asociată (diabet zaharat, hipertensiune arterială, ateroscleroză) şi factori de risc corelați (vasospasm, lipidemie).

Inițial, PIO a avut valori semnificativ crescute. Curba nictemerală a fost evaluată cu ajutorul unui aplanotonometru Goldmann, pe parcursul a 24 ore, la intervale fixe. Curba inițială a fost efectuată pentru a se determina profilul tensional nictemeral, iar orele de măsurare a PIO au fost standardizate: 9, 12, 15, 18, 21, 24 şi 6. În funcție de curba nictemerală, s-a calculat presiunea medie, valorile extreme şi diferența între acestea (variațiile tensionale nictemerale). La şase luni s-a repetat curba nictemerală la aceleaşi intervale orare. Valorile PIO au fost corectate în funcție de grosimea corneei centrale, după ecuația Herndon (Alcon Ocuscan RXP®). Lotul martor, format din 55 pacienți, a avut media tensiunii 24,11±2,65 mm Hg (tab. I).

TABELUL I

Evoluția presiunii intraoculare medii în intervalul orar, la prima internare şi la 6 luni (lotul martor)

	Orele							Media
	9	**12**	**15**	**18**	**21**	**24**	**6**	
PIO medie	25,02	25,65	25,07	23,96	22,82	22,47	23,80	24,11
DS	4,49	3,06	2,43	2,62	2,60	2,93	3,81	2,65
PIO medie	**16,19**	**16,68**	**16,30**	**15,58**	**14,83**	**14,61**	**15,47**	**15,66**
DS	**2,78**	**1,99**	**1,58**	**1,71**	**1,69**	**1,90**	**2,47**	**1,70**
Scăderea PIO (%)	35,29	34,97	34,98	34,97	35,01	34,98	35	35,05

2. Aspecte privind eficiența unor programe terapeutice. Tratamentul cu diverse topice antiglaucomatoase a determinat o scădere semnificativă a PIO ($p=6,85*10^{-38}$), valori aflate sub nivelul țintă, calculat individual pentru pacienții incluși în studiu, presiunea țintă fiind considerată cea maximă calculată după inițierea tratamentului, care determină încetinirea evoluției glaucomului.

Tratamentul cu topice antiglaucomatoase a inclus inhibitori ai anhidrazei carbonice (IAC), cum este dorzolamida care, similar cu ceilalți IAC, acționează asupra fluxului sangvin ocular, cu efect considerat neuroprotector (3, 4). Unele studii (1, 2, 3, 4) sugerează că modificările glaucomatoase pot apărea şi din cauza reducerii fluxului sangvin la nivelul nervului optic, iar IAC sistemici determină vasodilatația, această observație stând la aprecierea de rutină a rezervei de perfuzie cerebrală. Mecanismul poate fi în parte legat de cascada retenției CO_2 cu oxidul nitric şi Ca^{+2}, cu blocarea calciului (5). Inhibitorii anhidrazei carbonice ar putea creşte fluxul sangvin la nivelul retinei şi a nervului optic, având implicații în reducerea ischemiei în glaucomul acut, retinopatia diabetică şi alte boli vasculare (6). Atât brinzolamida cât şi dorzolamida ajung în concentrații suficiente pentru a dilata vasele retiniene. Un studiu realizat de Harris et al. (7) a sugerat că dorzolamida poate creşte viteza fluxului retinian fără să afecteze parametrii retrobulbari; acelaşi studiu a arătat o creştere a sensibilității la contrast după o lună de tratament cu dorzolamidă topică. Un studiu pe şobolani glaucomatoşi sugerează că dorzolamida poate avea proprietăți neuroprotectoare, dar nu este clar dacă rezultatele apar prin scăderea PIO sau este un efect independent de aceasta (8, 9).

Al doilea grup a fost tratat cu dorzolamidă, în asociere cu o prostaglandină sau în combinație fixă: dorzolamidă cu timolol 0,5% (Cosopt). PIO medie inițială a fost 24,73±2,78 mm Hg (tab. II).

TABELUL II

Evoluția presiunii intraoculare medii în intervalul orar, la prima internare şi la 6 luni
(al doilea grup tratat cu dorzolamidă, în asociere cu o prostaglandină sau în combinație fixă)

	Orele							Media
	9	**12**	**15**	**18**	**21**	**24**	**6**	
PIO medie	25,49	26,11	25,75	24,73	23,49	23,18	24,35	24,73
DS	4,51	3,14	2,74	2,72	2,73	3,04	3,75	2,78
PIO medie	**16,60**	**17,15**	**17,02**	**16,71**	**17,04**	**16,55**	**17,02**	**16,87**
DS	**1,86**	**1,54**	**1,67**	**1,86**	**1,50**	**1,74**	**1,82**	**0,6**
Scăderea PIO (%)	34,88	34,33	33,90	32,43	27,48	28,63	30,10	34,88

S-a observat o scădere medie semnificativă a PIO la 6 luni, cu o aplatizare a curbei, eficiența cea mai mare fiind înregistrată dimineața, atunci când PIO era inițial mai ridicată ($p=4,44*10^{-39}$).

Al treilea grup de pacienți a primit medicație locală cu topice antiglaucomatoase, asociată cu un vasodilatator cerebral (nicergolină 30 mg), administrat pe parcursul a 6 luni, cu acțiune asupra receptorilor alfa-adrenergici.

Nicergolina îmbunătățește fluxul sangvin, neurotransmiterea, metabolismul şi activitatea anti-oxidantă. Studii experimentale au arătat efectul hipotensor ocular, probabil prin stimularea transmiterii colinergice la nivel ocular (6, 10) (tab. III). S-a înregistrat o scădere semnificativ statistică a PIO la 6 luni ($p=2,43*10^{-41}$), ceea ce a însemnat pentru întregul lot o scădere medie de 31,79%.

TABELUL III

Evoluția presiunii intraoculare medii în intervalul orar, la prima internare şi la 6 luni (al treilea grup de pacienți a primit medicație locală cu topice antiglaucomatoase, asociată cu un vasodilatator cerebral)

	Orele							**Media**
	9	**12**	**15**	**18**	**21**	**24**	**6**	
PIO medie	25,89	26,13	25,60	24,55	23,64	23,40	24,65	24,84
DS	4,00	2,82	2,61	2,76	2,78	2,99	3,41	2,59
PIO medie	**16,93**	**17,09**	**17,00**	**17,22**	**16,78**	**16,60**	**16,96**	**16,94**
DS	**2,04**	**1,96**	**2,19**	**1,96**	**2,08**	**1,93**	**2**	**0,72**
Scăderea PIO (%)	34,62	34,59	33,59	29,85	29,00	29,06	31,19	31,79

*3. Unele rezultate asupra evoluției câmpului vizu*al. Inițial, s-au obținut două sau trei câmpuri vizuale, incluzând aria 24-2 standard şi perimetria în ax albastru – galben.

Într-o perioadă de 5 ani, au fost efectuate 21603 examinări de câmp vizual la 1637 pacienți dintre care 748 (45,67%) au fost anormale, iar 703 (43,0%) aveau indici corespunzători, dar la retestare anomaliile s-au evidențiat la 604 (85,9%). Pentru pacienții tratați cu topice antiglaucomatoase, fără a conține dorzolamidă, sensibilitatea medie a fost inițial de 33,9±1,29 dB iar după tratament a ajuns la 33,46±1,16 dB, cu o scădere de 0,44 dB, ceea ce arată că tratamentul topic a reuşit să încetinească evoluția bolii glaucomatoase, exprimată printr-o evoluție favorabilă a câmpului vizual (p=0,009).

La grupul tratat cu dorzolamidă, sensibilitatea medie inițială a fost 34,09±1,04dB iar după tratament, 34,35±0,89 dB, ceea ce a însemnat o creştere a sensibilității medii cu aproximativ 0,26 dB, rezultat care poate pune în discuție curba de învățare a analizei funcției vizuale sau modificarea sensibilității medii datorate creşterii fluxului sangvin ocular, atât la nivelul capului nervului optic, cât şi la cel retinian. Cum şi alți autori (3, 4) au implicat acest ultim mecanism ca fiind mai plauzibil, pacienții fiind selectați omogen, efectul ar fi trebuit să fie vizibil la toate grupurile incluse în studiu. Evoluția favorabilă a câmpului vizual a avut valori semnificative (p=0,044).

Grupul tratat cu nicergolină a avut o evoluție favorabilă semnificativă, de la 33,95± 0,99 dB înaintea tratamentului, la 34,46± 1,12 dB după acesta, deci o creştere de 0,51 dB (p=0,000525). Evoluția favorabilă poate fi explicată prin efectele nicergolinei asupra fluxului sangvin, urmat de scăderea directă a PIO sub acțiunea nicergolinei. Această evoluție a fost remarcată şi în alte cercetări (9, 10), unele folosind electrofiziologia retinia-

nă (PEV) ca metodă de explorare cantitativă.

CONCLUZII

Tratamentul diversificat al glaucomului a determinat scăderea semnificativă a PIO la fiecare din cele trei subgrupuri, totalizând 165 de pacienți cu GUD. Studiul a evidențiat că anumite programe terapeutice au determinat o evoluție mai lentă a degradării câmpului vizual, față de evoluția naturală acceptată de aproximativ 1 dB/an sau au indus o creştere a sensibilității medii, prin activarea altor mecanisme decât cele primare de scădere a PIO.

Studiul a arătat că deşi PIO a fost scăzută semnificativ, în proporții asemănătoare (35,05% la grupul 1; 34,88% la 2 şi 31,97% la al treilea), evoluția parametrilor a fost diferită, mai favorabilă la pacienții la care influența asupra fluxului sangvin ocular a fost mai mare. Răspunsul optim a fost constatat la pacienții tratați general cu nicergolină (-0,44 dB la grupul 1; 0,26 dB la 2 şi 0,51 dB la 3).

Rezultatele privind posibilitatea încetinirii evoluției nefavorabile a bolii glaucomatoase sunt obținute în variate studii clinice care au dovedit existența unor noi mecanisme etiopatogenice, precum şi noi orientări de tratament, unele acționând prin scăderea PIO, altele prin creşterea fluxului sangvin ocular.

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HEPATOTOXICITY – CURRENT CONCEPTS AND MECHANISMS

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HEPATOTOXICITY-CURRENT CONCEPTS AND MECHANISMS (Abstract): Liver is the main organ with critical metabolic functions, contributing to the biotransformation of numerous molecules. It has an essential role in converting and clearing of active agents and it is extremely vulnerable to the toxins. The use of chemicals (drugs, abuse substances, poisons, industrial and occupational agents, herbal remedies and dietary supplements) is an important cause of hepatic cells damages, most of them being involved in the development of liver injury. Three types of mechanisms may be responsible for the occurrence of liver toxicity: overdose, drug allergy and idiosyncratic reaction. This review aims to highlight the category of chemicals responsible for the architecture damage and liver function disturbances. The influence of various agents on the liver function may be accompanied by a multitude of manifestations, represented by the increase in liver enzymes activity, hepatocytolysis or liver failure. It is very important to recognize the commonly involved compounds, identify their adverse effects, especially during the drug associations, identify their mechanisms of action, predict and limit their harmful effects in contact with the body. Considering the clinical repercussions of chemical-mediated liver injuries, this review brings into attention several potential hepatotoxic compounds and their pathophysiological mechanisms. **Keywords:** HEPATOTOXICITY, ORGANIC SOLVENTS, ACETAMINOPHEN, ALCOHOL, METALLOIDS.

The purpose of the review is to illustrate the primary mechanisms of the liver toxicity induced by a wide variety of endo-exogenous agents. In this article we listed the most common causes of hepatocytolysis described in the medical literature: drugs (acetaminophen, antibiotics, statins), occupational chemical agents from the group of organic solvents (CCl_4, toluene, styrene), metalloids (As), alcohols (white spirit, ethyl).

Hepatotoxicity

Hepatotoxicity represents the chemical damage or malfunction of the liver due to an overload of drugs or chemicals. This may result not only from direct toxicity of the primary compounds but also from an

immunologically-mediated response or a reactive metabolite (1).

There are several causes for hepatotoxicity: usage of drugs with potential toxic effect for the liver when other medication is not available (e.g. anti-tuberculosis agents), overdosage by not respecting the medical prescription, for suicidal purpose, self-medication (2). Consumption of medications, herbal and dietary supplements causing liver damage has an annual incidence rate of approximately 20 cases per 100,000 per year. If in the United States and Europe the antibiotics produce the most cases of hepatic damages, in Asia, herbal and dietary supplements are especially responsible for most liver disturbances (3).

The acetaminophen is one of the most commonly utilized drugs worldwide. It can be found under the name of N-acetyl-p-aminophenol (APAP) in the USA, Paracetamol in Europe and other countries around the world. Paracetamol is the most incriminated drug in self-poisoning in Europe. In the United States, it became the first cause of acute liver failure, up-ranking viral hepatitis. Usually, toxic doses causing hepatic failure are over 150 mg/kg, yet a study suggests that lower doses of APAP may confer acute liver injury and some patients have presented with acute liver failure despite consumption of "safe" doses of APAP (3, 4). It is also known that the antituberculosis drugs may induce hepatotoxicity as a secondary effect (5).

Literature data reveal that the administration of carbon tetrachloride ($CCl_{4)}$ in laboratory animals resulted in liver injury, with a variety of manifestations consisting of steatosis, centrolobular necrosis, inflammation, fibrosis, and liver cancer (5).

Other experimental researchers show that combination of CCl_4 and acetaminophen resulted in liver damage, due to the potentiation of their hepatotoxic effects. Liver damage was biochemical and histopathological objected by the increase of alanine aminotransferase, aspartate aminotransferase activity, elevated pro-inflammatory cytokine levels, hepatic oxidative stress and characteristic architectural disturbances (6).

White spirit is the main volatile solvent contained in the polyurethane gloss paint. Also, it was observed because of accidental excessive inhalation of the paint vapor, nausea, somnolence, and hepatotoxicity (7).

Styrene is a colorless aromatic hydrocarbon available in liquid form and highly volatile. In its pure form, it gives a sweet smell. The primary source of exposure in the environment is from plastic materials, rubber industries, packaging materials, insulations, and fiberglass and carpet industry. Experimental research demonstrated that oral administration (using an esogastric device) of different doses of styrene dissolved in corn oil, for 42 days, determined dose-dependent increase in plasma superoxide dismutase (SOD) and protein carbonyl values. Moreover, it was registered an increase in lipid peroxidation (LPO) and reactive oxygen species (ROS) levels, as well as an intensification of the styrene influenced glucose-6-phosphatase (G6Pase). On the other hand, it was observed a decrease in ferrous reducing antioxidant power (FRAP) and in total thiol molecules (TTM) values in the liver tissue (8).

Toluene

Toluene (methyl-benzene) is a colorless liquid, with high vapor pressure and an aromatic odor, naturally found in crude oil and tolu tree and mostly used in the paint

industry as a solvent, and in the fuel and chemical industry. Cresols are known to be more toxic than others upon exposure (benzaldehyde, benzoic acid, cresols or methylphenols, methyl-catechol and methylresorcinol). Exposure to these substances often occurs by inhalation of contaminated air or by accidental ingestion. The toluene is metabolized by the liver. It has been established that the inhalation of toluene by the rats caused hepatic injury observed by increased levels of aminotransferase and malondialdehyde. It has been observed that liver cell apoptosis, mediated by increased activity of Bcl-2-associated X protein (Bax) and caspase-3 enzymes, is also present (9).

Ethanol

Alcoholic liver disease (ALD) is a major health problem worldwide, for which no successful treatments are available. The pathogenesis of ALD is characterized as steatosis, inflammation, fibrosis, cirrhosis, which eventually leads to hepatocellular carcinoma (HCC) (10).

The Complement system plays a crucial role in microbial defense and clearance of apoptotic cells, emerging evidence suggesting that it is an important contributor to alcoholic liver disease.

The studies have demonstrated that the complement has an important role in the development of the alcoholic liver disease. It was observed that the complement 1q (C1q) has a beneficial influence on the recovery of hepatic damages produced by ethanol.

Following a study, where was used a group of genetically modified C57BL / 6J (WT) female rats, which were fed during 25 days with a liquid diet containing ethanol, it was observed that prolonged exposure to alcohol was accompanied by the presence of liver damage, steatosis and an increased expression of proinflammatory cytokines (11).

Chronic alcohol intoxication is associated with an intensified oxidative stress. However, the mechanisms by which ethanol triggers an amplification in the production of reactive oxygen species (ROS) and the involvement of mitochondria in the development of oxidative stress hasn't been sufficiently studied. The biochemical and proteomic data obtained in various experimental researchers suggest that one of the main causes of the increase in ROS generation is represented by an enhanced oxidation of glutamate in response to long-term alcohol exposure. In the sequence of glutamate oxidation, liver mitochondria from alcoholic rats generated more superoxide anion and hydrogen peroxide (H_2O_2) than in the presence of other substrates, as well as in control organelles. In the alcoholic rat mitochondria, the rates of H_2O_2 production and nicotinamide adenine dinucleotide (NAD) were reduced in the presence of glutamate. They were almost twice higher than in the control group. The proteomic study revealed a higher content of glutamate dehydrogenase in liver mitochondria of rats with chronic exposure to alcohol. Simultaneously, the content of mitochondrial catalase decreased in comparison with the control group. Each of these factors stimulates the production of ROS in addition to ROS generated by the respiratory chain complex I. The results are demonstrating that glutamate contributes to alcohol hepatotoxicity by increasing the oxidative stress at the mitochondria level (12).

Cocaine

Cocaine-induced liver toxicity is occa-

sionally described in humans, and interesting interactions with other "recreational" drugs are rarely presented (13). Hepatotoxicity appears usually hours to a few days after an overdose and includes acute hepatic necrosis. Serum aminotransferases and lactate dehydrogenase levels are excessively increased initially, associated with a lower increase in alkaline phosphatase. When the prothrombin time is abnormally raised may suggest disseminated intravascular coagulation. Liver histology shows centrolobular necrosis and fatty change (14).

On the other side, cocaine toxicity it is considered as a cause of fever of unknown origin. Young males with unexplained transaminase elevation must be evaluated for recreational drug use. Multiple drug interactions can alter the histological pattern of liver injury in susceptible patients. Recreational drug use is an important cause of anicteric liver injury (13).

Arsenic

Liver is a well-known affected organ by the arsenic exposure. The mechanism by which arsenic causes liver injury is incompletely understood. However, several viable mechanisms have been identified experimentally. A mechanism by which arsenic induces liver injury involves aberrant lipid metabolism, oxidative stress, altered cytokine serum levels, conducting to inflammation. The concentrations of arsenic required to cause direct hepatotoxicity are high and therefore concerns exists especially in areas of high (ppm) arsenic contamination. Some experimental studies suggested that arsenic, around these concentrations, may sensitize the liver to damage from a second consecutive hepatotoxic injury. These findings are alarming, based on the increasing rate of liver diseases (e.g. nonalcoholic fatty liver disease - NAFLD) in the developed world, wherein arsenic contamination is generally below levels required to cause direct hepatotoxicity (15).

STATINS

The HMG-CoA reductase inhibitors statins are lipid-lowering drugs currently recommended for patients with high cholesterol levels to reduce the risk of developing cardiovascular diseases. Besides their beneficial effect, they also have major side effects like hepatotoxicity and myopathy. In a large prospective study conducted for several years (2004-2012), in the United States it was noted that, of the 1188 cases reported with drug-induced hepatotoxicity, 22 cases were attributed to a statin. The authors suggested that the latency period for the occurrence of the hepatocytolysis ranging from 34 days to 10 years (16).

Herbs and dietary supplements

Multiple experimental types of research and clinical trials reported liver toxicity with toxic compounds from vegetal products, herbal remedies and dietary supplements (for example, poison from *Amanita phylloides* species of mushrooms, pyrrolizidine alkaloids from chaparral leaf, germander and comfrey, or other mixtures of plants).

CONCLUSIONS

Toxic hepatitis is frequently a result of exposure to various chemical substances, like drugs, poisons, industrial and occupational agents, herbal remedies and dietary supplements and it is well-known that it has the potential to affect the daily life quality of patients.

The most important pathogenic mechanisms of hepatotoxicity are: up-regulation

of proinflammatory cytokines activity, intensification of reactive oxygen species production, lipid peroxidation, various immunological, enzymatic and metabolic dysfunctions.

Occupational exposure, as well as the domestic one, at many singular chemical agents or on the mixture of different compounds with hepatotoxic potential, requires advanced investigations procedures, coherent evaluation, efficient preventive and effective curative methods.

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GENETIC AND EPIGENETIC BIOMARKERS FOR EARLY DETECTION, THERAPEUTIC EFFECTIVENESS AND RELAPSE MONITORING IN BLADDER CANCER

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GENETIC AND EPIGENETIC BIOMARKERS FOR EARLY DETECTION, THERAPEUTIC EFFECTIVENESS AND RELAPSE MONITORING IN BLADDER CANCER (Abstract): Urinary bladder cancer is the fifth most common cancer in the Western world and is responsible for about 3% of all cancer-related deaths. Because most advanced invasive or metastatic cancers have low cure rates, risk assessment and early detection of the clinically occult premalignant phases of neoplasia are a particular importance. Many tumor biomarkers for bladder cancer have been evaluated for use in detecting and monitoring bladder cancers tissue specimens, bladder washes, and urine specimens but, none of the biomarkers reported to date has shown sufficient sensitivity and specificity to detect the entire spectrum of bladder cancers in routine clinical practice. The limitations of established prognostic markers requires us to identify better molecular parameters that could be of interest in predicting the prognosis of bladder cancer patients, in particular, the high-risk patient groups that are at risk of progression and recurrence. Methylation is an important molecular mechanism in the development of bladder cancer and could be used as a prognostic and diagnostic biomarker, because hypermethylation of several gene promoters was detected in urine sediment DNA from bladder cancer patients. Aberrant patterns of epigenetic modification could be, in the near future, crucial indicators in cancer diagnosis, prognosis, and additionally could be good targets for developing novel therapies while maintaining quality of life. **Key words**: BLADDER CANCER, BIOMARKERS, EPIGENETICS, EPIGENETIC THERAPY

Carcinogenesis is a complex process involving a series of steps in the transformation from a normal to a malignant cell. In an individual's lifetime, a gradual accumulation of mutations or "hits" to the involved genes results in the sporadic neoplasm, whereas the inherited form results from the transmission of one or more of the necessary mutations to successive generations (1). Despite the great advances in the last decade of identifying and characterizing relevant molecules individually, a more comprehensive approach may be required to fully understand the complex network of genes, their protein products, and their various cellular interactions.

Genomics-based approaches are ideally suited to identify and study the complexities of the normal cellular machinery and its multiple step perturbations that result in dysregulation of cellular proliferation, migration, adhesion, differentiation, apoptosis, DNA replication, DNA repair, angiogenesis, and cellular responses to growth factors and drugs (2).

Biomarkers are cellular, biochemical and molecular (proteomic, genetic and epigenetic) alterations by which normal, abnormal, or simply a biological process can be recognized or monitored. They are used to measure and evaluate normal biological processes, or pharmacological responses to a therapeutic intervention. Biomarkers are measurable in biological media, such as: tissues, cells, and fluids. Identification of early disease-stage biomarkers has potential value for the prevention, early detection and treatment of several cancers, including bladder cancer, too.

To diagnose bladder cancer cystoscopic examination represents the gold standard, but this method is costly, incurs substantial patient discomfort, and has variable sensitivity. The development of highly reliable, noninvasive tools for bladder cancer diagnosis would facilitate early detection and help to define the role of molecular biomarkers in prognostic evaluation at the time of initial diagnosis of patients with bladder cancer (17).

More than 90% of bladder cancers are transitional-cell carcinomas and approximately 60% of these are low grade, non-muscle-invasive transitional-cell carcinomas. After endoscopic resection, the majority of bladder cancer patients develop cancer recurrences, 16–25% of which are high-grade cancers. Approximately 10% of patients with non-muscle invasive bladder cancers subsequently develop invasive or metastatic cancer. Almost 25% of patients with newly diagnosed bladder cancer have muscle-invasive disease, the vast majority being cancers of high histological grade. Almost 50% of patients with muscle-invasive bladder cancer already have occult distant metastasis (6).

Patients with bladder cancer are monitored for cancer recurrence or progression by periodic cystoscopy and urine cytology, the frequency of which varies depending on the risk factors associated with the disease.

In cases of bladder cancer, life-long surveillance is required to detect subsequent tumor recurrence. Current patient-monitoring protocols generally consist of cystoscopic evaluations and urine cytology every 3–4 months for the first 2 years and at longer intervals in subsequent years. Voided urine cytology is a highly specific, non-invasive adjunct to cystoscopy (23). It has good sensitivity for the detection of high-grade bladder cancers but poor sensitivity for low-grade disease.

In the USA, Food and Drug Administration (FDA) has already accepted urinary tests for monitoring patients with bladder cancer, including the bladder tumor antigen *stat* test, bladder tumor antigen TRAK test, the fibrinogen–fibrin degradation products test, UroVysion, ImmunoCyt, and the nuclear matrix protein 22 assay (22).

The identification of major cancer susceptibility genes such as VHL and WT1 has provided important insights into the molecular basis of cancer, germline mutations in such genes are rare and account for an increased cancer risk in only a small segment of the population common polymorphic variants, which alone do not cause cancer, can in certain environments or in concert with other genetic alterations influence cancer development or progression (7, 8).

CHROMOSOMAL CHANGES IN BLADDER CANCER

Studies of cytogenetics have identified changes in the structure and copy number of chromosomes in transitional cell carcinoma of the urinary bladder cancer(18). Studies made on loss of heterozygosity (LOH) have demonstrated that the loss of 17p, 3p, 13q, 18q or 10q is found in high grade , high stage bladder cancer(19).Loss of 9q was also observed in high grade cancer, fact that suggests that the loss of 9q could be a primary event in the tumor genesis of bladder.

GENETIC AND EPIGENETIC BIOMARKERS IN BLADDER CANCER

Tumor genesis is a multiple step process that results from the accumulation and interplay of genetic and epigenetic mutations.

Whereas genetics refers to the study of information inherited on the basis of gene sequence, epigenetics is the study of inherited reversible changes in gene function or other cell phenotypes that occur without any change in DNA sequence.

DNA methylation represents an epigenetic mechanism used for the long-term silencing of gene expression.

Many studies have been made concerning the biological roles in bladder cancer development and progression. Researchers have studied some prognostic biomarkers such as : proto-oncogenes and oncogenes , tumor-suppressor genes, cell cycle regulators, and cell-adhesion molecules. Some correlations have been made between tumoral biomarkers and tumor progression. Biomarkers like : p53, retinoblastoma (Rb), epidermal growth factor receptor (EGFR), E-cadherin appear to be of prognostic value with regard to bladder cancer metastasis, recurrence and cancer specific survival (20).

Inactivation of gene expression by the abnormal methylation of CpG islands can act as a 'hit' for the generation of cancer. Many tumor-suppressor genes contain CpG islands and show evidence of methylation-specific silencing.

The hypermethylation of CpG islands around the promoter region and decreased expression of the tumor suppressor genes RUNX3, RASSF1A,p16, RARâ, and E-cadherin have been reported in transitional cell carcinomas of the bladder (12). It has been recently recognized that aberrant hypermethylation events can occur early in tumorigenesis, predisposing cells to malignant transformation (3).

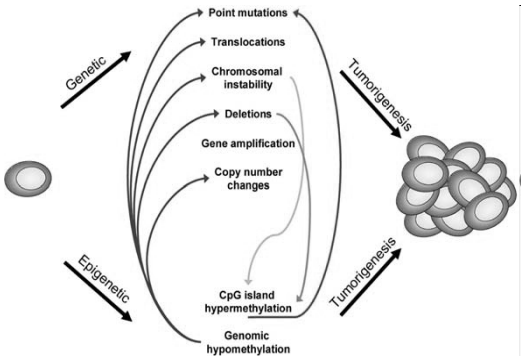

Fig. 1. Genetic and epigenetic changes in bladder cancer tumorigenesis

Promoter hypermethylation of CpG islands is strongly associated with tumor development, stage, recurrence, progression, and survival in transitional-cell carcinomas of the urinary bladder.The presence of methylation has been significantly associated with advanced-stage carcinomas, high tumor progression rates, and increased mortality rates compared to tumors without methylation (21). The methylation status could therefore be useful as a diagnostic and prognostic biomarker for bladder cancer and could also be used as a therapeutic target for bladder cancer in the clinical setting (16).

EPIGENETIC ALTERATIONS DETECTED FROM URINE

Some epigenetic events occur early in the disease process and in this case molecular diagnosis may allow their detection before symptomatic or overt radiographic manifestations. Because of this, from a clinical point of view the most promising application for methylation analysis is the detection of cancer or the utilization of methylation as a prognostic biomarker (10, 11).

The screening of bodily fluids such as the urine may ultimately provide a truly non-invasive diagnostic modality, thereby limiting the need for the current imaging techniques that provide anatomical details with-

out a definitive pathological correlation (13). The hypermethylation of several gene promoters has been reported in DNA isolated from bladder cancer tissues and urine sediment. These studies revealed that the detection of aberrant promoter methylation in urine is feasible and appears to be more sensitive than conventional cytology (4).

Recent studies have reported the feasibility of detecting DNA hypermethylation in voided urine and urine sediment and its potential role as a tumor biomarker for bladder cancer early detection.

Using a quantitative real-time polymerase chain-reaction assay the researchers examined the urine sediment DNA from 175 patients with bladder cancer and 94 age-matched control subjects for the promoter hypermethylation of nine genes (APC, $p14^{ARF}$, CDH1, GSTP1, MGMT, CDKN2A, RARβ2, RASSF1A, and TIMP3). The combined methylation status of four genes (CDKN2A, $p14^{ARF}$, MGMT and GSTP1) presented 69% sensitivity and 100% specificity (14, 15).

In another research study has been examined the methylation profile of four tumor-suppressor genes(APC, RASSF1A and $p14^{ARF}$) from matched sediment DNA in urine specimens obtained before from 45 bladder cancer patients, before surgery as well as normal controls (5, 9). Hypermethylation of at least one of the three tumor suppressor genes was found in the matched urine DNA from 39 from 45 patients, with a 87% sensitivity and 100% specificity.

The detection of DNA hypermethylation in voided urine represents a promising biomarker of the early detection and surveillance of bladder cancer.

DRUG DEVELOPMENT AND EPIGENETIC THERAPY

Unlike genetic alterations such as mutations, epigenetic changes such as DNA methylation are potentially reversible. This property of DNA methylation patterns makes epigenetic changes an attractive target for cancer therapy, and represents the basis of epigenetic therapy.

5-Azacytidine and 5-aza-deoxycytidine (decitabine), nucleoside analog inhibitors of DNA-methyltransferases (DNMTs) have been widely used *in vitro* cell culture systems to reverse abnormal DNA hypermethylation and restore silenced gene expression (25).

These drugs can be used therapeutically either alone or in combination with other therapeutic modalities such as chemotherapy, immunotherapy or radiation therapy.

CONCLUSIONS

A better understanding of the molecular mechanisms involved in the carcinogenesis and progression of bladder cancer has identified a large number of molecular biomarkers, each presenting a potential diagnostic and prognostic value.

The convergence of molecular biology, biotechnology, computer science, and statistics has spawned the field of genomics, a discipline that studies the complexity, diversity, evolution, regulation, and structure of the biological information stored as a digital code in the chromosomes. Medical applications of this rapidly changing field enable a greater understanding of the genetic and epigenetic events that contribute to the complex biological processes underlying human carcinogenesis.

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ACHIEVEMENTS WITHIN THE MILLENNIUM DEVELOPMENT GOAL NUMBER 5 - MATERNAL HEALTH - IN ROMANIA

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ACHIEVEMENTS WITHIN THE MILLENNIUM DEVELOPMENT GOAL NUMBER 5-MATERNAL HEALTH -I N ROMANIA (Abstract): Among the eight Millennium Development Goals (MDGs) adopted by the United Nations in 2000, Goal Number 5 focused on Improve the Maternal Health. **Aim:** This paper intends to assess the progress recorded in maternal health in Romania, from the perspective of the MDG number 5, at the target date of year 2015. **Material and methods**: The research represent a descriptive study (1990-2015) and an analysis of the related achievements, from the perspective of the MDG number 5. The specific targets and indicators recommended by the United Nations were: Target 5.A "Reduce by three quarters, between 1990 and 2015, the maternal mortality ratio" and Target 5.B "Achieve, by 2015, the universal access to reproductive health". **Results**: A significant downward has been recorded in the maternal deaths, approximately ten times, from 262 cases in 1990 to 28 cases in 2015. The proportion of the births attended in 2015 by skilled health personnel was 99%. The estimated prevalence rate for any contraceptive method pointed out that just two of three Romanian women of reproductive age, currently use contraceptive methods (69%). The Romanian adolescent birth rate was and remains high, 36 cases aged 15–19 years per 1,000 women in that age group. The antenatal care coverage decreased from 94% to 76%, along the studied interval. The estimated prevalence rate regarding the unmet need for family planning in 2015 was 9.5%. **Conclusions**: An important progress has been made in Romania, in achieving the MDG no. 5 - Improve Maternal Health - since 1990. The target 5.A. was achieved, whereas the target 5.B. revealed that in spite of the progress made, Romania still lags far behind the other countries of European Union. **Keywords**: MILLENNIUM DEVELOPMENT GOALS, MATERNAL HEALTH, MATERNAL DEATHS.

Women play a critical role in a society. They have their own job or duty, being involved in the postmodern society in many fields of activity: education, delivering health care, social services or acting as agents of social change. In the same time, they give birth, raise children and take care of their family. Women are worldwide living longer and healthier lives, despite large health inequities within and between countries (1).

In September 2000, at the beginning of the new millennium, 189 heads of state adopted the United Nations (UN) Millennium Declaration, with eight Millennium Development Goals (MDGs). The plan

was to support the countries to work together to improve the peoples live, to fight poverty in its many dimensions. That vision has represented the development framework for the entire world for 15 years, up to 2015 (2).

Each MDG had specific targets for 2015, and a number of indicators for monitoring the progress. All goals and their targets are measured in terms of progress since 1990.

- Goal No.1 – Reduce Severe Poverty
- Goal No.2 – Increase Compulsory Education Graduation Rate by 2012
- Goal No.3 – Promote Gender Equality and Empower Women
- Goal No.4 – Reduce Child Mortality
- Goal No.5 – Improve Maternal Health
- Goal No.6 – Combating HIV/AIDS, Tuberculosis and Other Diseases
- Goal No.7 – Ensure Environmental Sustainability
- Goal No.8 – Develop Global Partnerships for Development

Out of the eight development goals, the goal number 5 "Improve maternal health" is directly linked to woman's health.

We are now at three years after the target date – 2015 – by which the Millennium Development Goals have to be achieved. It is generally agreed that the MDGs have been a success worldwide and will be continued up to 2030 as Sustainable Development Goals (SDGs) (3). The paper intends to assess the progress recorded in maternal health in Romania, from the perspective of the MDG number 5, at the end of 2015 year.

MATERIAL AND METHODS

This research represents a descriptive study about the maternal deaths among Romanian women, in the interval 1990-2015 and an analysis of the related achievements, from the perspective of the MDG number 5. In the United Nations Millennium Campaign, Romania committed herself to reduce maternal deaths (4).

In order to help track progress toward this commitment, following specific targets and indicators recommended by the United Nations and the World Health Organization were used (2, 3):

Target 5.A. Reduce by three quarters, between 1990 and 2015, the maternal mortality ratio

Indicator 5.1.: Percent reduction in maternal mortality ratio, 1990–2015 (75%)
Indicator 5.2.: Births attended by skilled health personnel (90%)

Target 5.B. Achieve, by 2015, universal access to reproductive health

Indicator 5.3: Prevalence rate for contraceptive methods

Indicator 5.4: Adolescent birth rate (15-19 years) per 1,000 women in that age group

Indicator 5.5: Antenatal care coverage: at least one visit (100%)

Indicator 5.6: Unmet need for family planning (0%)

The comparison allows establishing the status (achieved or making progress) for each target. The phenomenon of maternal mortality has to be understood within the context of the pro- natalist policy promoted in Romania by the former communist regime before 1990, with dramatically impact on women's health (5). In order to perform an accurate and complete analysis, two supplementary indicators have been used: births rate and abortion rate.

The data sources were represented by the National Institute of Statistics, yearbooks of the Ministry of Health and Romanian Government, World Health Statistics.

RESULTS

Romanian fertility dramatically decreased at the beginning of 1990s after the fall of the former regime. The births rate dropped from values of 16.0 in 1989, respectively 13.6 live newborns per 1,000 inhabitants in 1990 to 9 in 2015. A similar tendency was recorded for abortions, as shown in table I, the total number of the performed abortions in 1990 being ten times higher compared to 2015 (6, 7).

TABLE I

Evolution of the abortion rate (1990-2015)

Year	Number of abortions	Abortion rate (per 1,000 live births)
1990	992,265	3,152.6
1995	502,840	2,124.9
2000	257,865	1,099.5
2005	163,359	735.8
2010	101,915	480.3
2015	70,885	352.6

Target 5.A.

A significant downward has been recorded in the maternal deaths, as described in table II (7). In 1990, the indicator 5.1 pointed out that the number of the deaths generated by complications related to delivery, pregnancy and confinement was approximately ten times higher (262 cases), compared to 2015 (28 cases).

TABLE II

Evolution of the maternal mortality ratio (1990-2015)

Year	Number of maternal deaths	Maternal mortality ratio (per 1,000 live births)
1990	262	0.83
1995	113	0.48
2000	77	0.33
2005	37	0.17
2010	51	0.24
2015	28	0.14

The indicator 5.2. calculated as the proportion of the births attended in 2015 by skilled health personnel was 99%, compared to the target of 90% (8).

Target 5.B.

The indicator 5.3. was calculated as the proportion of married or in-union women of reproductive age (15-49 years) who have their need for family planning satisfied and currently use contraceptive methods (%). The estimated prevalence rate for any contraceptive method increased from 57.3 to 69.8 percent between 1990 and 2015 (9).

The indicator 5.4 revealed in 2015 a Romanian adolescent birth rate of 36 cases aged 15-19 years per 1,000 women in that age group. This value was relatively stable along the interval 1990-2015. In any West European country, the rate doesn't exceed 10 births per 1,000 women aged 15-19 years, whereas in Eastern Europe the level varies between 25 and 40 is close to 50 (10).

The antenatal care coverage (Indicator 5.5.) describes the access and utilization of health care during pregnancy. The percentage of Romanian women aged 15–49 years who utilized antenatal care provided by skilled health personnel for reasons related to pregnancy at least once during pregnancy of the total live births decreased from 94% (urban 96%, rural 91%) to 76% (urban 74%, rural 79%) along the studied interval. The level recorded in Eastern Europe was of 95.7% (11).

The percentage of married or in-union Romanian women of reproductive age (15-49 years) who want to stop or delay birth child, but doesn't use any contraceptive method, in 2015 was 9.5%, Confidence Interval $_{80\%}$=5.3-15.7%.

The estimated prevalence rate regarding the unmet need for family planning (Indica-

tor 5.6.) in Europe was 9.7%, Confidence Interval $_{80\%}$=8.2-11.4% (12).

DISCUSSION

In Romania, the year 1990 marked the transition to democratic governance. The births rate was high before 1990, caused by the lack of the contraceptive methods and restrictive access to abortion. The policy was abolished at the end of 1989 and consequently, births rate dropped in the next year 1990 (5). The decline continued in the following 25 years, with a decrease rate 1990-2015 of 43.7%.

The most important consequences of the pro-natalist policy were: the abortions performed illegally and in precarious conditions, the high number of maternal deaths, children abandonment in case of unplanned or undesired pregnancy and infant deaths.

After 1990, the maternal mortality and abortion rate dropped as a result of implementing the family planning and reproductive health programs (6, 13). Many information and education campaigns regarding contraception and development of the family planning services conducted to an eight times lower maternal mortality ratio in 2015 (0.14 per 1,000 live births), compared to 1990 (0.83 maternal deaths per 1,000 live births) (7).

The target of a three-quarters reduction in 25 years of the 1990 maternal mortality ratio (MDG 5A) and that of achieving universal access to reproductive health (MDG 5B) together formed the two targets for MDG 5: Improve maternal health.

Both indicators 5.1 and 5.2 pointed out the accomplishment in Romania of the target 5.A "Reduce by three quarters, between 1990 and 2015, of the maternal mortality ratio".

The target 5.B "Achieve, by 2015, universal access to reproductive health" among Romanian population was not entirely met. Despite the lack of an explicit national value for the indicator 5.3, the favorable status is reflected by a level of "Prevalence rate for contraceptive methods" as increased as possible. By comparison with European countries, Romania is very close to the average prevalence rate recorded at continental level, of 69.6%. In the same time, the estimated prevalence rate for any contraceptive method of 69%, pointed out that just two of three Romanian women of reproductive age, married or in-union, have their need for family planning satisfied and currently use contraceptive methods (9).

An important medical and social issue is represented by the adolescent girls who are very vulnerable when they face the risks of a pregnancy and childbirth. In many countries births before age 18 has remained quite stable across age groups of women.

The indicator 5.4: "Adolescent birth rate (aged 15-19 years) per 1,000 women in that age group" was at least two times higher among Romanian young girls, then any other West European country.

Women attended during pregnancy by a physician, nurse or midwife may receive services vital to their health and that of their future children. The antenatal care can often help women prepare for delivery and understand warning signs during pregnancy and childbirth.

In 2015, the access and utilization of health care at least once during pregnancy was performed in one of four cases, pointing not an insufficient progress, but a genuine regress. Women' addressability to healthcare, especially in rural area, is still poor. The Romanian population coverage is not universal. Social Health Insurance is compulsory but covers only around 86% of

the population (14). Pregnant women, even uninsured, are entitled to a minimum benefits package that includes care during pregnancy.

Shortage of human resources (physicians and nurses), especially in rural area, combined to low health expenditure, both under the European Union average reduce the accessibility of the pregnant women to proper antenatal care. In rural area, the antenatal care coverage (Indicator 5.5) has to be improved much more, compared to urban area (15).

In 2015, one of ten married or in-union Romanian women of reproductive age (15-49 years) want to stop or delay birth child but doesn't use any contraceptive method. Despite the similar values for the indicator 5.6. characterize, in average, the whole European region, the Millennium Development Goal number 5 specifies that at the end of 2015, the unmet need for family planning have to be 0% (12).

CONCLUSIONS

An important progress has been made in Romania, in achieving the Millennium Development Goal No. 5 - Improve Maternal Health - since 1990.

The target 5.A - To reduce by three quarters, between 1990 and 2015, the maternal mortality ratio - was achieved. At the target date (2015), both indicators regarding the reduction of the maternal mortality ratio and the births attended by skilled health personnel have been attained.

The target 5.B - Achieve, by 2015, the universal access to reproductive health-revealed that in spite of the progress made, Romania still lags far behind the other countries of European Union. Within this MDG, three indicators regarding the adolescent birth rate, the antenatal care coverage and the unmet need for family planning represent a priority for health authorities and have to be optimized as Sustainable Development Goals.

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NEWS

A NEW HOPE FOR AN OLD HEART? CAN INTERFERON-FREE THERAPY ALSO REVERSE THE HCV-INDUCED CARDIOMYOPATHY IN PATIENTS WITH END-STAGE HEART FAILURE?

Several studies have previously confirmed an association between hepatitis C virus (HCV) infection and cardiomyopathy, but none could really establish a pathogenic relationship. These issues have arisen because the classic, interferon-based therapy for HCV infection has severe cardiac side-effects, thus limiting its use in patients with advanced heart failure. The aim of this prospective large-scale study was to assess the benefits of using interferon free regimens in HCV-infected patients with non-valvular, non-ischemic cardiomyopathy, the standard treatment (consisting of ombitasvir, paritaprevir, dasabuvir and paritaprevir) being well-tolerated, free of cardiac side-effects. The authors were encouraged to conduct a large study because they observed that this treatment was very effective in a patient with rapidly progressive heart failure (NYHA III/IV class), who was already listed for heart transplantation. In order to have an acceptable hepatic functional status prior to the transplant, the patient received the interferon-free regimen and, surprisingly, after her complete HCV clearance, her cardiac function improved significantly to NYHA II class, heart transplant being no longer considered. The conclusion was that HCV was etiopathogenetically involved in myocardial dysfunction for at least two reasons: the interferon-free therapy does not eliminate any other cardiotropic virus (e.g. Coxsackie B3), nor does it have *"per se"* any antifibrotic or cardiac anti remodelation action. These promising findings need to be further confirmed on larger patient cohorts in this ongoing study (Poller W, Haghikia A, Kasner M *et al*. Cardiovascular Involvement in Chronic Hepatitis C Virus Infections - Insight from Novel Antiviral Therapies. *J Clin Transl Hepatol* 2018; 6(2): 161-167).

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EVALUAREA CROMOZOMIALĂ A CUPLURILOR CU TULBURĂRI DE REPRODUCERE – STUDIU RETROSPECTIV PE UN LOT SELECŢIONAT DE 266 CUPLURI

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CHROMOSOMAL EVALUATION IN COUPLES WITH REPRODUCTIVE DISORDERS – RETROSPECTIVE STUDY OF A SELECTED GROUP OF 266 COUPLES (Abstract): Reproductive Disorders (RD), manifested by the biological inability to conceive (primary sterility) or inability to carry a pregnancy to full term (infertility), affect 10-15% of reproductive-aged couples. The genetic etiology of RD is represented, in the majority of cases, by the chromosomal abnormalities. **Aim**: To retrospectively analyze the karyotype results in a selected group of couples with RD. **Material and method**: The present study was performed in 266 couples with RD: 80 (30,07%) with primary sterility (ST), 149 (56,01%) with Recurrent Spontaneous Abortions (RSA) and 37 (13,90%) with Stillborn Children (SC). A GTG-banded karyotype was performed on both partners of each couple. **Results**: We identified a chromosomal abnormality in 43 individuals (16,16%): 20 cases (7,51%) with ST, 13 cases (4,88%) with RSA and 10 cases (3,75%) with SC. The affected partner was female in 23 cases (8,64%) and male in 20 cases (7,51%). A X chromosome (numerical or structural) abnormality was detected in 18 cases (6,76%), most frequent X chromosome monosomy mosaicism in female and trisomy XXY in male; a balanced structural chromosomal abnormality (BSC) was detected in 23 couples (8,64%); in other two males with ST, the karyotype result was 46,XX. **Conclusions**: The results of our study are similar to other reported studies and underline the major etiologic role of chromosomal abnormalities in RD and the importance of chromosomal analysis for the etiologic diagnosis and genetic counseling of these patients. **Key words**: REPRODUCTIVE DISORDERS, CHROMOSOMAL ABNORMALITIES, KARYOTYPE

Tulburările de reproducere umane (TR) se caracterizează prin incapacitatea unui individ/cuplu de a concepe natural (sterilitate primară) ori prin incapacitatea de a menţine o sarcină sau de a da naştere unui copil viu, sănătos (infertilitate) (1,2).

Sterilitatea primară (ST) este definită ca fiind imposibilitatea unui individ de a forma gameţi viabili sau inabilitatea unui cuplu de a concepe un copil după un an de viaţă sexuală normală, regulată, în absenţa folosirii oricărui mijloc contraceptiv (2,3,4). ST reprezintă o problemă medicală importantă şi frecventă, afectând 10-15% dintre cuplurile cu vârstă reproductivă (2,3).

Infertilitatea (pierderile de sarcină) este caracterizată prin sarcini finalizate cu producerea de avorturi spontane (AS)/avorturi spontane recurente (ASR) (= 2 AS) sau naşterea unor copii morţi (NM) (2,3,4).

Avorturile spontane (AS) reprezintă pierderea inexplicabilă a unei sarcini, înainte de împlinirea a 20 de săptămâni complete de gestaţie (18 săptămâni după fertilizare) sau, dacă nu este cunoscută vârsta gestaţională, pierderea unui făt cu greutatea sub

400 grame (4). Se estimează că aproximativ 15-20% din sarcinile recunoscute clinic, se elimină prin AS, dar frecvenţa reală este probabil mai mare, 2/3 dintre AS producându-se pe parcursul unor sarcini nedecelate clinic (avorturi biochimice) (2, 3).

Avorturile spontane recurente (ASR) se caracterizează prin apariţia a 2 sau mai multe avorturi spontane consecutive la acelaşi cuplu (2, 3).

Moartea fetală intra-uterină (nou-născuţi morţi, NM), definită ca fiind moartea *in utero* a unui făt cu vârsta gestaţională mai mare de 20 de săptămâni, are o frecvenţă de circa 6,5/1000 nou-născuţi (4).

Cauzele TR sunt multiple : genetice, malformative, endocrine, imunologice sau de mediu (1, 2). TR pot afecta ambii parteneri ai cuplului, etiologia putând fi feminină, masculină sau de cuplu (2). Incidenţa factorilor etiologici implicaţi în producerea TR umane a fost apreciată într-un raport al Societăţii Americane de Medicină Reproductivă din 2006 : 25% cauze masculine, 27% cauze ovulatorii, 22% cauze uterine/ tubare, 9% endometrioza şi 17% cauze idiopatice (2).

Rolul factorilor genetici, reprezentaţi în principal de anomaliile cromozomiale, în producerea TR umane, a fost dovedit fără echivoc (5).

Cauzele genetice ale sterilităţii primare (ST) sunt reprezentate de anomaliile cromozomiale (numerice şi structurale ale gonozomilor şi anomaliile de structură echilibrate ale autozomilor) şi, mai rar, de boli monogenice sau multifactoriale (2, 6).

Circa 50-60% dintre avorturile spontane recurente (ASR) precoce (0-8 săptămâni) sunt determinate de o aneuplodie prezentă la făt, în timp ce odată cu creşterea vârstei gestaţionale, incidenţa anomaliilor cromozomiale scade, acestea fiind decelate în aproximativ 1/3 (35%) din ASR din trimestrul II de sarcină şi doar la 5% dintre nou-născuţii morţi din trimestrul III de sarcină (5,7,8).

In circa 5% dintre avorturile recurente, la produşii de avort au fost identificate aneuploidii segmentale (monosomii parţiale, trisomii parţiale sau asocierea de trisomii şi monosomii parţiale), care în cea mai mare parte au fost rezultatul unei malsegregări a cromozomilor derivativi, în cazul unei anomalii cromozomiale de structură echilibrată (ACSE) prezentă la unul dintre părinţi (9,10).

Scopul studiului a fost analiza retrospectivă a rezultatelor analizei cromozomiale, în cazul unui lot selecţionat de 266 cupluri cu tulburări de reproducere (TR).

MATERIAL ŞI METODĂ

Pacienţii

In perioada ianuarie 1998 - decembrie 2009 au fost evaluate clinic în cadrul Centrului de Genetică Medicală Iaşi, 266 cupluri cu tulburări de reproducere : 80 cazuri (30,07%) cu sterilitate primară (ST), 149 cazuri (56,01%) cu avorturi spontane recurente (ASR) şi 37 cazuri (13,90%) cu nou născuţi morţi (NM).

Metode

În cazul fiecărui cuplu cu TR a fost efectuată analiza cromozomială folosind un marcaj în benzi de tip G (450-550 benzi/ metafază).

S-a urmărit evidenţierea unor *anomalii cromozomiale numerice sau structurale*. Analiza cromozomială s-a bazat pe o cultură stimulată, de scurtă durată a limfocitelor T şi a fost realizată conform tehnicii Moorhead (1960) (11), adaptată în cadrul Laboratorului de Citogenetică al Universităţii de Medicină şi Farmacie „Gr. T. Popa" Iaşi. Analiza a cuprins mai multe etape : recoltarea sângelui periferic pe heparină sodică ; punerea sângelui în cultură (mediul de cultură este RPMI 1640 (9 ml), suplimentat cu ser de viţel fetal (1ml) şi glutamină (0,1 ml)) ; hipotonizarea, fixarea, etalarea şi colorarea. Metafazele au fost examinate cu microscopul Nikon, iar imaginile au fost captate cu un sistem automat de cariotipare

TABELUL I

Anomalii cromozomiale decelate la cele două sexe în cazul a 266 cupluri cu tulburări de reproducere (TR)

		Femeie	Bărbat
Tip anomalie cromozomială	**Număr (%)**	**Număr (%)**	**Număr (%)**
Aneuploidii gonozomale	**14 (5,26)**	7 (2,63))	7 (2,63)
Anomalii structurale echilibrate	**23 (8,64)**	10 (3,75)	13 (4,88)
- translocații robertsoniene	**3 (1,12)**	3 (1,12)	**-**
- translocații reciproce	**2 (0,75)**	1 (0,37)	1 (0,37)
- inserții	**3 (1,12)**	1 (0,37)	2 (0,75)
- inversii	**15 (5,63)**	5 (1,87)	10 (3,75)
Anomalii structurale gonozomale	**4 (1,50)**	4 (1,50)	-
Alte anomalii	**2 (0,75)**	-	2 (0,75)
Număr total anomalii	**43 (16,16)**	**21 (7,89)**	**22 (8,27)**
Variante polimorfice normale	**3 (1,12)**	**1 (0,37)**	**2 (0,75)**

Cytovision (Applied Imaging).

Pentru fiecare caz au fost analizate un număr de minim 16 metafaze. În situaţiile în care au fost depistate anomalii, numărul de celule analizate a fost crescut la 32, 64 sau chiar 96.

REZULTATE

În cazul a 43 de cupluri (16,16%), cariotipul a relevat prezenţa unei anomalii cromozomiale (numerice sau structurale) la unul dintre parteneri: 20 cazuri (7,51%) la cuplurile cu ST, 13 cazuri (4,88%) la cuplurile cu ASR şi 10 cazuri (3,75%) la cuplurile cu NM. In 23 cazuri (8,64%) anomalia a fost decelată la femeie, iar în 20 cazuri (7,51%, la bărbat (tab. I).

Cele mai frecvente anomalii decelate au fost anomaliile cromozomiale de structură echilibrate (ACSE), prezente la 23 cupluri (8,64%), urmate de anomaliile de număr şi de structură ale cromozomului X, identifi-

TABELUL II

Anomalii cromozomiale decelate la 80 de cupluri cu sterilitate (ST)

Tip anomalie cromozomială	**Nr. Total (%) ***	**Femei**	**Bărbaţi**
		Nr. Cazuri (%)	**Nr. Cazuri (%)**
Aneuploidii gonozomale	**8 (10)**	**2 (2,50)** 1 – 45,X/47,XXX 1 – 45,X	**6 (7,50)** 6 – 47,XXY
Anomalii structurale gonozomale	**4 (5)**	**4 (5)** 1- 46,X,i(Xq) 1- 46,X,del(X)(p22.2? pter) 2- 45,X/46,X,i(X)(q10)	-
Anomalii structurale echilibrate **-** Inversii	**6 (7,50)**	**1 (1,25)** 1-46,XX,inv(9)(p13;q21.2)	**5 (6,25)** 2-46,XY,inv(9) (p13;q21.2) 1-46,XY,inv(9)(p13;q22) 2- 46,XY,inv(16)(p11.1;q21.1)
Alte anomalii	**2 (2,50)**	-	**2 (2,50)** 2- 46,XX
Nr total anomalii	**20 (25)**	**7 (8,75)**	**13 (16,25)**
Variante polimorfice normale	**1 (1,25)**	-	**1 (1,25)** 1-46,XY,9qh+

* procentele au fost calculate în raport cu numărul de cupluri cu ST

TABELUL III

Anomalii cromozomiale decelate la 149 cupluri cu avorturi spontane recurente (ASR)

Tip anomalie cromozomială	Nr. total (%)*	Femei	Bărbaţi
		Nr. cazuri (%)	Nr. cazuri (%)
Aneuploidii gonozomale	**4 (2,68)**	**4 (2,68)** 4 - 45,X/46,XX	-
Anomalii structurale echilibrate	**9 (5,38)**	**4 (2,68)**	**5 (3,35)**
- translocaţii robertsoniene	**1 (0,67)**	**1 (7,69)** 1 - 46,XX,rob(13;14)(q10;q10)	-
- translocaţii reciproce	**1 (0,67)**	**1 (7,69)** 1-46,XX,t(4;7)(q31.3;q31.2)	-
- inserţii	**1 (0,67)**	-	**1 (7,69)** 1- 46,XY,ins(5;4)(q13;q27q31)
- inversii	**6 (4,02)**	**2 (1,34)** 1-46,XX,inv(9)(p13.3l;q21.2) 1- 46,XX,inv(10)(p11.22;q21.2)	**4 (2,68)** 1- 46,XY,inv(1)(p11;q12) 1-46,XY,inv(1)(p13;q23) 1- 46,XY,inv(9)(p13q21.2) 1-46,XY,inv(15)(p11.2;q13)
Nr total anomalii	**13 (8,72)**	**8 (5,36)**	**5 (3,35)**
Variante polimorfice normale	**2 (1,34)**	**1 (0,67)** 1- 46,XX,9qh+	**1** 1- 46,XY,1qh+

* procentele au fost calculate în raport cu numărul de cupluri cu ASR

cate în cazul a 18 cupluri (6,76%), majoritatea lor fiind reprezentate de monosomia X în mozaic, la femeie şi de trisomia XXY (sindromul Klinefelter) la bărbat (tab. II, III şi IV). În alte 2 cazuri a fost decelat un cariotip 46,XX la bărbat, iar la 3 cupluri (1,12%) au fost decelate variante cromozomiale polimorfice, considerate normale: 9qh+ (2 cazuri) şi 1qh+ (1 caz) (tab. I, II , III).

La 20 de cupluri cu sterilitate (ST) (25% dintre cuplurile cu ST) a fost decelată o anomalie cromozomială, frecvenţa acestora fiind de aproape două ori mai mare la bărbaţi (13 cazuri- 16,25%), faţă de femei (7 cazuri - 8,75%). Cele mai frecvente anomalii decelate în aceste cazuri, au fost anomaliile de număr şi de structură a cromozomului X (12 cazuri- 15%), urmate de ASCE, reprezentate de inversii (6 cazuri - 7,50%) (tab. II).

La 13 (8,72%) dintre cele 149 de cupluri cu avorturi spontane recurente (ASR), a fost decelată o anomalie cromozomială, 8 (5,36%) dintre acestea fiind prezente la femeie. Cele mai frecvente anomalii decelate au fost ACSE (9 cazuri - 5,38%), majoritatea fiind inversii (6 cazuri - 4,02%), urmate de aneuplodii gonozomale (monosomia X în mozaic, 4 cazuri - 2,68%). (tab. III).

În cazul cuplurilor cu nou-născuţi morţi (NM), au fost decelate 10 cazuri cu anomalii (27,02%), cele mai frecvente fiind ACSE (8 cazuri-21,62%), urmate de aneuploidii gonozomale (2 cazuri- 5,40%) (tab. IV).

DISCUŢII

Ponderea anomaliilor cromozomiale decelate la lotul selecţionat al cuplurilor cu TR, a fost mai mare (16,16%), decât cea citată în alte studii similare: 8,9% - Pal (12), 9,04% - Nazmy (13), 5,53% - Espinoza et al (14) şi 1,97% Clementini et al (15,) probabil, datorită selecţiei clinice riguroase făcută anterior investigaţiei citogenetice. Per total, ponderea ACSE a fost mai mare (8,64%), faţă de cea a anomaliilor

TABELUL IV

Anomalii cromozomiale decelate la 37 cupluri cu nou-născuţi morţi (NM)

Tip anomalie cromozomială	Număr (%)*	Femei	Bărbaţi
		Nr. cazuri (%)	Nr. cazuri (%)
Aneuploidii gonozomale	**2 (5,40)**	**1 (2,70)** 1- 46,XX/47,XXX/48,XXXX	**1 (2,70)** 1- 46,XY/47,XYY
Anomalii structurale echilibrate	**8 (21,62)**	**5 (13,51)**	**3 (8,10)**
- translocaţii robertsoniene	**2 (5,40)**	**2 (5,40)** 1- 46,XX,rob (14;21)(q10;q10) 1-46,XX, rob (13;22)(q10;q10)	-
- translocaţii reciproce	**1 (2,70)**	-	**1 (2,70)** 1- 46,XY,t(6;8)
- inserţii	**2 (5,40)**	**1 (2,70)** 1-46,XX,ins(7;6)(p13;q25.2q25.3)	**1** 1- 46,XY, ins (9;21) (p22;q22? qter)
- inversii	**3 (8,10)**	**2 (5,40)** 1 - 46,XX,inv (9)(p22;q22) 1- 46,XX,inv(9)(p13;q21.2),1qh+	**1 (2,70)** 1- 46,XY,inv (9)(p13;q21.3)
Nr total	**10 (27,02)**	**6 (16,21)**	**4 (10,81)**

* procentele au fost calculate în raport cu numărul de cupluri cu NM

numerice şi structurale ale cromozomului X (6,76%), însă aneuploidiile şi anomaliile de structură ale cromozomului X au fost principala cauză a sterilităţii la cuplurile cu ST, în timp ce ACSE au reprezentat principalul factor etiologic genetic în cazul cuplurilor cu ASR şi NM (tab. II, III şi IV). Datele obţinute sunt concordante cu cele din literatura de specialitate (2, 5, 6).

Din totalul de 43 de anomalii cromozomiale decelate la cuplurile studiate, în 48,83% din cazuri, anomalia a fost prezentă la femeie, iar în 51,16% din cazuri, la bărbat, aceste rezultate fiind concordante cu cele prezentate în studii similare de Espinoza et al (54,7% la femei şi 54,3% la bărbaţi) (14) şi Clementini et al (51,82% la femei şi 48,78% la bărbaţi) (15).

Inversiile (15 cazuri - 5,63%) au fost cele mai frecvente anomalii cromozomiale structurale echilibrate (ACSE) decelate, urmate de translocaţii (5 cazuri - 1,87%). Cea mai frecventă inversie a fost cea a cromozomului 9 (2,63% - 7 cazuri), frecvenţă similară cu cea întâlnită în populaţia generală (1-3%) (tab. II, III şi IV). Inversia cromozomului 9 a fost întâlnită la toate cele trei categorii de cupluri cu TR (ST, ASR, NM), atât la femeie (4 cazuri), cât şi la bărbat (5 cazuri) (tab. II, III şi IV).

Implicarea inversiilor pericentrice ale cromozomului 9, inv(9)(p11q12) sau inv(9)(p11q13) în producerea TR este controversată, acestea fiind considerate variante normale. Numeroase studii efectuate de-a lungul timpului au încercat să elucideze mecanismul patogenic, luându-se în considerare necesitatea identificării purtătorilor inversiei inv(9) produse *de novo* sau a cazurilor familiale de inversie a cromozomului 9 (16, 17, 18, 19). Studiile privind organizarea heterocromatinei din regiunea pericentromerică şi din regiunea proximală a braţelor scurt (p) şi lung (q) ale cromozomului 9, au relevat o omologie între cele două regiuni 9p12 şi 9q13-21.1, în care se produc cel mai frecvent rupturi (*hotspots*), implicate în producerea recombinărilor cromozomiale (20). Multe secvenţe sunt duplicate în regiunea pericentromerică, fiind identificate blocuri mari de duplicaţii segmentale in interiorul şi între cele două regiuni pericentromerice 9p11–12 şi 9q11–12/13. Astfel, cele două regiuni cromozomiale conţin secvenţe identice în

proporţie de 97%, ceea ce favorizează recombinările cromozomiale aberante (21).

Cele mai frecvente translocaţii decelate, au fost translocaţiile robertsoniene (1,12% - 3 cazuri), toate fiind identificate la femei. Aceste rezultate au fost similare cu cele găsite în alte studii, raportul dintre purtătorii de translocaţii robertsoniene găsit de Espinoza et al fiind în favoarea femeilor (F : M =3,5 : 1) (14), la fel şi cel găsit de şi Orozco Quinono et al (F : M= 5,5 : 1) (22).

La 2 dintre bărbaţii cu ST şi azoospermie a fost identificat un sindrom de inversiune sexuală (bărbaţi 46,XX), determinat cel mai probabil de translocaţia genei SRY de pe cromozomul Yp pe cromozomul Xp în timpul *crossing over*-lui din meioza paternă.

Datele noastre sunt, în ansamblu, concordante cu alte studii şi relevă importanţa analizei cromozomiale la cuplurile cu TR, mai ales în situaţia în care, din ce în ce mai multe cupluri apelează la tehnici de reproducere asistată (fertilizare in vitro, FIV), ale căror protocoale impun obligatoriu efectuarea cariotipului la ambii membri ai cuplului, înaintea efectuării tehnicii respective.

CONCLUZII

În concluzie, rezultatele studiului nostru retrospectiv, concordante cu datele din literatură, evidenţiază rolul major pe care îl au anomaliile cromozomiale, în producerea TR umane, subliniind în acelaşi timp importanţa analizei cromozomiale pentru diagnosticul etiologic, managementul şi sfatul genetic acordat acestor cupluri, inclusiv în contextul aplicării în viitor a unei tehnici de reproducere asistată (FIV).

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NOUTĂŢI

Mecanisme non-imunologice ale inhibitorilor de calcineurină explică efectul lor antiproteinuric în glomerulopatiile genetice

A fost raportat că tratamentul cu ciclosporina A (CyA) în combinaţie cu corticosteroizi, inhibitori de enzimă de conversie şi blocanţi ai receptorilor de angiotensină, a dus la scăderea rpoteinuriei la 3 pacienţi cu sindrom nefrotic (NS) datorat mutaţiei *WT1*. tratamentul cu inhibitori de calcineurină a indus o remisiune parţială a proteinurie la câţiva alţi copii cu forme genetice de NS, cum ar fi mutaţii ale genelor podocinei şi fosfolipază C epsilon. CyA a fost raportată de asemenea a fi eficientă la pacienţii cu sindrom Alport. Date recente au arătat că efectul antiproteinuric al CyA în aceste cazuri se poate datora unor mecanisme non-imune. CyA exercită un efect antiproteinuric prin prevenirea degradării proteinei sinaptopodina organizatoare a actinei şi prin downregulation a TRPC6. aceste emcanisme conduc la stabilizarea citoscheletonului de actină în podocitele renale. Acest effect benefic al CyA este interesant dar rezultatele e termen lung privind functionarea si nefrotoxicitatea încă lipsesc. (Bensman A, Niaudet P, Non-immunologic mechanisms of calcineurin inhibitors explan its effects in genetic glomerupopathies, *Ped Nephrol* 2010 ; 25 : 1197-1199).

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THE USE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN PHARMACEUTICAL ANALYSIS

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THE USE OF HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY IN PHARMACEUTICAL ANALYSIS (Abstract): The aim of the study was to provide a review and strategy for the development and validation of a modern analytical method with nearly unparalleled accuracy in the pharmaceutical analysis. High performance liquid chromatography is an analytical method used to separate, identify and quantify components dissolved in a liquid solvent with a high analytical resolution. Because of its versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals. Characteristics that should be considered during validation of analytical methods include specificity, linearity, range, accuracy, precision, detection limit, quantification limit, robustness. **Keywords:** HIGH PERFORMANCE LIQUID CHROMATOGRAPHY, PHARMACEUTICAL SUBSTANCES, VALIDATION.

The advancement of pharmaceutical research has brought to the attention of specialists a large number of pharmaceutical substances that require very high purity parameters. Degradation and contamination of pharmaceutical products begins during the synthesis process of the active molecule and it continues during formulation, packaging, transport and storage. During all those stages, the control of purity may be done using high-throughput analytical methods. The biological pathway of the active molecules can also be tracked from the moment of administration, using quantitative methods with high sensitivity that may also be used to confirm the proposed mechanism of action, dose and schedule of administration, efficacy, safety and association between benefit and risks of the drug.

High Performance Liquid Chromatography (HPLC) is one of the most commonly used techniques in biomedical analysis for separation, identification, and quantification of each component in a mixture (1, 2). It was introduced for the first time in 1980 as the official method for bulk drug analysis (United States Pharmacopoeia, 1980), due to its high sensitivity and specificity. It has become one of the most widely used or even the main method for official analysis in various pharmacopoeias such as The United States Pharmacopoeia and The European Pharmacopoeia (3-19).

Conventional regulations, such as those of the European Medicines Agency

(EMEA), the Food and Drug Administration (FDA), and the International Conference of Harmonization (ICH), state that validation of the method is essential in drug analysis.

The development of high-performance liquid chromatography methods generally includes the following steps: selection of the HPLC method and chromatographic system, selection of initial conditions, selectivity and system optimization, and method validation (20).

In order for the analysis procedure to be applied, it must undergo verification steps confirming its scientific validity. Validation is meant to demonstrate that the proposed method corresponds to the intended use. The statistical processing of the results of an analysis gives the possibility of obtaining the most probable value that is closest to reality (21-22).

For HPLC analysis with UV-VIS detection there are several validation schemes, which consider the parameters described next.

Specificity/Selectivity

A method is specific if it can be applied to a single analyte, while a method is selective if it can accurately and specifically quantify the analyte or analytes in the presence of other compounds. In the case of pharmaceutical substances, selectivity implies the ability to separate the analyte of degradation products, metabolites, excipients and other compounds present in the sample.

For HPLC methods, selectivity can be achieved through a good separation between the analyte and other components of the sample. Peak resolution should be greater than 1.5. Interferences are tested on multiple samples, from different sources, and the peak corresponding to interference with the same retention time must be less than 20% of the standard quantification limit and less than 5% then that of the internal standard used (3, 4, 21, 22).

Selecting the detection wavelength

For HPLC analysis with UV-VIS spectrophotometric detection, there is a peak in the chromatogram at the retention time corresponding to the analyte. The UV-VIS absorption spectrum of that peak is recorded, and by analyzing it the absorption maxima are highlighted.

The detection wavelength is selected as the one with the maximum absorbance value, considering the potential presence of various interferences. That value will be used for all subsequent determinations.

Identifying the peak corresponding to the analyte

Due to the fact that a particular compound behaves identically during any HPLC analysis if all operating conditions are identical, the detection of the analyte in different samples is based on the comparison of the retention time of the peak from the chromatogram of the sample with that obtained in the chromatogram of the reference substance. A difference of no more than ± 5% of is acceptable (23).

In order to increase the accuracy of the identification, the absorption spectrum of peak from the sample chromatogram must be compared to that of the reference solution analyzed under the same conditions in terms of stationary and mobile phase composition, flow, temperature, detection wavelength, reference wavelength.

Accuracy of identification could be quantified by choosing a threshold factor for overlapping spectra. If the accuracy of the identification is equal to or greater than that factor, the peak is considered to be identified and it is assigned to the analyte (22).

Assessment of peak purity

The evaluation of peak purity excludes the coexistence of several substances for the same peak. The control of purity in pharmaceutical analysis is based on the comparison of three spectra recorded during elution of the peak. Among those, two spectra are recorded at the increasing and decreasing inflection points of the peak and one at the tip of the peak. If those spectra are not the same, in theory, the peak contains a spectral impurity. It may be caused by one or more components separated simultaneously, by peaks that are not properly separated, or it may be due to baseline noise (3, 4, 21).

There is also the possibility of identical spectra due to peaks containing impurities at a very low concentration compared to the analyte or if the impurity and the analyte have the same or almost the same spectrum and similar elution time (21).

Linearity of the response function

Linearity is the ability of the method to produce results directly or indirectly proportional to the concentration of the analyte in the sample over a certain range of concentrations.

In order to evaluate the linearity of the method, the relationship between the signal provided by the detector (absorbance, height or area of the peak) and the concentration of the analyte in the sample is determined. That relationship may be linear, exponential, logarithmic, polynomial, or other, and it must be a monotonous, incremental or decreasing function over the entire concentration range studied (3, 4, 22).

The statistical method most commonly used to find the best correlation between experimental data and the calibration curve type is the linear regression method. The parameters that must be calculated using regression analysis are correlation coefficient (r), regression coefficient (r^2), vertical intercept, slope and standard deviation of the regression line, least squares regression. The correlation coefficient indicates a linear relationship if its value exceeds 0.999 (22-26).

Calibrating the detector response

In order to obtain reliable results, it is necessary to calibrate the detector response (UV-VIS spectrophotometer). To that purpose, duplicate series of solutions are prepared in a matrix identical to that of the sample to be analyzed, for the entire working concentration range. For each concentration, the mean value of the peak area is calculate and then plotted against the theoretical value. A linear regression curve confirms the truthfulness of the detector response (27-29).

Detection and quantification limit

The Detection Limit (LOD) is the minimum amount of a component that can be highlighted against a blank sample with a 1% confidence level.

The Quantification Limit (LOQ) is the minimum quantity of a component that can be quantified with certainty.

One of the most commonly used methods to estimate LOD and LOQ is based on the standard deviation and the slope of the regression line using the equations:

$$\mathrm{LOD} = \frac{3 \cdot \sigma}{S} \quad \text{and} \quad \mathrm{LOQ} = \frac{10 \cdot \sigma}{S}$$

where: σ is the standard deviation of the regression line and S is the slope of the regression line (27).

The calculated values of the detection and quantification limit must be confirmed through independent analysis of an appropriate number of samples that have concentrations close to both limits (27-29).

Working range

Routine analysis in the pharmaceutical industry is conducted in 80% to 120% concentration range relative to the actual concentration of the analyte (24).

Precision

Precision provides data on random errors and characterizes the degree of concordance amongst the analytical results on repeated application of the analytical method on the same homogeneous sample or sample prepared under identical conditions. It estimates how close the measured values are to each other for a number of measurements under the same conditions.

The ICH rules define accuracy as being composed of three components: system precision, method precision, intermediate precision and reproducibility (23, 30).

The precision of the chromatographic system is the ability to detect small changes concerning analyte concentration as a result of multiple determinations (injections) from a homogeneous sample under established working conditions and during one day of tests. It is estimated using the coefficient of variation, a small value of which is related to a more precise result.

The precision of the method is expressed by the value of the relative standard deviation (RSD) for the results of multiple measurements of a sample by the same analyst in the same laboratory using the same equipment within a short time using the same procedure on identical samples in the same conditions of analysis (30, 31).

Intermediate precision assesses the dependence of the method in conditions other than those in which that particular method was developed. The purpose of intermediate precision evaluation is to ensure that the method will lead to the same results, if samples are analyzed in the same laboratory using the same procedure under the same analytical conditions by different analysts using different equipment within a given time frame. It is expressed through the value of relative standard deviation (RSD), evaluated in the exact same conditions as the precision of the method (32, 33).

Reproducibility is defined as interlaboratory precision in collaborative studies. In other words, reproducibility depends on the conditions chosen for which the results are obtained using the same procedure, on identical samples, in different laboratories, using different equipment and reagents of different origin, by different operators.

Accuracy

Accuracy is a validation feature that evaluates how close the analytical results are to the true value. The accuracy usually assessed for HPLC assay methods for pharmaceutical substances by adding a known amount of analyte to a placebo solution with concentrations ranging from 80-120% or even 75-125% of the actual concentration in the pharmaceutical form (24, 30, 31).

Replicate samples are evaluated for each concentration level studied. Accuracy evaluation calculate the percentages based on the experimental results obtained. The interval in which the results of the recovery test are accepted is between 98 and 102% or 95 and 105% (23, 34-36).

Robustness and stability of the method

The robustness of an HPLC method is evaluated by measuring the ability of the method to remain unaffected by small, deliberate variations of some method parameters (mobile phase composition, column temperature, column age, etc.) or environmental factors (room temperature, air humidity, etc.), in other words, the stability

of the method. The stability of an analytical method is the degree of reproducibility of results obtained for the same sample under different conditions, such as different laboratories, different analysts, different apparatus, different reagent lots, different days (31-34, 37).

CONCLUSIONS

In conclusion, the validation of an HPLC method used for pharmaceutical analysis must be performed by each laboratory according to the method-specific tests, to ensure that the method exhibits accuracy, precision, specificity, reproducibility and stability over the entire concentration interval for the analysis of the analyte. Validation of the method must be performed prior to application and it is essential in all pharmaceutical research phases.

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TECHNOLOGICAL PARTICULARITIES OF THE IMPLANT-SUPPORTED DENTAL PROSTHESES. CASE REPORT

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TECHNOLOGICAL PARTICULARITIES OF THE IMPLANT-SUPPORTED DENTAL PROSTHESES. CASE REPORT (Abstract): Complete edentulous is a pathological condition characterized by the loss of all teeth and their supporting periodontal structures on one or both jaws, accompanied by functional consequences. The biggest challenge for both, dentist and patient, is the transition from dentate to completely edentate status. The indication for dental implant insertion to a totally edentate patient should be considered with a lot of discernment because it is usually addresses to elderly people, often presenting an associated general pathology, which may affect the final therapeutic success. The purpose of this article is to present the clinical and technological steps in the metal-ceramic bridges in a patient with bimaxillary complete edentulous. Prostheses were fixed on dental implants in order to achieve a complex oral rehabilitation. The precise knowledge of the algorithm and technological parameters of each step allows to avoid mistakes and to obtain the appropriate biologic and mechanical prosthetic constructions. **Keywords:** COMPLETE EDENTULOUS, COMPLETE DENTURE, DENTAL IMPLANTS.

Complete edentulous is the most serious mutilation of the dento-maxillary system. This disease can usually be treated by a removable restoration which is based on different clinical and biological criteria; sometimes this therapeutic variant is difficult due to the serious, progressive and irreversible alteration of residual tissue components and the decrease in adaptive natural mechanisms. By losing all the teeth, oral homeostasis is seriously affected, and morpho-functional recovery through prosthetic treatment requires the correlation of numerous factors that ultimately ensure the success of this treatment. Complete dentures are restorations which demand a high degree of knowledge and skill from the dental team. Each working step must yield the maximum result, the final goal being an increased quality of life for the patient (1). Currently, treatment of complete edentulous follows three main directions: the classical complete denture, (base on the residual elements of denture bearing area for retention, support and stabilization), removable partial denture supported by dental implants and fixed prostheses supported by dental implants (2, 3). The long-term success rate of full-arch, implant-supported restorations is one of the best in medicine. Clinical studies on the implant supported fixed prostheses have shown that long-term results are clearly superior to removable prostheses, due to the design

and the coating treatment of the implants.

In order to choose a prosthetic solution on implants, the following aspects should not be omitted from the assessment of the patient condition: informing patients about the benefits of implant insertion and possible associated risks, the medical history, evaluation of the general condition of the patient, evaluation of the oral status by performing a rigorous examination, determination of the number and position of future implants after careful radiological analysis. General contraindications for implants include major immune deficiencies, major heart diseases, severe hypertension, endocarditis, insulin-dependent diabetes, liver cirrhosis, chronic obstructive pulmonary disease, frequent illness requiring periodic or rhythmic use of steroidal anti-inflammatory drugs, cancer patients who had undergone, are undergoing or will undergo chemotherapy, uncontrollable endocrine diseases, psychotic disorders and drug use (4). Local contraindications include poor oral hygiene, oral mucosal disorders, local inflammatory processes, insufficient bone support, poor-quality of the bone, post-radiation therapy in the cervical-orofacial area. In general, local conditions are not an absolute contraindication, but only a timing of the implant solution.

The decision of choosing a fixed prosthetic construction supported on implants in complete edentulous should be determined with great discernment and attention because it is usually addressed to elderly people, who often have an associated pathology, that can affect the success of the prosthetic treatment, due to insufficient bone support or presence of absolute general contraindications.

CASE REPORT

We present the case of a 64-years-old patient with masticatory and physiognomic disturbances as a result of an extended bimaxillary partial edentulous.

Following clinical and radiological examination we established that the first step of the treatment should be the extraction of compromised teeth on both arches, transforming partial edentulous into complete one (fig. 1).

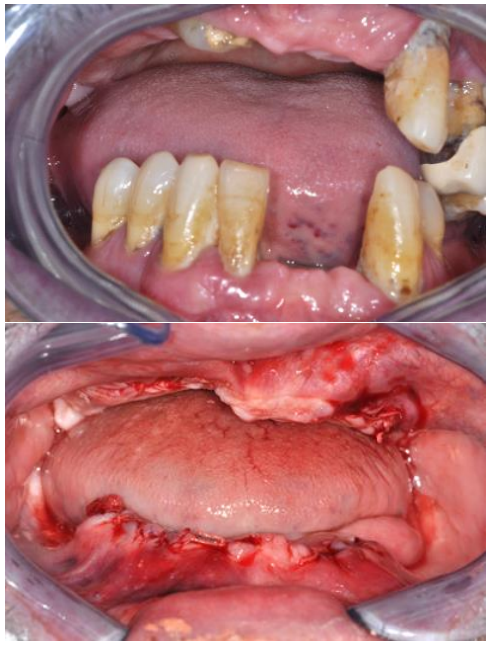

Fig. 1. Initial clinical situation and after teeth extraction

After complete cicatrization, bone capital was carefully analyzed again, performing a radiological examination by Cone-beam computed tomography (CBCT). This innovative technique for digital dental X-rays is commonly used in implantology, because it is more accurate than a panoramic dental X-ray and can target the area to be observed to avoid exposing other parts of the skull to X-ray. This examination explores the condition of the bones and the function of the temporomandibular joint.

The final therapeutic solution consisted in the insertion of 11 implants into the maxilla and 8 implants into the mandible, on which will be applied metal-ceramic bridges. The impression was taken using the indirect technique, with impression copings attached to the implant, which are removed together with the set impression. The analogues are attached to the impression copings while they are embedded in the impression tray (fig. 2).

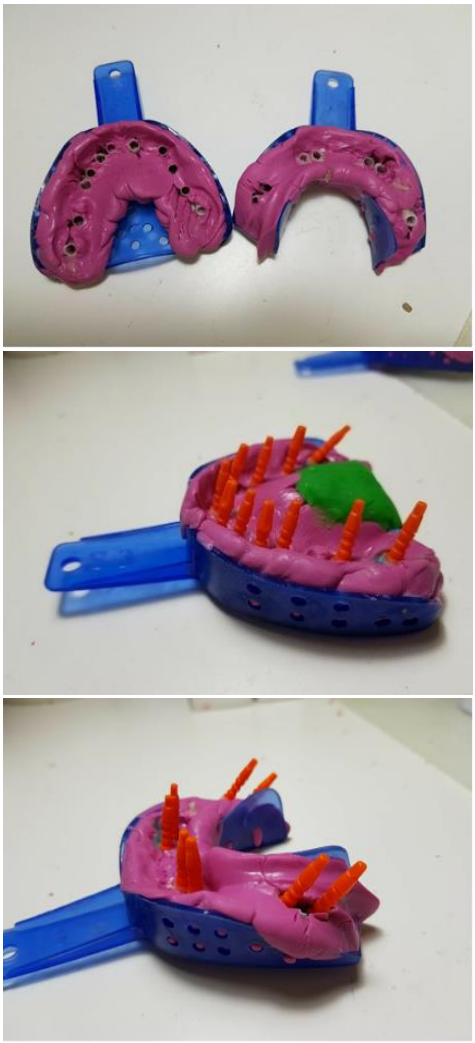

Fig. 2. The impressions

Dental impressions are checked and disinfected, and then sent to the dental laboratory, where the technician realize casts with removable dies for each impression, and then mount them into a dental simulator. The next step is the building metalic framework of metal-ceramic bridge. For the wax pattern a special blue inlay wax was used, and for metallic framework was used a cobalt-chrome dental alloy (Remanium CS/Dentaurum/Germany).

To enhance the bond between the metallic infrastructure and the aesthetic component, the framework was sandblasted with 50 μm Al_2O_3 particles. The ceramic layers were applied to the metallic framework and sintered at a preset temperature (960ºC for the opaque and dentine, in the vacuum condition, and 930ºC for glaze, in atmospheric conditions).

Fig. 3. Ceramic layers application

After completing all the steps, the prosthesis were verified on the die, then in the oral cavity, looking for adaption to the implant structures (fig. 4).

Fig. 4. The prosthesis on the die and in the oral cavity

DISCUSSION

Treating a completely edentulous patient with fixed prostheses implant-supported is a solution that has many advantages for the patient: much easier adjustment, immediate resumption of functions, superior esthetic appearance, and high mechanical strength. Prosthetic appliances involves treating dysfunctions, stimulating compensatory mechanisms, and achieving a new homeostasis balance between the elements of the oral system (5). Prosthetic treatment will not only aim to restore the dental arch continuity, but also the correct restoration of occlusion and of the fundamental mandibula-cranial relationships and mandibular dynamics. In order to achieve a correct and complete dental treatment, a clinical and paraclinical assessment of the initial situation is required. It is necessary to establish a correct diagnosis based on the clinical information and to develop a treatment plan based on the restoration of the balance between all the affected elements (6, 7). The treatment of complete edentulism should be the result of a comparative analysis of the benefits and disadvantages for each clinical case. The correct assessment of the initial clinical situation, preparation of the prosthetic area, preprosthetic and prosthetic treatments, and selection of the most appropriate therapeutic solution prevent failures and allow a good longevity of the restorations.

CONCLUSIONS

In order to achieve long-term therapeutic success in implant-supported oral rehabilitation some surgical, prosthetic, and periodontal aspects as well as the optimal timing of implant insertion must take into consideration. The selected implant type must provide mechanical strength, the implant material must be biocompatible, and its configuration must allow the aggregation of a suitable prosthetic superstructure and the transfer of the occlusal forces to the bone substrate, as physiologically as possible.

The prosthetic aspects should be considered from the very beginning before planning implant insertion. These aspects are essentials for the choice of implant system, number of implants to be inserted, their position, and insertion axis. In the fabrication of the prosthetic superstructure, the bio functional, gnathological and esthetic principles must be respected. The advantages of an implant-supported pros-

thesis is due to the fact that the patient gets easily accommodate to the artificial substitute, the comfort is superior, and the restoration of the functions is easily achieved.

The success of this solution is reached if the indication for implant therapy was correctly established and bridges were realized according to the clinical particularities of the case. These conditions are accomplished if there is a good collaboration between the implantologist, prosthetic specialist and dental technician.

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NEWS

INCREASED INTEGRATION OF EXTRACELLULAR DNA IN THE ENVIRONMENTAL METAGENOME IN THE PRESENCE OF MUTAGENS LIKE BROMOACETIC ACID

A study by Mantilla-Calderon D *et al.* demonstrated that bromoacetic acid can increase transformation rates in microorganisms. Bromoacetic acid is a drinking water disinfection byproduct. Natural transformation allows for the uptake and integration of foreign DNA, which is a form of horizontal transfer of genes. This can be responsible for the acquisition of virulence and antibiotic resistance. The model microorganism used in the study was *Acinetobacter baylyi* ADP1. The transformation rate in this study was dependent on concentration of bromoacetic acid and its ability to cause DNA damage. Thus, transcription of recA gene was enhanced by 20 to 40%. This, in turn, facilitates the integration of extracellular DNA within the acceptor bacterial cell during DNA repair (Mantilla-Calderon D, Plewa MJ, Michoud G, Fodelianakis S, Daffonchio D, Hong P. Water disinfection byproducts increase natural transformation rates of environmental DNA in *Acinetobacter baylyi* ADP1. *Environ Sci Technol* 2019; 53(11): 6520-6528.

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ELASTOGRAPHY, PROGRESSION FACTOR IN LIVER ULTRASOUND

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ELASTOGRAPHY, PROGRESSION FACTOR IN LIVER ULTRASOUND (ABSTRACT): Elastography is a method which,using ultrasound, obtains images and measurements of tissue elasticity when applying a force on it. As an imaging method it was developed to quantify objectively the pathological changes related to the presence of an abnormal tissue, compared to the surrounding tissues, giving information about the elasticity/stiffness of the examined tissue, the degree of fibrosis, the degree of stiffness compared to tumor free tissue. The tissue analysis can be done through a compression technology "*eSie touch* elasticity imaging" (with applications for the surface elastography) or *ARFI* technology – "acustic radiation force imaging" (the diffusion impulse of the acustic force). The *ARFI* method allows valid, accurate and flexible evaluation of liver stiffnessand it is correlates with the fibrosis stage. The liver elastosonography, through new technologies available, has reached the level of the fibroelastoscanner and magnetic resonance imaging. The ultrasound elastography application quickly advances, starting with the researches in this field. At present, it has analised the most various fields of application, from the breast, prostate, thyroid, pancreas imaging to the study of abdominal lymphnodes and peripheral vessels, gastrointestinal stromal tumors, primary and secundary liver tumors, the evaluation of uterine cervix, from cardiology to gastroenterology and urology, both in adults and in pediatrics. **Key words**: ELASTOGRAPHY, ULTRASOUND EVALUATION, IMAGING METHOD

The ultrasound evaluation is a non invasive, unpainful, repetitive investigation method which allows to have a good exploration of the tissues and organs, especially in children, where the patient's compliance is different from the adult's, often much lower.

Beside the simultaneous multichannel processing of information, the panoramic and harmonic ultrasound, the *elastography* is a new way of representing the ultrasonography information, a noninvasive evaluation concept of tissues nature (1,2). The method consists of applying a longitudinal pressure on a tissue and displacement measuring reflectance of the tissue nuclei. The representation of these displacements through colors, allows an information extract, which, in the end, facilitates the differentiation with high probability of benign from malignant tissue (2).

The method initially started and deve-

loped as applications on breast, but now it has applications in liver, prostate, thyroid, abdominal lymphnodes, peripheral vessels, myocardial and other directions of clinical application (3).

The ultrasound elastography reffers to ultrasound methods which can produce images or measurements of tissue elasticity - the ability to resist to deformation, deformations of tissues to the application on its strengths. The force applied to the tissue can be of several types (4) :

- the force applied by the ecografist's hand ;
- the force applied by an external mechanical device ;
- the force given by the physiological movements of the human body- breath, the cardiac activity, pulsed movements of large arteries ;
- the force applied by a certain ultrasound issued by the ultrasound probe which makes the exploration itself.

The response to the application of this force will vary according to the tissue elasticity.

The elastography as an imaging method was developed in order to objectively quantify the pathological changes related to the presence of an abnormal tissue, compared to the surrounding tissues (5). This new method of diagnosis gives informations related to the elasticity/stiffness of the examined tissue, degree of tissue fibrosis (eg. pre-stage liver cirrhosis), respectively the degree of stiffness of a tumor tissue compared to the free one, with promising results to differentiate between benign and malignant lesions or in assessing the tissue changes over time (4).

The manual palpation or the subjective "palpation" through ultrasounds was used for many years in order to give a qualitative assessment of the tissue stiffness, but the need to obtain a direct image led to the development of elastography (4).

The mechanical properties of the soft tissues which depend on their content, include elastic modulus (Young'modulus), Poisson's report and the shear properties of the tissue**.** These properties totally differ from the elastic modulus which governs the propagation of the ultrasounds. For example, the normal breast tissue is stiffer than the one made of fibroadenoma, and the breast cancers have shown how to shear up to seven times higher than in the normal tissue**.** The imaging potential resulted from this high contrast led to the development of some ultrasound investigation methods (4).

For these different approaches there have been developed :

1. The application of low frequency vibration energy and concomitant Doppler detection of the wave disturbance ;
2. Image of local response to a load applied to estimate the parameters by direct comparison between pre and post data compression ;
3. The calculation of these parameters.

This new ultrasonographic diagnosis method is not available using the conventional sonographic image and it represents the most important development in the ultrasounds technique since the appearance of the Doppler images. The information related to the tissue stiffness is complementary and independent of the information related to the acoustic impedance provided by the B--mode image (grayscale) and the one related to the vascular flow provided by the Doppler in age. Technically described since the last decade, the method was introduced just at the beginning of 2008, as an application of the latest ultrasound devices which include a wide range of analytical applications related to tissue strain or request and which allow visual qualitative and quantitative measurements of the tissue stiffness/ elasticity (6).

The extent of the tissue analysis can be done by two different technologies :

- Compression elastography "*eSie touch* elasticity imaging" (with applications

for the surface elastography – Real time exploration especially the breast. The aquisition of radiofrequency (RF) signals durind compression or because movements of inspiration/expiration, allows obtaining images generated by the tissue tension, which is proportional with its stiffness (4) ;

- An other example is *ARFI* – "acustic radiation force imaging" (the diffusion impulse of the acustic force)in which an acustic impulse is triggered in the tissue in order to assess/ interogate the mechanical properties of the tissue stiffness/ elasticity. The waves propagate like waves sideways when throwing a stone in the water. It has 2 possibilities of evaluation :

– *Virtual touch tissue imaging* – a method through which a diagram of the tissue stiffness is obtained, with a gray scale (reverse as the classical examination 2D) ;

– *Virtual touch tissue quantification* - through which it is appreciated the speed of the transverse propagation of the waves triggered, allowing the quantification of the tissue elasticity.

The *ARFI* method does not impose a certain degree of compression on the tissue, done by the examiner and which can vary (by hand fatigue), but it takes over the patient's respiratory movements, which means rhythmic and constant movements**.**

By means of the elastography using compression it can assess just the superficial tissues, while with the ARFI method the tissue elasticity can be assessed starting with a depth of 1.5 cm up to 8 cm.

Unlike the conventional sonography B--mode which gives us anatomical details based on differences in the acustic impedance, the Virtual Touch image describes properties reffering to the tissue stiffness . In this respect, the Virtual Touch image is closer to a tissue evaluation through a physical palpation than through the conventional ultrasound evaluation.

There are three steps in the process of Virtual touch acquisition :

- First an ultrasound image B-mode is obtained ;
- Then in a very short time (aproximatively 100 microseconds) a push impulse is given ;
- The issued impulse produces a displacement force, depending on the tissue's properties where it moves (so, in a soft tissue the waves move quickly, but in a stiff tissue it moves less or not at all). After the pushing pulse stops, the tissue relaxes and returns to its original shape. The captured ultrasound waves at a time are compared with the reference image, thus calculating the tissue displacements resulted from the pushing force (6).

VIRTUAL TOUCH TISSUE IMAGING (VIRTUAL TOUCH)

An image of the Virtual Touch soft-ware is actually a qualitative gray-scale map of the relative tissue stiffness (elastograma) for a user defined ROI (the region of interest). This information is calculated by examining the relative displacement of the tissue elements due to an acoustic pulse push. Thus, on elastograma the shining regions correspond to the tissue which is more elastic (less stiff) and the dark regions correspond to the stiff tissue.

If an image of the Virtual Touch soft--ware and a conventional image in B-mode are displayed together, the apparent limits of the tissue can differ, because the production of images is done through different mechanisms (6).

THE ADVANTAGES OF THE VIRTUAL TOUCH SOFT

The elastography methods previously available needed manual compression of the tissue or were based on the physiological needs of the body (cardiac activity, respiratory movements, and so on). These approaches

could limit the depth of the image location and easing the artifacts.

The application of the Virtual Touch soft allows an image by the independent combination of the multiple axial lines, obtained from the information of the tissue dislocation, starting with the left axial line within ROI, the tissue being described through a conventional ultrasound signal. Then, a push impulse is applied along this line, the captured conventional radiation being applied along the same line in order to obtain the signal of the dislocated tissue. Thus we can calculate the differences in tissue position every point along the axial line, between relaxation and compression (6).

The calculated differences are related to the maximum tested at a spatial location of tissue due to the tissues elastic properties in that area. The more elastic a tissue is, the bigger its dislocation is. The process above is repeated for each axial line within ROI as it is with a conventional B-mode scanning. Finally, all the calculated dislocations all over ROI are transformed in elastograma, describing the relatively tissue stiffness.

Generally, the stiffer the tissue is, the bigger the wave velocity is as it passes through this region. Overall, the measured speed of the wave is an intrinsic tissue property, which can be reproduced. The cut waves are generated and pass the tissue perpendicular, as some small waves resulted by throwhing a pebble in a pond. In contrast with the classical ultrasound waves axially oriented, the cut waves do not interact directly with the convertor. Besides this, unlike the conventional ultrasound waves, the cut waves are attenuated aproximately 10.000 times faster and thus needing a higher measurement sensitivity. Still, as the wave which is cut in front passes through the tissue, the generated dislocations are detectable, using ultrasound captive waves. Observing the front wave in more locations, and correlating these measurements with the past tense, the speed of the cut wave can be quantified (6).

VIRTUAL TOUCH TISSUE QUANTIFICATION

The *ARFI* technology can be used to measure a numerical value of the wave speed, by implementing the Virtual Touch tissue quantification.

For the Virtual Touch tissue quantification, first, it is identified an anatomical area using a ROI situated on a conventional image obtained through ultrasounds. An impulse of acoustic pushing is applied laterally, inducing a wave-cut which goes through ROI. The captured radiation, sensitive to more than 1/100 sound wave length, is applied adjacent to the push impulse. These radiations are continuously transmitted until the wave cut front is detected. The time between the cut-wave generation and the peak detection is used to calculate the speed of the cut wave. Multiple measurements are done for a given spatial location, for the reported value to ensure the quality measurement (6).

The tissue image and the Virtual Touch quantification is the first and the only implementation of the acoustic radiation image generated by the pulse force, which can be used with the conventional ultrasound in researches about the density and elasticity of the liver tissue. By this way, the previous elastography evaluations which were difficult and with variable results, become correct and practical (6).

It is important the fact that the technique of the Virtual Touch programme brings new information about the tissue, which can be used for diagnosis and in the therapeutic clinical applications.

CLINICAL APPLICATIONS OF THE ELASTOGRAPHY

The researches related to the ultrasound elastography quickly advances and the techniques utility too. At present, it is analised in the most various fields, from cardiology

to urology and gastroenterology, in adults and children too. Elastography begins trial in clinical practice and as a tool for cancer diagnosis (7).

Elastography was used in different studies to obtain images in vivo of the prostate and in the breast imaging, to view the elasticity of different tissues and to determine different degrees of stiffness, being able to distinguish the malignant tumor tissue from the benign tumor tissue. The researchers managed to classify correctly the lesion of the prostate using this technique (8,9).

The usefulness of this technique has been interes aroused also in the exploration of malignant tumors of the cervix and thyroid (10,11,12,13,14,15).

Elastography may be used in transvaginal ultrasound assessment of the female pelvis (16). There are only a few reports in the literature on the in-vivo use of real-time transvaginal elastosonography in the field of gynecology, being a promising tool that can provide detailed mapping and characterization of uterine fibroids, that could improve the gynecological ultrasound evaluation of size, volume and delineation of uterine fibroids before surgery or embolization (16,17).

The combination of elastography with highly sensitive conventional B-mode sonography has the potential to further improve the diagnosis of metastatic enlarged cervical lymphnodes (18).

Thyroid elastography is useful to make a correct differential diagnosis between malignant and benign thyroid nodules (10,11, 12,13,14,15).

This method of ultrasound exploration is used in the evaluation of superficial lymphnodes stiffness, too (10,11,12,13,14,15).

USING ELASTOGRAPHY IN HEPATOLOGY

Elastography of the liver through new technologies available, has become comparable to fibroelastoscanner (for assessing liver fibrosis) and to the nuclear magnetic resonance.

Normal liver tissue is soft and flexible. With inflammation, however, many of the cells die and are replaced by collagenous fibrils and the tissue gets stiffer. The process is often slow-extending over decades in many cases. Precise, noninvasive measurement of liver stiffness, a simple application of elastography, promises to be a safe, inexpensive method to monitor the progress of liver patients (19).

Real-time elastography is a noninvasive method based on use of ultrasounda promising in preoperative assessment of liver fibrosis in chronic hepatitis, in nonalcoholic fatty liver disease (NAFLD) and in cirrhosis (20,21).

Elastography imaging of the small liver nodules in cirrhosis might be used as a non-invasive tool for accurate diagnosis, as well as selection of patients for curative therapy, but not for screening. Its utility in patients with non-vascularised nodules, can help to eliminate the false negative diagnosis of contrast enhanced imaging of the liver and to improve their outcome. Real-time elastography is a promising sonography--based noninvasive method for the preoperative assessment of liver fibrosis both in liver diseases with considered fibrosis and in cirrhosis, having a highly sensitivity and specificity. It is considered fibrosis if the media of 3-5 successively determinations in the same area is over 2 (21,22).

Real-time elastography is a promising method for the non-invasive diagnosis of early hepatocarcinoma. Blue color at elastography and hypervascular aspects are independent predictors of hepatocarcinoma (22, 23).

THE ARFI ELASTHOGRAPHY METHOD IN HEPATHOLOGY

The new ultrasonographic method of *ARFI*

elastography allows valid, accurate and flexible evaluation of liver stiffness. Elastography of the left liver lobe is also possible which may be especially helpful in obese patients (24).

ARFI allows SWV quantification (SWV--shear wave velocity), in strong correlation with the fibrosis stage. Steatosis does not influence SWV (24,25). The maximal performance of the method consists of the prediction in severe fibrosis and cirrhosis. The diagnostic accuracy is strongly comparable to transient elastography only for the prediction of severe fibrosis and cirrhosis, whereas for earlier stages, TE performs better (25).

Both *ARFI* and TE have excellent predictive values in the presence of the cirrhosis, even if the liver elasticity determined by TE better correlates with the histological fibrosis in patients with chronic liver disease HCV and HBV (with hepatitis C, respectively B), compared to the values obtained through *ARFI* (26).

ARFI elastography has very good accuracy for the assessment of liver fibrosis and is superior to other noninvasive methods (APRI Index, FibroMax) for staging liver fibrosis (27).

Images obtained with ARFI elastography provided additional qualitative information regarding the stiffness and tumor margin of liver tumors. By measuring shear wave velocity, the quantification of stiffness was made possible and showed the potential to differentiate malignant hepatic tumors from hepatic hemangiomas (28).

CONCLUSION

Using conventional real-time ultrasound equipment with modified software (for example *virtual touch tissue imaging, virtual touch tissue quantification şi eSie touch elasticity imaging*), elastography has been reported to be useful for differentiation and characterization of various malignant tumors, such as breast, prostate, thyroid, pancreas, lymphnodes, gastrointestinal stromal tumors, primary and secundary liver tumors, the evaluation of uterine cervix (23).

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DIGITAL DENTISTRY

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DIGITAL DENTISTRY (Abstract): The age of full digital dentistry is a new concept, specific to the latest trends of aesthetics, providing superior innovation power in order to reach this goal. In today's world, technological advancements are far from being limited to specific fields. Oral diseases affect a large category of people physically and psychologically, influencing how they look chew, speak, integrate into the social life. Dentistry is witnessing phenomenal innovations, transforming people's smiles and lives in each and every way. Prosthodontic dentistry deals with the science and art of restoring teeth, both functionally and esthetically. The diversity of cases exposes the practitioners to a wide range of clinical situations and treatment solutions. The replacement of lost teeth with prosthetic appliances may sometimes be a problem for the clinicians, due to the esthetic requests. Among the multiple choices of techniques available for a suitable rehabilitation, the CAD/CAM techniques represent an ergonomic and minimal discomfort solution for the patient, representing a revolutionary method of oral rehabilitation. All stages of each dental treatment should benefit from the very latest technology and best products so the final result being characterized by efficiency and durability. **Keywords:** DIGITAL DENTISTRY, CAD/CAM TECHNOLOGIES, INTRA ORAL IMPRESSION.

Visionaries have long dreamed of digital intra oral impression, then sending the optical impression by e mail, and having their work designed and executed by a trained dental technician in his remote laboratory. The contribution of the dental team on treatment planning, managing the oral health and the interaction with the patient's demands will remain relevant regardless of technology changes. Professional exchanges are important for the advancement of digital dentistry. The CAD/CAM technologies, are constantly moving in new directions, to provide exciting, innovative products and systems, with the highest quality standards. Thanks to CAD/CAM technologies, perfect clinical restorations can be achieved, with no biocompatibility secondary reactions and excellent esthetic appearance, through a digital cooperation between dentists and dental labs. CAD/CAM technology allows the realization of a well-fitting, aesthetic, and durable prosthetic appliances. CAD/CAM complements earlier technologies used for these purposes by any combination of increasing the speed of design and creation; increasing the convenience or simplicity of the design, creation, and insertion processes; and making possible restorations and appliances that otherwise would have been infeasible. Other goals include reducing unit cost and making affordable restorations and appliances that

otherwise would have been prohibitively expensive. However, to date, chairside CAD/CAM often involves extra time on the part of the dentist, and the fee is often at least two times higher than for conventional restorative treatments using lab services. CAD/CAM is one of the highly competent dental lab technologies. CAD/CAM used in dentistry is based either on subtractive processes such as CEREC technology or additive processes, such as 3 D printing to produce restorations from 3d models to the final prosthetic substitute.

The 1980s represented the first use of CAD/CAM in dentistry. The technique was efficiently used in dental offices and dental laboratories. Chairside CAD/CAM restoration differs from conventional dentistry in that they are fixed in the same day, conventional prosthesis being cemented or bonded in place after a longer period of time. CAD/CAM systems use an optical camera to take a virtual impression, generating a digital cast. A 3D image is forwarded to a software program that can turn the captured images in order to produce and design the prosthesis.

Combining CAD/CAM software with 3D images from 3D imaging system means greater safety and security from any kind of intraoperative mistakes.

Typically, CAD/CAM dental restorations are milled from solid blocks of ceramic or composite resins or zirconia. If the CAD/CAM restorative material is Zirconia the restoration is “radio-opaque”, blocking X-rays. Only alumina, lithium disilicate materials are “radio-lucent” (1, 9). According to the used material, CAD/CAM pieces may have some aesthetic drawbacks, whether they are created at the dental practice or into a dental laboratory fabricating center. Depending on the dentist or technician, CAD/CAM restorations can be layered and baked or sintered in order to get a deeper more natural look.

Stains and glazes can be added to the surfaces of the milled ceramic crown or bridge to correct the otherwise monochromatic appearance of the restoration. The restoration is then adjusted in the patient’s mouth and fixed in place (2, 3).

CAD/CAM has improved the quality of prostheses in dentistry and found a way to standardize the production process. It has increased productivity and the chance to work with new materials with a high level of accuracy. It has also found a way to decrease chair time for the patient by the use of intra-oral scanning systems which allow the dentist to send electronic impressions to the lab. CAD/CAM requires a large initial investment. Despite the CAD/CAM being a major technological advancement, it is important that the dentists’ technique is suited to the methods of CAD/CAM and milling technology. This includes correct tooth preparations with a continuous preparation margin (which is easy registered by the scanner); avoiding the use of no shoulder preparations and parallel walls and the use of rounded incisor and occlusal edges to prevent the concentration of tension. Similar to the conventional restorations, CAD/CAM pieces present different aesthetic value, many presenting only one shade. In order to create a higher aesthetic appearance feldsphatic porcelain is fused to glass-infiltrated aluminum oxide (alumina) or zirconium-oxide (zirconia) (11). For the veneered crowns and bridges, the porcelain is layered onto a metal framework and often the color is influenced by the presence of the dark oxide lines related to the gum line. So, metal-free restorations are typically more aesthetically according to the patient expectations.

Fit accuracy varies according to the

CAD/CAD system utilized and from user to user. Some systems are designed to attain higher standards of accuracy than others and some users are more skilled than others (4).

There are different systems in use: the 3 Shape TRIOS reinvents the digital impression registration. Featuring color digital impressions, shade measurement and HD photos, TRIOS@ PACKS precision and speed into an easy to use, powder-free scanning solution. Enhanced detail capture makes it simple to identify preparation margins and evaluate the clinical situation, while shade measurement instantly identifies teeth shades as we scan. HD photos realize the documentation for each case, esthetic capture and visualize the solution for the patient. The 3 Shape Dental System offers technology-leading dental scanners for 3 D scanning of both physical impressions and gypsum models. The high-speed scanners have the capability to provide the accuracy and detail required for full-arch bridges and implant bars. The full integration with the design software ensures the optimal scan-to-design workflow. 3Shape Dental System™ is known throughout the dental industry as the most powerful CAD CAM design system in the world, supporting familiar workflows, providing the market's most comprehensive dental indication support (5, 6).

An important aspect is represented by the presence of the Sirona Connect portal, which links dentists and dental technicians in a very modern way, so they can exchange data conveniently and securely via the portal. The portal interface is integrated into the dentist's and technician's software, regardless of which software version is being used by either party, thus greatly facilitating workflow.

The 3D scanners and the CAD CAM software solutions are designed for the creation, processing, analysis and management of 3D data for application in complex manufacturing processes.

The benefits of CAD CAM systems are related to:

- the registration of a digital impression and ordering the restoration online, quickly and easily via the global portal as the Sirona one
- high cost-effectiveness of investment due to:
- extremely wide range of applications and material versatility
- maximum precision for all results combined with easy handling
- high flexibility and future security due to open CAD/CAM interfaces, a diversity of possibilities of integration and the possibility to process additional materials.

It is estimated that many new systems are expected to be present on the domain in the next few years. In the last decades the use of 3D printing techniques for medical and dental applications highly developed. Additive manufacturing is gaining rapid potential in nearly all dental fields It differs from formative and subtractive manufacturing as in the additive manufacturing process the object is "printed" by adding the building material layer by layer The application of the 3D printing in dentistry offers the possibility of individualized prosthetic appliances, savings on small scale productions, eased sharing and processing of patient image data and educational upgrading.

The entire process of additive manufacturing technology can basically be divided into four steps: creating a digital 3D model designed with a software or using intraoral scans or computed tomography data, processing and slicing of the 3D model into many two-dimensional layers, printing the 3D end product layer by layer, post-processing of the printed object. This basic

workflow can be applied for the different printing technologies, using a wide range of materials as polymers, metals or ceramics.

Advancement in digital workflow makes it possible to directly print the prosthesis from silicone providing acceptable esthetics and reducing the number of appointments for the patient at the same time.

Minimizing the treatment time and number of appointments is not only favorable for the patient but also precludes the complications and it is possible to achieve the preferred treatment outcomes with expedition of the whole procedure. Another advantage is represented by the leading of an excellent intraoral fit of the prosthetic appliance (7, 8).

Although clinical studies presented patient satisfaction using this technique, further groundwork is required with regards to the stability and comfortable design of the 3D-printed prosthetic restorations.

The importance of 3D documentation in prosthetic has been noticed since the last decade. Plaster models have now been replaced by digital information and data. This does not only solve the bulk storage problems often faced by clinicians, but also opens a new horizon of education and research. Rescuing the patient from repeated exposure to ionizing radiation, 3D-printed models have been used to establish new theorems and relationships between alveolar area and the remaining teeth. In the future it will be possible to use real patient additively manufactured dental models based on intraoral scans or cone beam computed tomography (CBCT) for training fixed and removable prosthetic to dental students (10).

Dental specialists should seek an entire ethical equilibrium of how to get and maintain optimal oral health, being open-minded, compassionate but also expecting the unexpected.

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PLĂMÂN MONOBLOC DREAPTA-STÂNGA. CAVITATE PLEURALĂ UNICĂ. PLĂMÎN HIPOPLAZIC STÂNG. CAZ CLINIC

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UNIQUE MONOBLOCK LUNG RIGHT-LEFT, UNIQUE PLEURAL CAVITY (Abstract): A 34 years old female with WPW syndrome has been under surgical treatment in our clinic with left basal chronic pleural empyema. During the surgical intervention multiple anatomical anomalies were detected, like unique pleural cavity, monoblock lung left-right, hypoplasic left lung, no scissures on the left side, no left pulmonary arteria with an aberrant arteria for the left lung derived from the right pulmonary arteria, the same for the bronchis. The pleural-pulmonary shelling proceeded without incidents and it has been finalized with the drainage of the whole pleural cavity. **Key words**: MONOBLOCK LUNG, UNIQUE PLEURA

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF NITRO-L-ARGININE METHYL ESTER DERIVATIVES WITH THIAZOLIDIN-4-ONE SCAFFOLD

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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF NITRO-L-ARGININE METHYL ESTER DERIVATIVES WITH THIAZOLIDIN-4-ONE SCAFFOLD (Abstract): This paper is focused on the evaluation of the anti-inflammatory potential of some novel nitro-L-arginine methyl ester derivatives with thiazolidin-4-one structure, synthesized by our research group. **Materials and methods**: The evaluation of the anti-inflammatory potential was performed using *in vitro* assays: human red blood cell membrane stabilization (HRBC) and inhibiting bovine serum albumin denaturation (BSA) assays. **Results**: The obtained results showed that both stability of the erythrocyte membrane and inhibition of bovine serum albumin (BSA) denaturation, as parameters of anti-inflammatory effect evaluation, depend on the structure and concentration of the tested compounds. The most active derivatives were the compounds in which the aromatic ring from 2-position of thiazolidine-4-one scaffold is unsubstituted (**2a**) and halogen-substituted (with 4-chloro – **2c**, and 4-bromo– **2e**). **Conclusions**: The most favorable influence was obtained by the aromatic substitution with *chloro* and *bromo* in para position, the corresponding derivatives being the most active derivatives compared to diclofenac, used as anti-inflammatory standard. **Keywords**: L-ARGININE, THIAZOLIDINE-4-ONE, ANTI-INFLAMMATORY ACTIVITY, *IN VITRO* ASSAY.

INTRODUCTION

L-Arginine is considered as a semi-essential or conditionally essential amino acid depending on the stage of development and the individual's health (1). This amino acid is a precursor for polyamines, proline, glutamate, creatine, agmatine, and urea and, also functions as a precursor for protein synthesis and as substrate for several enzymes. It plays a critical role in several physiological processes, exerting antioxidant and anti-inflammatory effects and also serving as a precursor for nitric oxide (NO), a well-known vasodilator (2). Literature indicates that thiazolidin-4-one is a significant scaffold with medicinal relevance. Thiazolidin-4-one ring is susceptible for chemical modulations in positions 2, 3 and 5. Such modifications facilitate the search for novel compounds with desired activity (3). Upon modification

with various substituents, it exhibits a broad spectrum of biological activities, including antioxidant, antimicrobial, anticancer, and anti-inflammatory effects (4, 5).

Inflammation is the result of an oxidative stress that attacks the cells and prevents them from functioning normally and is a component of several types of diseases; therefore, the investigation of the anti-inflammatory action has an important role in the biological action assessment of different substances.

Based on these considerations, the aim of this paper was the evaluation of the anti-inflammatory properties of new compounds derived from the methyl ester of nitro-L-arginine (H_3C-OCO-Larg-NO_2), using *in vitro* assays, based on spectrophotometric methods.

MATERIALS AND METHODS

Nitro-L-arginine methyl ester (H_3C-OCO-Larg-NO_2) derivatives with thiazolidin-4-one scaffold (**2a-j**) (fig. 1) were synthesized by our research group (6). The standard reagents used for the anti-inflammatory activity tests: saline phosphate buffer pH 7.2, sodium chloride (NaCl), dimethylsulfoxide (DMSO), bovine serum albumin (BSA), sodium diclofenac, were purchased from Sigma Aldrich Company and Fluka Company, and they were used as received, without further purification. **Apparatus**: The absorbance measurements were read using microplate reader Tecan Austria GmbH Sunrise Remote with Magellan software and UV-Vis Spectrophotometer GBC Cintra 2020 V4166 with Cintra software.

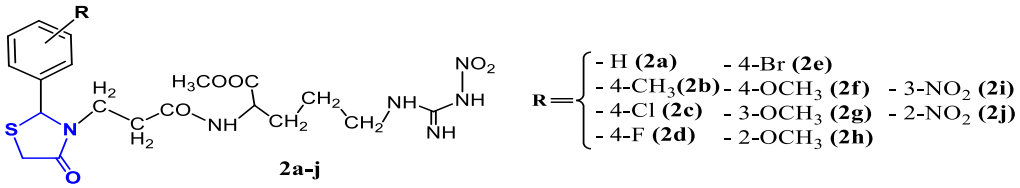

Fig. 1. Structure of H_3C-OCO-Larg-NO_2 derivatives with thiazolidin-4-one scaffold **(2a-j).**

1. Human red blood cell membrane stabilization (HRBC) assay. In order to obtain the erythrocyte suspension 10% (v/v) (HRBC suspension), blood from healthy volunteer was collected on citrate anticoagulant, and then washed (3 times) with 0,9% NaCl, centrifuged at 3000 rpm, followed by the removal of the supernatant. For each tested compound **(2a-j)** and reference drug (sodium diclofenac) a stock solution of 1 mg/mL was prepared by dissolving in DMSO. To prepare serial dilutions, from each stock solution different volumes (30 µL, 60 µL, 120 µL) were measured and 0.85% NaCl was added up to a volume of 1000 µL.

Finally, a volume of 250 µL from serial dilutions was measured, and added to a mixture of 500 µL phosphate buffer (pH = 7.4), 450 µL hyposaline solution (0.36% NaCl) and 300 µL HRBC solution (10% v/v). The hemolysis control sample was prepared by adding instead of 450 µL hyposaline solution (0.36% NaCl solution), 450 µL bi-distilled water, to which was added 250 µL 0.85% NaCl, 500 µL phosphate buffer (pH = 7.4) and 300 µL HRBC solution (10% v/v) - this representing the value of 100% hemolysis ($A_{\text{hemolysis control}}$) (7).

All samples (tested compounds - **2a-j**, reference – sodium diclofenac and hemolysis control) were incubated at 37°C for 30

min, and then centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant was measured at 560 nm. The capacity of the tested compounds (**2a-j**) to maintain the stability of the erythrocyte membrane, expressed as a percentage of hemolysis inhibition (%), was calculated using the following formula (1):

% of hemolysis inhibition (% erythrocyte membrane stabilization) = $(A_{\text{hemolysis control}} - A_{\text{sample}}) / A_{\text{hemolysis control}} \times 100$ (9).

in which: $A_{\text{hemolysis control}}$ – absorbance of the hemolysis control;
A_{sample} – sample absorbance.

For each serial dilution a corresponding DMSO control was prepared using different volumes of DMSO (30 µL, 60 µL, 120µL) which were completed up to a volume of 1000 µL with 0.85% NaCl solution, then processed similarly to samples and which were used as a spectrophotometer reading blank for the samples. All determinations were performed in triplicate.

2. Bovine serum albumin denaturation (BSA) assay. For this assay a 1% (w/v) bovine serum albumin (BSA) solution in phosphate buffer (pH 7.2) was used. For tested derivatives (**2a-j**) as well as for the reference substance (sodium diclofenac) a stock solution of 1 mg/mL was prepared by dissolution in DMSO. To prepare serial dilutions, from each stock solution, different volumes (50 µL, 100 µL, 200 µL, 400 µL) were measured, and 0.85% NaCl solution was added up to a volume of 1000 µL.

Finally, a volume of 25 µL from serial dilution was measured, in a 96 well plates, and 225 µL of 1% BSA was added. A mixture of 25 µL 0.85% NaCl and 225 µL of 1% BSA was used as a control for albumin denaturation ($A_{\text{denaturation control}}$).

All samples (tested compounds **- 2a-j**, reference - sodium diclofenac and control) were incubated for 20 min at 37°C, then 5 min at 72°C, and finally cooled for 10 min. After that, the turbidity of each sample was read at 416 nm. The capacity of the tested compounds to inhibit the denaturation (precipitation) of serum albumin was calculated using the following formula (2):

% of inhibition of protein denaturation=$(A_{\text{denaturation control}} - A_{\text{sample}} / A_{\text{denaturation control}}) \times 100$ (8).

in which: $A_{\text{denaturation control}}$ – absorbance of the denaturation control;
A_{sample} – sample absorbance.

For each serial dilution a corresponding DMSO control was prepared using different volumes of DMSO (50 µL, 100 µL, 200 µL, 400 µL), which were completed up to a volume of 1000 µL with 0.85% NaCl solution, then processed similarly to samples and which were used as a spectrophotometer reading blank for the samples.

All determinations were performed in triplicate.

It was established that the lower absorbance, means a greater ability of the

sample to inhibit the thermally induced denaturation (precipitation) of proteins, respectively of BSA (11, 12).

RESULTS AND DISCUSSION

1. Human red blood cell membrane

2. stabilization test (HRBC assay)

The HRBC assay allow to evaluate the ability of the compounds to protect the erythrocyte membrane from lysis induced by hypotonicity (10). The effect of the tested compounds (**2a-j**) at different concentrations (5 µg/mL, 10 µg/mL, 20 µg/mL), on erythrocyte membrane stability, expressed as hemolysis inhibition (%), in reference with diclofenac, used as standard drug, are represented graphically in figure 2.

From the analysis of the obtained results it is observed that for all the studied compounds, the stability of the erythrocyte membrane increases with the concentration; the highest effect being reach at the concentration of 20 µg/mL. It was also noted that at concentration higher than 20 µg/mL, the stability of erythrocyte membrane slightly decreases. This could be explained in relation with the saturation degree of the permeability of erythrocyte membrane which can cause the lysis of the red blood cells' membrane. At 20 µg/mL the best results were obtained for compounds **2a**, **2c** and **2e**, for which the hemolysis inhibition (94.5% - **2a**, (93.46% - **2c**, 92.97% - **2e**) was similar with diclofenac sodium, at the same concentration (92.8%).

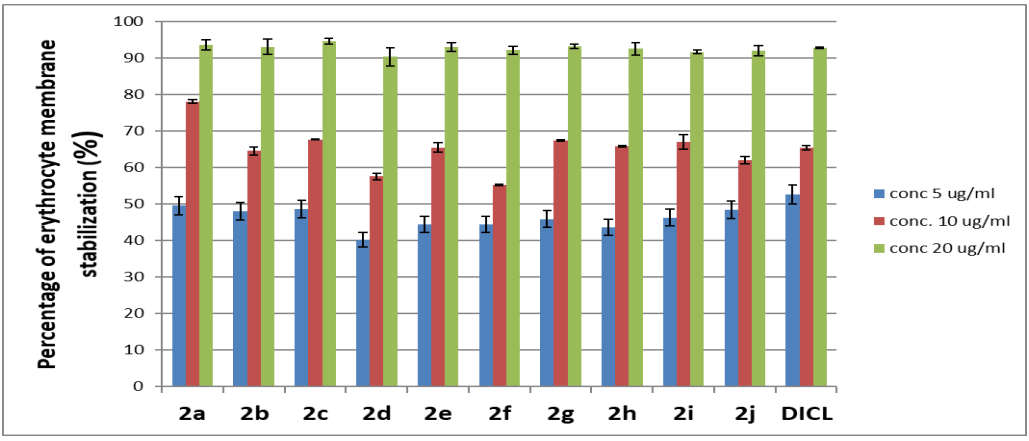

Fig. 2. Percentage of erythrocyte membrane stabilization of the compounds (**2a-j)** tested at different concentrations

3. *Bovine serum albumin denaturation (BSA) assay.*

Recent data suggest that tissue protein denaturation is among the causes of inflammatory and rheumatic diseases, through generation of autoantigens that can lead to *in vivo* protein denaturation. Upon heating, bovine serum albumin (BSA) undergoes a denaturation (precipitation) process with the release of antigens responsible for a number of hypersensitivity reactions. Thus, the agents that can prevent protein denaturation deserve to be studied later for the development of new anti-inflammatory agents (10). It is considered that any compound that has a percentage of protein denaturation inhibi-

tion of more than 60% can be further evaluated as a potential anti-inflammatory agent. A number of non-steroidal anti-inflammatory drugs, such as indomethacin, diclofenac, ibufenac, aspirin, flufenac acid, in addition to their ability to inhibit protaglandin synthesis in the inflammatory process (cyclooxygenase inhibition), have also shown appreciable ability to prevent BSA denaturation.

The inhibition percentage of BSA denaturation for tested compounds (**2a-j**) at different concentrations (5 μg/mL, 10 μg/ mL, 20 μg/mL, 40 μg/mL), in reference with sodium diclofenac, used as standard drug are represented graphically in figure 3.

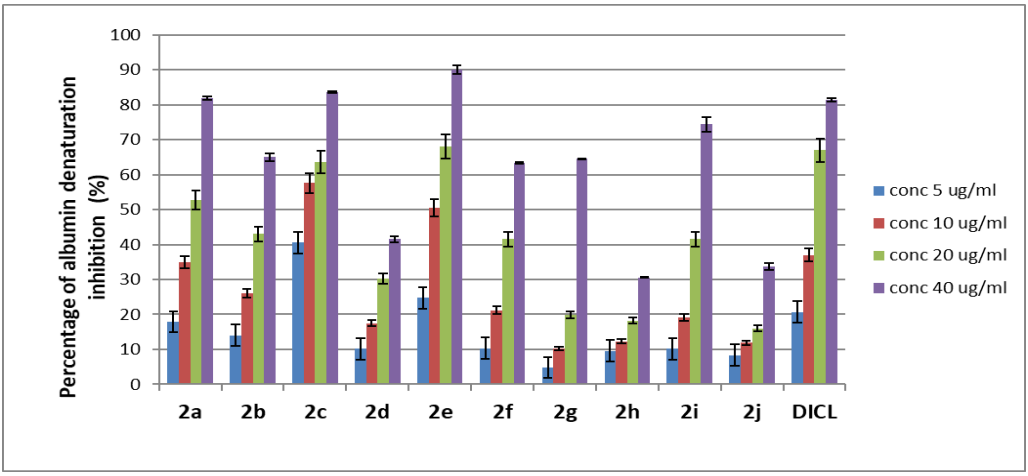

Fig. 3. The effect of the tested compounds (**2a-j**), at different concentrations, on inhibiting the BSA denaturation

From the analysis of the obtained results it is observed that for all studied compounds the inhibition of BSA denaturation increases with concentration, respectively the highest percentage of inhibition was obtained at the concentration of 40 μg/mL. At this concentration, the most active compounds were **2a**, **2c**, **2e**, for which the BSA inhibition (81.9% - **2a**, 83,65% - **2c**, 90.1% - **2e)** was higher than diclofenac (81.4%), used as a positive control at the same concentration of 40 μg/mL.

For both assays, HRBC assay and BSA denaturation assay, respectively, the 50% inhibitory concentration (IC_{50} μg/mL) was calculated from the dose response curve obtained by plotting percentage inhibition *versus* concentrations (tab. I).

The results confirmed that compounds **2a, 2c, 2d** which showed the best stability of the erythrocyte membrane and BSA inhibition denaturation, were slightly better than the positive control, sodium diclofenac. These results support that the compounds can prevent lysosomal membrane damage and thus impede tissue damage through hydrolytic enzymes provided by lysosomes. The lysosomal enzymes released during inflammatory condition produce a variety of disorders and they are related to acute or chronic inflammation. The anti-inflammatory agents act by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal membranes (14, 15).

TABLE I.

IC_{50} value of tested compounds (2a-j) for in vitro anti-inflammatory effects.

No.	Compound	IC_{50} (µg/mL) – BSA assay	IC_{50} (µg/mL) – HRBC assay
1.	**2a**	12.21 ±0.34	4.17±0.18
2.	**2b**	26.36 ±0.41	6.23±0.54
3.	**2c**	9.22 ±0.22	4.05±0.11
4.	**2d**	55.4 ± 0.73	5.96±0.81
5.	**2e**	12.50±0.91	5.06±0.42
6.	**2f**	27.74±0.18	6.09±0.48
7.	**2g**	33.51±1.16	6.45±0.54
8.	**2h**	71.52±2.13	7.86±0.18
9.	**2i**	25.14±0.78	6.64±0.41
10.	**2j**	58.41±1.41	7.95±0.78
11.	**Diclofenac**	13.25±0.81	5.25±0.37

CONCLUSIONS

The anti-inflammatory potential of the new nitro-L-arginine methyl ester (H_3C-OCO-Larg-NO_2) derivatives with thiazolidin-4-one scaffold (**2a-j**) was evaluated by two *in vitro* methods - inhibition of BSA denaturation (BSA assay) and erythrocyte membrane stability (HRBC assay). The obtained results showed that both the inhibitory capacity of BSA denaturation and the stability of the erythrocyte membrane increase with concentration, respectively the best results were obtained at the concentration of 40 µg/mL in the BSA assay, respectively at the concentration of 20 µg/mL for the HRBC assay. The most active compounds were **2a, 2c, 2e**, which showed the best anti-inflammatory effect in comparation with sodium diclofenac, used as positive control.

CONFLICT OF INTEREST.

Authors declare that there is no conflict of interest.

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DIGESTIVE HEALTH AND FACTORS THAT IMPACT IT: AN OBSERVATIONAL CASE-STUDY OF THE PEDIATRIC PATIENTS HOSPITALIZED FOR GUT DISEASES AT A PEDIATRIC HOSPITAL FROM TIMISOARA, ROMANIA

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DIGESTIVE HEALTH AND FACTORS THAT IMPACT IT: AN OBSERVATIONAL CASE-STUDY OF THE PEDIATRIC PATIENTS HOSPITALIZED FOR GUT DISEASES AT A PEDIATRIC HOSPITAL FROM TIMISOARA, ROMANIA (Abstract): The **aim** of the study was to investigate whether personal, perinatal and early nutritional factors have an impact on digestive health. **Materials and methods:** An observational case-study was performed on a sample of 248 pediatric patients (51.2% males), admitted into a pediatric hospital over the course of two years. The gathered data (the prespecified gut diseases, personal, perinatal factors and early nutrition) from the patients` files were used to build a data base. Statistical analysis (binomial logistic regression) was performed with the aid of SPSS20 Program. Two statistically significant age-groups (0-3 years, 3-18 years) were considered. **Results:** Differences were revealed between the two significant age-groups: 0-3 years (predominance of: enterocolitis, gastroenteritis-GE, cow milk allergy-CMA, acute dehydration syndrome-ADS and bacterial diseases, females, caesarian section, breast feeding 0-2 months) and 3-18 years (predominance of: GE, ADS and parasitic diseases, males, natural birth, breast feeding over 12 months). The outcome variables were gut diseases (enterocolitis, GE, CMA, ADS), the independent variables were personal (gender, age, residence), perinatal (gestational age, delivery type), early nutrition (duration of breast feeding, milk formulas, diversification age). Statistically significant associations were demonstrated between: natural feeding 0-2 months (NF-2)-CMA (OR=13.51, p=0.007-entire sample, OR=7.48, p=0.002-age group 0-3 years), NF-2-enterocolitis (OR=5.47, p=0.005-age group 0-3 years), NF-2-GE (OR=0.185, p=0.009-age group 0-3 years). Age, residence and delivery type also related to gut diseases. **Conclusions:** There is an association between gut diseases as dependent variables and personal (age, residence), perinatal (type of birth) and early nutrition (NF-2) independent variables. **Keywords:** GUT DISEASES, PERSONAL FACTORS, EARLY NUTRITION, OBSERVATIONAL CASE-STUDY.

INTRODUCTION

Gut diseases are frequently found and they differentiate within the child population depending on etiology, localization and effects. In a study performed in The United States the results showed that two-

thirds of the surveyed Americans are burdened by gastrointestinal symptoms (1). There are gaps in our knowledge in understanding of infection/immunity throughout the human life cycle, focusing on focusing on early childhood growth (2). In the western region of Romania there is an increased burden of digestive pathology in pediatric patients, with an unknown area regarding factors that impact digestive health. In the performed study we aimed to investigate digestive health and factors that impact it in a population of pediatric patients hospitalized for gut diseases at a pediatric hospital in Timisoara, Romania, over the course of 2 years of study.

MATERIALS AND METHODS

The study was performed on a sample of 248 pediatric patients (51.2% males, aged between 1 day and 18 years) hospitalized at the "Pius Brinzeu" Clinical Emergency County Hospital Timisoara, Bega Clinic - Pediatric for gut diseases. The enrollment period was between January 1st, 2018 and December 31st, 2019. Each patient was hospitalized only once. It was a non-probability purposive sample, children with gut diseases being selected. Two age groups (0-3 years - 133 patients: 48.9% males), and 3-18 years - 115 patients: 53.9% males) and their recorded pathology gastroenteritis –(GE), enterocolitis, cow milk allergy (CMA), and acute dehydration syndrome (ADS) were considered in this analysis. The main inclusion criterion for the study sample was the hospitalized pediatric patients with diarrhea, vomiting or/and dehydration due to infectious or allergic disorders with location in the intestinal tract. Diagnoses of these intestinal diseases were collected from the children-patients` files. The exclusion criteria were: more than one admission and incomplete files.

The method consisted of an observational case-study (combined approach: cohort and cross-sectional) of the hospitalized pediatric patients for gut diseases during these two years of study. The primary medical records were gathered for this investigation. The gathered data (certain gut diseases, personal, perinatal factors and early nutrition) from these patients' files were used to build a data base. The agreement of the County Hospital Leadership was asked for and received in order to access primary medical records while I agreed to keep them confidential and to respect the personal data protection law. The medical data were already considered for medical and statistical purpose, with the requested and received individual consent of the patients. The agreement (no. 10/ 02.04.2019) of the "Victor Babes" University of Medicine and Pharmacy's Ethical Commission was requested and obtained for this statistical clinical study. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments.

Statistical analysis was performed by the aid of software *SPSS version 20* (3). Binomial logistic regression used the dependent variable that consisted of only two nominal categories (absence/presence of one specific gut disease), and the independent nominal (categorical: personal - gender, age-groups according to developmental stages criterion and residence, peri-

natal factors - type of birth and gestational age, and early nutrition - duration of breast feeding, milk formulas and diversification age) variables that can be analyzed as possible predictors.

RESULTS

Gut diseases - by localization and etiology

Of the studied population 35% had enterocolitis and 29.9% gastroenteritis. At the age group *0-3 years* the most frequently were patients with enterocolitis (51.8%) and patients with GE (28.6%) and at the age group *3-18 years* the most frequently were patients with GE (31.3%).

Main gut diseases by *etiologies* were CMA (20.2%), bacterial (14.5% with: *E. coli* and *Helicobacter pylori*), parasitic (10.5% with: *Giardia lamblia, Toxocara canis and Enterobius vermicularis*) and viral (6.9% of patients with: Rotavirus and Adenovirus) diseases. At the age group *0-3 years* the most frequently met were CMA (34.6%) and bacterial diseases (15% of patients). At the age group *3-18 years* most frequently were found parasitic diseases (18.3%).

A generalized effect was ADS (31% of patients: 16.5% with gut diseases and 14.5% as result of other diseases). ADS was found in 27.8% patients aged 0-3 years, and in 34.8% patients aged 3-18 years.

Personal factors (gender, age-groups and residence)

The *gender* distribution in the two statistically significant samples chosen by *age groups* was: 0-3 years - 48.9% males, and 3-18 years - 53.9% males.

The most frequently admitted pediatric patients came from *urban* (58.9%), majorities of them living in Timisoara (50.4%). Patients who came from *rural* (38,7%) were less numerous and majorities of them came from Timis County (31%). Distribution on the statistically significant age groups of the pediatric patients considering urban/rural environment was: *0-3 years* (54.1% from urban, 48.9% living in Timisoara and 49.2% from rural environment, 38,3% living in Timis County) and *3-18 years* (64.3% from urban, 52.2% living in Timisoara and 33.9% from rural environment, 22.6% living in Timis County).

Perinatal factors (gestational age and delivery type)

Most frequently found *gestational ages* were 40 weeks (41.9% pediatric patients) and 38 weeks (20.6% pediatric patients). There was a high rate of caesarean *delivery* (41.5% pediatric patients). Distribution of the pediatric patients on the focused age-groups was as follows: *0-3 years* (natural delivery - 48.1%, caesarian - 48.1%, gestational age 38 weeks - 27,1%, gestational age 40 weeks - 34,6% patients), *3-18 years* (natural delivery - 53.9%, caesarian - 33.9%, gestational age 38 weeks - 13.9% and gestational age 40 weeks - 48.7%).

Early nutrition (duration of natural feeding, milk formula, diversification age)

Most frequent duration of the *breast feeding* was over 1 year (21.8%), 0-2 months (20.2%) and 2-6 months (19.8% of patients). Most used *milk formulas* were Nan, Aptamil and Humana (29.9%), followed by powder milk (15.3% of patients). Most frequently *diversification age* was at the 6th month (46.4%). Distribution of the pediatric pa-

tients considering *early nutrition* on the focused age groups was: *0-3 years* (breast feeding 0-2 months at 24.1% and breast feeding over 12 months at 20.3% of patients) and *3-18 years* (breast feeding over 12 months at 23.5% and between 2-6 months at 20%) for breast feeding.

Distribution of the pediatric patients for artificial feeding with *milk formulas* was: at age groups *0-3 years* - Nan, Aptamil, Humana (42.9%), powder milk (16.5%) and at age groups *3-18 years* - Nan, Aptamil, Humana (14.8%) and powder milk (13.9%).

Distribution of the pediatric patients considering *diversification* age was: at age group *0-3 years* (at the 6th month - 46.6%, at the 5th month 33.1% of patients) and at age *group 3-18 years* (at the 6th month - 46.1% and at the 5th month - 15.7% of patients).

High frequency (15% pediatric patients) of missing cases (no information) for early nutrition variables represents a limit of this study.

Prediction of gut diseases for entire sample

Residence resulted as a predictor for parasitic diseases with a decrease of these diseases in Timisoara (OR=0.11, p=0.001) and Timis County (OR=0.07, p=0.001). The model was 88.7% accurate.

A predictor for CMA was breast feeding with duration 0-2 months (OR=13.51, p=0.007, model - 84.2% accurate).

Prediction of gut diseases for age group 0-3 years

At the age group 0-3 years, binomial logistic regression indicated the breast-feeding duration 0-2 months as predictor for: GE (OR=0.185, p=0.009, model - 71.5% accurate), Enterocolitis (OR=5.476, p=0.005, model - 66.1% accurate) and CMA (OR=7.48, p=0.002, model - 67.9% accurate).

Association GE - gender (OR=3.619, p=0.019, model - 71.5% accurate) indicate a higher frequency of this disease at female than male patients. Associations of age with gut diseases indicate for Enterocolitis (OR=0.516, p=0.005, model - 62.3% accurate) and for CMA (OR=0.54, p=0.01, model - 66.2% accurate) a decrease with age increase.

Prediction of gut diseases for age group 3-18 years

At the age group 3-18 years delivery type was found as predictor for GE (OR=3.033, p=0.014, model - 68.1% accurate), which indicates an increase of GE for patients with natural delivery.

DISCUSSION

In the performed case-study a population of pediatric patients was investigated for gut diseases and factors that could impact them. First results indicated localization and etiology as being used in the diagnoses of this pathology. Therefore, enterocolitis, GE, CMA, microbial, viral and parasitic diseases were determined to be gut diseases. The highest frequencies of enterocolitis and CMA were found in pediatric patients aged between 0-3 years, and of GE in patients aged between 3-18 years. Previous studies have shown that Enterocolitis could be determined by sepsis or food allergies in infancy (4) and that the burden of acute gastroenteritis on children continues to be enormous (5). CMA is the most common food allergy,

especially in infancy (6). A generalized effect was ADS and predominated in patients aged between 3-18 years. In another study, most cases of ADS were clinically diagnosed (similar with our study) (7).

In the performed study, distribution of age-groups of the pediatric patients diagnosed with gut diseases was non-uniform when the information was collected from primary sources of evidence. More than half of the pediatric patients age was between 0 - 3 years. The necessity to adjust the investigation of GE to the age resulted in an empirical examination study of the 'pediatric gastroenteritis admission rate' indicator in Italy (8). In our study we considered age-groups (according to developmental stages criterion) when analyzed association with gut diseases for both samples.

The study involved a predominance of patients from an urban area for these two age groups. The loss of environmental biodiversity could be a public health threat as was concluded in another study (9). Caesarean delivery was more frequently (14%) at the age group 0-3years than at 3-18 years. Caesarean section is medically indicated. The concept of option arises from the fact that the regulations are more permissive in certain countries. The reasons why women opt for caesarean section were analyzed in another study and consisted of the fact that it is a pain-free method of birth and it is safe for both mother and baby (10). Early nutrition investigation of the pediatric patients in the performed study indicated natural (breast) and artificial feeding. Natural feeding duration was frequently found between 0-2 months for pediatric patients aged between 0-3 years, and over 1 year for pediatric patients aged between 3-18 years, suggesting a tendency to decrease the duration of breast feeding at 0-2 months. Human milk has potential effects on the infant, effects that will never be possible with the milk formula: it contains specific oligosaccharides that drive the growth of microbes within the gut (human microbiome), antimicrobial proteins/peptides that assure the immune protection (11). In the performed study, main milk formulas used for artificial nutrition were NAN, Aptamil, Humana and powder milk. These milk formulas predominated at the age groups 0-3 years and were used in the same measure with powder milk at the age group 3-18 years. Milk formulas proved to be useful against infections and to stimulate growth when they were symbiotic enriched (12).

In this study associations were found between gut diseases and certain personal (residence, age and gender), perinatal (delivery type) and early nutrition (breast feeding 0-2 months) factors. The most significant results of this study were obtained for association of gut diseases (GE, enterocolitis, CMA) with natural feeding duration (0-2 months) at the age group 0-3 years. Frequency of patients with GE decreases when frequency of patients with natural feeding duration (0-2 months) increases. On contrary, frequency of patients with Enterocolitis and CMA increase with frequency of patients with natural feeding duration (0-2 months) increase. Another study demonstrated that there are risks associated with all forms of infant feeding, including the breast feeding or use of manufactured milk formulas (13). CMA was proved to develop even when the child is breast feeding when the mother's diet con-

tains cow milk (14).

Other important predictors for gut diseases were age and residence. At the age group 0-3 years, Enterocolitis and CMA decrease with age increase. Residence was associated with parasitic diseases for entire sample, with a decrease of parasitic diseases in urban environment (9). Age proved to be an important factor in the study of digestive diseases in children (8). Delivery type was associated with GE, at the age group, 3-18 years, with an increase of GE for patients with natural delivery. Another study found that the mode of birth affects oral microbiota in infants, vaginally delivered infants having a higher number of taxa detected than infants delivered through caesarean section (15).

The discovered results were supported by research literature presented. A limit of this study is that it is an observational one.

CONCLUSIONS

The most frequently identified gut diseases in hospitalized pediatric patients are: enterocolitis, gastroenteritis and cow milk allergy. ADS is frequently found as a generalized effect of gut diseases. The most affected age group is 0-3 years. Personal factors (age and residence) are associated with gut diseases: age relates to enterocolitis and CMA. at the age group 0-3 years and residence relates to parasitic diseases for the entire sample. There is a weak association between delivery type and GE for age group 3-18 years. At the age group 0-3 years, there is a strong association between early nutrition factors (duration of breast feeding 0-2 months) and gut diseases: CMA, Enterocolitis and GE. As a general conclusion, there is an association between specific gut diseases and personal (age, residence), perinatal (delivery type) factors and early nutrition (natural feeding duration - 0-2 months).

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PRINCIPLES OF OVERDENTURE ON ONE-PIECE NARROW DENTAL IMPLANTS IN PATIENTS WITH DIFFERENT PATTERNS OF MANDIBULAR RIDGE RESORPTION

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PRINCIPLES OF OVERDENTURE ON ONE-PIECE NARROW DENTAL IMPLANTS IN PATIENTS WITH DIFFERENT PATTERNS OF MANDIBULAR RIDGE RESORPTION (Abstract). **Aim**: To highlight the particularities of mandibular overdenture on one-piece narrow dental implants in patients with two frequent and high-difficulty clinical situations, namely flat alveolar ridge (also known as severe ridge resorption, when mainly vertical bone resorption occurs) and narrow alveolar ridge (which in its most severe form has a knife-edge appearance, when mainly horizontal bone resorption occurs). Narrow implant overdenture is a treatment alternative that responds well to the most important reason of patient dissatisfaction with conventional denture, namely its instability, through a relatively minimally invasive surgical intervention followed by relatively simple prosthetic procedures. To obtain a good outcome, particularization according to patient features, such as the morphology of the alveolar ridge is recommended. Deciding upon implant type, number, site, and attachment system should be made in accordance with such factors as bone offer and morphology of the alveolar ridge, edentulous and prosthetic status in the maxilla, prosthetic space, and the need to improve denture stability and retention. **Keywords:** DENTURE, MINI DENTAL IMPLANT, MANDIBULAR EDENTULOUS, FLAT RIDGE, NARROW RIDGE.

Complete mandibular edentulism is frequently treated by complete dentures, treatment option that often does not ensure a good outcome in terms of proper prosthesis stability and retention, and patient satisfaction. This is linked to objective factors, such as increased treatment difficulty due to the loss of all teeth and consequent ridge resorption, and subjective factors, as psychologically rejection of conventional complete denture due to associating it with aging and limitation of social functioning. According to current perspective, the new minimal treatment standard for mandibular complete edentulism is stated to be the two-conventional implant overdenture, treatment alternative that addresses the main deficiencies of complete denture (1-3). The usage of the narrow dental implants is beneficial especially in the aged edentulous patients, with unfavorable morphological conditions for the conventional or implant prosthetic rehabilitation (4, 5). This treatment option is well-adapted to the

older patients' needs, as it addresses the main reason of dissatisfaction (increases prosthesis retention and stability), at moderate biological, financial and time costs, having also rather simple maintenance procedures and means of addressing possible complications (6).

The aim of this article was to highlight the particularities of mandibular overdenture on one-piece narrow dental implants in patients with two frequent and high-difficulty clinical situations, namely flat alveolar ridge (known also as severe ridge resorption, when mainly vertical bone resorption occurs) and narrow alveolar ridge (that in its most severe form has a knife-edge appearance, when mainly horizontal bone resorption occurs).

General coordinates of treatment planning and conduct

The following aspects are to be considered when planning for mandibular implant overdentures: accurate patient assessment, through anamnesis, clinical evaluation, panoramic radiographs, supplemented sometimes by lateral cephalometric radiographs and other investigations (7, 8); the number of narrow dental implants to be used, which vary, usually 2 to 4 implants being sufficient, a higher number being chosen when teeth or implant prosthesis are found in the maxilla, in younger patients (under 60 years), in those with a harder food diet, bruxism and a more obvious tendency to ridge resorption (fig.1); narrow dental implants sites, usually implants being placed in the interforaminal area, upon considering the bone density, width and height, and the prosthetic needs (a surgical template can be used for a more accurate localization of implant placement site); surgical technique of implant placement, by the usage of a flap technique (advantageous by the possibility to accurately evaluate bone width through direct assessment), or flapless technique (advantageous by the reduced invasiveness of the surgical procedure) (4, 9); loading protocol, which can be either an immediate loading protocol (i.e., immediate fixation of the metal rings in the overdenture base) or progressive loading protocol (i.e., soft lining materials were used as soft matrices during the osseointegration period); type of attachment system, which for one-piece narrow implants is usually O-Ring (e.g., miniSKY1 dental implants, Bredent; mini dental implants, IMTEC, later 3MESPE), some other variants being available (e. g., Locator, LODI implant system, Zest Anchors; magnet, Magfit, Aichi Steel Corporation)(10,11); overdenture design, which should aim to ensure adequate support, retention and stability, a complete coverage of the support area, in correspondence with the anatomical and functional borders, being usually preferred (a), should not exert pressure on the dental implants, when they are used only to increase retention, not for support (b), and should be made at correct intermaxillary relationship, providing a stable occlusion (c); previously made complete dentures can be either kept and transformed into overdentures (when considered satisfactory, this promoting a faster adjustment with the new treatment, aspect that should be considered especially in the elderly, known to have difficulty adapting to change, either preserved as interim prosthesis during the healing phase (when considered unsatisfactory, but not having severe deficiencies that may be risk factors for implant failure), either replaced before implant placement.

Fig. 1. Implant number varied according to the dentate status in the maxilla

One-piece narrow-dental implants for edentulous patient

Various types of dental implants can be used for implant overdenture, e.g. conventional or narrow, one-piece or two-piece design, for which different types of attachment systems are available. The one-piece narrow dental implants are mainly used in the completely edentulous elderly patients. Their narrow diameter is usually well-suited for them because of the severe bone loss and requiring minimally invasive surgical procedures. In this context, it should be mentioned that there isn't a well-defined cut-off point for differentiating the mini-, from narrow-, from conventional-diameter dental implants. Generally, there are considered narrow implants those with a diameter below 3.75mm. Therefore, the 2.8 mm dental implants, as mini SKY 1, are included in either mini-, narrow-, or hybrid-dental implant category. The narrow dental implants are generally manufactured in different lengths (e.g., miniSKY1 is available in lengths of 10 mm, 12 mm and 14 mm, and MDI form 3M ESPE in lengths of 10 mm, 13 mm, 15 mm and 18 mm), suiting both vertical and horizontal ridge resorption. The one-piece design, usually encountered, has some advantages, among which is the possibility to verify the primary stability of the implants through horizontal percussion, or to easily address implant loss by placing another implant (not requiring major interventions or replacing the prosthesis). Also, the use of O-ring attachment system has the benefit to allow an implant divergence, less of 30 degrees. The usage of the implants mainly targets increasing prostheses' retention and stability, and generally are not to be used for support.

Particularities of treatment conduct in patients with flat alveolar ridge

These patients usually have wider alveolar ridges that allow placement of larger diameter dental implants, but have a reduced bone height, which associates the need of use of shorter dental implants. This clinical situation is encountered more frequently in patients with the following characteristics: often old patients, with expectations mainly related to a solution for improving prosthesis retention by interventions that required minimal surgery, at moderate costs; patients complaining of poor denture retention and stability, and occasionally pain described as appearing under the dentures, main reason of dissatisfaction with conventional denture; many of them are long-time mandibular denture wearers, some of them having teeth or

fixed conventional or implant prosthesis in the maxilla (these being risk factors for severe ridge resorption);usually eating a soft diet, motivated by the masticatory deficiencies related to the ill-fitting dentures; the attached mucosa occasionally presents alterations related to the long-period of denture wearing, as reduced thickness, it becoming painful under the pressure exerted by the denture; soft peripheral tissue may exhibit changes that increase treatment difficulty, such as: herniation of sublingual glands; displacement of muscle and ligaments attachments closely to the ridge crest; posterior position of tongue during rest position; tongue hyperactivity (that is related to patient's functional particularities, prolonged wearing of ill-fitting dentures or involution phenomena); hypertonia and shortening of the lower lip *orbicularis oris* muscle that enhances mandibular denture instability; there can be encountered abnormal sagittal and horizontal ridge relationships, that can be related to factors such as the skeletal class, the pattern of the bone resorption of the jaws (centripetal in the maxilla, respectively centrifugal in the mandible) and the anterior hyperfunction syndrome (combination syndrome), all contributing to the facial appearance known as pseudo-skeletal class III facial appearance or the old man's prognathism (12); these patients generally have oral conditions unfavorable to obtain a good denture retention, support and stability, with a high degree of treatment difficulty according to Prosthodontic Diagnostic Index (PDI) classification System of American College of Prosthodontists (ACP) (13), the usage of dental implants being considered appropriate in order to ensure an adequate functionality with the prosthesis.

Planning and conducting he treatment in these patients by narrow dental implant overdenture, without bone augmentation procedures, usually has the following pattern: a number between 2 and 4 narrow dental implants is usually sufficient; shorter dental implants of less than 12mm or 10 mm are frequently needed (14); dental implant placement site is in the inter foraminal region; in cases with severe ridge resorption, sometimes adequate bone quantity is found only nearby the mental symphysis, in these cases without bone augmentation the implant number being limited to 2 implants, placed in the most anterior part of the mandible (fig. 2); dental implants can be usually placed by a flapless technique; generally the bone density is assessed as being favorable in regard to ensuring a good primary stability for immediate implant loading; the vertical prosthetic space is generally sufficient for O-Ring attachment system, difficulties in this respect being encountered in patients with a hypodivergent class II skeletal pattern, in the context of the counter clock wise rotation of the mandible, that occurs in edentulous patients.

Fig. 2. In patients with severe ridge resorption, implants are placed more frequently closer to the mental symphysis

Particularities of treatment conduct in patients with narrow alveolar ridge

These patients usually have unfavorable conditions for conventional denture and implant placement, due to the reduced width of alveolar bone and sometimes the sharp ridge crest, covered by a painful mucosa. Narrow dental implants are indicated especially in cases with narrow-ridges, considering their diameter is well suited to bone width (at least 4 mm width). Narrow alveolar ridges are encountered more frequently in patients with the following characteristics: have more frequently a class II hypodivergent skeletal pattern, which associates tongue and lip muscle hyperactivity; are usually recent edentulous patients with low-density trabecular bone, and first time denture wearers; present to the dentist usually dissatisfied with denture instability and complaining about pain related to denture pressure; alveolar ridge morphology is usually high and thin, with a greater depth of the vestibular sulcus; different morphological aspects of the alveolar ridge are observed, characterized by the relatively frequent presence of cortical bone irregularities, such as exostosis, covered by thin mucosa, painful under pressure, which are linked to intolerance of conventional dentures; in previous wearers of ill-fitting dentures in which tooth loss is mainly linked to periodontal disease, there was rather often encountered flabby ridges and mucosal hyperplasia (12).

Planning and conducting edentulism rehabilitation in these patients by narrow dental implant overdenture has usually the following pattern: the number of implants is generally between 2 and 4 narrow dental implants, according to bone offer, the degree of overdenture retention needed and clinical situation on the opposite jaw (fig. 3); if the clinical situation requires, the number of implants can be increased later, after the stabilization of bone level after

Fig. 3. Patient with narrow mandibular ridge, with two implants placed for increasing denture's retention

tooth extraction; implant scan usually have higher length, of more than 12 mm or 14 mm; implant diameter is usually smaller, corresponding to ridge width; more frequently the implants are placed by a flap technique, chosen in order to correct bone and mucosa alterations, and to adequately assess bone offer and the limits of implant placement (to prevent cortical perforation); progressive implant loading is usually preferred due to rather frequently encountered reduced bone density, for which soft silicone or acrylic materials may be used during the osseointegration period for amortization of occlusal forces or peri-implant soft tissue conditioning; the healing process is slower compared to the one of the patients with flat ridge considering that in these patients usually a flap technique is used for implant placement; the vertical prosthetic space may be decreased, in relation to the skeletal class, therefore implants with other type of attachment system (e.g., magnets) can be used; overdenture risk fracture is higher because of the reduced width and thickness of overdenture base, especially when there are placed more than

two implants; dental implants are especially beneficial in recent edentulous patients with narrow-ridges, through minimizing the bone resorption, which has the highest rate after tooth loss and is enhanced by ill-fitting dentures.

In both clinical situations, rehabilitation by recreating the correct intermaxillary relationship, with a stable occlusion, correct relationship of the implant with the overdenture base which should not exert pressure on the dental implants, as well as compliance with the recommendations after implant placement (as having a soft diet for the next following days, and maintain a good oral hygiene) are required.

CONCLUSIONS

We can conclude that narrow implant overdenture is as a treatment alternative that responds well to the most important reason of patient dissatisfaction with conventional prosthesis, namely the mandibular denture instability, through a relative minimal invasive surgical intervention followed by relatively simple prosthetic procedures. Aspects as accurate selection of narrow implant number and placement sites, correctness of overdenture execution, proper maintenance and monitoring are important prognostic factors. To obtain a good outcome, particularization according to patient features, such as the morphology of the alveolar ridge, is recommended. Deciding upon implant type, number, site, and attachment system should be made in accordance with factors as bone offer and morphology of the alveolar ridge, edentulous and prosthetic status in the maxilla, prosthetic space, and the need to improve denture's stability. Prospective studies must be implemented to gain knowledge on best treatment coordinates for different clinical situations.

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NEWS

COULD CALCIUM HOLD THE KEY TO FIGHTING A DANGEROUS HOSPITAL INFECTION?

Clostridium difficile (*C. difficile*) is an anaerobic gram-positive pathogen that is the leading cause of nosocomial bacterial infection globally. *C. difficile* infection (CDI) typically occurs after ingestion of infectious spores by a patient that has been treated with broad-spectrum antibiotics. While CDI is a toxin-mediated disease, transmission and pathogenesis are dependent on the ability to produce viable spores. These spores must become metabolically active (germinate) in order to cause disease. *C. difficile* spore germination occurs when spores encounter bile salts and other co-germinants within the small intestine, however, the germination signaling cascade is unclear. Here we describe a signaling role for Ca^{2+} during *C. difficile* spore germination and provide direct evidence that intestinal Ca^{2+} coordinates with bile salts to stimulate germination. Endogenous Ca^{2+} (released from within the spore) and a putative AAA+ ATPase, encoded by *Cd630_32980*, are both essential for taurocholate-glycine induced germination in the absence of exogenous Ca^{2+}. However, environmental Ca^{2+} replaces glycine as a co-germinant and circumvents the need for endogenous Ca^{2+} fluxes. *Cd630_32980* is dispensable for colonization in a murine model of *C. difficile* infection and *ex vivo* germination in mouse ileal contents. Calcium-depletion of the ileal contents prevented mutant spore germination and reduced WT spore germination by 90%, indicating that Ca^{2+} present within the gastrointestinal tract plays a critical role in *C. difficile* germination, colonization, and pathogenesis. These data provide a biological mechanism that may explain why individuals with inefficient intestinal calcium absorption (*e.g.*, vitamin D deficiency, proton pump inhibitor use) are more prone to CDI and suggest that modulating free intestinal calcium is a potential strategy to curb the incidence of CDI (Travis J. Kochan, Madeline J. Somers, Alyssa M. Kaiser *et al.* Intestinal calcium and bile salts facilitate germination of Clostridium difficile spores. *PLOS Pathogens* 2017; 13(7): e1006443 DOI: 10.1371/ journal.ppat.1006443).

Redea Maria Andreea

THE ASSESSMENT OF THE RISK FACTORS FOR ATHEROSCLEROSIS AMONG POPULATION FROM THE NORTH-EASTERN REGION OF ROMANIA

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THE ASSESSMENT OF THE RISK FACTORS FOR ATHEROSCLEROSIS AMONG POPULATION FROM THE NORTH-EASTERN REGION OF ROMANIA (Abstract): **Aim**: This study investigates the factors that influence the risk of atherosclerosis for the population included in the research and to assess the relationship between these factors and atherosclerosis. **Material and methods**: A case-control study was conducted on cardiovascular risk factors that contribute to the occurrence of atherosclerosis which included a number of 207 patients of both sexes, selected from urban and rural areas, with permanent residence in the county of Iasi, aged between 20 and 80 years, during the period April-June 2014. **Results:** Analyzing the risk factors there was found a statistical significance for the values of the total cholesterol and triglycerides, as well as for other risk factors such as obesity (assessed by body mass index), blood pressure, smoking and physical inactivity. For other risk factors involved in the occurrence of atherosclerosis, such as: ischemic personal history, family history of coronary heart disease, diabetes, type of diet, history of infection with *Chlamydia pneumoniae*, *Cytomegalovirus*, *Helicobacter pylori* there were obtained insignificant statistical values. **Conclusions:** The results of this study provide information with practical implications that can be used by clinicians when they faced people with cardiovascular risk factors. **Keywords**: CARDIOVASCULAR DISEASES, ATHEROSCLEROSIS, RISK FACTORS, ROMANIA, PREVALENCE.

Atherosclerotic cardiovascular diseases are the leading cause of death worldwide (1). Atherosclerosis (ATS) is considered a chronic inflammatory disease related to age, with a long and slow asymptomatic phase. Recent data shows that it begins to develop early in life and presents clinical manifestation in many patients in a relatively advanced stage. The consequences of ATS responsible for the occurrence of cardiovascular diseases are among the leading causes of morbidity and mortality worldwide. It should also be noted that the coronary heart disease due to ATS is increasing. The diag-

nosis and treatment are a priority partly because the sudden death is a consequence of primary coronary artery disease in 50% of men and 64% of women (2).

Increased susceptibility to atherosclerosis is conditioned by the accumulation of the risk factors (RF): hypercholesterolemia, hypertension smoking (3).

The risk factors for atherosclerotic cardiovascular diseases includes (4, 5): *individual characteristics* (unchangeable = non-modifiable RF): age, sex, family history of atherosclerosis at a young age (for women < 65 years, for men < 55 years), previous personal history of atherosclerosis; *the biochemical/physiological factors*: hyper-LDL-cholesterol, hypo-HDL-cholesterol, hyper-triglycerides, high blood pressure (HBP), diabetes mellitus (DM) or impaired glucose tolerance, obesity, thrombogenic factors; *lifestyle characteristics*: diet rich in saturated fats, smoking, alcohol, physical inactivity (6).

The aim of this study was to investigate the factors that influence the risk of atherosclerosis for the population included in the research and to assess the relationship between these factors and atherosclerosis.

MATERIAL AND METHODS

A case-control study was developed to assess the risk of various factors among the study population. The case-control study on cardiovascular risk factors that contribute to the occurrence of ATS included a number of 207 patients of both sexes, selected from urban and rural areas, with permanent residence in the county of Iasi, aged between 20 and 80 years, during the period April – June 2014. Patients were divided into two groups, the group of cases which included 78 patients with ATS, recruited from people who visited the family doctor office, and the control group, which included a total of 129 persons selected from the subjects who were present in the Ambulatory of the "Dr. I. Czihac" Emergency Army Hospital from Iasi. The inclusion criteria were: age over 20 years, and the diagnosis of ATS confirmed by angiography or computed tomography. During the visit to the family doctor for each patient enrolled in the study was filled up a questionnaire with the following data: date of birth, age, gender, ethnicity, residence, education level, economic situation perceived by the patient, personal history, lipid profile (the total cholesterol value, the amount of LDL and HDL cholesterol and the amount of triglycerides), body weight, height, blood pressure value, the presence of diabetes, smoking, physical activity, type of food, the inflammatory markers C-Reactive Protein (CRP), evidences of the medical history of infections with *Chlamydia pneumonia*, *Cytomegalovirus*, and *Helicobacter pylori*.

A pilot study was conducted to validate the questionnaire used for the assessment of the risk factors for ATS. A number of 40 subjects were included in the study: 20 cases, recruited from people who visited the family doctor office and 20 controls, chosen from the subjects who were present in the Ambulatory of the "Dr. I. Czihac" Emergency Army Hospital from Iasi, during a period of 3 months, between January and March 2014.

In both studies, the information was collected using a questionnaire specially designed for this purpose, and then were included in an Excel database that has been further processed using Excel and Epi Info 7 programs. The variables under the study were analyzed and the values within the statistical confidence interval (CI) 95% ($p < 0.05$) were considered values with statistical significance.

RESULTS

Characteristics of the subjects under the study. The study included a number of 207 participants of which 78 cases (37.68%) were cases with ATS and 129 (62.31%) were controls without a disease with clinical confirmation. Regarding the gender distribution, was found a preponderant presence of the disease in males (51.28%), because they have a higher risk of getting the disease.

The most affected patients were within the age group of 70-79 years (41.02%), followed by those aged 60-69 years (28.21%) (fig. 1).

Most of the cases were from urban areas (55.13%) compared with 44.97% from rural areas. A possible explanation could be that people in rural areas have a higher addressability to the doctor and an easier access to specialized medical services.

If we refer to the level of training, the cases with secondary education have been recorded in more than a half of the cases (51.28%), followed by those with higher level of training (28.21%) and those with primary education (20.51%).

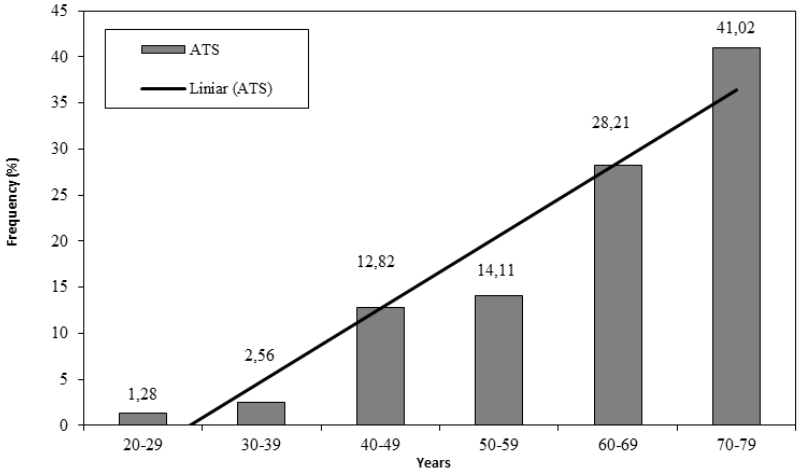

Fig. 1. The prevalence of atherosclerosis, according to different age groups

The assessment of the relationship between different risk factors and ATS. The following risk factors were studied: personal history of ischemic heart disease, family history of coronary heart disease, the amount of LDL cholesterol, HDL cholesterol, triglycerides, body mass index, blood pressure, diabetes, smoking, sedentary lifestyle, type of diet, C Reactive Protein (CRP), history of infections with *Chlamydia pneumoniae*, *Cytomegalovirus*, *Helicobacter pylori.*

Analyzing the risk factors there was found a statistical significance for the values of the total cholesterol (TC) and triglycerides, as well as for other risk factors such as obesity (assessed by body mass index), blood pressure, smoking and physical inactivity. For other risk factors involved in the occurrence of ATS, such as: ischemic personal history, family history of coronary heart disease, diabetes, type of diet, history of infection with *Chlamydia pneumoniae*, *Cytomegalovirus*, *Helicobacter pylori* there were obtained insignificant statistical values (tab. I).

TABLE I

The relation between the risk factors and the atherosclerosis among the population under the study

The studied factor	Cases		Controls		OR	X^2	p	Statistical significance
	No.	%	No.	%				
Obesity	67	85.9	54	41.9	2.8	13.5	0.01×10^{-2}	SS
High cholesterol	60	76.9	49	37.9	5.5	31.1	$<0.01\times10^{-5}$	SS
High LDL-cholesterol	57	72.2	42	32.6	5.2	30.5	$<0.01\times10^{-5}$	SS
Smoking	57	67.1	58	44.9	0.6	3.0	0.04	SS
High triglycerides	50	64.1	29	22.5	6.3	35.9	$<0.08\times10^{-5}$	SS
High Blood Pressure	48	61.5	41	31.8	3.5	18.0	0.01×10^{-3}	SS
Sedentarism	43	55.7	52	40.3	1.8	4.5	0.01	SS
Low HDL- cholesterol	34	43.6	103	79.8	0.2	28.9	$<0.01\times10^{-4}$	SS
Poor economic situation	34	43.6	30	23.2	2.6	9.6	0.001	SS
Type of food	42	53.9	59	45.7	1.4	1.6	0.1	NS
Infections with *Ch. Pneumoniae, Cytomegalovirus, H. pylori*	29	36.7	43	33.3	1.2	0.4	0.3	NS
Family history of ischemic heart disease	23	29.5	33	25.6	1.2	0.4	0.26	NS
Personal history of ischemic heart disease	17	21.8	26	20.2	1.1	1.1	0.36	NS
Diabetes	14	17.9	29	22.4	0.7	0.5	0.24	NS
CRP	4	5.1	4	3.1	1.7	0.5	0.24	NS

For the subjects included in the study, it was observed an average of the cholesterol value above the normal value, as well as for the other biochemical indicators (LDL-cholesterol and triglycerides). Regarding the variation coefficient, which shows us the scatter of the value series, the lower the value (as is the case with cholesterol) the closer it is to the average (tab. II).

TABLE II

Biochemical investigations among subjects included in the study

	Mean	Standard Deviation	Variation coefficient (%)
Total cholesterol	211.19	42.15	10.96
LDL-cholesterol	126.68	50.07	39.52
HDL-cholesterol	61.06	15.44	25.28
Triglycerides	137.34	69.47	50.58
Body Mass Index	26.71	5.4	20.21

Retrospective and prospective epidemiological research regarding the risk factors among the studied subjects. Of the total number of ATS cases included in our study, it was observed that the most patients had high total cholesterol levels (76.92%), followed by increased LDL-cholesterol values (72.15%) and low HDL-cholesterol levels (56.41%). The most affected age group by the increased values of the cholesterol, triglycerides and LDL-cholesterol was 70-79 years of age, and the

least affected was 20-29 years (fig. 2).

Another risk factor involved in the occurrence of ATS is the high triglyceride levels (normal values being between 50-150mg/dL). Laboratory investigations found that the most patients diagnosed with ATS had elevated triglycerides levels (>150mg/dL) (64.11%).

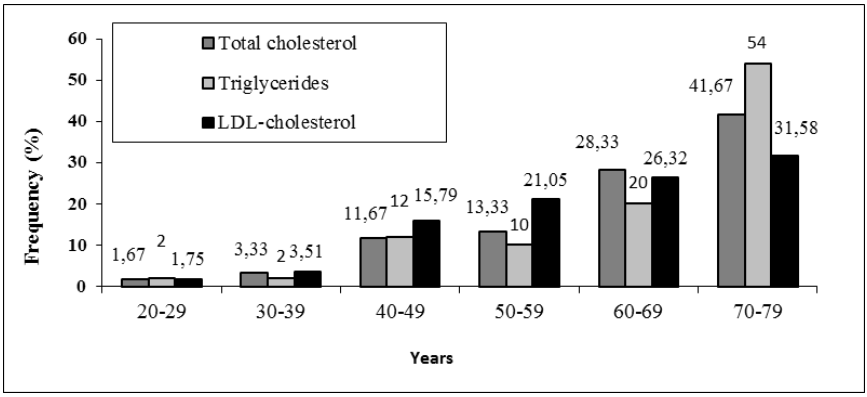

Fig. 2 The distribution of atherosclerosis cases with high values for the total cholesterol, LDL-cholesterol and triglycerides according to age groups

To assess the obesity as a risk factor for ATS, we calculated the Body Mass Index (BMI) for all patients included in the study. The most common classes of obesity were II degree, corresponding to a BMI values between 35.00 and 39.99 Kg/m2 (37.44%) and I degree (BMI between 30.00 and 34.99) (22.82%). Following the analysis of ATS cases, the obesity has been found to affect most the age groups 70-79 years and 60-69 years, and then decreases with age (fig. 3).

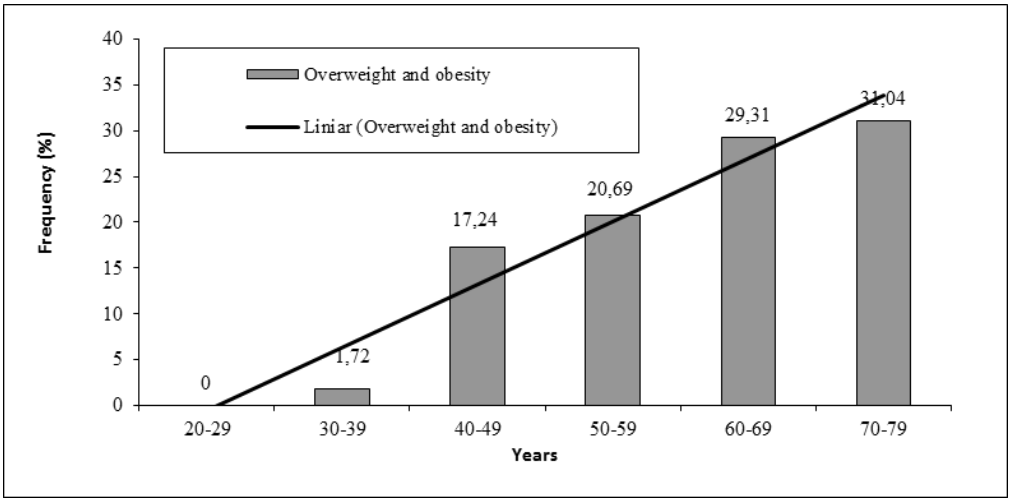

Fig. 3 The distribution of atherosclerosis cases among overweight and obese according to the age groups

Assessing another risk factor for ATS, namely high blood pressure (HBP), it was found to be present in most of the cases (61.54%). The most affected by hypertension among the atherosclerosis cases included in the study were those in the 70-79 age group (39.58%), followed by the age group 60-69 years (31.25%) (fig. 4).

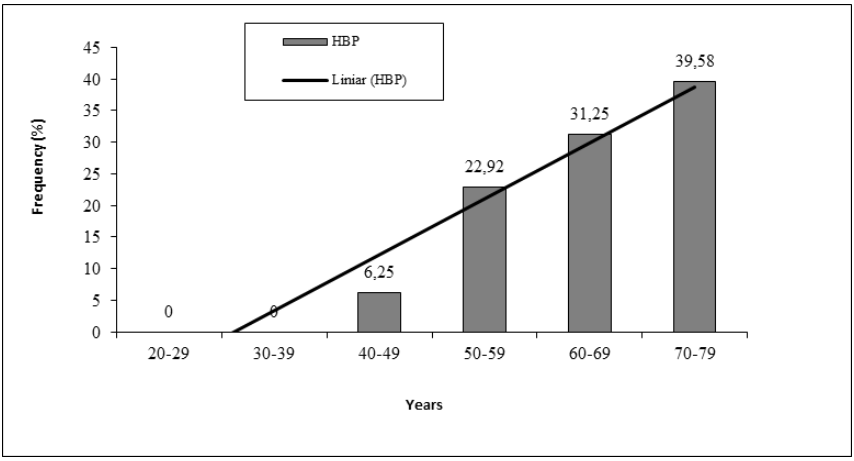

Fig. 4. The distribution of subjects with atherosclerosis and HBP according to the age groups

Smoking, another risk factor for ATS, was present in 67.09% of the investigated cases, the most frequently affecting the age group of 40-49 years (40.35%) and the age group of 50-59 years (35.09%) (fig. 5).

Another risk factor involved in the occurrence of ATS is the sedentarism. In the current study, it was observed that more than a half of the patients (55.7%) were sedentary (walking <30 min/day), more frequently within the 70-79 age group (30.23%) (fig. 6).

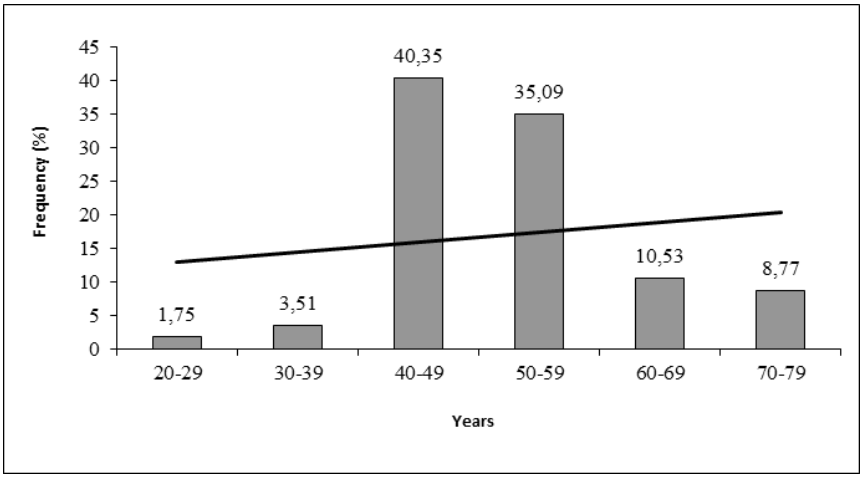

Fig. 5 The distribution of atherosclerosis among smokers according to the age groups

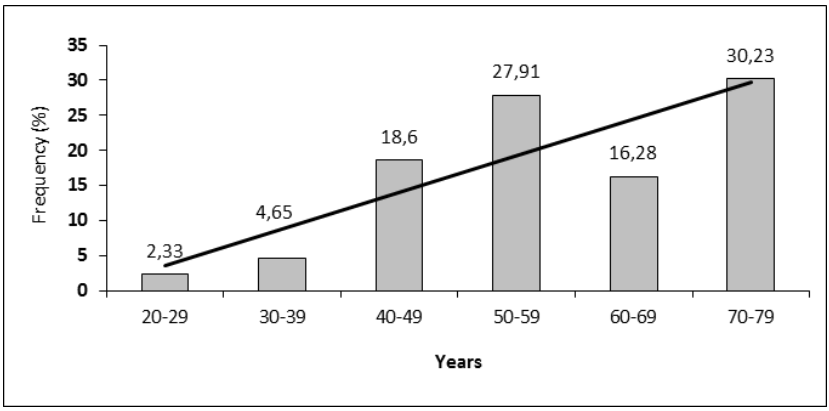

Fig. 6. The distribution of atherosclerosis among sedentary subjects according to the age groups

DISCUSSION

It has been noted that infectious factors play an important role in the development and progression of ATS, leading to chronic infections and persistent inflammatory process. Both the acute and chronic inflammation causes endothelial dysfunction. The main microorganisms that have been studied were *Chlamydia pneumoniae*, *Cytomegalovirus* and *Helicobacter pylori* (7). Also, some studies have reported that seropositive to *Chlamydia pneumoniae*, *Cytomegalovirus* and *Helicobacter pylori* is associated with coronary artery disease (8, 9). Infectious agents are considered risk factors or enhancers of other factors contributing to the occurrence of ATS (10).

In a study of the rural Chinese population, it was found that the most common risk factors involved in the occurrence of peripheral cardiovascular disease, more precisely in atherosclerosis, were high blood pressure and increased LDL-cholesterol / HDL-cholesterol ratio (11). Our data were similar to those found by Liang et al, the high blood pressure and high cholesterol levels being identified as risk factors with statistical significance.

There are several cohort studies that support a strong correlation between HDL-cholesterol and coronary risk. Some US epidemiologic studies involving many subjects (like the Framingham Heart Study, the Lipid Research Clinics Primary Prevention Trial) showed that low HDL-cholesterol levels increase the risk of coronary heart disease and argue that every 1 mg/dL increase in HDL-cholesterol level reduces the risk of coronary heart disease by 2% in men and 3% in women. The Framingham Heart Study supported the role of HDL-cholesterol as an independent factor for the coronary artery disease. The main mechanism by which HDL-cholesterol is considered to reduce the cardiovascular risk consists in the reverse transport of cholesterol, a way to remove the free cholesterol from macrophages into the arterial wall and to return to the liver for bile excretion (12). Our study did not found an increased risk for atherosclerosis when low values of HDL-cholesterol were registered ($OR = 0.2$, $p < 0.01 \times 10^{-4}$).

Various epidemiological studies have shown that around 25% of US adults are estimated to have high levels of triglycerides (> 150mg/dL), leading to an increased risk of cardiovascular disease. It is well known that lowering the high level of low-density lipoprotein (LDL-cholesterol) remains the primary target of treatment to reduce the

risk of cardiovascular diseases. Also, a series of epidemiological studies including many subjects have shown that increased levels of triglycerides are associated independently with an increased incidence of cardiovascular events, even in patients treated with appropriate statins (13, 14). This fact was observed in the current study, which found that subjects diagnosed with ATS had elevated levels of both triglycerides and LDL-cholesterol. The same aspect was identified by Fukuda N et al. during an experimental trial conducted in 2013 involving 15 rabbits. The postprandial hypertriglyceridemia was found to be a risk factor in promoting the ATS (15).

The early measurements of BMI, cholesterol, triglycerides and the blood pressure were found to be more strongly associated with the presence and severity of coronary calcifications (16). In the current research obesity has been shown to be a risk factor in the development of atherosclerosis. Also, there are researchers shown that obesity induces a pro-inflammatory state due to the secretion of adipokines that are an essential event in the occurrence of cardiovascular disease (17). The risk factors for ATS and diabetes are based on inflammatory and genetic backgrounds that can be accounted for by the excess of fat. Longevity has increased in last past years and is related to a pro-inflammatory response with cardiovascular consequences. If lifestyle would include an increase in physical activity as well as a calories restricted diet, the low degree of obesity would have a limited effect on chronic inflammation, and the risk of atherosclerosis and diabetes mellitus could be reduced (17).

In a study of the prevalence of risk factors for atherosclerosis among the adults from the Philippines, which was conducted in 2008, the following results were obtained analyzing the 7,700 eligible subjects who participated in the study: the prevalence of dyslipidemia - 72%, high blood pressure - 24.6%, diabetes mellitus - 3.9%, obesity - 4.9%, smoking - 31%, and a history of coronary, cerebrovascular and peripheral disease - 1.1%, 0.9% and 1.0%, respectively. Following this comparative study, it was found that the prevalence of the risk factors for ATS was higher in 2008 than in 2003, indicating a need for active collaboration intervention with all government agencies and medical societies in the Philippines in order to prevent or control the action of these factors (18). A similar situation was found in our study, which showed that the economic situation of patients, high level of cholesterol, triglycerides, blood pressure, smoking, and sedentarism can influence the development of ATS.

Smoking is another risk factor with a major contribution in the development of cardiovascular and atherosclerotic diseases, but our study did not identified smoking as a risk factor for ATS ($OR = 0.06$, $p = 0.04$). Another research involving 299 subjects, from which 34 (11%) smokers, 73 (24%) former smokers and 192 (4%) who never smoked showed that smoking was associated with subclinical atherosclerosis in diabetic persons and was influenced by the duration of diabetes to promote the progression of ATS (19).

CONCLUSIONS

The actual research identified as risk factors with statistical significance for ATS the following conditions: obesity, high cholesterol, high LDL cholesterol, high triglycerides levels, high blood pressure, sedentarism, and poor economic situation. The findings of this study provide information with practical implications that can be used by clinicians when they faced with people with risk factors.

Most of the cases come from urban are-

as. One possible explanation might be that the urban population has a greater addressability to the doctor and easier access to the specialist medical services. So, the future cardiovascular preventive strategies should address this issue.

This study showed that patients with high blood pressure have an increased risk of developing ATS due to hyperreactivity to risk factors. Although sometimes it is difficult to manage the systolic blood pressure in the elderly, the total cardiovascular risk can be reduced by action on other risk factors such as smoking, obesity, sedentarism, and high cholesterol levels. If the lifestyle would include an increase in physical activity as well as a calorie restricted diet and a diet rich in fruits, vegetables and fish, the low degree of obesity could have a limited effect on chronic inflammation and the risk of ATS and consequently the risk of cardiovascular disease may be reduced.

The measures used to prevent cardiovascular events in patients with coronary artery disease involve a multifactorial approach including the management of all the modifiable risk factors (especially high cholesterol, high LDL-cholesterol, high triglycerides levels, obesity, and high blood pressure). Prevention of cardiovascular disease should be a priority not only in research but also in clinical practice, especially in preventive medicine.

ACKNOWLEDGEMENTS

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NEWS

RISK OF ACUTE MYOCARDIAL INFARCTION WITH NSAIDS IN REAL WORLD USE: BAYESIAN META-ANALYSIS OF INDIVIDUAL PATIENT DATA

Michèle Bally (epidemiologist in the Department of Pharmacy and Research Center, Centre hospitalier de l'Université de Montréal, Quebec, Canada) and her research team conducted a systematic review followed by a one stage bayesian individual patient data meta-analysis which shows that the use of nonsteroidal anti-inflammatory drugs (NSAIDs) is associated with a significantly increased risk for myocardial infarction (MI). A cohort of 446,763 individuals (61,460 with acute myocardial infarction) was included in the study. The results highlighted that taking any dose of NSAIDs for one week, one month, or more than a month was associated with an increased risk of MI. If the subject used the NSAIDs for one to seven days the probability of increased MI risk was 92% for celecoxib, 97% for ibuprofen, and 99% for diclofenac, naproxen, and rofecoxib. The corresponding odds ratios (95% confidence intervals) were 1.24 (0.91 - 1.82) for celecoxib, 1.48 (1.00 - 2.26) for ibuprofen, 1.50 (1.06 - 2.04) for diclofenac, 1.53 (1.07 - 2.33) for naproxen, and 1.58 (1.07 - 2.17) for rofecoxib. A greater risk of MI was reported for higher dose of NSAIDs, but the use for longer than one month, did not appear to exceed the risks associated with shorter durations. The study concluded that all NSAIDs, including naproxen, considered by some as one of the safest drugs in this class, were found to be associated with an increased risk of acute MI. The risk of MI with celecoxib was comparable to that of traditional NSAIDs and was lower than for rofecoxib. Also, the risk was greatest during the first month of NSAIDs use and with higher doses. (Bally M, Dendukuri N, Rich B, Nadeau L, Helin-Salmivaara A, Garbe E et al. Risk of acute myocardial infarction with NSAIDs in real world use: bayesian meta-analysis of individual patient data. *BMJ* 2017; 357 : j1909. doi.org/10.1136/bmj.j1909).

Mioara Matei

THE RISK OF ITERATIVE FRACTURES IN PATIENTS WITH OSTEOGENESIS IMPERFECTA - CASE REPORT

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THE RISK OF ITERATIVE FRACTURES IN PATIENTS WITH OSTEOGENESIS IMPERFECTA-CASE REPORT (Abstract): *Osteogenesis imperfecta* is characterized by connective tissue involvement and is due to quantitative and qualitative changes of collagen type I. The curative treatment is not possible, but the introduction of antiresorptive bisphosphonate therapy and multidisciplinary approach of this pathology has allowed a considerable improvement in quality of life for these patients. The case presented is of a patient who inherited the disease from the paternal side and has a very rich medical history represented by the existence of 18 fractures. Leg fracture occurred at the age of 20 years has not been treated with centromedullary implants due to the accentuated tibial curves and tibial medullary canal obliteration, so osteosynthesis with plate was don. Removal of osteosynthesis material 18 months postoperatively resulted in the emergence of iterative fracture after 3 months, necessitating a reintervention by the same technique with bone consolidation. **Keywords**: BLUE SCLERA, COLLAGEN, BONE FRAGILITY, OSTEOSYNTHESIS.

Osteogenesis imperfecta (O.I.) is a hereditary disease which involves the connective tissue and is due to quantitative and qualitative changes of collagen type I. The clinical expression of this alteration of connective tissue can be various, but manifests most commonly by fragility of the bone associated with multiple fractures and deformities of anatomical segments, blue stained sclera, impairment of the internal ear segment with disturbing of hearing to complete extinction, developmental abnormalities of the teeth. The disease was detected beginning with the 17th century and was originally classified by Silence (1), based on clinical and radiological modifications, in four types and later, with the introduction of molecular genetics techniques, classification has been extended to 8 main types. The most frequently encountered are the type I and IV. Type I is characterized by the mildest clinical manifestation. The transmission of the disease is usually autosomal dominant (80-90%) but an autosomal recessive transmission is also possible, even an appearance of a spontaneous mutations that make possible its emergence in the absence of a family history.

Prenatal diagnosis is possible in case of risk pregnancies in the weeks 10-12, by analyzing the collagen produced by fetal cells, interruption of pregnancy being a

good option in this case, considering the poor prognosis of these children. The fetal ultrasound is the second diagnostic method, as it can visualize the fractures already established (2).

Clinical manifestations can vary from simple osteopenia to fractures in utero and are those who raise the suspicion diagnosis, the genetic analysis being the diagnosis of certainty. Most patients fall into type I and most often the stature is normal, distortions are minimal and the intellect is unaffected. Type II is a form that is manifested soon after birth and often incompatible with life. Type III and IV have a more severe clinical expression than type I and are characterized by triangular faces, short stature and important bony deformities. The peculiarity specific for type V is represented by the interosseous membrane calcification in forearm and fractures healing by hypertrophic calluses. The disease affects both sexes in equal proportions, all races and ethnic groups being exposed (3), and has an estimated incidence of 6-7 cases per 100,000 people worldwide (4) of which about half are affected with OI type I or IV (5). The first 4 types of the disease are due to mutations of collagen type I alpha 1 chain (COL1A1) and collagen type I alpha 2 chain (COL1A2). Bone fragility is due to decreased amount of collagen type I. Patients of types V to IX show mutations in cartilage associated protein gene (CRTAP), prolyl 3-hydroxylase 1 (LEPRE1), peptidylprolyl isomerase B (PPIB) and FK506 binding protein 10 (FKBP10) genes. Although curative treatment is not possible, multidisciplinary approach of this pathology has allowed a considerable improvement in quality of life. The introduction of antiresorptive bisphosphonate therapy resulted in a considerable reduction in the number of fractures and their further complications (6). The correction of deformities by orthopedic techniques, the maintenance of joint mobility through physical therapy, introducing modern analgesic medication, are other important therapeutic resources (7).

CASE REPORT

The patient, female aged 20 years is taken in our evidence starting with July 2014, when she was presented after a minor right leg injury. Radiological examination revealed a fracture of both bones of the right leg in the distal part and a particular aspect of them represented by a thin structure of both tibia and fibula, deformation of skeletal parts by accentuated curves and post fracture sequels, disappearance of spinal peroneal canal and narrowing until the obliteration of the tibial canal, a marked osteoporosis especially in the ankle and foot (fig. 1).

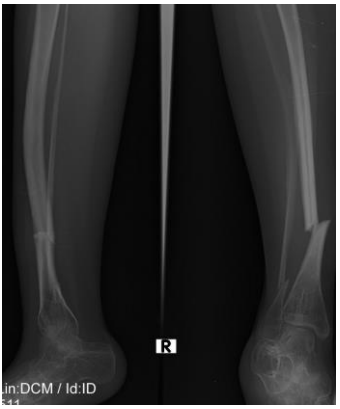

Fig. 1. Fracture in distal part of right leg

The patient's *medical history* included a total of 18 fractures, with onset around the age of 1 year, when the first fracture of the lower limbs occurred and was suspected of *osteogenesis imperfecta*, diagnosis made in the context of a family history. The disease

had a paternal transmission, patient's grandfather experiencing a mild form of the disease and presenting a normal waist without having suffered any fractures, and who, besides the existence blue whites, installed hearing problems after the age 40 years. In her father's case, the disease evolved more aggressively, due to the emergence of 6 fractures before puberty and their end after the age of 14. He also has been affected by hearing disorders occurred after age 23 years. In the father's family, the disease manifested in a different form in his sister also, who developed hearing impairment around the age of 20 years, while fractures occurred in adulthood, after 35 years. The patient's sister also presents the characteristics of the disease, but in a mild form, suffering only one fracture until now.

Physical examination reveals a waist of 146 cm and a weight of 39 kg. The patient presents blue sclera and slight modifications of the dentition. Musculoskeletal examination shows the hyperlaxity of articulations in both upper and lower limbs as well as an inequity of 4 cm length between the lower limbs due to underdevelopment of the left femur that was installed after its fracture at the age of 9 years, probably by affecting the cartilage of growth. Thorax examination reveals the presence of kyphoscoliosis, without generating breathing difficulties or auscultation pathological phenomena, the patient maintaining her muscle tone through daily exercise using treadmill.

Cardiological evaluation revealed no pathological elements and neurological examination was normal, the patient not showing any hearing changes at that moment. Bone Mineral Density (BMD) was measured via dual energy X-ray absorptiometry, and indicated a T score value of -2.8 and a Z score f-2.6, indicating the presence of osteoporosis even under chronic treatment with antiresorptive medication from the age of 9 years. At the moment of presentation, the regimen for increasing the bone mineral density contained Ibandroic acid 150 mg / month, Alpha D_3 0.5µg / day and Coral D 1cp / day.

Thoracic radiograph confirms the presence of moderate kyphoscoliosis, normal ribs and no post fractural changes (fig.2), pelvic radiograph captures bilateral *coxa valga* with cervical shaft angle of 160 degrees (fig. 3) and radiographs of the limbs identify deformities and osteoporosis in all skeletal parts (fig. 4). On the basis of the clinical and radiological changes captured, the patient has been included in the osteogenesis imperfecta type I.

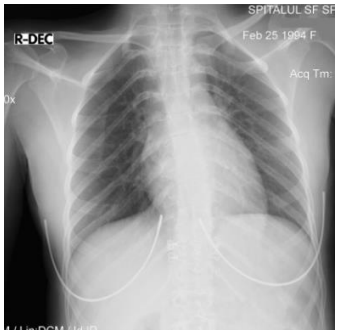

Fig. 2. Thoracic radiograph

Fig. 3. Pelvic radiograph

Fig. 4. Left femur deformation

Laboratory tests being totally normal, it was opted for the surgical treatment of the fractured leg, which was implemented in the second day posttraumatic, under general anesthesia, spinal being a difficult choice in the context of existing changes in the lumbar spine. The tibial intramedullary canal extremely narrow and even disappeared in the distal portion, made intramedullary osteosynthesis impossible and an open reduction of the fracture by anterolateral approach and osteosynthesis with dynamic compression plate (DCP) placed on the lateral side of the distal tibia were practiced.

The postoperative radiological control showed anatomical reduction of the fracture and correct positioning of the implant (fig. 5)

Fig. 5. Postoperative result

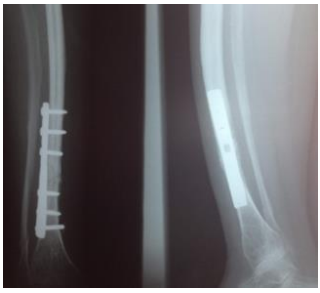

Fig. 6. Result after 12 weeks postoperative

Postoperative evolution was favorable, it was applied a splint for 3 weeks, the partial support being resumed in 8 weeks postoperatively and the integral support in 12 weeks postoperatively, after radiological assessment confirmed the fracture healing (fig. 6)

Clinical and radiological periodic reassessments have captured a favorable evolution with consolidation of the fracture, so that after 18 months postoperatively was chosen the ablation of the plate, followed by avoiding any support for 4 weeks. The patient returned to our service following a minor injury occurred 3 months after the removal of the plate, when she was diagnosed with an iterative fracture in the right leg and the therapeutic approach was represented by the resuming the osteosynthesis with DCP plate and healing of the bone (fig. 7).

Fig. 7. Iterative fracture of the right leg and osteosyntesis with DCP plate

DISCUSSION

Osteogenesis imperfecta type I is the mildest clinical expression of this disease and fortunately has the maximum prevalence. In most cases, this version has family history and autosomal dominant mode of transmission, but the possibility of this form of the disease to be a consequence of a de novo genetic mutation has also been demonstrated. In the mild forms of the disease, the fragility of bone system and joints hyperlaxity are due to an insufficient amount of collagen and the number of fractures in patients who have the mutation is 15 times higher compared with healthy children, existing the risk of being confused with a parental abuse (8).

Exceeding the period of puberty makes the fractures frequency decrease. The longevity of these patients is not directly influenced by genetic mutation, but recurrent fractures and their complications can alter life expectancy. Serious forms that occur when collagen is not only insufficient, but also nonfunctional, can be diagnosed prenatally by specialized monitoring of risk pregnancies and fetal's DNA-analysis.

Regarding the case presented with osteogenesis imperfecta type I, the patient has a consistent family history and the transmission was autosomal dominant. If one parent is affected by this genetic mutation, each child has a 50% chance to be born with this disease but the clinical expression may be totally different from that of their parents (9). The diagnosis was a clinical one and was suspected initially because of the modified color of sclera, later confirmed with the appearance of early fractures (starting from one year), of joints and skin hyperlaxity and bone deformities. No complex investigations such as quantitative and qualitative analysis of type I procollagen molecules obtained by biopsies from cultured skin fibroblasts or identification of genetic mutations in the blood cells DNA were conducted. According to studies, false negative results are obtained in 13.6% of cases. In the present case, the patient had multiple fractures, occurred starting with one year of age and produced by minor trauma. Administration of calcium preparations during childhood had not the desired favorable effect and the frequency of fractures decreased only after overcoming puberty.

Clinical examination reveals the existence of a triangular face, blue sclera, joints hypermobility and no hearing damage. Radiological investigations confirm the presence of osteoporosis in all skeletal parts, including the spine, where was an evidence of a moderate scoliosis caused by unequal legs. The patient reported no history of rib fractures and thoracic radiographs confirmed the normal appearance of the ribs. The actual therapeutically management makes possible to achieve a quality of life close to normal, addressing osteogenesis imperfecta type I in multidisciplinary team: antiresorptive medication administration (bisphosphonates orally and intravenously) under the supervision of the endocrinologist, correction of deformities and treatment of fractures by orthopedic surgery, physiotherapist and physician's intervention of recovery to preserve joint mobility and maximizing physical performance. Bisphosphonate therapy was administered with caution in children at first, because it was considered a risk to disrupt the processes of mineralization and bone growth, emphasizing such deformities.

Research published by Bianchi ML showed that administration of alendronate to children affected by osteogenesis imperfecta does not interfere with normal bone growth (10).

CONCLUSIONS

Our case is very suggestive for osteogenesis imperfecta type I, starting from the rich family history and especially the clinical manifestations and radiological evaluations. Insufficient amount of type I collagen is responsible for the existence of bone fragility and increased risk of fractures fallowing minimal trauma. Antiresorptive bisphosphonate therapy is effective for increasing bone strength, DNA tests conducted in patients who stopped the medication period indicate decreased bone mineral density. Stabilization of fractures in these patients is difficult due to the poor quality of bone tissue and to the atypical form of the skeletal parts because the long bones of patients are thin for their length and body weight and this condition tend to be more severe with age (11). Osteosynthesis with plates and screw provides a low resistance because of the failure of each screw and because of the stress riser effect at the plate ends (12).

Although the centromedullar implant would have represented the ideal solution for stabilizing a fracture occurred on a bone with little resistance, we had to use plates that we could adapt intraoperatively to the tibial shape, because the small size and conformation of tibial canal did not allow the placement of a nail. Even if the radiological image showed normal aspect of consolidation and tibial cortices, removal of osteosynthesis material 18 months after implantation favored the reappearance of fracture in the old fracture place, with its recurrence 3 months after the ablation of the plate. Multidisciplinary approach of the patient with osteogenesis imperfecta significantly improves patient's quality of life.

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DETECTION AND PREVENTION OF FRAILTY IN INDEPENDENTLY LIVING PRE-ELDERLY AND ELDERLY IN NORTHEASTERN ROMANIA

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FRAILTY DETECTION AND PREVENTION IN INDEPENDENT LIVING PRE-ELDERLY AND ELDERLY IN NORTHEASTERN ROMANIA (Abstract): Frailty is the central feature of old age, a new concept in medicine, being the leading cause of decreased quality of life, dependency and death in elderly people. The older we get, the more fragile the basic balance, and increasingly lower aggression can be sufficient to disrupt this balance. **Aim:** To identify frailty through the Romanian version of the Groningen fragility indicator (GFI) questionnaire among the independently living elderly in the northeastern region of Romania. **Material and Methods:** GFI questionnaire was applied to 60 persons aged 65 to 95 years, randomly selected from general practices in Iasi town and Iasi country in the interval March-April 2014. **Results**: A significant percentage of the subjects were considered frail, particularly women, and divorced or widowed. According to the general practitioners, 65% of the older persons were frail without significant gender and marital status-related differences, with a significantly higher degree of frailty in those the those aged over 75. **Conclusions**: These findings are a red flag to the health system, and given the major social impact the detection of frailty and early intervention should be a priority. **Keyword:** FRAILTY, ELDERLY POPULATION, PREVENTION, STATE OF VULNERABILITY, SARCOPENIA.

Frailty is a clinical state of increased vulnerability and decreased ability to maintain the homeostasis, which is characterized by a decline in physiologic reserve and function across multiple organ systems. Currently, a consensus was reached regarding the description of frail elderly person, which includes: weight loss, decreased muscle mass and strength, impaired balance and mobility, physical inactivity and cognitive impairment (1). In the clinical picture, sarcopenia is considered a key element of frailty.

Several studies have proposed different tools for measuring fragility. Some are complex, others are simple (2). The frailty index seems to be the most appropriate instrument to measure frailty, but this instrument is complex, the psychometric properties need to be explored more extensively and they were not validated in a primary healthcare setting.

Therefore, it is difficult to determine which the best screening instrument is, and

that is why there is an attempt to establish a frailty detection protocol including the assessment of all systems involved, which to be easy to implement, reproduce, and whose validity could not be challenged. Such an instrument does not currently exist, and the numerous questionnaires and even laboratory assessments of the musculoskeletal, neuro-endocrine, immune, and inflammatory systems did not prove to meet all these criteria; frailty is best detected through comprehensive geriatric assessment. Additionally, multiple studies have shown the importance of assessing frailty in the primary care system (3-5).

In Romania frailty is detected since 2013 following a training course delivered by Professor Heuvel and the initiation of a pilot project in a community of independent elderly (6). The results of the study showed that the Romanian version of the Groningen frailty indicator (GFI) is a valid and feasible instrument for assess in frailty. The present study implements the detection of frailty by the Romanian version of GFI in the independently living older persons in northeastern Romania.

MATERIAL AND METHODS

The study was conducted in March-April 2014. The data were collected by using the Romanian version of GFI questionnaire (fig.1) in a group of 60 persons aged 65 to 90, randomly selected from the general practices in Iasi town and Iasi country. Informed consent was obtained from all study subjects in the general practice waiting room or at home. At the same time, the GPs were asked to rate the subjects as fragile or robust based on clinical judgement without having access to the questionnaire data. The obtained data were statistically analyzed using *SPSS 13.0* software.

RESULTS

Data on patients' age did not reveal significant gender differences. In women, the age ranged from 65 to 95 years, mean age 74.29 ± 7.74 years.

The distribution by marital status showed a slightly higher percentage of married persons (65%).

The questionnaire consisted of 15 questions related to different functions: physical (questions 1-4), sensory (questions 6-9), cognitive (questions 5, 10), social (11-13) and psycho-affective (questions 14, 15).

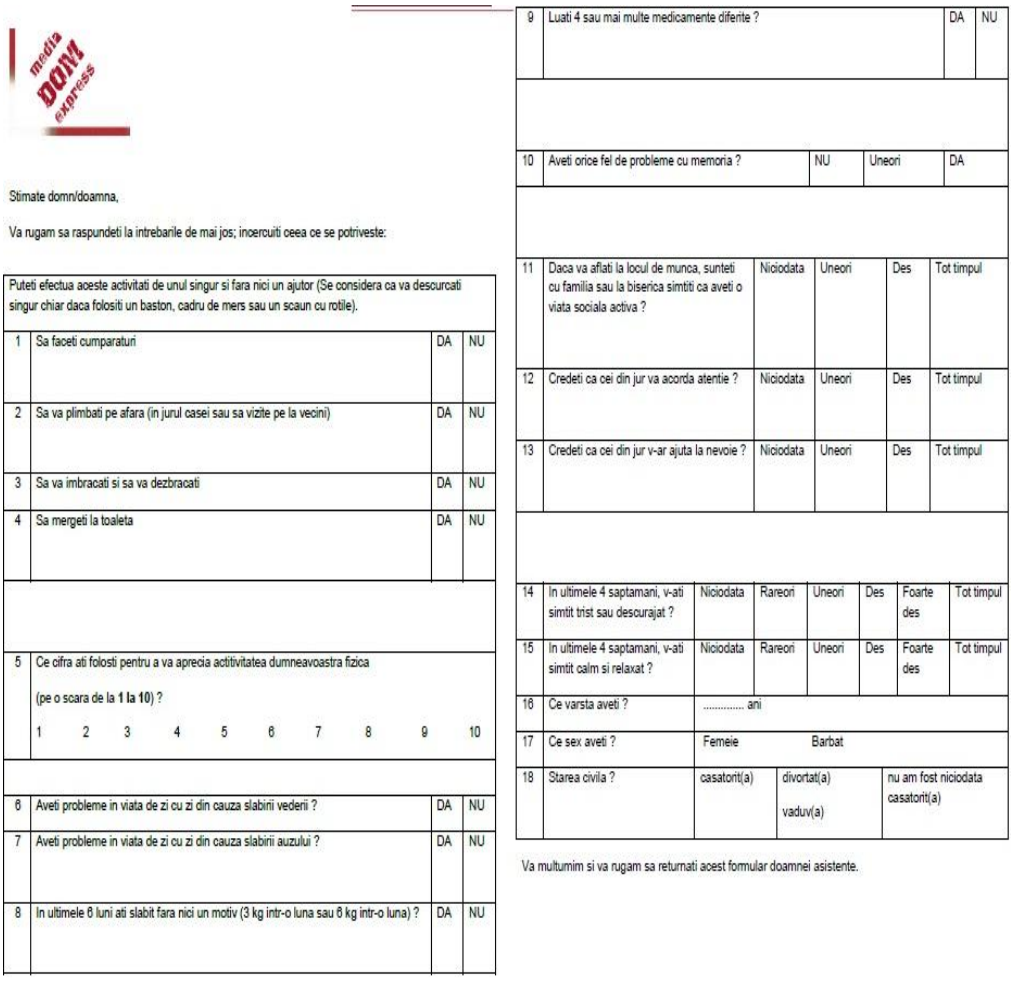
The items can be grouped into three subscales that define daily physical activities (questions 1-4), health-related problems (questions 5-10) and psychosocial activities (questions 11-15).

A percentage of 63.3% of the subjects responded affirmatively to question number 1, without significant percentage gender and marital status differences, but only 50% of the subjects aged over 75 could do the shopping themselves ($p=0.046$). The percentage of subjects who could walk independently was 83.3% with no significant percentage differences between sexes, age groups or marital status (tab. I).

In terms of sensory functions, 48.3% of the subjects experienced difficulties in their activities of daily living due hearing impairment, significantly more common in men and in subjects over 75 years ($p=0.004$; $p=0.010$);

Only 15% of the respondents mentioned unintentional weight loss in the last 6 month, most of them being older than 75 years or unmarried ($p=0.049$; $p=0.040$), most likely suffering from depression due to the absence of a life partner.

Sixty-one-point seven percent of the subjects were taking 4 or more drugs, but statistically there were no significant differences by gender, age group or marital status (tab. II).



media DOM express

Stimate domn/doamna,

Va rugam sa raspundeti la intrebarile de mai jos; incercuiti ceea ce se potriveste:

Puteti efectua aceste activitati de unul singur si fara nici un ajutor (Se considera ca va descurcati singur chiar daca folositi un baston, cadru de mers sau un scaun cu rotile).

1	Sa faceti cumparaturi	DA	NU
2	Sa va plimbati pe afara (in jurul casei sau sa vizite pe la vecini)	DA	NU
3	Sa va imbracati si sa va dezbracati	DA	NU
4	Sa mergeti la toaleta	DA	NU
5	Ce cifra ati folosti pentru a va aprecia actitivitatea dumneavoastra fizica (pe o scara de la **1 la 10**) ? 1 2 3 4 5 6 7 8 9 10		
6	Aveti probleme in viata de zi cu zi din cauza slabirii vederii ?	DA	NU
7	Aveti probleme in viata de zi cu zi din cauza slabirii auzului ?	DA	NU
8	In ultimele 6 luni ati slabit fara nici un motiv (3 kg intr-o luna sau 6 kg intr-o luna) ?	DA	NU
9	Luati 4 sau mai multe medicamente diferite ?	DA	NU
10	Aveti orice fel de probleme cu memoria ?	NU	Uneori	DA
11	Daca va aflati la locul de munca, sunteti cu familia sau la biserica simtiti ca aveti o viata sociala activa ?	Niciodata	Uneori	Des	Tot timpul
12	Credeti ca cei din jur va acorda atentie ?	Niciodata	Uneori	Des	Tot timpul
13	Credeti ca cei din jur v-ar ajuta la nevoie ?	Niciodata	Uneori	Des	Tot timpul
14	In ultimele 4 saptamani, v-ati simtit trist sau descurajat ?	Niciodata	Rareori	Uneori	Des	Foarte des	Tot timpul
15	In ultimele 4 saptamani, v-ati simtit calm si relaxat ?	Niciodata	Rareori	Uneori	Des	Foarte des	Tot timpul
16	Ce varsta aveti ?	..... ani					
17	Ce sex aveti ?	Femeie	Barbat				
18	Starea civila ?	casatorit(a)	divortat(a) vaduv(a)	nu am fost niciodata casatorit(a)			

Va multumim si va rugam sa returnati acest formular doamnei asistente.

Fig. 1. The used questionnaire.

TABLE I

Statistical significance of affirmative responses to questions related to physical functions by gender and marital status

Physical functions	Yes n (%)	Demographic structure					
		Sex	p	Age	p	Marital status	p
Question 1	38 (63.3)	F 65.7% M 60.0%	0.856	<75 75.0% ≥75 50.0%	**0.046**	C 61.5% NC 66.7%	0.911
Question 2	53 (88.3)	F 91.4% M 84.0%	0.634	<75 93.8% ≥75 82.1%	0.162	C 89.7% NC 85.7%	0.966
Question 3	56 (93.3)	F 94.3% M 92.0%	0.861	<75 96.9% ≥75 89.3%	0.511	C 94.9% NC 90.5%	0.914
Question 4	56 (93.3)	F 94.3% M 92.0%	0.861	<75 96.9% ≥75 89.3%	0.511	C 94.9% NC 90.5%	0.914

TABLE II

Statistical significance of questions related to sensory function by age, gender and marital status

Sensory functions	Yes n (%)	Demographic structure					
		Sex	p	Age	p	Status marital	p
Question 6	44 (73.3)	F 74.3% M 72.0%	0.921	<75 68.8% ≥75 78.5%	0.572	C 74.4% NC 71.4%	0.951
Question 7	29 (48.3)	F 31.4% M 72%	**0.004**	<75 31.3% ≥75 67.9%	**0.010**	C 46.2% NC 52.4%	0.850
Question 8	9 (15.0)	F 11.4% M 20.0%	0.582	<75 9.4% ≥75 21.4%	**0.049**	C 7.7% NC 28.6%	**0.040**
Question 9	37 (61.7)	F 62.9% M 60.0%	0.861	<75 62.5% ≥75 60.7%	0.901	C 56.4% NC 71.4%	0.388

The mean values for self-rated physical activity on a scale of 2 to 9 indicated a moderate level of physical activities, no significant differences by gender, age groups and marital status being recorded.

Only 23.3% of the subjects reported memory impairment and 50% occasional memory problems, statistically insignificant.

Both men and women aged under 75 years reported an active social life regardless of marital status (p=0.050). The respondents believed they would receive help from their entourage when they need it, without percentage differences by gender or marital status, but with a significantly increased frequency in subjects aged under 75.

About the psycho-affective component, the divorced or widowed subjects felt sad or discouraged in the last 4 weeks more frequently than the married ones, aspect which is not significantly influenced by on gender or age group (tab. III).

TABLE III

Statistical significance of responses related to the affective component

Question 14	Female		Male		< 75 yrs		≥75 yrs		Married		Divorced/ widower	
	n	%	n	%	n	%	n	%	n	%	n	%
Never	4	11.4%	3	12.0%	5	15.6%	2	7.1%	7	17.9%	-	-
Rarely	4	11.4%	3	12.0%	6	18.8%	1	3.6%	5	12.8%	2	9.5%
Sometimes	15	42.9%	8	32.0%	9	28.1%	14	50.0%	12	30.8%	11	52.4%
Often	7	20.0%	9	36.0%	8	25.0%	8	28.6%	13	33.3%	3	14.3%
Very often	3	8.6%	-	-	1	3.1%	2	7.1%	-	-	3	14.3%
Always	2	5.7%	2	8.0%	3	9.4%	1	3.6%	2	5.1%	2	9.5%
p	0.533				0.218				**0.020**			

Per the general practitioners, 65% of the subjects were frail, without significant percentage differences by gender or marital status, but significantly more frail were the patients aged over 75.

The results obtained by processing the questionnaire responses show that 80% of the subjects were frail, higher percentages being recorded in females than in males (82.9% *vs* 76%), but the percentage distri-

bution is not statistically significant (p=0.294).

There is slightly higher frequency of frailty among people aged over 75 years under 75 years (92.9% *vs* 68.8%) (p=0.045), with a significantly higher percentage in the divorced or widowers (95.2% *vs* 71.8%) (p=0.028) (tab. IV) (8).

TABLE IV
Statistical significance of the questionnaire results and GP rating by age group, gender and marital status

Demographic structure	GP Review			Questionnaire results			GP rating *versus* Questionnaire results
	fragile	robust	p	fragile	robust	p	
Female	65.7%	34.3%	0.891	82.9%	17.1%	0.294	0.172
Male	64.0%	36.0%		76.0%	24.0%		0.537
< 75 yrs.	50.0%	50.0%	**0.020**	68.8%	31.3%	**0.045**	0.203
≥75 yrs.	82.1%	17.9%		92.9%	7.1%		0.419
Married	59.0%	41.0%	0.294	71.8%	28.2%	**0.028**	0.341
Single	76.2%	23.8%		95.2%	4.8%		0.186

DISCUSSION

The use of these subscales allowed a more accurate identification of the needs of the elderly (social, medical, emotional) by categorizing them as frail or robust.

Based on a descriptive analysis of daily activities, in our study 50% of the elderly aged over 75 years could do the shopping themselves, with no differences by gender or marital status, and maintained an optimal level of autonomy.

Compared to GP's ratings, the questionnaire revealed a higher frequency of frailty in the studied population, but the ratio between sexes, age groups and marital status was similar (6). We believe that these results do not reflect reality, because the methodology for completing the questionnaire did not comply with the rules necessary for completing close to the truth (questionnaires filled out with the help of qualified personnel, which does not manipulate the response, under stress conditions, in limited time).

In agreement with the literature, the two subscales that define the daily physical activity and psychosocial component proved to have good internal consistency, meeting the criteria for validation (Cronbach's alpha value = 0.778, respectively 0.630) (7).

The research based on questionnaire can never be completely objective. Both the researchers and the respondents are human beings, with different psychological, emotional and social needs. Thus, the participants must have the feeling that they are collaborators to the study, without being subjected to psychological abuse.

CONCLUSIONS

In our study the percentage of subjects identified as frail with the help of the used questionnaire was high. Based on the obtained results, it could be stated this instrument of research is a valid one, and that it could be applied on larger population group, even at national level.

Another focus is on the development of appropriate method for administering the

questionnaire, to prevent potential false positive or false negative results.

Therefore, identifying the frailty status through the questionnaire among elderly in primary care requires complex geriatric evaluation in the geriatrics ambulatory.

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NEWS

REVERSING THE NON-VITAMIN K DEPENDING ANTICOAGULANTS: A NEW ANTIDOTE ON THE HORIZON?

The newer non-vitamin K dependent anticoagulants (NOACs) have provided a new tool in the armamentarium of physicians treating nonvalvular atrial fibrillation and thromboembolism. The major limiting factor and the concern that precluded their use was lack of reversal in emergent situations. Andexanet alfa is a modified factor Xa molecule which reverses the anticoagulant effect of both direct and indirect factor Xa inhibitors. A recent multicenter, prospective study showed that an initial bolus and subsequent 2-hour infusion of andexanet substantially reduced anti–factor Xa activity in patients with acute major bleeding, gastrointestinal or intracranial, associated with factor Xa inhibitors, with effective hemostasis occurring in 79% (Stuart J. Connolly, Truman J. Milling, Jr., John W. Eikelboom, M.D., et al , *Andexanet Alfa for Acute Major Bleeding Associated with Factor Xa Inhibitors*, *N Engl J Med* 2016; 375:1131-1141September 22, 2016DOI: 10.1056/NEJMoa1607887).

Ionuţ Valentin Şimon

BILATERAL SYNCHRONOUS MALE BREAST CANCER OF RARE HISTOLOGIC TYPE. CASE REPORT

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BILATERAL SYNCHRONOUS MALE BREAST CANCER OF RARE HISTOLOGIC TYPE. CASE REPORT (Abstract). The particularity of the presented case is bilateral synchronous male breast cancer of uncommon histologic type – invasive cribriform carcinoma. In the practice of our surgical unit it is the first and only case of synchronous bilateral male breast cancer. The patient was followed up regularly for the last 4 years after a modified radical bilateral mastectomy with axillary lymph node clearance followed by adjuvant chemotherapy and endocrine therapy and he showed no signs of local recurrence or metastatic disease. **Keywords:** MALE BREAST CANCER, SYNCHRONOUS CANCERS, INVASIVE CRIBRIFORM BREAST CANCER, MORBIDITY.

Male breast cancer (MBC) is a rare disease (0.5-1% of all breast cancers (1)). Only 2% of all reported cases of MBC are bilateral (2). Men diagnosed with breast cancer are older (mean age at diagnosis is 67 years) than women with this disease, and have a poorer survival rate (5-year-survival - 67%). Klinefelter syndrome is found in approximately 7.5% of men with breast cancer. The risk of breast cancer in individuals with Klinefelter syndrome is up to 20–50 times higher compared to men with normal karyotype. BRCA2 mutation carriers have an 80–100 times higher lifetime risk of MBC. Up to 20% of men with MBC have a positive family history of breast cancer (3). Conditions such as hyperestrogenism, hyperprolactinemia, alcohol intake and tobacco smoking, occupational risks (especially working in hot environments) are slightly increasing the risk of MBC (4). Gynecomastia, a very common disorder in young men, does not increase the risk for MBC (5). First described by Page et al. in 1983, invasive cribriform carcinoma (ICC) has a low incidence (0.3% to 3.5% of all invasive breast carcinomas) (6,7). There are 3 forms of ICC of the breast - pure, classical, and mixed. ICC usually presents as a slowly growing, frequently occult tumor mass, often difficult to detect on conventional examinations. Most of these tumors have positive estrogen and progesterone receptors (8). Metastatic disease in patients with ICC is rarely reported (7). Five-year survival rate is

nearly 100% for pure and classical types (⩾50% ICC), 88% for mixed types (<50% ICC), and 78.3% for the infiltrating duct carcinoma controls (8).

CASE PRESENTATION

A 61-year-old male presented to our unit with an unpainful, insidiously growing left breast mass and a bloody discharge from the right nipple that occurred 2 months ago. His past personal medical and family history was unremarkable, except for rheumatic fever (in childhood). He had no pertinent occupational exposure. No liver dysfunction or testicular failure was found. Routine laboratory data showed no pathologic changes. Tumor marker CA15.3 was within normal range. The levels of other tumor markers (α-fetoprotein=1,26 ng/ml, PSA=1,81 ng/ml, Prolactin=310.9 µUI/ml) were also normal. Physical examination of the right breast revealed no palpable masses and clinically negative right axilla. Palpation of the left breast revealed a 3 cm irregular, solid lump in the central quadrant, fixed to the subjacent tissues. Left axilla was also clinically negative. Other physical assessments were normal. No genetic tests were performed.

Fig. 1. Right breast ultrasound appearance.

Breast ultrasound revealed in the right breast (fig.1) two nodular, retro areolar, well defined hyperechoic masses of 1.1 x 0.9 cm and 0.74 x 0.65 cm, respectively and possibly another 0.37 x 0.34 cm mass adhering to the subjacent muscular structures, absence of axillary lymph nodes. The left breast (fig. 2): retro areolar and external 2.6/2.1 cm mixed lump with the presence of a 1.9 x 1.7 cm solid mass with Doppler signal present; left axilla contained no detectable lymph nodes. Mammography showed: in the right breast a 0.7 x 0.6 cm homogeneous opacity with precise contour; in the left breast a 3 x 3.1 cm solid mass, which contained on the lateral wall a 1.4 x 1.4 cm solid irregular mass adherent to the subjacent pectoral muscle. No distant metastases were identified (thoracic radiography and abdominal ultrasonography).

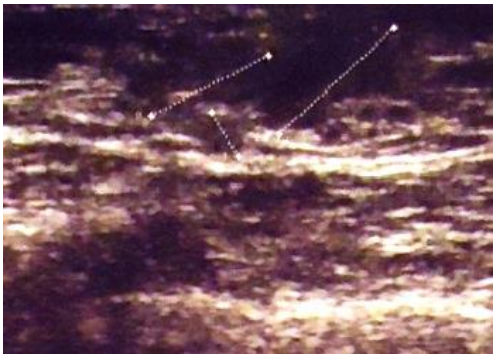

Fig. 2. Left breast ultrasound appearance.

After adequate preoperative preparation, an excisional biopsy the of breast tumors was performed. Extemporaneous analysis of the specimens revealed a bilateral invasive ductal carcinoma. A bilateral Madden-type modified radical mastectomy with axillar lymphadenectomy was performed. Postoperative course was favorable. The patient was discharged from our unit 8 days after surgery, in good condition, with no complains.

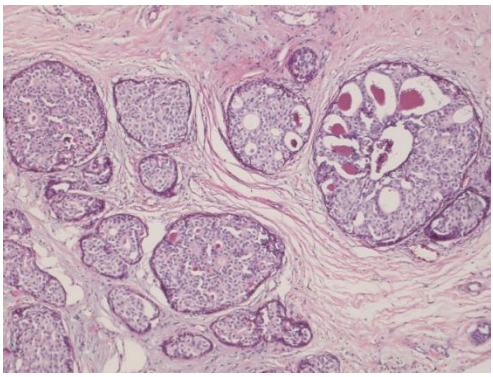

Fig. 3. Invasive cribriform carcinoma of the breast in male X 10.

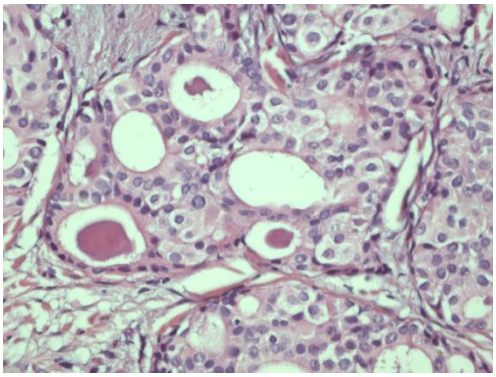

Fig. 4. Invasive cribriform carcinoma of the breast in male X 40.

Pathological examinations revealed: right breast – invasive cribriform carcinoma (fig. 3, 4) with lymph node metastasis pT1cN1aG1, 1 of 22 lymph nodes with metastatic carcinoma, ER (estrogen receptor) 90%, HER2 negative, PR (progesterone receptor) 80%; left breast – invasive ductal mixed with invasive cribriform carcinoma without lymph node metastases pT1cN0G1, ER 90%, PR 90%, HER2 negative. The patient has undergone both adjuvant chemotherapy (6 cycles of cyclophosphamide and doxorubicin/epirubicin) and hormonal therapy (tamoxifen). The 4-years regular follow up showed no signs of local recurrence or metastatic disease.

DISCUSSION

The management of MBC is mainly developed based on treatment guidelines elaborated for breast cancer in women due to the lack of large population studies and randomized clinical researches of MBC. An age- and stage-matched comparison between male and female breast cancer revealed that there is no significant sex-related difference in overall survival (3). There are studies that concluded that men with breast cancer have lower risk of death than comparable female patients (9). The main known prognostic factors for MBC are tumor size (85% survival rates when tumor sizes are less than 2 cm) and lymph node involvement (50% higher risk of death than those with negative axillary nodes) (10). Incidence of bilateral MBC is approximately 2%, and synchronous tumors are exceedingly rare (11). The importance of early diagnosis is overwhelming in MBC when surgical treatment may be radical, curative and sometimes sufficient. The significance of neoadjuvant chemotherapy in MBC remains uncertain and is used in few cases in order to obtain operability. Surgery is the mainstay of treatment for breast cancer in men. Usual surgery for MBC is modified radical mastectomy with axillary clearance or sentinel node biopsy. In rare locally advanced cases with muscle involvement a radical mastectomy should be performed with partial or total resection of pectoral muscles. Lumpectomy in initial MBC stages in selected patients followed or not by adjuvant radiation therapy has a little better cosmetic outcome and is performed in few surgical centers (2). Due to the presence of ER and PR in most patients with MBC hormone

therapy is indicated with a significant benefit in overall survival, local recurrence and metastatic disease control. Adjuvant radiation therapy is indicated in MBC with large tumors, cutaneous involvement, invasion of areola or subjacent muscles, axillary lymph-node metastases, involved margins on mastectomy specimen. Postoperative radiation therapy is also recommended in patients with multifocal, high grade tumors, high tumor proliferation rate, lymph, vascular and perineural invasion. Chemotherapy should be considered in men with intermediate or high-risk early breast cancer, especially those with hormone-receptor-negative disease as it reduces the risk of recurrence and death. HER-2 amplification in MBC is rare (2 to 15%). The effectiveness of targeted treatment with trastuzumab in MBC is uncertain due to the lack of controlled prospective studies, and may be used in patients with HER-2 positive breast cancers (3).

CONCLUSIONS

For our unit, the here presented clinical case of synchronous, bilateral male breast cancer of uncommon histological type – invasive cribriform carcinoma is an exceptional finding because, until now, there a no similar cases reported in literature. Due to its very low incidence in men, breast cancer is rarely suspected both by patients and medical personnel.

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MANAGEMENT OF BILATERAL FEMORAL NECK FRACTURE IN A NONAGENARIAN PATIENT - CASE REPORT

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MANAGEMENT OF BILATERAL FEMORAL NECK FRACTURE IN A NONAGENARIAN PATIENT-CASE REPORT (Abstract): Simultaneous bilateral femoral neck fracture is rare injury. Cases with this type of fracture have been reported in the literature since the 1950s, following the introduction of electroconvulsive therapy which generates violent hip muscle contractions. In young patients' simultaneous bilateral femoral neck fracture results from high energy trauma (car accident or fall from height) in a normal bone. Pathological changes in bone structure occurring in chronic kidney disease, vitamin D deficiency, osteomalacia, osteoporosis, metabolic imbalances and administration of corticosteroids explain the occurrence of this particular type of fracture following low-energy trauma. We present the case of a 90-year-old female patient who suffered a simple fall from her own height resulting in a Garden IV bilateral femoral neck fracture. Our therapeutic option in this patient was bilateral uncemented bipolar hemiarthroplasty in a single session using a single tray of sterile surgical instruments and two sterile drapes. Postoperative outcome was very good, allowing the initiation of functional recovery on the first postoperative day. Uncemented hemiarthroplasty proved to be a good choice in such a patient in the associated diseases may trigger the risk of cardiovascular disturbances specific to bone cement implantation syndrome. **Keywords:** BILATERAL HIP FRACTURE, HEMI-ARTHROPLASTY, CEMENT IMPLANTATION SYNDROME, LOW ENERGY TRAUMA, SINGLE STAGE REPAIR.

Bilateral femoral neck fracture resulting from low energy trauma is uncommon and only few cases have been reported in the literature. It is more commonly associated with electroconvulsive therapy and tonic-clonic seizures and may occur in young adults following high energy trauma. The presented case reflects our experience in managing an elderly patient (nonagenarian) with significant cardiovascular comorbidities and who benefited from surgery in a single session.

CASE REPORT

A 90-year-old patient presented to the emergency department following a low energy trauma following same-level fall at home, resulting initially in partial and soon after total functional impairment. The patient was known with type 2 diabetes treated with oral antidiabetic drugs and diabetic sensori-motor polyneuropathy. Cardiovascular pathology was represented by grade III hypertension and chronic ischemic heart disease associated with significant carotid

atheromatous disease and a recent ischemic stroke. Initial clinical examination performed in the emergency department raised the suspicion of a bilateral hip fracture because the legs appeared shortened and significant externally rotated. Classical radiological examination of the pelvis confirmed the clinical suspicion of bilateral hip fracture with bilateral intracapsular femoral neck fracture Garden IV (fig 1).

Fig. 1. Anteroposterior radiograph of the pelvis showing bilateral femoral neck fractures

Immediately after admission the patient was examined by the anesthetist in order to assess if the patient can withstand general or spinal anesthesia. Cardiological assessment recommended to delay surgery and changes in therapeutic doses to normalize high blood pressure and heart rate. Surgery could be performed 48 hours following trauma and anesthetic consultation considered general anesthesia safer than spinal anesthesia regarding the expected duration of surgery and severity of cardiovascular disease. The first option of the surgical team was uncemented bipolar hemiarthroplasty in a single session in both hips. The patient was placed in supine position and the approach was the one usually used by the operating team, namely anterolateral. First we performed right hip replacement and then using the same tray of sterile instruments we performed a new draping and hemiarthroplasty in the contralateral hip using the same size prosthetic components in the right hip. Postoperative surveillance was done in the anesthesia and intensive care unit, and transferred to the orthopedic unit on the first postoperative day. After postoperative radiological assessment which confirmed a sufficient bone stock and the correct calibration and placement of prosthetic components, functional recovery first through passive and then active hip movements, with resumption of walking using a walker and full weight bearing since the second postoperative day (fig. 2).

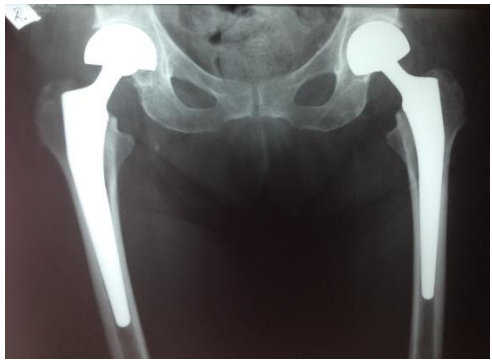

Fig. 2. Postoperatively radiograph of the pelvis showing bilateral uncemented hemiarthroplasty

The occurrence of these bilateral fractures following a low energy trauma accurately described by the patient excluding the possibility of an episode of seizure, raised the suspicious of significant changes in bone structure and density and a DXA scan of the forearm and spine was performed. Serological tests showed: mild hypothyroidism (TSH = 5.02 UUI/ml), PTH = 22.63 pg/ml, and vitamin D deficiency (7.91 ng/ml) for which therapy with

Vigantol was initiated. The severe changes captured by DXA scan of the lumbar spine, including multiple vertebral compressions and secondary scoliosis made the results uninterpretable. The occurrence of hip fracture automatically included the patient into the severe osteoporosis group although the obtained forearm values suggested osteopenia (*T score -1.5*).

Postoperative course was favorable both in terms of surgical wound healing and rehabilitation program and resumption of walking using a walker and full weight bearing. The patient was discharged on postoperative day 7 and referred to a specialized rehabilitation and physiotherapy unit. Clinical and radiological assessment at 3 months postoperatively showed a good function of both hip joints that allows independent walking over short distances using an walker. Radiological assessment of the pelvis revealed prosthetic components correctly calibrated and positioned and preservation of good quality bone in the proximal femur bilaterally.

DISCUSSION

Bilateral femoral neck fracture is extremely rare; a small number of cases being published in the literature. The introduction of electric shock therapy in the treatment of mental disorders occasionally resulted in bilateral femoral neck fractures due to the strong muscle contractions. The first reports of this fracture were published in the 1960s in this kind of patients (1).

High-energy trauma specific to polytrauma and electrocution are the main cause of these fractures when they occur in normal structure bone. Bone loss specific to primary bone disease, osteomalacia, osteoporosis or secondary changes in chronic kidney disease explain the occurrence of bilateral lesions following simple fall from the same level (2).

Transient osteoporosis of pregnancy was involved in the occurrence of bilateral femoral neck fracture during pregnancy in a 35-year-old patient who was not a victim of trauma (3).

Being a rare fracture it is often underdiagnosed as the clinical and radiological exams are focused on the hip whose symptoms are more dramatic. For this reason, X-ray of the pelvis and both hips can capture this rare lesion in confused patients suffering from neurological or psychiatric illnesses or following seizures.

Several variants of therapeutic approach have been presented in the literature starting with less invasive interventions such as orthopedic reduction followed by osteosynthesis up to simultaneous total hip arthroplasty. Sood A. et al. published in 2009 a similar case in which the bilateral fracture was the result of low energy trauma (fall from own height) in which their surgical option was bilateral cemented hemiarthroplasty through the anterolateral approach in supine position in a single session (4). In our patient, the intraoperative finding of sufficient bone stock and significant cardiovascular comorbidity that could favor the occurrence of rhythm disturbances, hypoxia and hypotension specific to bone cement implantation syndrome were strong arguments in favor of uncemented arthroplasty (5). Although the number of published cases of bilateral femoral neck fracture is limited, a high mortality rate of about 50% in elderly patients with associated comorbidities and of only 25% in young patients is reported (6). In most cases the cause is delayed surgery and rapid development of complications related to bedsores, of which deep vein thrombosis and pulmonary infection are most often fatal. In this respect, treatment guidelines for hip fractures rec-

ommend osteosynthesis or arthroplasty within 48 hours after trauma (7).

Surgery should be done as soon as the medical conditions are safe (8). In our patient the 48-hour preoperative interval was essential for adjusting the treatment for her heart disease and obtaining adequate blood pressure and heart rate so that her life not to be endangered in the perioperative period. McBryde et al. reported the safety and benefits of bilateral hip resurfacing arthroplasty (9). Arguments in favor of the supine position and anterolateral approach were the preference of the surgical team and avoidance of patient repositioning thus shortening the duration of the two successive surgeries.

CONCLUSIONS

The surgical management of our case, a nonagenarian patient with significant associated diseases, was a challenge and the treatment was adapted to its particular conditions. Uncemented hemiarthroplasty proved to be a good choice in such a patient where the associated diseases may trigger the risk of cardiovascular disturbances specific to bone cement implantation syndrome. Our therapeutic option in this patient was a bilateral uncemented bipolar hemiarthroplasty in a single session using a single tray of sterile surgical instruments and two sterile drapes. The possibility of treating both hips in the same session, thus minimizing the adverse effects of anesthesia, was a great advantage in a patient of extreme age and cardiovascular comorbidities. Early diagnosis and rapid identification and correction of imbalances that may delay surgery were the two steps essential for a successful treatment.

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REDUCE CHILD MORTALITY AS A MILLENNIUM DEVELOPMENT GOAL IN ROMANIA

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REDUCE CHILD MORTALITY AS A MILLENNIUM DEVELOPMENT GOAL IN ROMANIA (Abstract). **Aim:** To assess the efforts made in Romania towards achieving the Goal 4 from MDGs - Reduce Child Mortality. **Materials and methods:** A descriptive study about the deaths among Romanian children under five, between 2002 and 2015, from the perspective of the MDGs. To help track progress toward this commitment, following specific targets and indicators were developed: Target 1- Halve the mortality rate in children aged 1-4 years between 2002-2015; Target 2 - Reduce infant mortality by 40% between 2002 and 2015; Target 3- Eliminate measles by 2007. The comparison allows establish the status (achieved or not) for each target. **Results:** From 2002, the under-five mortality rate recorded a continuous descendent trend till now (20.8 to 10.3 under five deaths per 1000 inhabitants in 2013). The infant mortality rates declined from 17.3 to 8.5 deaths per 1,000 live births (2002-2013). Eliminating measles by 2007 - was achieved one year later, because of the measles epidemic in 2005 and 2006. High vaccination rates have been maintained, with the proportion of children 1 year old vaccinated against measles reaching and being maintained at between 94-98%. **Conclusions:** Substantial progress has been made in Romania, in achieving the Millennium Development Goal no. 4. All the three targets were achieved. However, infant mortality still remains above the average of European Union (4 infant deaths per 1,000 live-births). **Keywords:** MILLENNIUM DEVELOPMENT GOALS, UNDER-FIVE MORTALITY, INFANT MORTALITY, ROMANIA.

In September 2000, 189 countries from the total of 193 member states within the United Nations signed the Millennium Declaration, with the scope to eradicate the extreme poverty in all its forms by 2015. The commitment was based on a set of time-bound and quantified goals and targets, called the Millennium Development Goals, focused on combating poverty in its many dimensions - including reducing income poverty, hunger, disease, environmental degradation and gender discrimination (1,2).

These goals were created to improve the health status and wellbeing of people worldwide.

The Millennium Development Goals (MDGs) include 8 goals, 21 targets and 60 indicators for measuring progress between 1990 and 2015, when the goals are ex-

pected to be met (3):

- Goal No.1 – Reduce Severe Poverty
- Goal No.2 – Increase Compulsory Education Graduation Rate by 2012
- Goal No.3 – Promote Gender Equality and Empower Women
- Goal No.4 – Reduce Child Mortality
- Goal No.5 – Improve Maternal Health
- Goal No.6 – Combating HIV/AIDS, Tuberculosis and Other Diseases
- Goal No.7 – Ensure Environmental Sustainability
- Goal No.8 – Develop Global Partnerships for Development

The target in the Goal No.4 was "*Reduce by two-thirds, between 1990 and 2015, the under-five mortality rate*" and the included indicators were:

4.1 Under-five mortality rate;

4.2 Infant mortality rate;

4.3 Proportion of 1-year old children immunized against measles.

The use of infant mortality rates as a measure of overall health status for a given population is based on the assumption that it is particularly sensitive to socio-economic changes and to health-care interventions (4).

The infant mortality rate is commonly used as an indicator of the level of health in a community. The vast majority of these deaths are preventable with current technology, as evidenced by the extremely low child mortality risks prevailing in rich countries.

The purpose of this article is to assess the efforts made in Romania towards achieving the Goal 4 from MDGs - Reduce Child Mortality.

MATERIALS AND METHODS

This research represents a descriptive study about the deaths among Romanian children under five, between 2002 and 2015, from the perspective of the MDGs.

In the United Nations Millennium Campaign, Romania committed herself to reduce child mortality (5). To help track progress toward this commitment, following specific targets and indicators were developed:

Target 1: Halve the mortality rate in children aged 1-4 years between 2002-2015;

Indicator: Mortality rate in children aged 1-4 years;

Target 2: Reduce infant mortality by 40% between 2002 and 2015;

Indicator: Infant mortality rate;

Target 3: Eliminate measles by 2007;

Indicator: Proportion of 1-year-old children immunized against measles.

The comparison allows establish the status (achieved or not) for each target. The data sources were represented by the National Institute of Statistics, yearbooks of the Ministry of Health and Romanian Government.

RESULTS

Target 1

The risk of a Romanian child not reaching his or her fifth birthday was nearly six times greater in 1970 (66 deaths per 1000 inhabitants) than nowadays (6,7).

Under five years of age mortality rate recorded in 1990 a still high level of 38 children aged 1-4 per 1000 inhabitants. Between 1990-2000, under-five deaths overall declined to 22.2 per 1000 inhabitants. From 2002, as specified in target 1, the indicator recorded a continuous descendent trend till now (20.8 to 10.3 under five deaths per 1000 inhabitants in 2013), as presented in fig.1 (8).

Target 2

The infant mortality rate is the second

of three indicators used to monitor achievements towards the Fourth Goal of the eight Millennium Development Goals. In the last four decades (1970-2010), the infant mortality rates have significantly declined.

From a high of 49.4 deaths per 1,000 live-births in 1970, infant mortality has dropped to 29.3 in 1980, respectively 26.9 in 1990 (32 deaths per 1,000 live births in rural areas). In 2000 it was 18.6 and subsequently, declined at 9.8 (2010). Throughout the current decade is 8.5 deaths per 1,000 live-births (2013). The evolution of the infant deaths between 2002 and 2015, as specified in the target 2, is described in fig. 2.

Since year 2000, the infant mortality pattern has changed, the perinatal conditions replacing the respiratory diseases as a first cause of infant death. In 2013 the leading causes of death among children under one year were:

- perinatal conditions (38.8%);
- respiratory diseases (23.5%);
- congenital anomalies (23.5%).

Infant death may occur in neonatal or post-neonatal period. In Romania, the ratio between neonatal and post-neonatal deaths changed, in favor of the first. Thus, in 1990 the values for neonatal and post-neonatal rates were 8.9 and respectively, 18 deaths per 1,000 live births, whereas in 2012 they declined to 4.8 and respectively, 4.2 (8).

Target 3

The target referring to eliminating measles by 2007 was reached later, in 2008, because of the measles epidemic in 2005 and 2006. However, the number of vaccinations has been significantly increased, the epidemic has been controlled and in 2008 and 2009 no new cases of measles have been reported. High vaccination rates have been maintained, with the proportion of children 1 year old vaccinated against measles reaching and being maintained at between 94-98% (9).

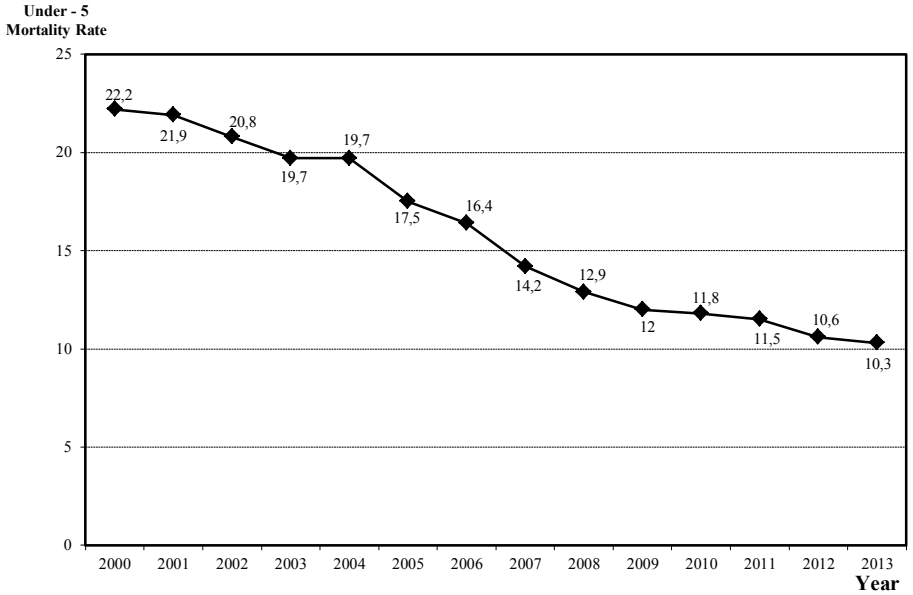

Fig. 1. Evolution of Under-five mortality rate in Romania, 2000-2013 (Romanian Statistics Annuary, 2014)

DISCUSSION

In Romania, mortality of children aged between 1 and 4 years dropped between 2002 and 2013 by 47.8%. The national target of halving the indicator (2002-2015) was achieved.

The United Nations worldwide target of a two-thirds reduction in under-five mortality (1990-2015) has already been achieved since 2009. Annual rate of reduction (1990-2013) was 5%.

Large reductions in infant mortality rates have occurred in Romania. The second target of the Millennium Development Goal no. 4 was achieved in 2012, but the indicator still remains well above the average of European Union (4 infant deaths per 1,000 live-births).

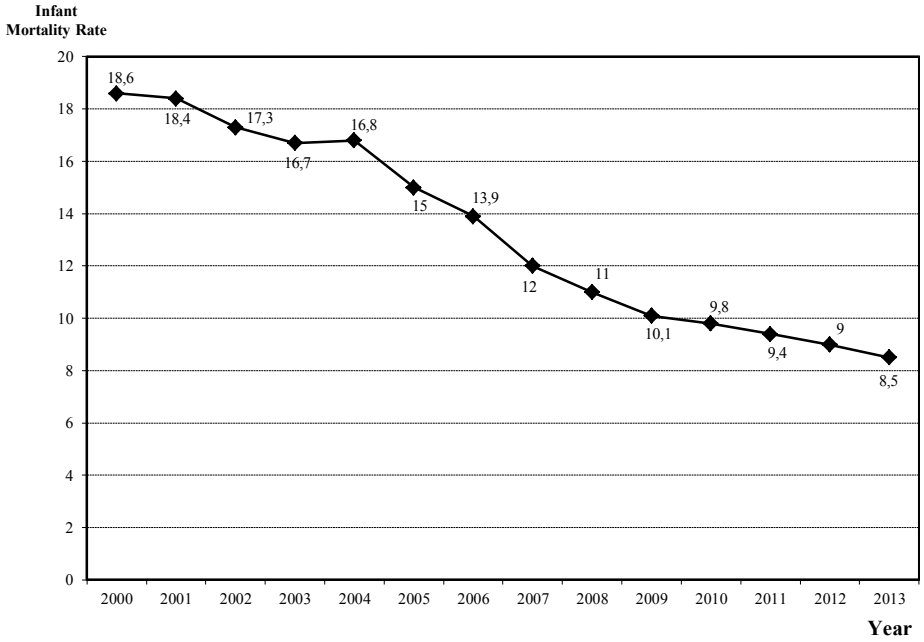

Fig. 2. Evolution of infant mortality rate in Romania, 2000-2013 (Romanian Statistics Annuary, 2014)

Neonatal mortality represents a significant proportion of child mortality (approximately 45%) and the child health Goal will not be reached unless neonatal mortality is addressed. The causes of neonatal death are different from the causes of death among older children. Health child programs and policies have to be focused on reducing neonatal mortality.

In order to maintain in the future the control of measles (target no. 3), the proportion of one-year-old children immunized against this disease must be kept within 96±2%

CONCLUSIONS

Substantial progress has been made in Romania, in achieving the Millennium Development Goal no. 4 - reducing child deaths - since 1990.

The global target no. 1 to reduce by two-thirds, between 1990 and 2015, the under-five mortality rate was achieved. The

national target no.1 - to halve mortality of children aged between 1 and 4 between 2002 and 2015 - was also, achieved.

The target no. 2 - Reduce infant mortality by 40% between 2002 and 2015 - was achieved. However, with regard to both indicators concerning child mortality, in spite of the remarkable progress made, Romania still lags far behind the other countries of European Union.

The target no. 3 - eliminate measles by 2007 - was achieved one year later (2008). These important gains will have to be maintained in the future.

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NEWS

INTERNAL HERNIAS AFTER BARIATRIC SURGERY

Hernias are among the most common long-term complications after bariatric surgery. Incisional hernias occur very often after conventional open bariatric surgery. The incidence of internal hernias after bariatric surgery (a special challenge) is more common than for other upper gastrointestinal tract operations. These hernias are not limited to laparoscopic procedures but are also observed after conventional open gastric bypass surgery. As the incidence is significantly increased after laparoscopic interventions, there seems to be a close association with minimally invasive procedures. The clinical symptoms of internal hernias without complete obstruction are non-specific and the correct diagnosis is often not straightforward. In addition to the aspects of prevention of internal hernias in laparoscopic surgery, this article discusses the diagnosis and treatment, taking into account the various forms of internal hernia after bariatric operations (Karcz W et al. Internal hernias after bariatric surgery. *Der Chirurg* 29.08.2015 86 : 9 : 855-860).

Roxana Gherasim

ETIOLOGY AND RESISTANCE PROFILE OF ISOLATED STRAINS FROM SEVERE SYSTEMIC INFECTIONS IN PATIENTS WITH IMMUNODEPRESSION- EXPERIENCE OF THE IASI INFECTIOUS DISEASES HOSPITAL 2011-2014

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ETIOLOGY AND RESISTANCE PROFILE OF ISOLATED STRAINS FROM SEVERE SYSTEMIC INFECTIONS IN PATIENTS WITH IMMUNODEPRESSION- EXPERIENCE OF THE IASI INFECTIOUS DISEASES HOSPITAL 2011-2014 (Abstract): Microbial resistance is an increasingly serious threat to global public health and it is linked to patient's age, immune status, and also antibiotic overuse or misuse and repeated hospitalizations. The high incidence of infections caused by multidrug-resistant bacteria requires rethinking the first-line therapeutic schemes. **Aim**: Retrospective study of the etiologic and antibiotic resistance profile of the bacterial strains isolated from immunocompromised hosts diagnosed with severe systemic infections, aimed at gaining a better understanding of the potential infectious sources and optimizing the antibiotic therapy. **Material and methods**: 178 cases of severe sepsis associated with immunosuppression (caused by diabetes, malignancies, liver cirrhosis, chronic alcohol abuse, kidney failure) were admitted to the "St. Parascheva" Infectious Diseases Hospital Iaşi in the interval January 2011- December 2014. **Results**: The mean age of the study patients was 61 years, most patients being female (53%) and living in urban areas (51%). The causal agents were: *Escherichia coli* (20.2%), *Klebsiella pneumoniae* (16.8%), *Pseudomonas aeruginosa* (14.6%), *Methicillin-Sensitive Staphylococcus aureus* (MSSA) (11.2%), *Enterococcus spp.* (10.1%), *Methicillin-Resistant Staphylococcus aureus* (MRSA) (8.9%), *Proteus spp.(5%), Acinetobacter baumanii* (5%), *Streptococcus pyogenes* (1.6%), *Staphylococcus epidermidis* (1.1%) and *Citrobacter* (0.5%). As to the resistance profile the following were found: 100% susceptibility of MRSA and *Enterococcus species* to vancomycin, as well as for the *Enterococcus* species; 27% of *E. coli* strains were resistant to beta-lactams and 20% of *Klebsiella pneumoniae* to carbapenems. Antibiotic therapy associated two or three drugs with an immediate result and a favorable outcome in 84.2% of the cases. **Conclusions:** The etiological agents implicated in the occurrence of severe sepsis in patients with acquired immunosuppression were Gram-positive bacteria (GPB) as well as Gram-negative bacteria (GNB) with moderate resistance to usual antibiotics. The infections caused by GNB were predominant in immunocompromised patients, but also in those with associated urinary and respiratory tract infections and chronic indwelling urinary catheters. In our severe sepsis patients Gram positive bacteria caused mainly skin and joint infections.
Keywords: ANTIBIOTHERAPY, BACTERIAL RESISTANCE, SEVERE SEPSIS.

Infections caused by multidrug-resistant germs are a current public health problem worldwide which affects all medical specialties, especially intensive care, surgery, pediatrics, neonatology, gynecology and oncology. Under these circumstances, in selecting an appropriate antibiotic therapy, clinicians must consider the current resistance spectrum of the strains circulating in a certain geographic area. Equally important are the efficacy, side effects, and high cost of antibacterial medication, especially of the latest generation one. Infections are an aggravating factor in the case of immunosuppressed patients, in which making a diagnosis is difficult because of the predominance of subclinical forms and the therapeutic choices must include specific antibiotics with maximum effectiveness and minimum side effects. Recent studies have demonstrated an increasing frequency of infections with GPB and especially with *MRSA*, with and estimated incidence of 67%, and *Enterococcus spp.* with an estimated incidence of 12% (1,2,3). *Klebsiella pneumoniae* is one of the most highly resistant bacteria, which is, in over 50% of cases, refractory to treatment with third-generation cephalosporins, fluoroquinolones and aminoglycosides. The resistance to carbapenems is also on the rise in Europe (4). The resistance of *E.coli* to third-generation cephalosporins ranges from 25 to 50% (5). *E.coli* is also a common GNB cause of sepsis and represents the most common cause of nosocomial urinary tract infections (6,7,8).

METHODS

The cases of severe sepsis admitted to the "St. Parascheva" Infectious Diseases Hospital Iaşi in the interval January 2011-December 2014 were retrospectively studied. The 178 patients included in the study were diagnosed by physical examination, laboratory tests able to evaluate the immune system (complete blood count, inflammation tests, liver and kidney function tests, blood glucose level) and bacteriological tests (identification test by classical diagnosis algorithm and antibiograms by Kirby-Bauer method and CLSI- Clinical Laboratory Standards Institute- standards). The data were collected from the observation papers of the studied patients and were processed using Microsoft Office Excel 2013. During the course of disease we followed the resistance of the involved microorganism to classical antibiotics, antibiotic treatment schemes and disease course under treatment (monitored by physical exam, repeated laboratory tests, temperature curve, days of hospital stay, and the need of intensive care procedures in unstable patients).

RESULTS

The 178 patients included in the study accounted for 2.1% of all sepsis cases admitted during the study period. The mean age of the study patients was 61 years (range 20-91 years), most patients being female (53%) and living in urban areas (51%). The causative agents of the infectious episode were varied (tab. I).

Methicillin-sensitive S. aureus (*MSSA*) was the main Gram positive coccus implicated in the etiology of severe sepsis in immunodepressed patients (11.2%). In these cases the systemic infections with *MSSA* was associated with skin (60%) and joint infections (40%). *MRSA* (*methicillin resistant S.aureus*) was present in a lower percentage compared to *MSSA* (8.9% vs 11,2%). Susceptibility of *MRSA* to vancomycin, teicoplanin, linezolid, ciprofloxacin,

rifampicin and ceftaroline was preserved. Fifty-six percent of *MRSA* strains were sensitive to cotrimoxazole. None of the *MRSA* strains showed resistance to vancomycin or linezolid and these were the first therapeutic options. *MRSA* infections were treated with combinations of glycopeptides (vancomycin) with quinolones (ciprofloxacin), fifth-generation cephalosporins with quinolones (levofloxacin) or lincosamides (clindamycin) with quinolones (ciprofloxacin).

TABLE I
Causative agents implicated in severe sepsis

Causative agent	No. of patients	%
Escherichia coli	36	20.2
Klebsiella pneumoniae	30	16.8
Pseudomonas aeruginosa	26	14.6
Methicillin-sensitive S. aureus (MSSA)	20	11.2
Enterococcus spp.	18	10.1
Methicillin-resistant S. aureus (MRSA)	16	8.9
Proteus spp.	9	5.0
Acinetobacter baumannii	9	5.0
Enterobacter spp.	8	4.4
Streptococcus pyogenes	3	1.6
Staphylococcus epidermidis	2	1.1
Citrobacter	1	0.5

Another GPB with special implications in the etiology of severe sepsis in immunodepressed patients was *Enterococcus sp.*, with a prevalence of 10.1% and a resistance to ampicillin of 50%. No vancomycin-resistant strain was found. The presence of *Enterococcus* frequently worsened the immunodepressed status of patients suffering from diabetes, biliary disease, liver cirrhosis, malignancies.

The most commonly isolated Gram-negative pathogens were *Escherichia coli* (20.2%), followed by *Klebsiella pneumoniae* (16.8%) and *Pseudomonas aeruginosa* (14.6%). The incidence of severe sepsis with *E.coli* was higher in patients with preexisting urinary system disorders with associated urinary tract infections (30%). Twenty-seven percent of *E.coli* strains were resistant to ampicillin and 13.8% to third-generation cephalosporins. Gentamycin resistance among *E.coli* strains isolated was of 36.1%. *Klebsiella pneumoniae.* caused severe sepsis in 30 cases (16.8%) and was isolated from blood culture, but also from distilled beverages (23.3%), urine (20%) and tracheal aspirates (10%). Twenty percent of *Klebsiella* strains were extended spectrum beta-lactamase (ESBL+)-producing bacteria, and 12.2% were carbapenemase producing-bacteria.

Pseudomonas aeruginosa was isolated

from 14.6% of cases (26/178), especially from patients admitted to Intensive Care Unit (ICU). Sepsis induced by *Pseudomonas* began with respiratory tract and ear, nasal and throat (ENT) infections (53.8%), urinary (27%) and soft tissues infections (19.2%).

Acinetobacter baumannii and *Enterobacter spp.* affected a similar percentage of the study patients (5% vs 4,4%). *A.baumannii* was present in patients who developed ventilator-associated pneumonia during hospital stay; 75% of them had positive tracheal aspirate cultures. Antibiogram revealed a 100% sensitivity to colimycin; 62.5% of *Acinetobacter* strains maintained their sensitivity to carbapenems.

Proteus mirabilis caused severe sepsis in patients with immunodepression due to chronic liver diseases (liver cirrhosis) and kidney diseases (stage IV and V kidney failure). *Proteus mirabilis* and *Proteus vulgaris* were isolated from urine in 62.5% of cases; 72% of the strains were resistant to ampicillin and 45% showed resistance to aminoglycosides (gentamycin).There were also cases of sepsis caused by *Streptococcus pyogenes* (1.6%), *Staphylococcus epidermidis* (1.1%) and *Citrobacter* (0.5%). Of all study patients, 36 (20.2%) required admission to Intensive Care Unit (ICU), because they had at least two organ dysfunctions. The average hospital stay was 12 days and the outcome was favorable in 84.2% of the cases (150 patients).

DISSCUSIONS

The etiology of severe sepsis in immunodepressed patients is varied and complex. It usually includes germs with a lower pathogenic potential. These patients are more exposed to nosocomial multidrug-resistant infections in comparison to immunocompetent hosts and the risk is due to repeated contacts with hospital environment (9,10). In the last years the incidence of MRSA infections among patients admitted to ICU reached almost 35%, no significant differences compared with those who did not require these maneuvers (25%) being found (10). In our study the prevalence was of 8.9%, lower than the prevalence rates reported in the literature, most probably due to the fact that the infections were community-acquired. The resistance mechanism of MRSA strains is most frequently based on the expression of a modified penicillin binding-protein (PBP), known as PBP 2a, which is responsible for resistance to all beta-lactam antibiotics and other classes of chemotherapeutic agents (quinolones, macrolides, tetracyclines). Future biological molecular tests are necessary to confirm the mechanism of resistance (10). In order to fix this problem, the clinicians had to use reserve antibiotics: glicopeptids (vancomycin/teicoplanin), oxazolidinones (linezolid), streptogramins, with the mention that the last ones are not available in our country (11). The treatment of the study patients was based on the administration of vancomycin/linezolid. *Enterococcus* strains are also implicated in the initiation of severe sepsis and septic shock, with a significant rate of vancomycin resistance (30%) in some countries (11). In our study we found no vancomycin-resistant *Enterococcus* strains (VRE). *E.coli* strains resistant to third-generation cephalosporins have been reported in the European countries, mainly in Italy (23%) and Germany (15%) (12,13). In our study, *E.coli* resistance to third-generation cephalosporins was of about 13.8%, similar to that previously mentioned; on the other

hand a higher resistance to aminoglycosides (36.1%) was recorded. In 2012 a significant increase in carbapenem-resistant *Klebsiella pneumoniae* was recorded (15% respectively) (11,12,13), consistent with our findings (*Klebsiella* ESBL+ strains 20% and carbapenemase-producing strains 12.2%), in which the administered antibiotic was colimycin. The carbapenems are reserve antibiotics for more than a decade and are extremely important in the case of severe infections caused by such GNB as *Pseudomonas aeruginosa, Acinetobacter* or *Enterobacteriaceae* species (14,15). *Acinetobacter sp.* causes nosocomial infections so that patients at higher risk for unfavorable course will be difficult to treat due to the high rates of resistance to carbapenems. In our study *Acinetobacter* resistance to carbapenems was of 37.5%, higher than the resistance rates recorded in Europe. A rate of carbapenem-resistant *Acinetobacter species* of over 25% was found in 8 of the 18 EU countries reporting data on *Acinetobacter*, while five of them reported a *Klebsiella pneumoniae* resistance to carbapenems of over 5% (15). The rate of bacterial resistance significantly differs from one country to another. The rate of *Acinetobacter* resistance to carbapenems has already exceeded that of *Klebsiella pneumoniae.* The same is true in the present study, in which *Acinetobacter* resistance to carbapenemes is almost three times higher than that of *Klebsiella pneumoniae.* Fortunately, the nosocomial infections caused by *Acinetobacter* are seven times more rare than those due to the two *Enterobacteriaceae species* (15).

CONCLUSIONS

In our study, the etiology of severe sepsis in patients with immune deficiency included gram positive bacteria (*MRSA, MSSA, Staphylococcus epidermidis, Enterococcus sp, Streptococcus pyogenes*), as well as gram negative bacteria (*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Acinetobacter baumanii, Pseudomonas aeruginosa, Citrobacter, Enterobacter sp.*). The resistance of these microorganisms to usual antibiotic treatment had often complicated the disease course, requiring the rethinking of therapeutic schemes, although the incidence of MRSA sepsis was below that reported in the other European countries (8.9%) and glycopeptide susceptibility has been preserved by 100% of the studied strains. Of the GNB *Escherichia coli* and *Klebsiella pneumoniae* were the most common causative agents, with a moderate resistance to beta-lactam antibiotics (20%) and aminoglycosides (36,1%), but with total susceptibility to colimycin. In this context, an appropriate antibiotic therapy can improve the chances of healing and, at the same time, may reduce costs and length of hospital stay.

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ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISMS IN CORONARY ARTERY DISEASE- A CONTROVERSIAL ROLE

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ANGIOTENSIN CONVERTING ENZYME GENE POLYMORPHISMS IN CORONARY ARTERY DISEASE-A CONTROVERSIAL ROLE (Abstract): Coronary artery disease (CAD) is the most prevalent cause of morbidity and mortality worldwide. It is a multifactorial disease, which is influenced by genetic and environmental factors. The major risk factors of CAD are age, male gender, hypertension, diabetes, hyperlipidemia, smoking, family history of CAD and obesity. Genetic factors add further information in predicting CAD. The relationship between genetic risk factors and the development of CAD is not well understood, likely due to the complex interrelation of genetic and environmental risk factors. One of the currently explored genetic factors is angiotensin converting enzyme (ACE) polymorphisms, which may have an influence on the progression of coronary artery disease. We present the results of several recently published studies (2012,2013) on the association between traditional cardiovascular risk factors and novel genetic risk factors (polymorphisms of ACE gene: insertions/deletions). The results of these studies are controversial. Most of them show an association between DD polymorphisms and D allele and the severity of stenotic coronary lesions, evaluated using the Gensini score (Gensini>6). However, others found no association. These differences may be due to geographical discrepancies, ethnical characteristics, small number of subjects included and variability of used techniques. We can therefore draw the conclusion that an international study on a large number of patients, using the same technique for genetic determination and the same statistical software should be performed. **Keywords**: ANGIOTENSIN CONVERTING ENZYME, GENETIC POLYMORPHISMS, CORONARY ARTERY DISEASE, STENOSIS.

Cardiovascular disease (CVD) is the major cause of morbidity and mortality in Westernized societies (1). It is well known that the etiology of this devastating disorder involves both genetic and environmental factors. Sequence variants of the components of the renin-angiotensin-aldosterone system are suggested to have significant influences on cardiovascular homeostasis. Both gene targeting and transgenic studies in mice have clearly suggested a critical role of the angiotensin converting enzyme (ACE) gene in blood pressure regulation. Thus, the ACE gene has been

recognized as a top candidate gene for cardiovascular research. The relationship between genetic risk factors and the development of coronary artery disease (CAD) is not well understood, likely due to the complex interrelation of genetic and environmental risk factors (3).

CARDIOVASCULAR RISK FACTORS

Risk factors for CAD were officially described after the discoveries of the Framingham study:

1. Conventional risk factors: age (over 45 years for men, over 55 years for women), family history of heart disease, race (higher incidence in African-Americans);

2. Modifiable risk factors: smoking, high total cholesterol level (especially LDL-cholesterol), hypertension, lack of physical activity, obesity, diabetes, metabolic syndrome, stress or depression;

3. Nontraditional risk factors (novel risk factors): inflammatory markers such as C-reactive protein, coronary calcium, lipoprotein (a), interleukin-6, fibrinogen, homocysteine, small-dense low-density lipoprotein, insulin resistance;

4. Genetic factors, which represent the latest discoveries in the field of cardiology: ACE gene polymorphisms (4).

ANGIOTENSIN CONVERTING ENZYME POLYMORPHISMS

ACE is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. Several different names refer to this enzyme in the scientific literature. ACE converts the inactive decapeptide angiotensin I (Ang I or Ang 1-10) to the active octapeptide and potent vasoconstrictor angiotensin II (Ang II or Ang 1-8), which is the main active product of the renin–angiotensin system (RAS). Long-term regulation of blood pressure and blood volume in the body is controlled by RAS.

Angiotensin II is a potent vasoconstrictor. It also acts on the adrenal cortex, causing the release of aldosterone, which stimulates tubules in the kidneys, allowing them to reabsorb more sodium and water from the urine. These effects directly act to increase the amount of fluid in the blood, making up for a loss in volume, and to increase blood pressure. Angiotensin II also mediates cell growth and proliferation by stimulating various cytokines and growth factors (5). Furthermore, angiotensin II may induce endothelial dysfunction by reducing nitric oxide bioavailability (6). These findings emphasize the importance of angiotensin II in cardiovascular pathophysiology and motivate exploration of the role of RAS in atherosclerosis and other cardiovascular outcomes. ACE also plays an important role in another hormonal system, the kallikrein kinin–cascade. ACE metabolizes bradykinin, which is a strong vasodilator, forming the inactive metabolite bradykinin 1-5. Therefore, ACE plays a prominent role in blood pressure regulation through this pathway as well (7, 23).

The ACE gene is located on chromosome 17q23. It spans 21 kb, and comprises 26 exons and 25 introns (8). It contains a polymorphism due to an insertion (I) or a deletion (D) of a 287 base pair (bp) *alu* sequence in intron 16, resulting in the 3 genotypes of insertion/insertion (II), insertion/deletion (ID), and deletion/deletion (DD). (8, 9, 10). The I/D polymorphism is associated with circulating and tissue ACE levels. Individuals homozygous for the D allele had higher tissue and plasma ACE concentrations than heterozygotes and II homozygotes (11). *ACE* polymorphism has

attracted a great deal of information as a potential cause of genetic variation in cardiovascular physiological function (12). Consequently, the *ACE* I/D polymorphism have been associated with various cardiovascular diseases, such as atherosclerosis (13), hypertension (14), and coronary artery disease (CAD) (15). *ACE*, as a candidate gene, can associate with traditional risk factors in predicting the severity of CAD (3, 24).

GENETIC STUDIES

The relationship between ACE polymorphisms and other cardiovascular risk factors (CRF) is not well understood in CAD, likely due to the complex interrelation of genetic and environmental risk factors. Several studies were aimed to investigate the associations of CAD risk factors and *ACE* polymorphisms in patients with CAD.

Patients included in the studies were referred for coronary angiography as a result of various clinical indications.

The experimental protocol was approved by the Ethics Committees, the study procedures being in accordance with the principles of the *Declaration of Helsinki II*, and all subjects provided written informed consent prior to enrollment.

Physical examination was performed on and a detailed family history was obtained for all subjects. The severity of CAD was evaluated according to the number of stenotic vessels on coronary angiography. The coronary angiography findings were reviewed by independent operators. The angiography results were reported as Gensini scores, which were computed by assigning a severity score to each coronary stenosis according to the degree of luminal narrowing and its importance based on location. Reduction in the lumen diameter and the angiographic appearance of concentric lesions and eccentric plaques were quantitatively evaluated. Gensini scores higher than 6 were considered a criterion for having CAD in the patients.

Informations about the conventional risk factors for CAD (1. hyperlipidemia, 2. hypertension, 3. diabetes mellitus, 4. family history of CAD, 5. smoking, 6. obesity) were obtained from all subjects. All studies used well defined inclusion criteria, according to strict definitions of the risk factors.

Hyperlipidemia was defined based on low-density lipoprotein cholesterol(>100 mg/dL for subjects with CAD) and/or high-density lipoprotein cholesterol (<40 mg/dL for men and <50 mg/dL for women) and triglyceride levels(>150 mg/dL), or prior requirement of lipid lowering medications. Hypertension was defined as blood pressure level above 140/90 on 3 different occasions or requirement of pharmacological intervention to lower blood pressure. Diabetes mellitus was defined as fasting venous blood glucose ≥126 mg/dL on 3 separate occasions or requirement of pharmacological intervention for diabetes mellitus. Family history of CAD was defined as having a first degree relative with established CAD before age 50, or before menopause. Cigarette smoking was assessed by asking participants about prior and current history of smoking habits. Body mass index (BMI) was calculated as weight (kg)/height (m^2). Overweight or obesity were defined based on the BMI levels (>25-29.9 and ≥30 kg/m^2 respectively).

CAD was defined as a documented coronary stenosis of at least 70% by quantitative coronary angiography in one or more major (more than 2 mm in diameter by

quantitative coronary angiography) coronary arteries. The patients included were divided into groups according to the number of diseased vessels (coronary obstruction of at least 70%) as determined by the number of diseased major coronary arteries (left anterior descending, left circumflex, and right coronary artery). Patients were grouped into 0, 1, 2, 3, or 4 vessel disease groups. Some studies selected patients without significant coronary obstruction by coronary angiography (less than 30%) as controls.

All studies extracted the DNA, used polymerase chain reaction (PCR) amplification and different SPSS programs for statistical analysis.

ANALYSIS OF THE STUDIES

Zhou Y.F. et al (16), enrolled 603 patients in three groups: 260 subjects were enrolled in the elderly control group (Male, n = 150, Female, n = 110), whose age were from 75 to 85 years (81.6 ± 4.55 years). They were admitted to the hospital for medical examination and routine treatment from the October 2006 to March 2013. They did not have hypertension, diabetes, coronary heart disease (CHD) or any other CVD. Two hundred and five patients aged 74 to 86 years (79.8 ± 6.28) years were enrolled in elderly hypertensive group (male, n = 103, female, n = 102). One hundred and thirty eight patients aged 75 to 83 years (79.12 ± 5.21 years) were enrolled in elderly diabetic hypertension group (male, n = 70, female, n = 68). The result was that in Chinese elderly population DD genotype and D allele in ACE gene were associated with hypertension and lipid levels. However, they were not risk factors for type 2 diabetes.

Guney A.I. et al. (2), enrolled 343 patients with CRF and angiographically proven CAD. Patients were grouped into 0, 1, 2, 3, or 4 vessel disease groups. Patients without significant coronary obstruction by coronary angiography (less than 30%) were selected as controls (140 patients). Their results showed that the frequency of the DD genotype was significantly higher in patients. D allele frequency was higher among CAD subjects when compared to the control group. The number of stenotic vessels were found to be statistically associated with a high frequency of DD polymorphism and D allele and a low frequency of I allele in patients, especially in male patients. The control group displayed II and ID genotypes more frequently than did the patients. The *ACE* I/D genotype was associated with hyperlipidemia and smoking history. They considered that the DD polymorphism and D allele may affect the severity of CAD, while I allele may have a protective effect. In conclusion, the *ACE* I/D genotype may interact with conventional risks criteria in determining the risk of CAD.

Dhar S. et al. (17), included 217 clinically diagnosed cases with CAD from the outdoor of Cardiology Department of Ramakrishna Mission Seva Pratishthan. 255 age and sex matched medically diagnosed healthy controls were taken for the study which were negative for Treadmill test (TMT_ve). All the samples were diagnosed by biochemical tests and other medical tests like electrocardiogram, echocardiography, or treadmill test.

The results of this study outlined that both DD [OR: 2.16; 95%CI: (60.60e67.40)] and ID [OR: 1.48; 95%CI: (93.28e97.72)] genotypes of the ACE gene showed significant associations in the development of CAD. Coexistence of diabetes and hyper-

tension was found to be a risk modifier of the disease. Tobacco intake in various forms elevates the risk of the disease among the cases with risk genotypes. Therefore, ID and DD genotypes of ACE gene came out to be predisposing factors for the CAD cases in our study population.

Poorgholi L. et al. (18) performed a cross-sectional study on a total of 1050 individuals referred to hospital for coronary angiography. 676 CAD-positive patients (documented by coronary angiography and Gensini scores higher than 6) and 374 CAD-negative patients were evaluated for ACE gene I/D polymorphism via the Polymerase Chain Reaction Amplification method. Patients' age, sex, smoking status and its duration as well as family history of CAD, hypertension, and diabetes mellitus were recorded.

The results showed that 504 (74.6%) of the CAD-positive patients were male, and the mean age of this group was 60 (60 ± 10). In the CAD-negative individuals, the mean age was 56 (56 ± 10) and 196 of them were male (52.4%). After the analysis of all groups and gender subgroups, neither genotype nor allele frequency was significantly different between the CAD-positive nor CAD-negative groups (p values for genotypes and allele frequencies were 0.494 and 0.397, respectively). Therefore, ACE gene I/D polymorphism were not associated with an increased risk of CAD in an Iranian population.

Sahin S.et al. (19), investigated whether the insertion/deletion (I/D) polymorphism in the ACE gene and serum ACE levels are associated with traditional risk factors of CAD. They enrolled 250 individuals without CAD and 750 individuals suffering from CAD who were angiographically diagnosed. Biochemical risk factors, the ACE (I/D) gene polymorphism, and ACE serum levels were compared. Compared to the control group, the CAD group showed significantly higher serum ACE levels ($P < 0.001$). The highest ACE levels were found in those with the DD genotype. Other genotypes also presented statistically significant differences. They observed a significant difference between the control and coronary patient groups regarding the levels of total cholesterol, triglyceride, high-density lipoprotein-cholesterol, and low-density lipoprotein-cholesterol ($P < 0.05$). ACE (I/D) genotypes and serum ACE levels may be associated with risk factors and the development of CAD.

DISCUSSION

We presented the results of several recently published studies (2012,2013) on the association between traditional CRF (hypertension, diabetes, hyperlipidemia, obesity, smoking habit, family history) and novel genetic risk factors (ACE deletion/insertion polymorphisms).

In all studies, DNA was extracted from peripheral leukocytes and amplified by polymerase chain reaction (PCR). Data were analyzed with different statistical softwares, such as *SPSS 11.5, SPSS 13.0, SPSS 14.0, SPSS 18.0, SAS 9.2* packages.

Inclusion criteria were similar between studies, but some studies chose healthy subjects for comparison (16) while others used patients without significant stenosis (less than 30%) for the second group (2, 18).

The results of those studies were controversial. Most of them showed an association between genetic risk factors (DD genotype, D allele) and the severity of stenotic coronary lesions, evaluated using the Gensini score (Gensini>6). Gensini

score is based on the number of stenotic coronary artery segments, degree of luminal narrowing and the regional importance of lesion location in the coronary tree. However, Poorgholi L. et al. (18), found no association between ACE I/D polymorphism and the progression of coronary artery disease in an Iranian population.

There are multiple implications of ACE gene in CAD. The presented studied focused on the development and severity of stenosis. Other directions scientists are working on are left ventricular dysfunction and in-stent restenosis, in the context of ACE gene polymorphisms. In-stent restenosis occurs after treatment of coronary artery stenosis in 12% to 32% of coronary interventions with stents. Experimental and clinical studies have suggested that the deletion/insertion (D/I) polymorphism of the ACE gene plays a role in this. Jorgensen E. et al.(20), performed a study on 369 patients after stent implant and concluded that the D/I polymorphism is not an independent predictor of coronary in-stent restenosis in general, but it may be of clinical importance in patients treated with ACE inhibitors or angiotensin receptor antagonists. Martinez R.A. et al. (21) also stated genetic variations of the ACE gene could be a genetic factor related to coronary artery disease in the Mexican mixed racial ancestry individuals, but do not support its role as a risk factor for developing restenosis after coronary stenting. However, the meta-analysis by Wang S et al. (22), showed that the DD genotype of ACE I/D polymorphism was significantly associated with increased risk of restenosis, particularly for PTCA stent.

CONCLUSIONS

We believe that these differences may be due to geographical discrepancies, ethnical characteristics, rather small number of study subjects and variability of the used techniques. We can therefore draw the conclusion that an international study on a large number of patients, using the same technique for genetic determination and the same statistical software should be performed.

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THE EFFECTS OF RIBOFLAVIN AND METHYLENE BLUE ON NOCICEPTION AND VISCERAL PAIN

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THE EFFECTS OF RIBOFLAVIN AND METHYLENE BLUE ON NOCICEPTION AND VISCERAL PAIN (Abstract) **Background**: Methylene Blue (MB) can prevent electron leaking, increase mitochondrial oxidative phosphorylation, and reduce ROS overproduction under pathological conditions, currently being trace evidence that it can alter pain perception in humans by local administration during certain surgical procedures. Riboflavin or vitamin B2 (B2) constitutes a part of the vitamin B group, which in recent studies shows a growing implication in the treatment of some pathology that imply pain management. **Aim**: To investigate the effect of one dose of Riboflavin and Methylene Blue on nociception and visceral pain in mice. **Methods**: A total of 48 BALB/c male mice were divided into 3 groups: MB Group, B2 Group and C Group. MB (5 mg/kg b.w.), B2 (100 mg/kg b.w.) or an equivalent volume of saline was administered intraperitoneally. Mice were tested before (baseline) and after drugs administration over a 4h period. Nociception was evaluated by means of Hot Plate Test (HPT) and TFT (Tail Flick Test). Visceral pain was evaluated 2h after administration. **Results**: Four hours after MB administration we recorded an analgesic effect on the hot plate test ($p<0.05$ at 30, 60 and 240 min). No significant effect on the TFT was noticed. B2 vitamin had an antinociceptive effect as compared to control group only for HPT that persisted for 2h but had no effect on TFT. Both MB and B2 vitamin have shown an analgesic effect ($p<0.01$) on visceral pain when compared to the control group but the pain inhibition was more important after riboflavin administration. **Conclusions**: Even if the exact mechanisms are not clarified by our study, we demonstrated that both ATP modulators (MB & B2 vitamin) have analgesic effect on visceral pain and nociception. **Keywords:** METHYLENE BLUE, RIBOFLAVIN, PAIN

Methylene Blue (MB) is a synthetic redox compound containing a central thiazidic ring system (1) that had a wide usage from chemotherapy to dementia, antimalarial, and not least contributed to the discovery of the neuroleptic drug families due to its oxidation-reduction facilities (2).MB improves mitochondrial respiration and the metabolic rate stimulating the mitochondrial complex IV, enhancing it by 30% and increasing the oxygen consumption to 70% (3).

MB is inhibiting cGMP- mediated processes and neuronal NOS (4,5), and can

influence NO-synthases (ROS), mono-amino-oxidase A (MAO) and disulfide-reductases (6).

MB is able to interfere with peripheral and central nervous systems by modifying the glutamatergic, monoaminergic and cholinergic neuronal communication (7), and can also prevent leaking of electrons, increasing mitochondrial oxidative phosphorylation and reduce ROS overproduction (8).

In humans MB proved its analgesic effects after local administration either alone (intradermal anesthetic) in association with propofol injections (9) or in chronic low-back pain (10). In animals models it being reported that it could have antinociceptive effects.

Riboflavin (RF) also known as vitamin B2 constitutes a part of the vitamin B group that is essential for cellular functions and development. It plays an important role in oxidation-reduction reactions, reducing oxidative stress and is involved in mediating the immune responses (11).

RF has a modulating role on mitochondrial oxidative stress in the brain (12), and is documented that although most of the conditions were associated with B2 deficiency, it can be used to alleviate migraine as a prophylactic drug (13) or in diseases associated with metabolic disorders (14).

RF also demonstrated in recent studies that it can influence experimental nociception in mice by possibly influencing the inflammatory mediators (15) (TNF α, IL-1 or IL-6) and increase the effect of non-steroidal drugs in rats (16).

The purpose of our study was to assess the effects of both MB and RF on nociception and visceral pain in mice as long as both substances modulate serotonergic, noradrenergic, dopaminergic systems as well as NO and ROS all implicated in nociception modulation.

MATERIAL AND METHODS

Animals. The experiments were conducted on 48 BALB/c male mice (28-34 g), housed at 21 ± 2 °C under a 12-h light/ dark cycle, with access to food and water ad libitum. All animals were habituated to the testing laboratory for at least three days. The experimental protocols and procedures described in this article complied with the European Communities Council Directive 86/609/EEC and followed the International Association for the Study of Pain's (17) and the University of Medicine and Pharmacy "Gr. T. Popa" ethical guidelines for investigations of experimental pain in conscious animals.

Drugs. The drugs used in these experiments were: methylene blue 1% (Tis Farmaceutic, Romania), acetic acid (Sigma, Germany) and riboflavin (≥98%, Sigma, Germany).

Both MB and RF were freshly diluted in saline and administered intraperitoneal (i.p.).

The Hot-Plate Test (HPT). The hot-plate test was used to measure nociceptive response to heat according to the method described by Woolfe and MacDonald (18), with minor modifications. Briefly, the mice were placed into an open Plexiglas tube, on the hot-plate apparatus (Ugo Basile, model-DS 37, Italy) 55 ± 0.1°C and the time between placing the animal on the hot-plate and the occurrence of licking, shaking of hind paws or jumping off the surface was recorded as response latency. An automatic 15 s cut-off was used to prevent tissue damage. Animals displaying baseline latencies of more than 10 s were excluded from the study (20).

The Tail Flick Test (TFT). The tail flick test (19), was used to measure the latency for tail flick reflex following exposure to a heat stimulus. The heat source of the Tail-Flick unit (Ugo Basile, Italy) was focused on the distal portion of the tail at 4-5 cm from the tip and the reaction time necessary for the mouse to remove its tail was defined as the tail-flick latency. Animals displaying baseline latencies of more than 6 s were excluded from the study. The cut-off time was set at 12 s to avoid tissue damage (21).

Antinociception on the hot plate and tail flick tests was quantified as percentage of maximal possible effect (%MPE):

%MPE = [(post-treatment latency-baseline latency)×100 / (cut off latency-baseline latency)].

The Acetic Acid Induced Writhing Test (Visceral pain). Was performed as previously described by us, after acclimatization in an acrylic observation chamber for at least 20 minutes, the mice received 0.1 ml /10 mg b.w i.p. injections with 1.0% (v/v) acetic acid (20).

The antinociceptive activity in the writhing test was expressed as the percentage of inhibition of nociceptive behavior using the ratio:

% pain score = (control mean - treated mean) x 100/ (control mean)

Study design. A total of 48 mice were divided into three groups as follows: Group MB, mice receiving a single intraperitoneal dose of methylene blue (5 mg/kg b.w); Group RF that received a single dose of 100 mg/kg b.w. riboflavin and Group C that received an equivalent volume of saline in the same manner.

The TFT, HPT tests were performed at baseline and thereafter 30, 60, 120, and respectively after 240 minutes after administration. The writhing test was performed 2h after administration.

The doses used in this study are in range with literature data and have been proved a maximum antinociceptive effect from the doses tested in our lab (data not shown).

Statistical analysis. The data obtained were expressed as the mean value ± SEM. Statistical evaluations were performed using SPSS v 20.0 software with one-way ANOVA. Post hoc comparisons were performed using Tukey`s Test. The significance level was set at $p<0.05$.

RESULTS

The Tail Flick Test. Administered i.p. neither MB 5mg/kg b.w. or B2 100 mg/kg b.w. did not influence the reaction of the mice to thermal stimuli over the time period allocated for the experiment , with no statistical significance as compared to the control group.

The Hot Plate Test. A single dose of riboflavin produced a significant analgesic effect ($p<0.05$) that started 30 minutes after i.p. administration (fig. 1) and maintained for 2h (MPE of 30.8% reaching a maximum of 56% at 60 minutes) showing a decrease in effect after 240 min (MPE= -51.7). MB demonstrated the same analgesic effect ($p<0.05$) that occurred at the same time as for B2, with a MPE between 29% and 38%, the effect lasting over 4h%. Although the MPE was higher in B2 Group, the analgesic effect lasted longer in the MB Group.

The Writhing Test. Intraperitoneal administration of vitamin B2 showed a significant analgesic effect on acetic acid induced pain ($p<0.01$) same as MB ($p<0.05$) when compared to control group (fig. 2). The pain inhibition score was higher for

riboflavin (77%) as compared to the one MB demonstrated (23.2%) Tukey's multiple comparisons test showing strong significance between the two.

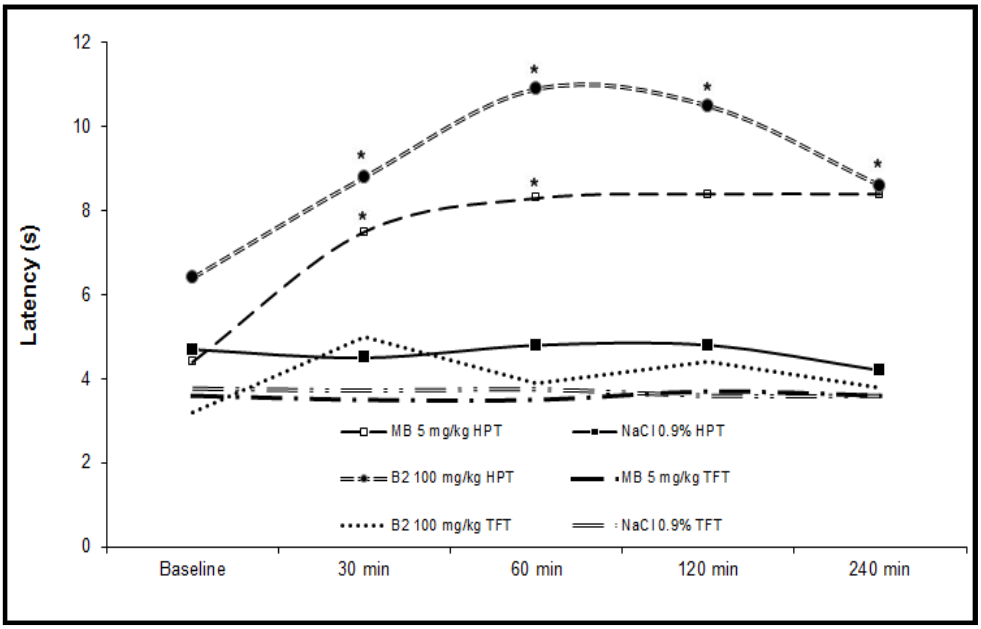

Fig. 1. The effects of B2 and MB on Hot Plate. The results are expressed as mean± S.E.M. * $p < 0.05$.

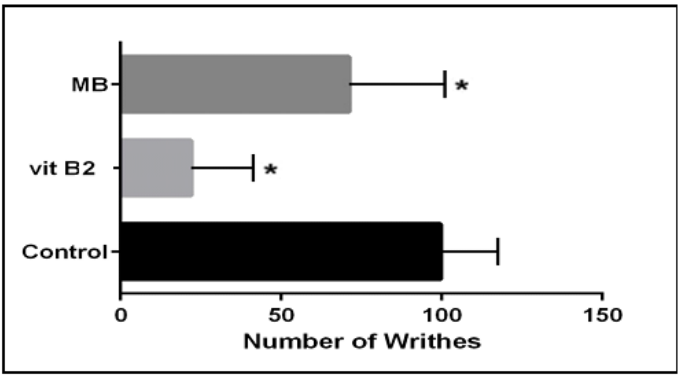

Fig. 2. The effects of B2 and MB on visceral pain in mice. The results are expressed as mean± S.E.M. * $p < 0.05$.

DISCUSSION

This study demonstrates, concordant with literature data, that methylene blue (22) and riboflavin (23) modify pain perception. Thus, our study is one more proof that ATP modulators had an analgesic effect on the hot plate test and visceral pain and a neglect effect of the thermonociception evaluated by the tail flick test.

The substances used by us are mitochon-

drial linked and have proved their ability to change the cellular ATP level and REDOX status of the cells. Thus, MB acts as an antioxidant and as an artificial electron donor to complex I-IV of the mitochondria, increasing ATP production and riboflavin has been shown to improve both complex I (NADH dehydrogenase) and complex IV (cytochrome c oxidase) activity. Riboflavin serves as a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD) coenzymes that participate in oxidative-phosphorylation reactions at the cellular level. A unique feature of FAD and FMN is their function as prosthetic groups in many enzyme systems and as catalysts of oxidation-reduction reactions.

There is a growing body of evidence that extracellular ATP has an important role in pain signaling (both the periphery and in the CNS). It is supposed that ATP is involved in acute pain; as long as ATP is released from damaged cells it excites directly primary sensory neurons by activating their receptors, by neither blocking nor suppressing the expression of P2X/Y receptors in sensory neurons or in the spinal cord, showing an effect on acute physiological pain. There is also evidence that endogenous ATP and its receptor system might be activated in pathological pain states, particularly in neuropathic pain. Thus, ATP is implicated in synaptic transmission of innocuous mechano-receptive and nociceptive input in the superficial dorsal horn; the purinergic ATP receptors being expressed in dorsal horn neurons and in DRG cells (24).

In our study we used two models of acute pain to evaluate the effects of methylene blue and riboflavin namely thermonociception models of pain (tail flick and hot plate tests) and visceral pain.

The hot plate and tail flick tests are commonly used for evaluating thermal pain sensitivity, heat selectively stimulating thermoreceptors and nociceptors. The tail-flick is considered mainly a spinal reflex, at least in its shorter latency form, persisting after section or cold block of upper parts of the spinal cord (25) but as with all reflexes, it is subject to supraspinal structures control (26).

By contrary, the hot plate test is supposed to have supraspinally integrated responses. The paw licking behavior is affected only by opioids but the jumping/shaking reaction time is increased equally by less powerful analgesics such as acetylsalicylic acid or paracetamol (27). Thus we must keep in mind that mainly tail-flick and less the hot plate tests are predictive only for substances that are morphinomimetic in the strictest sense, with very few effects for partial agonist compounds and none at all for mild antalgics.

Visceral pain results from activation of sensory afferent nerves innervating internal organs (28). Viscero-sensory axons are almost exclusively thinly myelinated A-delta and unmyelinated C fibers while the receptors exhibit chemosensitivity, thermosensitivity and/or mechanosensitivity. The afferent fibres convey sensory information from the upper gastrointestinal tract to the CNS via vagal and splanchnic nerve pathways (29). A nociceptive stimulus evoked by i.p. injection of acetic acid or citric acid in mice (a model of inflammatory visceral pain) stimulates sensory afferents and the transmission of nociceptive information, centrally leads to the activation of descending antinociceptive mechanism to a noxious stimulus (30). It has been proposed that distension of visceral organs leads to re-

lease of ATP from the epithelium lining the organ; the released ATP then acts upon P2X receptors in subepithelial nociceptors to elicit visceral pain (31). However, the ATP inhibitors (32) were ineffective in reducing nociception in animal models of visceral pain.

Our behavioral data demonstrate that both substances known to stimulate cellular ATP level, decrease pain perception explored by hot plate test and visceral pain. As long as exogenous ATP proved its pronociceptive effect when administrated locall or in DRG, we suppose that the ATP modulator used act rather on the ATP-sensitive potassium channel (K_{ATP}) family imply in analgesia and/or on purinergic receptor imply in pain inhibition.

CONCLUSIONS

Even this study does not clarify the intimate mechanisms of action for methylene blue and riboflavin it is proving that ATP stimulators after one dose has analgesic effects. Long term administration effects and the analgesic neuromediation activated need further studies.

ACKNOWLEDGEMENTS

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PELVIC ENDOMETRIOSIS AND URETERO-HYDRONEPHROSIS: CLINICAL, IMAGISTIC AND SURGICAL IMPLICATIONS - CASE REPORT

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PELVIC ENDOMETRIOSIS AND URETERO-HYDRONEPHROSIS: CLINICAL, IMAGISTIC AND SURGICAL IMPLICATIONS - CASE REPORT (Abstract): Endometriosis represent the presence and proliferation of functional endometrial-like tissue outside the uterine cavity, and is a chronic, recurrent, debilitating desease, in which kidney implications are rare, but complex. **Objective:** Evaluation of clinical, imagistic and surgical implications of uretero-hydronephrosis secondary to recurrent pelvic endometriosis. **Material and methods:** 30 year old patient diagnosed with primary infertility and left ovarian endometriosis (treated with classically conservative surgical procedure), presented acute onset of obstructive urinary symptoms, requiring internal drainage of the upper urinary straight path (Cook probe) for uretero-hydronephrosis grade III. CT and MRI examination reveals the presence of a imprecise pelvic mass incorporating right distal ureter and uretero-bladder ostium. **Results:** In this clinical and laboratory context, classic surgery is practicing with right anexectomy and direct right uretero-vesical reimplantation, also with psoas bladder-hitch. Histopathological examination confirmed the presence of recurrent ovarian endometriosis with right distal ureteral invasion. **Conclusion:** Recurent endometriosis , although considered a benign condition, often present invasive characters, determining considerable anatomical destructions and severe symptoms. **Keywords**: PELVIC ENDOMETRIOSIS, URETERO-HYDRONEPHROSIS, CT , MRI.

Endometriosis is a hormonal and immune system disease in which cells similar to that which line the uterus (endometrium) grow outside the uterine cavity, being commonly found on other organs of the pelvis. Endometriosis is typically diagnosed during the reproductive years, and is more common in women who are experiencing infertility than in fertile women (1,2). It is considered a disabling and expansive condition in the pelvic region and beyond, including internal scarring, adhesions, pelvic cysts, chocolate cyst of ovaries, ruptured cysts, and bowel and ureteral obstruction resulting from pelvic adhesions (3). Less frequently, 10% of women in the reproductive age have involvement of the genitourinary tract by endometriosis, leading to hydronephrosis as a result of adhesions and scar formation (4). In this cases, symptomatology is represented by dysuria, suprapubic pain, hematuria. Medical therapy is palliative and definitive therapy is surgical (5,6). Here we present clinical,

imagistic, and surgical implication in a case of pelvic endometriosis complicated with uretero-hydronephrosis.

CASE REPORT

In April 2011, thirty year old female patient (nulliparous/ no history of pregnancy) was admitted to our clinic with complaints of ongoing pelvic pain (accompanied by lower back pain/abdominal pain), dysmenorrhea, dyspareunia, constipation,chronic fatigue, nausea and vomiting, headaches, with exacerbation of the symptoms in the last 6 months, during her menstrual cycles. Her menarche occurred at the age of 14, with heavy and irregular menstrual periods. She did not receive a hormonal theraphy before, and reports that for the past 2 years she has been unsuccessfully trying to conceive. At this moment, patient denied any urinary symptoms. A pelvic examination revealed a normal sized uterus that was fixed and slightly tender, but with exacerbation of painful sensation in the left adnexal region. Vaginal examination reveals a painful induration in the posterior fornix. Transvaginal ultrasonography demonstrated a heteroecogenic multicystic formation (45/39/37 mm), located in the left of uterus.

A magnetic resonance imaging (MRI) was performed, revealed the presence of tumoral mass with polycyclic contour and multicystic appearance, located between the posterior wall of the uterus and left anterolateral wall of rectosigmoidian junction (fig. 1).

We performed a classical laparotomy with ablation of tumoral formation (anatomopathological report confirming the presence of an endometriotic ovarian cyst)-preserving the left ovary - and lysis of intra-abdominal adhesions, for an severe pelvic adhesive disease to the posterior surface of the uterus, posterior cul-de-sac endometriosis, contributing to a distorted pelvic anatomy.

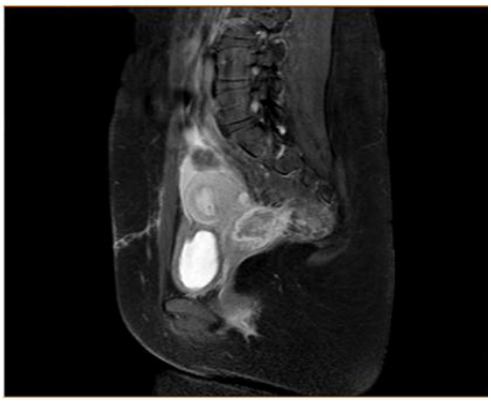

Fig. 1. Tumoral pelvic mass with polycyclic contour and multicystic appearance characterised by hyperintense signal on fat-saturated T1-weighted images – MRI.

The patient recovered well after her surgery and received Diphereline (Triptorelin Acetate) treatment - 3.75mg administered as one intramuscular injection repeated every 4 weeks (6 months). After surgery, the patient never presented for any clinical and laboratory reevaluation. In January 2013, the patient went to the emergency department for an acute episode of severe pelvic pain associated with abdominal cramps, vomiting, dysuria, and intermittent hematuria associated with the menstrual cycle. Renal functional tests (blood urea nitrogen/ creatinine/creatinine clearance rate) were abnormal, but whith normal urine culture. Pelvic ultrasonography revealed uretero-hydronephrosis grade II-III with no evidence of calculi, and the presence of a heteroecogenic multicystic formation (52/47/41mm), imprecisely delimited, located in the right of uterus. A contrasted abdominal computed tomography (CT) examination reveals the presence of severe obstruction of right distal ureter by an intraureteral enhanced soft

tumoral mass. In this context, urologists performed internal drainage of the upper urinary straight path (Cook probe) for uretero-hydronephrosis.

After intervension, a MRI examination reveals the presence of a imprecise pelvic mass incorporating right distal ureter and uretero-bladder ostium, characterised by hyperintense signal on fat-saturated T1-weighted images (fig. 2).

Fig. 2. Imprecise pelvic mass incorporating right distal ureter and uretero-bladder ostium, characterised by hyperintense signal on fat-saturated T1-weighted images – MRI.

The double-J stent was kept in 6 weeks postoperatively, and after this, classic surgery is practicing with right anexectomy and direct right uretero-vesical reimplantation, also with psoas bladder-hitch. Histopathological examination confirmed the presence of recurrent ovarian endometriosis with right distal ureteral invasion. She will be followed up with a cystoscopy and pelvic magnetic resonance imaging scan in 4 months to check for endometriosis recurrence.

DISCUSSION

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterus. The prevalence of endometriosis is unknown but estimates range from 2 to 10% of women of reproductive age (7). It predominantly affects women of reproductive age and is associated with pelvic pain and fertility problems (8). The aspects of the pathophysiology can basically be classified as underlying predisposing factors, inflammation, metabolic changes, formation of ectopic endometrium, and generation of pain and other effects (9). The ectopic endometrium can be limited to the superficial peritoneum in an otherwise normal pelvis (peritoneal endometriosis) but can also develop into ovarian endometriotic cysts (endometriomas) (10). In some cases, it involves the uterosacral ligaments, the rectosigmoid colon, the vagina, and the bladder and can cause a complete distortion of the pelvic anatomy (11). Urinary tract endometriosis (UTE) is observed in 1-2% of women with endometriosis, about 80% have urinary bladder involvement (12). In the evolution of this disease, recurrency is an essential feature. In our case, urinary symptoms were present at 20 months after the first intervention, in the context of hormonal treatment. It is difficult to say that was just a primary uretero-bladder

endometriosis, which occurs when the endometrial tissue is congenitally located within the urinary uretero-bladder wall. Most likely, in our case, was the case of a secondary endometriosis, due to iatrogenic lesion occurring after primary pelvic conservative intervention.

In this cases, symptomatology is not specific, being represented by dysuria, suprapubic pain, cyclic urgency, and rarely hematuria, symptoms similar to those of recurrent cystitis, which may be responsible for its delayed diagnosis (13,14). Some authors suggest that negative urinary cultures in symptomatic individuals may suggest urinary bladder endometriosis (15). Transvaginal ultrasonography is the first-line imaging study when endometriosis is suspected: it is powerful, simple, widely available, and cost-effective, being recommended for diagnosing endometriotic ovarian cysts (16). On the other hand, MRI has high specificity for the diagnosis of endometriomas as a result of its ability to detect aged hemorrhagic content. MRI is also less operator dependent than transvaginal ultrasonography and is more sensitive for detecting foci of deeply infiltrating endometriosis, because of its ability to completely survey the anterior and posterior compartments of the pelvis (17).

In women in the reproductive years, endometriosis is merely managed: the goal is to provide pain relief, to restrict progression of the process, and to restore or preserve fertility where needed. In younger women, like our patient, with unfulfilled reproductive potential, surgical treatment attempts to remove endometrial tissue and preserving the ovaries without damaging normal tissue. Conservative treatment is very important, but may increase the risk of recurrence, like urinary tract endometriosis, the second most commonly involved site by extrapelvic endometriosis (18). Surgical management of uretero-bladder endometriosis may includes urethral resection for obstructive lesions, combined with reconstruction of the bladder, and association of medical therapy.

CONCLUSIONS

It is a rare case of endometriosis with involvement of the urinary tract leading to renal failure because of the silent obstruction of the ureter. In this case, the surgical treatment is the best choice, the symptomatology beeing nespecific and the usual investigations such as CT, RMN and ultrasonography only suggesting this chronic condition.

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NEWS

TRANSORAL ROBOTIC SURGERY (TORS) WITH THE MEDROBOTICS FLEX™ SYSTEM: FIRST SURGICAL APPLICATION ON HUMANS

Head and neck surgery can be fraught with difficulties in accessing the pharynx and larynx. Minimally invasive surgery has developed with the recent advances in technology. Currently, a variety of high-definition multichannel videoendoscopes and robots became available. The authors present their experience in a new robotic surgical system-'The Medrobotics Flex™ System' at our tertiary referral unit. The aim of this prospective study eas to assess the safety, functionality and ease of use of this new device. Thus far, this is the first study in live human subjects who have undergonesurgery for the following conditions: (1) obstructive sleep apnoea involving the base of tongue, the tonsil and the velum; (2) vocal fold polyp; (3) carcinoma of the lateral edge of the tongue. No complications were recorded and the system provided good visualisation and access to these subsites without compromising safety or success. In summary, the authors found the Medrobotics Flex™ System to have certain other advantages including ease of set up and use besides being reliable and safe. (Remacle M, M N Prasad V, Lawson G, Plisson L, Bachy V, Van der Vorst S. Transoral robotic surgery (TORS) with the Medrobotics Flex™ System: first surgical application on humans. *Eur Arch Otorhinolaryngol* 2015 Feb 8. Epub ahead of print)

Radu Dănilă

THE USE OF TITANIUM MESH IN FACIAL CONTOUR RECONSTRUCTION

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THE USE OF TITANIUM MESH IN FACIAL CONTOUR RECONSTRUCTION (Abstract): Bone defects following trauma, or the radical resection of facial tumors are often associated with the loss of soft tissue support. The objectives of facial reconstructive surgery should not only be the restoration of the defect, but also the aesthetic and functional craniofacial rehabilitation with the restoration of facial symmetry. **Aim:** The purpose of this study is to assess the practicality of titanium mesh panels for reconstructing the craniofacial skeleton. **Material and methods:** Between January 2015 and December 2017 the authors used titanium mesh for a number of 26 patients in 20 trauma-related cases and six cases of post-ablative defects when titanium mesh was covered using loco-regional flaps. **Results:** All the facial dimensions were restored. None of the titanium meshes was exposed postoperatively. **Conclusions:** Titanium mesh has proven to be a useful material for the reconstruction of the craniofacial skeleton, capable of providing a true individualized treatment. **Keywords:** TITANIUM MESH, CRANIOFACIAL SKELETON, FRACTURE, SYMMETRY.

Posttraumatic defects of the facial skeleton, or bone defects following tumor removal are usually associated with soft tissue support deficit, resulting in facial asymmetry, enophthalmos, lagophthalmos with secondary conjunctivitis, the loss of tooth-bearing segments, and speech impairment (1). All lead to a lower quality of life for the patient. Furthermore, the restoration of the complex architecture of the craniofacial bone frame is a challenging task for the surgeon.

The purpose of the reconstruction is to restore the aesthetic appearance and the lost functions by providing continuity of the bone contours, which ensures support for the soft tissues and allows the restoration of facial symmetry (2).

There are many possibilities for facial bone reconstruction such as titanium plates, bone grafts, composite flaps and titanium mesh. Titanium mesh has proven to be a useful material for the reconstruction of the craniofacial skeleton, capable of providing a true individualized treatment.

MATERIAL AND METHODS

Between January 2015 and December 2017, the authors used titanium mesh for a number of 26 patients including 20 (76.92%) trauma-associated defects and 6 (23.08%) post-ablative defects. In the

trauma-related group there were 6 (23.08%) cases of comminuted mandibular fractures, 3 (11.54%) fractures of the atrophic mandible, 6 (23.08%) cases of complex midface fractures, 3 (11.54%) fractures of the zygomatic complex with comminution of the zygomatic-alveolar crest, and 2 (7.69%) patients with sequelae following nose-orbital-ethmoid (NOE) fractures associated with anterior wall of the frontal sinus fractures. In the 6 (23.08%) patients with post-ablative bone and skin defects following the removal of cheek region basal cell carcinomas, titanium mesh was associated with loco-regional flaps consisting of 3 (11.54%) frontal flaps with a medial pedicle, 2 (7.69%) frontal flaps with a lateral pedicle and one (3.85%) rotated cheek flap.

All the titanium mesh panels were modelled intraoperatively, with the exception of two (7.69%) cases where stereolithographic 3D printed models were used for the pre-operative shaping of the titanium-mesh panels. The resulting modelled titanium meshes were sterilized and used intraoperatively. The mesh was fixed in place with titanium screws using a minimum three-point fixation method for ensuring spatial stability of the material. For the fixation of mandible fractures, the titanium mesh was bent according to the shape of the basilar border so that it would contain all fragments and the adjacent ends of the fracture. More fixation screws were therefore needed for the immobilization of all fragments.

RESULTS

All the dimensions of the comminuted and atrophic mandibular fractures were accurately restored. The affected segments were sensibly equal with the opposing side when compared with the help of imagistic methods. The functionality was resumed in the cases of mandibular fractures, with adequate healing, absence of persisting mobility in the line of fracture and resumption of proper mastication and deglutition.

Regarding the restoration of facial bone contours the results were excellent for NOE fracture sequelae cases and good for the other fracture cases, ensuring improved symmetry. The projection of the zygomatic bone and midfacial contours were reconstructed due to the possibility of multiple fragment preservation and fixation. Titanium mesh fixation at the level of the zygomato-alveolar crest allowed improved stability and possibility to bridge bone defects or comminution to achieve proper fixation in the anatomically reduced position of the zygomatic bone. The facial appearance of patients improved in all cases. There were no complaints of postoperative diplopia or restricted eye movements. An adequate support for the soft tissues was achieved in trauma-related cases.

For defects following the removal of malignant tumors the plasty had excellent results when the defect involved only the inferior orbital margin, anterior wall of the maxillary sinus and the orbital floor. The extension of the defect to the zygomatic bone body increased the complexity of the shaping process for ensuring good postoperative results. Titanium mesh allowed proper reconstruction of the orbital floor, without complaints of postoperative diplopia. Secondary plasties were needed for further fine adjustments regarding the position of the soft tissue coverage in two (7.69%) cases.

When stereolithographic models were used for the pre-bending of the titanium meshes, not only the results were more accurate, but the operative time was shorter.

None of the titanium meshes was exposed postoperatively.

The good outcomes of titanium mesh reconstruction are illustrated by the help of a case portraying the treatment of a double angle fracture of an atrophic mandible in an edentulous patient (fig. 1-7).

Fig. 1. Patient with bilateral mandibular angle fracture. Note the impossibility of closing the mouth due to bilateral angle fractures.

Fig. 2. Lateral view of the patient with bilateral mandibular angle fracture.

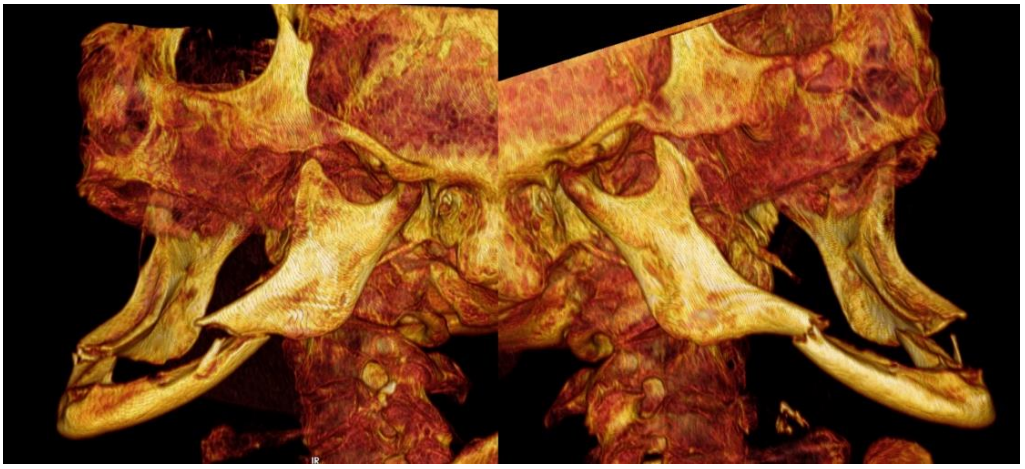

Fig. 3. Lateral left and right view of 3D reconstruction from Computed Tomography (CT) scan. Note the severe atrophy of the mandibular bone and the edentulous state.

Fig. 4. Aspect of the titanium mesh tray modelled after the inferior border of the mandible

Fig. 5. Intraoperative view of the right and left mandibular angle fracture sites fixated with titanium mesh sheet

Fig. 6. Postoperative frontal view of the patient demonstrating the restored contour of the mandible and the ability to close the mouth.

Fig. 7. Postoperative profile view of the patient demonstrating the restored contour and projection of the mandible and the ability to close the mouth.

DISCUSSION

Titanium is considered the most biocompatible metal due to its resistance to corrosion from bodily fluids, bio-inertness, capacity for osseointegration, and high fatigue limit. Titanium's ability to withstand the harsh bodily environment is a result of the protective oxide film that forms naturally in the presence of oxygen (3, 4). The titanium mesh couples the advantages of the material with the ones offered by the geometry of the mesh itself that is purposely designed to allow simple three-dimensional modelling without compromising the stability and support of the structure (5, 6).

Versatility, adaptability, stability and malleability are only a few of the advantages of the titanium mesh. Modelling it according to the defect and giving the titanium mesh a complex form often needed for the reconstruction, eventually results in a structured shape capable of resisting mechanical stress and providing an individualized treatment (7).

In cases of comminuted fractures of the facial skeleton, titanium mesh allows improved containment and fixation of the multiple bone fragments, reconstructs contours, and even bridges regions of missing bony substance. These objectives could not be fulfilled to the same degree by the simple use of multiple titanium plates. For post-ablative defects, titanium mesh can provide an alternative to more complex, time consuming methods of treatment involving the association of bone grafting or composite flap raising with titanium reconstruction plates.

Although the price for the titanium mesh is higher than individual plates, it is offset by using fewer mesh panels than titanium plates otherwise needed by the same procedure.

One of the disadvantages of using the titanium mesh is that for the placement of the implant, an extraoral access is frequently needed, leaving a visible scar. Additionally, the removal of the mesh, when this is needed, is difficult due to the connective tissue growing around and through the structure of the panel (3). At the same time, this behavior is an advantage when the titanium panels are used for the reconstruction of the sinus walls, preventing the intrasinus exposure of the titanium mesh.

Although a considerable number of treatment methods exist for comminuted and atrophic mandibular fractures, choosing the best procedure remains a challenge due to the high infection rate, unsatisfactory mandibular shape and loss of bone fragments.

In cases of mandibular fractures with severe atrophy of the mandibular bone, the use of the “classic” titanium reconstruction plates are not indicated since it involves the decollement of the periosteum form a large surface of the bone, which can lead to osteitis/osteomyelitis. Angle fractures of the atrophic mandibles are another therapeutic challenge since the mouth guard fixation with circum-mandibular wires is not effective for this region. In comminuted mandibular fractures, periosteal stripping can lead to decreased vascularity and loss of the intermediate bone fragments. Additionally, it is extremely challenging to provide adequate stability and fixation for the multiple fragments by using simple titanium plates or a reconstruction plate, while also ensuring the correct mandibular body length (8).

In our opinion, the advantage of using the titanium-mesh in cases of comminuted mandibular fractures, is that by modelling

it around the lower border of the body of the mandible in a "U" shape, and going up just a few millimeters on the lingual side, the resistance of the panel is greatly increased while also leaving the lingual periosteum attached on the bone fragments, ensuring their vascularization and thus a favorable healing. On the vestibular aspect of the mandible, the titanium mesh is modeled to the shape and form necessary to envelop and preserve all the bone fragments. The characteristic design with multiple holes makes the reduction and fixation with screws possible for all the bone fragments. The good results that we obtained in our study are consistent with the results of other authors that have used titanium mesh for the reconstruction of the mandibular contour due to its semi-rigid fixation properties and the need for limited periosteal stripping (7, 8).

Comminuted fractures of the midface can result in important facial deformation if not properly reconstructed. The three-dimensional analysis of CT (Computed Tomography) or CBCT (Cone Beam Computed Tomography) scans offers important information regarding the number of fragments, degree of displacement, the presence of soft tissue entrapment or foreign bodies (9) and helps select the most appropriate treatment method (10).

Restoring the face symmetry after zygomatic complex or midface fractures can be difficult if there is comminution at the zygomatic-alveolar process. The three-dimensional modeling of the titanium-mesh panel and fixing it in such a way to reestablish a correct and stable vertical dimension allows an accurate rehabilitation of the zygomatic geometry, symmetric with the opposing side. Other authors also underline the importance of titanium mesh fixation for achieving improved fixation and individualized treatment in complex midface fractures (11). Although the bone frame support is restored by using the titanium mesh, it is important to also address the soft tissues by repositioning sutures fixed to the periosteum or to the titanium mesh, placed at the end of the surgery. Omitting to perform soft tissue rearrangement might lead to tissue ptosis and an aged facial appearance (12, 13). Corrective surgery can be performed later by inserting barbed threads for soft tissue lift (14), or by autologous fat transfer in order to restore the lost volume of the midface (15).

Due to the complex geometry of the region, comminuted NOE fractures need a material capable of covering a larger surface. The titanium mesh can stabilize the smaller bone fragments and by modeling it, due to its malleability, it ensures an excellent amendment of the region. This is consistent with the favorable results achieved in our study regarding NOE sequelae treatment using titanium mesh. Repositioning of the detached medial canthal ligament by anchorage to the modelled titanium mesh is also possible and ensures a good definition of the bony contour in the paranasal region and a normal intercanthal distance (11). In our opinion, an adequate exposure of the sunken region for the placement of the titanium mesh could be the most challenging aspect to minimize scars and damage to the overlying soft tissues when addressing sequelae of NOE fractures.

NOE fractures are frequently associated with fractures of the anterior wall of the frontal sinus. The purpose of the treatment is to protect the intracranial components, the deterring of cerebrospinal fluid leak, and the rehabilitation of the facial contour.

The management of the comminuted fractures of the frontal sinus is difficult because the bone fragments are too small for fixing them with wires or plates. The titanium mesh is one of the most used material for the anatomic rehabilitation of comminuted frontal sinus fractures generating minimal inflammatory response and imaging artefacts (16).

In many cases of malignant tumors of the midface, the resection often implies a maxillectomy which leads to deformities such as diplopia, depression of the cheek, as well as articulatory, masticatory and swallowing disorders. The timing and method of reconstruction is in relation with the histology and considering the need for postoperative radiotherapy. If a delayed reconstruction is decided, the defect can be initially addressed using an obturator prosthesis and/or an epithesis (17). Frequently, the main treatment for basal cell carcinoma is surgical resection. For this reason, in our case series, the reconstruction was performed at the same time with tumor removal.

Composite flaps or multiple flaps are needed for restoring the complex anatomy of the maxillary bone and for achieving a restoration of both bone and soft tissue. In our opinion, titanium mesh allows a good restoration of midfacial contours following the removal of malignant tumors. The reconstruction implies the shaping of the titanium-mesh panel to rebuild the missing bony architecture, but also ensuring an adequate coverage of the titanium mesh with a soft tissue flap. Considering the extent of the defect, several flaps can be used for the reconstruction. The frontal flap with a medial or lateral pedicle is a good option in the absence of sufficient local tissues for a genian flap coverage. For more extended defects, free flaps are needed to achieve adequate closure (1).

The composite temporo-parietal ostheofascial flap is an option for reconstructing bone defects at the level of the zygomatomaxillary complex and orbital floor. In this case, titanium mesh can be used for fixation of the composite flap, but also for the reconstruction of the skull defect at the donor site (18). When performing the reconstruction of the resected orbital floor, it is important to adequately restore the shape of the inferior wall, but also the correct level of the transition area located at the intersection of the medial orbital wall and the orbital floor. This medial bony strut is considered important in establishing the proper orbital volume and it is usually preserved for best functional results when performing bone decompression surgery for Graves orbitopathy (19, 20, 21).

Although the titanium mesh can be modeled to fit the bone defect perfectly, this objective is arduous to accomplish by the manual intraoperative shaping of the mesh due to the reduced operative access and the long time needed (22). This detriment can be overcome with the help of a stereolithographic model that can be used for the preoperative shaping of titanium-mesh panels when faced with complex three-dimensionally shaped defects that require increased accuracy and elaborated shaping of the mesh that would be difficult to perform intraoperatively. The titanium meshes pre-modelled to the exact anatomy of the individual's mirrored healthy side are then sterilized and used intraoperatively.

From our experience, the procedure decreases surgery time and increases the accuracy of the reconstruction. Still, it is stated by some authors that the repeated bending of the material in trying to achieve

the optimal shape could decrease the mechanical strength of the mesh (7). This aspect is of interest when the titanium mesh is used to reconstruct the mandible since the masticatory forces involved in the mandibular region are greater than forces acting in other facial regions. Prospects involve the use of individualized 3D printed titanium mesh trays by direct metal laser sintering that could improve the accuracy of reconstruction even more by eliminating the manual modelling of the titanium mesh (7).

CONCLUSIONS

From our study we conclude that the titanium mesh panel is a material capable of providing an individualized treatment for reconstructing the craniofacial skeleton in cases where large defects need to be rectified, for ensuring stability in cases of comminuted fractures and for providing soft tissue support and the rehabilitation of facial symmetry. Furthermore, the use of stereolithographic models warrants the perfect fit of the individualized panels and shortens the operative time.

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NEWS

ETHYL GLUCURONIDE - A USEFUL BIOMARKER FOR ETHANOL INTAKE IN FORENSIC FIELD

Alcohol consumption has an important role in most of the forensic deaths, starting with acute alcohol intoxication as a cause of death and going on through many of the fatal accidents (road and occupational) where alcohol intake has its own influence. This is why ethyl alcohol determination in blood specimens is one of the most requested analyses in forensic field. A group of researchers from the Institute of Legal Medicine in Modena (Italy) investigated the role of ethyl glucuronide (EtG) in supporting medico-legal investigations, with the purpose to develop and validate a sensitive procedure for its quantitative determination in order to give a better interpretation of postmortem EtG concentrations in formulating medico-legal conclusions. They analyzed the distribution of this biomarker in different specimens, collected from 21 subjects, : central blood, peripheral blood and liver samples; in 13 of them urine was also available. The specimens were analyzed by liquid chromatography tandem mass spectrometry. EtG levels were higher in the liver samples compared to the blood sampled, and the urine samples (n=13) presented higher EtG levels than the corresponding liver samples, except for 4 cases. Due to the lack of controlled conditions such as the information on the time of alcohol intake and the ingested doses it was difficult to interpret the EtG levels, but the strength of the study was the simultaneous analysis of this metabolite in different matrices from the same subject. (Santunione AL, Verri P, Marchesi F, Rustichelli C, Palazzoli F, Vandelli D, Licata M, Silingardi E, The role of ethyl glucuronide in supporting medico-legal investigations: Analysis of this biomarker in different postmortem specimens from 21 selected autopsy cases, *Journal of Forensic and Legal Medicine* (2017), doi: 10.1016/j.jflm.2017.10.009).

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MULTIFACTORIAL ANALYSIS OF LOCAL AND LYMPH NODE RECURRENCES AFTER CONSERVATIVE OR RADICAL SURGERY FOR STAGE 0-II BREAST CANCER

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MULTIFACTORIAL ANALYSIS OF LOCAL AND LYMPH NODE RECURRENCES AFTER CONSERVATIVE OR RADICAL SURGERY FOR STAGE 0-II BREAST CANCER (Abstract): **Aim.** Multifactorial analysis of local and lymph node recurrences in stage 0-II breast cancer treated by conservative or radical surgery. **Material and methods**: In the interval January 1, 2005 - July 31, 2013, 477 breast cancer patients were assessed by imaging techniques at the Radiology Clinic of Iasi "Sf. Spiridon" Hospital and Radiology Service of the Iasi Regional Cancer Institute. Of these 229 (48%) patients underwent conservative surgery and 248 (52%) patients radical surgery. **Results:** Local recurrences were 2.8 times more frequent in conservatively *vs.* radically treated patients, and lymph node recurrences 2 times more frequent in patients treated conservatively. Breast tumors larger than 3 cm in diameter were at higher risk for local and distant recurrence in lymph nodes. **Conclusions:** Assessment in patients with local and lymph nodes recurrences of the relative risk for developing other types of recurrences (bone, pleuro pulmonary, liver, brain metastases) indicated that these are a risk factor for other types of recurrences, influencing the prognosis of patients. Local recurrences showed a higher relative risk for other types of recurrences than nodal recurrences. **Keywords:** LOCAL RECURRENCE, LYMPH NODE RECURRENCE, BREAST CANCER.

Conservative treatment is indicated breast cancer stages 0, I and II, with breast tumors less than 3 cm in diameter (1). The goal of conservative treatment is to decrease the risk of local recurrence to <1% per year and < 10% at 10 years.

The relative 5-year survival rate for breast cancer by tumor stages: stage 0 and stage I - 100%, stage IIA - 92%, stage IIB - 81% (the American Cancer Society). The main goal of clinical and imaging monitoring of patients operated for breast is the detection of recurrences with intent to improve survival (2-4).

This paper presents a complex, multifactorial analysis of local and nodal recurrences in patients operated for breast cancer stages 0-II and monitored in our institution.

MATERIAL AND METHODS

Between January 1, 2005- July 31, 2013 at the Radiology Clinic of "Sf. Spiridon" Emergency Hospital and Radiology Service of the Iasi Regional Cancer Institute 477 breast cancer patients were assessed by imaging methods. Patients enrolled in this

study had curable stage 0, I and II breast cancer; 229 (48%) patients underwent conservative surgery and 248 (52%) radical surgery. The ultrasound, mammographic, CT and MRI semiological patterns based on which a diagnosis of loco-regional and distant recurrences was made were evaluated.

For statistical processing we used *SPSS 16.00* for Windows to determine the frequency, multiple regression, enter method, regression coefficient, Pearson correlation (statistically significant at $-1 < r < 1$, no statistical significance when $r = 0$), relative risk (RR) assessment, and *odds ratio* (OR, statistically significant when OR> 1), Chi-Square Tests, ANOVA test, test of significance (significant at $p<0.05$) and confidence interval (CI).

RESULTS

At the time of surgery our patients were aged 21 to 86 years, mean age 57.02 ± 0.77 years; most patients belonged to age group 51- 60 years.

Postoperative treatment varied depending on pathological findings and immunohistochemical results. Thus, patients treated conservatively most commonly received chemotherapy associated with radiotherapy and hormone therapy (76.9%), while patients who underwent radical surgery most frequently received chemotherapy associated with hormone therapy (78.2%). Although radiotherapy is mandatory in the treatment of patients operated conservatively for breast cancer, 13 patients (5.67%) did not receive it (most often due to patient refusal).

Both local and lymph node recurrences were more frequent in patients treated conservatively. Of the 229 patients who underwent conservative surgery 31 patients (13.5%) had local recurrence and 11 patients (4.8%) lymph node recurrences. Of the 248 patients treated radically 12 patients (4.8%) had local recurrences and 6 patients (2.4%) lymph node recurrences (tab. I).

Most nodal recurrences occurred within the first 2 years after surgery in patients operated conservatively (81.9%), and after 3 to 7 years in the patients who underwent radical surgery (66.6%).

The location of nodal recurrences differed depending on the type of surgery. In the patients who underwent conservative surgery nodal recurrences were: axillary - 6 cases (54.5%), supraclavicular - 2 cases (18.1%), axillary and supraclavicular - 2 cases (18.1%), and mediastinal 1 case - (9%).

TABLE I
Distribution of cases by type of recurrence and minimum/maximum/average disease-free interval

Surgery type	Recurrence	No. cases (%)	Minimum disease-free interval (years)	Maximum disease-free interval (years)	Average disease-free interval (years)
Conservative	Local recurrence	31(13.5)	<1	13	2.8710
	Lymph node recurrence	11 (4.8)	<1	9	2.4545
Radical	Local recurrence	12 (4.8)	<1	8	2.921
	Lymph node recurrence	6 (2.4)	1	8	4.831

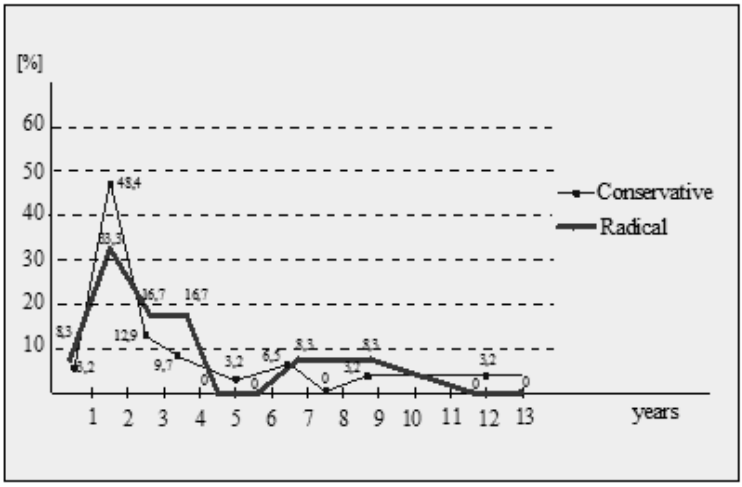

Fig. 1. Distribution of local recurrences according to disease-free interval

In patients receiving radical surgery lymph node recurrences were: supraclavicular - 3 cases (50%), axillaries - 2 cases (33.3%), and mediastinal - 1 case (16.6%).

For all recurrences the following data were extracted from the pathology reports after the first surgery for breast cancer: initial breast tumor size (in cm), number of removed lymph nodes, and the number of positive lymph nodes after the initial surgery (tab. II).

TABLE II

Correlation between recurrences and initial tumor size, number of excised lymph nodes and number of positive lymph nodes

Descriptive Statistics	Minimum	Maximum	Mean		Std. Deviation
	Statistics	Statistics	Statistics	Std. Error	Statistics
Tumor size (cm)	0.40	9.00	3.0482	0.21454	1.60544
Number of excised lymph nodes	0.00	27.00	11.9643	0.76233	5.70475
Number of positive lymph nodes	0.00	16.00	2.2453	0.43429	3.16170

In patients receiving conservative surgery, 17 local recurrences (54.8%) and 4 nodal recurrences (36.3%) were associated with the initial size of breast tumors larger than 3 cm; 12 local recurrences (38.7%) and only two nodal recurrences (18.1%) were associated with more than 5 positive axillary nodes.

In patients with radical surgery, 5 local recurrences (41.6%) and 5 nodal recurrences (83.3%) were associated with the initial size of breast tumors larger than 3 cm; 6 local recurrence (50%) and 3 nodal recurrences (50%) were associated with less than 5 positive axillary lymph nodes.

In patients who underwent conservative surgery there was a statistically significant association between local recurrences and

immunohistochemical data, hormone receptors (HR) and Her-2/neu protein, respectively (p =0.003, Pearson Correlation r = 0.195) for CI = 95%; thus most patients were HR-positive and Her-2/neu protein-negative (17 cases) as well as HR and Her-2/neu protein-negative (8 cases).

Nodal recurrences after breast cancer conservative surgery showed statistically significant associations with tumor stage (p = 0.004, Pearson correlation r = 0.188), 8 cases being stage II and 3 cases stage I, for CI = 95%. Statistical significant associations (p < 0.001, Pearson correlation r = 0.167) were found between lymph node recurrences and immunohistochemical data, for CI = 95%, most cases being HR- and Her-2/neu protein-negative (5 cases) and HR- and Her-2/neu protein- positive (four cases).

In the patients who underwent radical surgery the statistical correlations between local and nodal recurrences and immunohistochemical data were approximately similar.

TABLE III

The risk for other types of recurrences in patients with local recurrence

Risk Estimate	Conservative surgery, local recurrence			Radical surgery, local recurrence		
	Value	95% Confidence Interval		Value	95% Confidence Interval	
		Lower	Upper		Lower	Upper
Odds Ratio for bone MTS	**6.963**	2.454	19.756	**2.750**	0.555	13.631
Odds Ratio for lung MTS	**5.486**	1.389	21.659	**10.905**	2.415	49.245
Odds Ratio for pleural MTS	**20.276**	2.040	201.533	0.951	0.925	0.979
Odds Ratio for liver MTS	**4.375**	1.334	14.351	0.950	0.923	0.978
Odds Ratio for cerebral MTS	**13.067**	1.149	148.574	0.045	0.025	0.079
Odds Ratio for skin MTS	**0.132**	0.095	0.184	0.951	0.925	0.979
Odds Ratio for peritoneal MTS	**0.860**	0.816	0.906	0.951	0.925	0.979

TABLE IV

The risk of patients with nodal recurrence of having other types of relapse

Risk Estimate	Conservative surgery, lymph node recurrence			Radical surgery, lymph node recurrence		
	Value	95% Confidence Interval		Value	95% Confidence Interval	
		Lower	Upper		Lower	Upper
Odds Ratio for bone MTS	**3.007**	0.595	15.188	0.974	0.954	0.995
Odds Ratio for lung MTS	**13.250**	2.797	62.760	0.975	0.955	0.995
Odds Ratio for pleural MTS	**24.000**	3.028	190.250	**48.200**	2.626	884.653
Odds Ratio for liver MTS	0.949	0.920	0.979	**9.480**	0.929	96.712
Odds Ratio for brain MTS	**48.222**	3.993	582.395	0.976	0.957	0.995
Odds Ratio for skin MTS	0.952	0.924	0.980	0.976	0.957	0.995
Odds Ratio for peritoneal MTS	0.952	0.924	0.980	0.976	0.957	0.995

In patients presenting local recurrences, treated conservatively or radically, the relative risk for developing another type of recurrence was estimated by calculating the OR. Data were statistically significant at OR > 1 (tab. III).

In patients with lymph node recurrence, treated by conservative or radical surgery for breast cancer, the relative risk for developing another type of recurrence was estimated by calculation OR (tab. IV).

DISCUSSION

Numerous articles, retrospective and prospective studies have shown no differences in survival when conservative surgery for breast cancer (after proper staging) is compared with radical mastectomy. However, all studies conducted so far have reported a net difference in terms of local recurrences, which are more common after conservative surgery than after radical mastectomy (5-8).

In our study, local recurrences are 2.8 times more frequent after conservative than radical surgery (slightly elevated compared to literature data) (5). Most local recurrences were diagnosed 1 year after surgery, irrespective of the surgical approach (radical/conservative). The longest disease-free intervals were recorded in patients treated conservatively; supporting the fact that conservative surgery for breast cancer presents a long-term risk for the development of local recurrence (9).

Significant differences between patients treated by conservative or radical surgery for breast cancer were recorded in lymph node recurrences.

These were 2 times more frequent in patients treated conservatively, being most often axillary in the patients who underwent conservative surgery and supraclavicular in those who underwent radical surgery (10, 11). No differences were found in the longest disease-free interval (8 years in both cases), conservative and radical surgery being associated with a similar risk for nodal recurrences.

The most frequent local and lymph node recurrences occurred in patients with an initial breast tumor larger than 3 cm in diameter and more than 5 positive axillary nodes.

Immunohistochemical results showed that the risk of local or lymph node recurrence persists over time in hormone sensitive tumors (12, 13).

By calculating the risk of patients with local recurrence of developing in time other types of recurrences we found that patients treated by conservative surgery for breast cancer are at higher risk for other types of recurrence (bone metastases, pleuro pulmonary, liver or brain metastases) than those treated by radical surgery (bone or lung metastases) (14,15).

The same is true for patients with nodal recurrences. By calculating the relative risk of patients with nodal recurrence to develop in time other types of recurrence we found that patients treated by conservative surgery are at higher risk for other types of recurrences (bone, pleuro pulmonary or brain metastases) compared to patients who underwent radical surgery (pleural or liver metastases).

CONCLUSIONS

This study proves that local and lymph node recurrences are more frequent in patients treated by conservative surgery for breast cancer compared to those who underwent radical surgery. Diagnosis and treatment of neoplastic breast nodules smaller than 3 cm decreases the risk of local and lymph node recurrences.

Assessment of the relative risk for developing other types of recurrence in breast

cancer patients with local and lymph node recurrences (treated by conservative or radical surgery) showed that they are a risk factor for recurrence affecting the prognosis (local recurrences had a higher relative risk than nodal recurrences).

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DISPARITIES IN THE ACCESS TO PRIMARY HEALTHCARE IN RURAL AREAS FROM THE COUNTY OF IASI - ROMANIA

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DISPARITIES IN THE ACCESS TO PRIMARY HEALTHCARE IN RURAL AREAS FROM THE COUNTY OF IASI - ROMANIA (Abstract). **Aim:** To identify the factors that may conduct to various forms of social exclusion of the population from the primary healthcare and to analyze health disparities as population-specific differences in the access to primary healthcare in rural compared to urban residence areas from Iasi, the second biggest county, situated in the North--East region of Romania. **Materials and methods:** This research is a type of inquiry-based opinion survey of the access to primary healthcare in rural compared to urban areas of the county of Iasi. Data were collected by face-to-face interviews. There were taken into account the socioeconomic status (education level in the adult population, employment status, family income, household size) and two temporal variables (the interval of time spent to arrive at the primary healthcare office as a marker for the geographical access and the waiting time for a consultation). The study group consisted of two samples, from rural and urban area, each of 150 patients, all ages, randomly selected, who were waiting at the family doctor's practice. **Results:** The study has identified disparities related to a poor economic status assessed through the employed status ("not working" 15% in urban and of 20% in rural).The income calculated per member of family and divided in terciles has recorded significant differences for "high" (36.7% urban and 14.7% rural) and "low", respectively (14.6% urban and 56.6% rural). High household size with more than five members represented 22.6% of the total subjects in rural and 15.3% in urban areas. The assessment of the education level in the adult population (>18 years) revealed that in the rural areas more than a half (56%) of the sample is placed in the category primary and secondary incomplete, whereas the value for secondary complete and postsecondary was 37.3%. The proportion of respondents in the urban areas who have post-secondary education is five times higher than those in rural areas (15.4% vs. 2.7%). The reduced geographical access assessed as the interval of time spent to arrive at the primary healthcare office revealed, on average, 25 minutes in urban versus 75 minutes in rural areas. **Conclusions:** The research outcomes highlight the fact that the population living in rural communities from the county of Iasi, are disadvantaged in accessing the primary health services, with negative consequences on the health status. **Keywords:** ACCESSIBILITY, PRIMARY HEALTHCARE, RURAL, URBAN

A complete and actual definition of the accessibility refers to "the possibility of the user to obtain the needed health care or the health service in the right place and the right time, according to the perceived needs, and assumes the lack of geographical, economic, financial, social, cultural, organizational or language obstacles" (1).

In the Member States of the European Union, the health services access is legislatively stipulated and guaranteed in the EU Charter of Fundamental Rights, Article 33: "Everyone has the right of access to preventive health care and the right to benefit from medical treatment"(2).

The fair and as complete as possible access to a basic package of services is an essential criterion for a well-organized and operating at optimal parameters health system, along with other objectives such as: general coverage, continuity of health care, patient's ability to choose the provider and costs of medical care (3).

Health disparities or healthcare inequalities refer to gaps in the quality of health and health care across various population groups (4).

The improvement of the access to health services represents the first priority objective of the policy adopted by the Ministry of Health from Romania (5). A special concern is focused on primary care because they act as a first point of consultation for all patients within the health care system.

The aim of this study is to analyze health disparities as population-specific differences in the access to primary healthcare and to identify the main perceived barriers to health care access in rural compared to urban areas from Iasi, the second biggest county, situated in the North – East region of Romania.

MATERIALS AND METHODS

This study research represents an inquiry-based descriptive analysis of the degree of access to primary healthcare in rural compared to urban areas of the county of Iasi. Data on socioeconomic variables were collected by face-to-face interviews.

There were taken into account the socioeconomic status (education level in the adult population, employment status, family income, household size) and two temporal variables (the interval of time spent to arrive at the primary healthcare office as a marker for the geographical access and the waiting time for a consultation).

The study group consisted of two samples, from rural and urban area, each of 150 patients, all ages, randomly selected, who were waiting at the family doctor's office. The location was the city of Iaşi and five rural communities inside the county of Iasi.

The database and statistics were performed in *Epi Info* ^{TM} *7*.

RESULTS

The median age was 39 years, with limits ranging between <1 year and 87 years. Distribution by age groups is shown in table I.

TABLE I

Distribution by age groups and residence

Age group (years)	Urban		Rural	
	No.	No.	%	No.
< 10	14	9.3	10	6.7
10-19	15	10	26	17.3
20-29	22	14.7	16	10.7
30-39	28	18.7	27	18
40-49	33	22	30	20
50-59	18	12	14	9.3
60-69	9	6	7	4.7
70-79	8	5.3	12	8
>80	3	2	8	5.3
Total	**150**	**100.0**	**150**	**100.0**

Of the total sample, 24% of the subjects living in rural and 19.3% of those from urban areas are children under 20 years of age.

Gender distribution was similar in urban (male 43.6%; female 56.4%) and rural areas (male 44.7%; female 55.3%). The report male/female was approximately 5/6 in both residence areas.

The assessment of the education level in the adult population (>18 years) revealed that a proportion of 5.3% of the rural patients and 3.3% of the urban patients reported only an elementary school education (tab. II).

The socioeconomic status of the respondents is an important variable associated with the degree of access to healthcare.

TABLE II

Percent distribution of adult subjects by education level and residence

Education level	**Urban**		**Rural**	
	No.	**%**	**No.**	**%**
Elementary	5	3.3	8	5.3
Secondary incomplete	51	34	76	50.7
Vocational, complementary or apprentice level	15	10	20	13.3
Secondary complete	56	37.3	42	28
Post Secondary	23	15.4	4	2.7
Total	**150**	**100.0**	**150**	**100.0**

The employment status was assessed as working or not working (unemployed or pensioner). Comparing the distribution in the two areas of residence, we found a proportion of 15% for "not working" in urban (1% unemployed; 14% pensioner) and of 20% in rural (3% unemployed; 17% pensioner).

The income calculated per member of family was divided into terciles in order to have three categories for the socioeconomic variable:

- high (36.7% urban and 14.7% rural);
- medium (48.7% and 28.7%, respectively);
- low (14.6% and 56.6%, respectively).

The household size expressed as the number of persons that share the same dwelling, as well as the expenses related to it, represented another variable taken into account in assessing the accessibility to primary healthcare, particularly from financial point of view. A household may be made up of one person or a group of persons.

The average number was of 4.2 persons per urban household and 4.4 in rural, respectively.

Families with more than five members represented 22.6% of the total subjects in rural and 15.3% in urban areas (tab. III).

The interval of time spent to arrive at the primary healthcare offices was, on average, of 25 minutes in urban and of 75 minutes in rural areas.

The access within a city is easier, thus a proportion of 38.6% of the investigated patients answered they need less than 30 minutes to arrive at their family doctor (fig. 1).

The average time patients spent waiting to see their health provider varied from less than 15 minutes to more than 60 minutes. Patients sit a long time in the waiting room

either in a rural or an urban practice (fig. 2). Short waiting times (< 15 minutes) have been recorded in less than 10% (7.2% urban, 7.8% rural).

TABLE III

Distribution by household size and residence

Persons (no.)	Urban		Rural	
	No.	%	No.	%
1	5	3.4	12	8
2	20	13.3	23	15.3
3	23	15.3	16	10.7
4	46	30.7	38	21.4
5	33	22	33	22
6	10	6.7	9	6
>6	13	8.6	19	16.6
Total	**150**	**100**	**150**	**100**

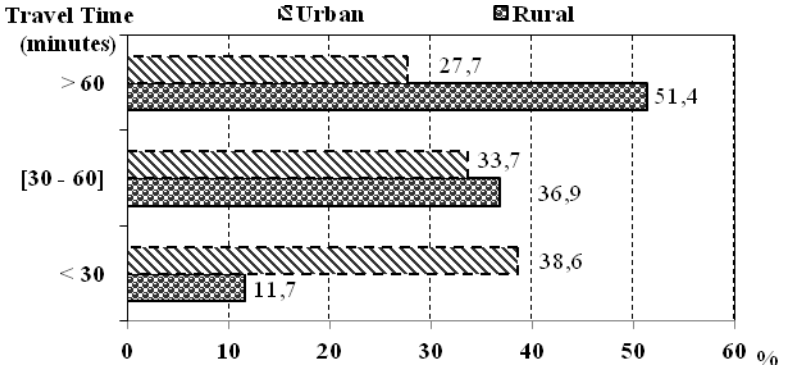

Fig. 1. Interval of time to arrive at the family doctor's office

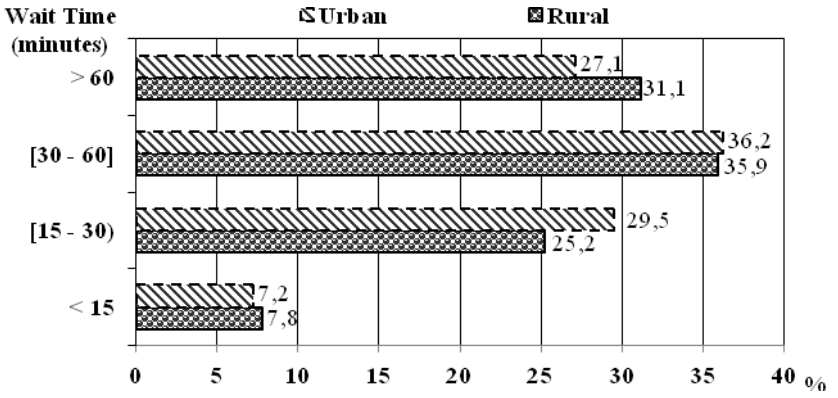

Fig. 2. Waiting time for a consultation at the family doctor's office

DISCUSSION

The comparison of the age group distribution between the two areas of residence highlights that the population who access the healthcare in rural areas is somewhat younger in rural, where one of four patients is under 20 years, compared with urban, where one of five is under 20 years, but neither difference is statistically significant. The proportion of the elderly (>60 years) who needed a consultation to a family doctor office was higher in rural (18%), compared to urban areas (13.3%).

Gender distribution shows a higher addressability among female compared to male patients, for both residence areas, but without significant differences ($p<0.05$).

The assessment of the education level in the adult population (>18 years) revealed that in the rural areas more than a half (56%) of the sample is placed in the category primary and secondary incomplete, whereas the proportion for secondary complete and postsecondary was 37.3%.

The differences in education between the urban and rural areas are more obvious when it comes to post-secondary education; the proportion of respondents in the urban areas who have post-secondary education is five times higher than those in rural areas (15.4% vs. 2.7%). The respondents in urban areas, not surprisingly, have a higher level of education than respondents in rural areas.

Low-income families were four times numerous among rural patients, compared to those living in urban areas (56.6% vs. 14.6%), whereas high-income rural families represented one third of the corresponding value from urban (14.7% vs. 36.7%). These differences are statistical significant ($p<0.01$)

In the same time, the proportion of the unemployed and pensioners was higher (20%) among people living in rural communities, than the value recorded in the city of Iasi (15%).

These aspects should be taken into consideration in the evaluation of the accessibility, being well-known the fact that the degree of poverty of a population correlates directly with a poor economic status and inversely with the educational level, and that both influences the optimal access to the healthcare (6).

Households made up of a single person are twice frequent in rural compared to urban environment (8% and 3.4%, respectively), the difference being significant ($p<0.01$). One of five households in rural (22.6%) had six or more persons; the corresponding percentage found in urban areas was of 15.3%.

The access to medical care in rural areas can be limited due to the dispersion of the primary care practitioners. The spread of the human communities correlates with the distance between health care facilities and dwellings and, in consequence, 51.4% of the patients of villages travel more than one hour to meet their family physician. The average interval of time to arrive at the primary healthcare practice was three times higher in rural compared to urban residence areas ($p<0.01$).

Another variable analyzed in this research was the waiting time to see a physician. A long time is unpleasant because the patient is often anxious about the appointment, in pain, or worried (7). One of three patients in both residence areas was waiting on average an interval of 30-60 minutes.

More than a quarter of the subjects of the two studied samples complained about long waits of more than 60 minutes.

In case of patients who often have been

kept waiting a long time by their healthcare providers, there is the risk to postpone the consultation if it is not a life-threatening illness or a severe injury. For waiting time, no significant differences have been recorded between the studied residence areas. Long waiting time reduces the accessibility to healthcare and leads to dissatisfaction among patients.

CONCLUSIONS

The identified factors that may conduct to various forms of social exclusion of the population from the primary healthcare in rural compared to urban residence areas from the county of Iasi are:

- *poor economic status* assessed through direct variables (unemployed status, low family income, high household size with more than five members) and an indirect variable – the low level of education;
- *reduced geographical access* assessed as the interval of time spent to arrive at the primary healthcare office.

These disparities represent in the same time inequities in the population's access to primary healthcare in rural environment.

The research outcomes highlight the fact that the population living in rural communities from the county of Iasi, are disadvantaged in accessing the primary health services, with negative consequences on the health status.

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EPIDEMIOLOGICAL EVALUATION REGARDING THE ROLE OF CYSTIC FIBROSIS AS A RISK FACTOR FOR CHILD MALNUTRITION

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EPIDEMIOLOGICAL EVALUATION REGARDING THE ROLE OF CYSTIC FIBROSIS AS A RISK FACTOR FOR CHILD MALNUTRITION (Abstract): Cystic fibrosis (CF) is the most common monogenic autosomal recessive disorder with progressive chronic evolution which is potentially lethal. Poor growth is a characteristic of children suffering from cystic fibrosis. A poor nutritional status is an independent risk factor for inadequate survival in cystic fibrosis and is associated with disease complications. The appropriate nutritional management is an important part of the treatment so that the patient with cystic fibrosis can achieve normal growth and development and maintain the best possible health status. A balanced diet supplemented with snacks high in fat and calories is necessary to increase the caloric intake in children with cystic fibrosis. Children with cystic fibrosis have higher caloric needs than healthy children of the same age and sex. Malnutrition in CF is multifactorial. Cystic fibrosis is a complex multisystem disorder affecting mainly the gastrointestinal tract and respiratory system. In the past, malnutrition was an inevitable consequence of disease progression, leading to poor growth, impaired respiratory muscle function, decreased exercise tolerance and immunological impairment. A positive association between body weight and height and survival has been widely reported. The energy requirements of patients with CF vary widely and generally increase with age and disease severity. Cystic fibrosis remains a paediatric disorder which is often underdiagnosed but which, if therapeutically managed properly (by means of drug therapy as well as by appropriate physiotherapy techniques), can lead to improved quality of life and, thus, to a bigger life expectancy. **Keywords:** CYSTIC FIBROSIS, MALNUTRITION, CHILDREN, NUTRITIONAL STATUS

Cystic fibrosis (CF) is the most common lethal inherited genetic condition affecting the Caucasian population. One in twenty-five individuals in the UK are carriers of the gene and the condition is inherited in an autosomal recessive manner, which results in an incidence of one in approximately 2,500 (1, 2). CF is less

common in Oriental and black populations. It is estimated that 8500 individuals in the UK have CF (2, 3). Cystic fibrosis is one of the genetic disorders commonly seen in the paediatric pathology. It is an autosomal recessive disease whose incidence in Caucasians is 1 : 2000 – 1 : 2500 in new-borns with a heterozygote frequency from 1 to 25 individuals. The frequency of the disease in the black race is 1/17,000 live new-borns, in mongoloids is 1/100,000 live new-borns and in the Republic of Moldova is 1:1,000-1:1,200 (4).

The study by Farrel combines a variety of methods to determine the prevalence of cystic fibrosis in the European Union. The results of literature reviews, surveys, and registry analyses revealed a mean prevalence of 0.737/10,000 in the 27 European Union countries, which is similar to the value of 0.797 in the United States, and only one outlier, namely the Republic of Ireland at 2.98 (5).

Cystic fibrosis is a complex multisystem disorder affecting mainly the gastrointestinal tract and respiratory system. Intestinal malabsorption occurs in approximately 90% of patients. In the past, malnutrition was an inevitable consequence of disease progression, leading to poor growth, impaired respiratory muscle function, decreased exercise tolerance and immunological impairment (3, 4). A positive association between body weight and height and survival has been widely reported. The energy requirements of patients with CF vary widely and generally increase with age and disease severity. For many young adults requirements will be 120–150% of the age-related estimated average requirement. To meet these energy needs patients are encouraged to eat a high-fat high-energy diet with appropriate pancreatic enzyme supplements. Many patients are unable to achieve an adequate intake as a result of a variety of factors including chronic poor appetite, infection-related anorexia, gastro-oesophageal reflux and abdominal pain. Oral energy supplements and enteral tube feeding are widely used

Improvements in respiratory and nutritional management have led to an impressive increase in life expectancy for individuals with CF. CF, which was once considered a life-threatening disease of childhood is now considered a life-limiting disease of adulthood rather than a terminal childhood illness (1, 2). The median predicted survival is now 35 years and there are now more individuals with CF aged>16 years in the UK than <16 years (2, 3).

Malnutrition in CF is multifactorial. There has been evidence of strong links between improved nutritional status and survival for >30 years. A study that compared patients of two North American CF clinics, one of which treated patients with the then traditional low-fat diet and the second treated patients with a high-fat diet, has found the patients treated with a high-fat diet to be taller and heavier (2, 6). Importantly, this improvement in nutritional status was deemed to be the main reason for a 9-year survival advantage. Since this early study a poor nutritional status has been shown to independently contribute to prognosis (2, 7, 8, 9). Growth failure and wasting are both highly significant independent prognostic indicators of survival. In patients with height < 5th percentile at age 5 years risk of death is significantly increased; this increased risk persists at age 7 years (2, 7, 8). Patients with >85% ideal body weight have a better prognosis at 5 years of age than those with < 85% ideal body weight (2,

8). There is also a positive association between nutritional status and lung function. Conversely, malnutrition results in poor growth, impaired respiratory muscle function decreased exercise tolerance and immunological impairment that results in increased susceptibility to infections (2, 10).

The aims of the nutritional management of CF are that children and adults should be adequately nourished, have normal weight, height, body composition and pubertal development and have optimal vitamin, antioxidant and essential fatty acid status. With increased life expectancy there are constantly new nutritional challenges emerging (2, 11). Pancreatic insufficiency usually develops in infancy and approximately 92% of individuals with CF are pancreatic insufficient by 1 year of age. Approximately 95% of patients in northern Europe will eventually be pancreatic insufficient as a result of an inadequacy of their own pancreatic enzyme secretions (2, 12). With the introduction of newborn screening within the UK and the early identification of milder mutations the percentage of patients with pancreatic sufficiency may increase. In individuals with CF malabsorption is secondary to pancreatic insufficiency. In patients with CF a host of other factors contribute to malabsorption, including a deficiency of pancreatic bicarbonate that reduces duodenal pH an increased loss of bile salts in the stool, an imbalance in the type of bile salts produced, abnormal ion transfer in the gut as a result of the basic defect in the CFTR (Cystic fibrosis transmembrane conductance regulator) impaired mucosal uptake and transport of long-chain fatty acid across the gut wall and altered gut motility (2, 13, 14, 15).

Growth failure is a common presentation of patients with pancreatic insufficient cystic fibrosis. However, full blown kwashiorkor is extremely rare. Cystic fibrosis is also considered to be rare in the South Asian population.

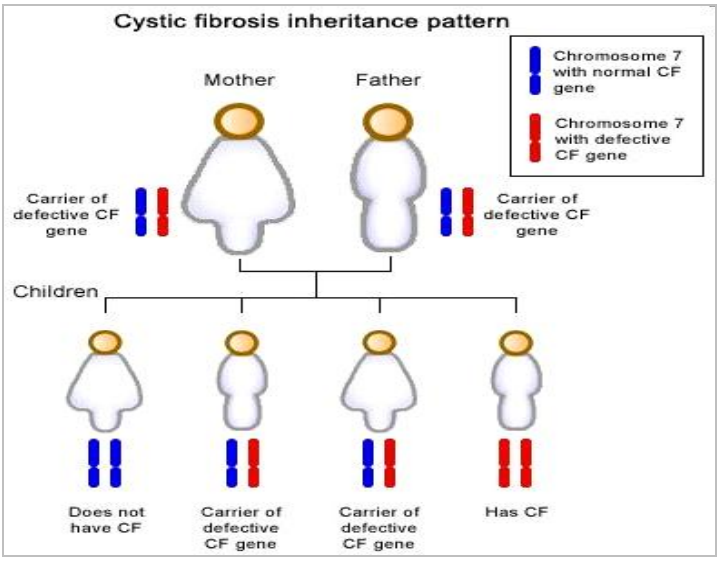

Fig. 1 Cystic fibrosis inheritance pattern

Protein energy malnutrition results in a group of disorders, including marasmus and kwashiorkor. Kwashiorkor, caused by decreased protein availability, results in oedema, muscle wasting, skin and hair changes, irritability, and anorexia (16, 17). In developing countries it occurs mainly as a result of primary protein calorie malnutrition. However, in developed countries, it usually arises as a result of chronic disease rather than primary nutritional deprivation (16, 18). Cystic fibrosis is frequently presents in infancy with failure to thrive. CF is the most common inherited autosomal recessive disease in the Caucasian population, but is considered rare in the Indian subcontinent population (16, 19) and in North Africa.

Epidemiological aspects. According to the National Centre for Cystic fibrosis in Timisoara (where a pilot study was conducted in the 2nd Paediatric Clinic of Timisoara in the '80s, on a number of 10,000 live births), the incidence of the disease in Romania is 1 : 2054 newborns. In terms of survival rate, it can be noted that, in Western Europe, life expectancy was situated around the age of 17-18 in the 70s, in 2005 it increased to about 32, and, at present, due to scientific progress, the patients who were the newborns with CF at the beginning of the 90s can survive well over 40 years of age (4).

The gene for cystic fibrosis is located on the long arm of the 7th chromosome, which encodes the regulatory protein of the cystic fibrosis trans membrane conductance regulator (fig. 1). The gene can undergo multiple gene mutations (about 1300) and it may have more than 300 polymorphic variants, among which the most commonly found in Europe is the Δ F 508 CFTR mutation. (20) F508del accounts for 70% of the cases worldwide3 and for 52–54% of CF cases in Greece. Frequencies vary from a maximum of 100% in the isolated Faroe Islands of Denmark, to a minimum of about 20% in Turkey.

In the last two decades, CF has been increasingly diagnosed in Latin America, the Middle East, and populations derived from the Indian subcontinent that have emigrated to Western Europe, thus implying the presence of CF in significant numbers among the citizens of India and Pakistan who have remained in their homelands (21).

In South Africa alone, it is expected that over 110 African CF babies are born annually. With an overall infant mortality rate of 52 per 1000, CF would only make up between 0.12 and 2.5% of the total.

In India, the CF incidence is estimated to be 1 in 40,000 to 100,000 live births. In Japan, the estimated incidence is 1 in 100,000 to 350,000 live births, but is likely to be higher than anticipated.

The incidence of CF in the Middle East varies according to the ethnic background and the degree of consanguinity. Consanguinity is claimed to be about 65% in the Arab world. Estimates range from 1 in 2,560 to 1 in 15,876 (20, 22). A few mutations in the Middle East are shared with many other regions in the world, i.e. F508del, N1303K, W1282X and 3120+1G >A.

In Uruguay, Ecuador, Colombia, Venezuela and Brazil, the presence of descendants of African origin is important, although its percentage does not exceed 10%. The incidence of CF ranges respectively from 1/3,900 to 1/8,500 neonates in Cuba and Mexico (23).

The incidence of CF, based on derivative populations, is about 1 in 3,500 in North America.

There are no accurate CF prevalence figures for northern African countries bordering the Mediterranean, although small CFTR mutation detection studies have been done in Algeria and Tunisia, showing largely European mutations such as F508del, G542X and N1303K, albeit at different frequencies. Some unique mutations were identified in these populations (20).

Reports from South Africa show the presence of CF in persons of pure African descent, thus demonstrating that the earlier observation of the presence of CFTR mutations in African Americans was not simply due to a mixture of European genes A small proportion of CF patients (<3 %) remains undiagnosed until adulthood (24, 25).

CF affects both females and males in all racial and ethnic groups, but it is common among Native American and Latino population, particularly in areas of Pueblo and Zuni (Colorado). In France, for example, there is a very high incidence of CF in Northwest Brittany and a lower incidence in the South Ethnic. Specific mutations are observed in some populations such as the Nordic mutation 394delTT, the 3905insT mutation in Switzerland, the R1162X mutation in Northeast Italy, and the Eastern Slavic CFTRdele2, 3 (21kb) mutation (20, 26).

Therapeutic aspects. Malnutrition may be aggravated by the presence of anorexia, resulting from gastro-oesophageal reflux and/or cough, respiratory infections and chronic psychosocial stress. The role of dietary treatment is to improve or maintain adequate nutritional status of the patient and break the vicious malnutrition-infection cycle (27). Despite CF being the most common lethal inherited genetic condition affecting the Caucasian population, it remains relatively rare, with approximately 8,500 individuals with CF in the UK (2, 3). Nevertheless, increasing numbers reach adulthood and go through the transitional process. Ensuring a seamless transfer is one of the greatest challenges facing both children's and adult services. Poor growth is a characteristic of children suffering from cystic fibrosis. A poor nutritional status is an independent risk factor for inadequate survival in cystic fibrosis and is associated with disease complications (28).

The appropriate nutritional management is an important part of the treatment so that the patient with cystic fibrosis can achieve normal growth and development and maintain the best possible health status.

A balanced diet supplemented with snacks high in fat and calories is necessary to increase the caloric intake in children with cystic fibrosis. Children with cystic fibrosis have higher caloric needs than healthy children of the same age and sex for the following reasons: higher energy consumption during respiration; energy use in fighting fevers and infections; few energy-producing foods are properly digested and absorbed into the body even if the treatment is optimal. In patients with cystic fibrosis what is recommended is the high calorie and high protein diets, which are rich in fats, and have a normal amount of carbohydrates as well as supplements of fat-soluble vitamins. The hyper-caloric diet can be achieved by: spontaneous intake, nutritional supplements (orally, by nasogastric gastrostomy tube) and parenteral nutrition (4, 16).

The recommended calorie needs is: 1,700–1,950 calories/day for preschool children; 2,300–3,000 calories/day for school children; 2,900–4,500 calories/day for adolescents. Dietary supplements are recom-

mended to malnourished patients with cystic fibrosis and to patients with suboptimal dietary intake despite all the measures taken. Consumption is recommended after meals or at bedtime. Dietary supplements available for patients with cystic fibrosis are: **Frebini** (special, energy enriched food for children between 1-12 years old), **Nutren junior** (especially designed for a nutritionally balanced diet and suitable for children between 1-10 years old), **Nutridrink** (a ready-made nutritional, liquid supplement with milk flavour and other 8 different flavours for increased compliance), **Infatrini** (a high-energy formula in a small volume, with an optimal ratio between macronutrients and micronutrients, and with prebiotics), **Cystilac** (nutritionally complete special formula with prebiotic blend).

CONCLUSIONS

Cystic fibrosis remains a paediatric disorder which is often under diagnosed but which, if is therapeutically managed properly (by means of drug therapy as well as by appropriate physiotherapy techniques), can lead to improved quality of life and, thus, to a bigger life expectancy.

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NEWS

Hp1404 - A NEW ANTIMICROBIAL PEPTIDE

Antimicrobial peptides are active against a variety of microbes including antibiotics resistant strains. In a study by Li Z *et al*, a novel cationic antimicrobial peptide Hp1404 was identified from the scorpion *Heterometrus petersii*. It is an amphipathic α-helical peptide with inhibitory activity against gram-positive bacteria including methicillin-resistant *Staphylococcus aureus*. At low concentration, it is able to penetrate the membrane of *S. aureus*, and at high concentration it disrupts the membrane directly. The bacterium does not develop drug resistance after multiple treatments at sub MIC concentration. Hp1404 has low toxicity to mammalian cells and balb-c mice and it can improve the survival rate of the MRSA infected balb-c mice in the peritonitis model. In conclusion, Hp1404 can be considered to have potential applications as an antibacterial agent (Li Z, Xu X, Meng Let al. Hp1404, a New Antimicrobial Peptide from the Scorpion Heterometrus petersii. *PLoS One*. 2014;9(5):e97539. doi: 10.1371/journal.pone.0097539).

Teodora Vremeră

PALLIATIVE CARE - INTEGRATION MODEL INTO ONCOLOGICAL ASSISTANCE FOR THE PATIENTS OF REGIONAL INSTITUTE OF ONCOLOGY IASI

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PALLIATIVE CARE - INTEGRATION MODEL INTO ONCOLOGICAL ASSISTANCE FOR THE PATIENTS OF REGIONAL INSTITUTE OF ONCOLOGY IASI (Abstract). **Aim**: This paper aims to present a retrospective analysis of activity from Palliative Care Compartment of Regional Institute of Oncology Iasi during 12 months of activity and to propose a model of integration of the palliative care in oncology. **Materials and methods:** Data were collected from 415 patients hospitalized to the Palliative Care Compartment using the computerized database, records and books of existing appointments. **Results:** Data analysis reveals that 170 cases (40%) were aged higher or equal to 70 years and a number of 142 patients (34%) were aged between 60-69 years. Results regarding the provenience of the patients shows that most of them are from urban areas. Gastrointestinal neoplasms were present at about one-third of the patients – 133 cases (32%) and 95 (71%) of these cases had metastases. Most patients - 114 cases (27%) required hospitalization for a period between 8 and 14 days. Palliative care has interdisciplinary relations with other specialties. In 2013 from other specialties were transferred to palliative care a number of 156 patients, most of them (87 cases) from Oncology Department. **Conclusions:** Integrating palliative care in oncology would increase the quality of life of patients, would relieve the other sections or hospitals by patients who need palliative care, would decrease hospitalization costs and would avoid performing aggressive maneuvers at the end of life. **Keywords:** PALLIATIVE CARE, CANCER, INTEGRATION, ONCOLOGY

Palliative care is a holistic approach to relieving the suffering of people with chronic and life ending illnesses. The science of palliative care has undergone rapid developments, most notably with advances in pharmacological interventions to help control chronic pain and distressing symptoms. This vital and essential aspect of optimizing patients'quality of life has undoubtedly contributed to the widening acceptance of palliative care (3, 7).

The World Health Organization (WHO) states that "palliative care is an approach that improves the quality of life of patients and their families facing the problem associated with life-threatening illness, through

the prevention and relief of suffering by means of early identification and impeccable assessment and treatment of pain and other problems, physical, psychosocial and spiritual". WHO sustain that PC interventions should start early in the course of illness, in conjunction with other therapies that are intended to prolong life. Therefore, in the care of advanced incurable cancer patients, there should not be a transition from oncology to palliative care but a continuous adaptation or change of goals (6).

Palliative care treats people suffering from serious and chronic illnesses such as cancer, cardiac disease such as congestive heart failure, chronic obstructive pulmonary disease, kidney failure, Alzheimer's, Parkinson's, amyotrophic lateral sclerosis and many more (3).

Palliative care is interdisciplinary in its approach and encompasses the patient, the family and the community in its scope. In a sense, palliative care is to offer the most basic concept of care – that of providing for the needs of the patient wherever he or she is cared for, either at home or in the hospital. Palliative care affirms life and regards dying as a normal process; it neither hastens nor postpones death. It sets out to preserve the best possible quality of life until death (1, 5).

In most of the world, the majority of cancer patients are in advanced stages of cancer when first seen by a medical professional. For them, the only realistic treatment option is pain relief and palliative care. Effective approaches to palliative care are available to improve the quality of life for cancer patients. The WHO ladder for cancer pain is a relatively inexpensive yet effective method for relieving cancer pain in about 90% of patients (7).

In Romania it is estimated that less than 7,000 of the 4 million people who need palliative care actually receive care. Romanians receiving palliative care report improved pain and symptom control, less anxiety, greater choice in place of death, an overall improvement in quality of life and great satisfaction with services provided. Many aspects of oncological care are under rapid development, but there is a high variability on access to high level quality palliative care and substantial regional (rural/urban) disparities. Recent successes focus on access to pain medication and the perceived need of HCP (Health Care Professionals) for improvements in education and access is growing (2).

In Romania there is no unique way of collaborating relationships with other health care providers for the specialized Palliative Care services providers. Each Palliative Care provider establishes relationships according to their own developing strategy definitely based on the financial backgrounds they have available at one point in their development (4).

The primary mission of Palliative Care services in Romania over the foreseeable future is to improve the quality of life of those people with illnesses not responsive to curative treatment and their families care.

MATERIALS AND METHODS

This paper aims to present a retrospective analysis of activity from Palliative Care Compartment of Regional Institute of Oncology Iasi during 12 months of activity (January 1 to December 31, 2013). Data were collected from 415 patients using the computerized database, records and books of existing appointments.

The inclusion criteria were represented by patient consent, the presence of oncological

diseases (which is also one of the hospitalization criteria) and age over 18 years.

The exclusion criteria were patient refusal, the absence of an oncological disease and age less than 18 years.

RESULTS AND DISCUSSION

Data analysis reveals that 170 cases (40%) were aged higher or equal to 70 years and a number of 142 patients (34%) were aged between 60-69 years. Was registered a number of 109 patients (26%) under the age of 60 years. Must be mentioned that the minimum age was 20 years, the maximum 92 years and the average age of patients was 66.2 years (fig. 1).

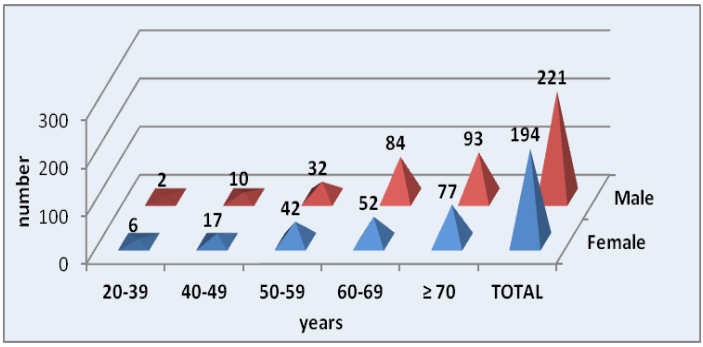

Fig. 1. Patient distribution by age and gender

The distribution based on gender shows a slightly higher preponderance of male patients, which represents a 53% (221 cases) compared with females who have a percentage of 47% (194 cases). This uneven distribution by gender shows a slight increase addressability of males to palliative care service.

Because the Regional Institute of Oncology is located in Iasi, most of the patients - 337 cases (81%) come from this county. The other counties of Moldova present a percentage of addressability between 1% and 5%. These percentages could demonstrates a poor dissemination of information outside of Iasi county regarding palliative care and a low addressability caused probably by the large distance between counties.

Results regarding the provenience of the patients show that most of them are from urban areas. We found a number of 295 patients (71%) coming from urban areas and 120 patients (29%) in rural areas, their ratio being 2.5:1 (fig. 2).

Depending on the oncological condition it was noted that the gastrointestinal neoplasm were present at about one-third of the patients – 133 cases (32%) and 95 (71%) of these cases had metastases. Was found that urogenital cancer was present in a number of 126 patients (30%), and 103 (82%) of these patients had metastases. Other localizations of cancer were respiratory, brain, bone, and skin, in a percentage that varied from 1% to 22% (fig. 3). In the other category was included a number of 35 patients (8%) who had a different type of cancer such as parotidian, thyroidian, glottic, amidgalian, tongue, non-Hodgkin lymphoma, but also the patients who had metastases with unknown starting point (tab. I).

Since most patients were in an advanced stage of illness, data analysis revealed that

more than a third – 302 cases (73%) had metastases. There is a possibility that this percentage to be higher, caused by their lack of diagnosis because they had no symptoms. Most patients who had metastases were among those with gastrointestinal, respiratory and genitourinary cancer. From the total of 302 patients who had metastases we found that 129 cases (43%) were female and 173 cases (57%) male (fig. 4)

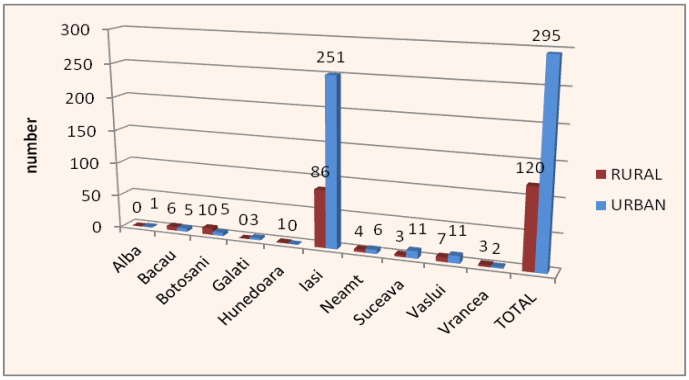

Fig. 2. Patient distribution by county and provenience

TABLE I

Patients' percentage by cancer localisation

Cancer Localization	MTS PRESENT	MTS ABSENT	%
Respiratory	63	27	22%
Gastrointestinal	95	38	32%
Genitourinary	103	23	30%
Cerebral	7	3	2%
Bone	11	4	4%
Cutaneous	4	2	2%
Other	19	16	8%

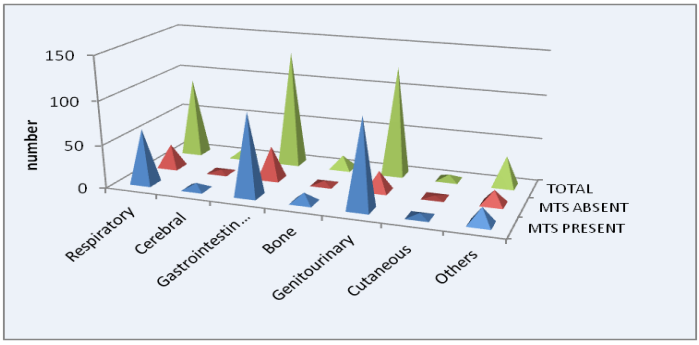

Fig. 3. Patient distribution by cancer localization and metastases

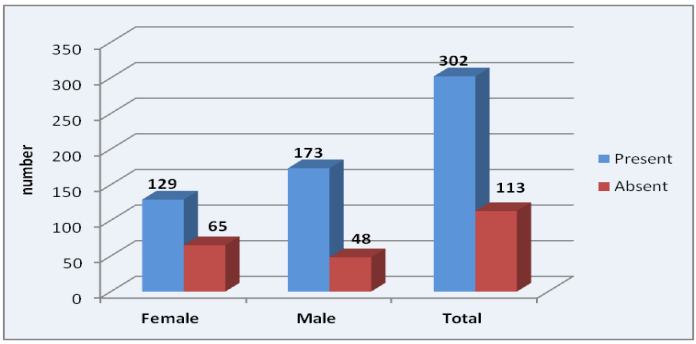

Fig. 4. Patient distribution by gender and metastases

Regarding the duration of hospitalization was noted a wide range of it, between 1 day and 166 days. This fact is explained by the death of the person immediately after admission, after the occurrence of an acute event on the background of severe chronic diseases and on the other hand by the need for a prolonged hospitalization for some complex oncological cases. It was found that a number of 151 patients (36%) had duration of hospitalization up to 8 days. Most patients - 114 cases (27%) required hospitalization for a period between 8 and 14 days (fig. 5). Was registered a total of 96 cases (23%) who required palliative care for a period between 15 and 28 days and a relatively high percentage (13%) for more than 28 days (tab. II). Must be mentioned that the average hospitalization of the patients hospitalized to palliative care was 15 days.

TABLE II

Patients percentage by days of hospitalization

Hospital days	Number of cases	%
≤ 3 days	64	16%
4-7 days	87	21%
8-14 days	114	27%
15-21 days	58	14%
22-28 days	38	9%
≥ 29 days	54	13%

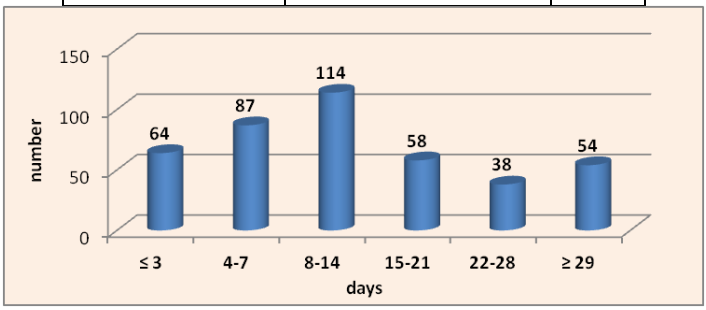

Fig. 5. Patient distribution by duration of hospitalization

Palliative care has interdisciplinary relations with other specialties. As Palliative Care Compartment is a part of Regional Institute of Oncology Iasi, most patients are from this hospital, but also from other hospitals or addressed by GPs.

In 2013 from other specialties within Regional Institute of Oncology were transferred to palliative care a number of 156 patients, most of them (87 cases) from Oncology Department. From Surgery, including plastic surgery, thoracic surgery, gynecologic surgery, oncologic surgery come to palliative care 41 patients, for different purposes such as thoracic drainage, intestinal occlusion and biopsy. The Radiotherapy and the Hematology send a number of 15, respective 13 cases, who needed palliative and supportive treatment.

Except these patients was registered a considerable number of oncologic cases that were hospitalized to palliative care from other hospitals (Neurosurgery Hospital, Pneumology Hospital, Saint Spiridon Hospital, Cuza-Vodă Hospital, Parhon Hospital and others). The family doctors also sent to the Regional Institute of Oncology those patients diagnosed with cancer that needed specialized palliative care.

Being in a interdisciplinary relation the palliative care sent to other specialties a total number of 30 cases, distributed as follows: 14 to Oncology, 9 to Surgery, 5 to Radiotherapy and 2 to Hematology (fig. 6).

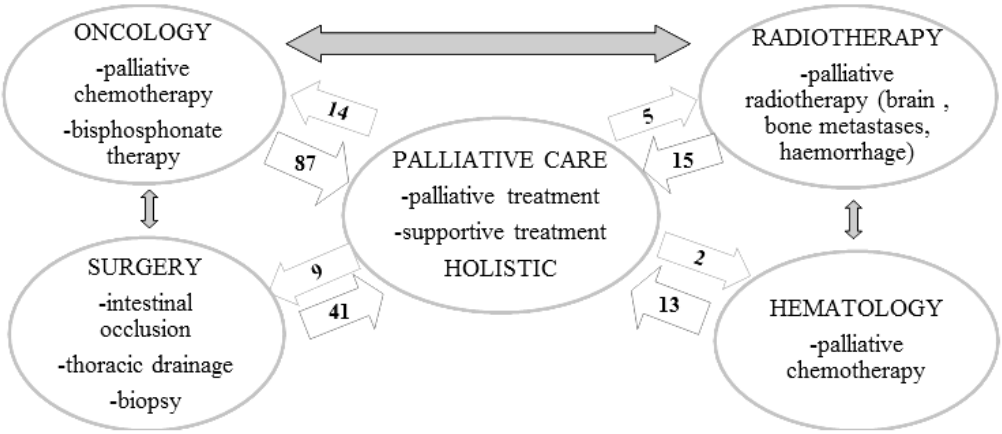

Fig. 6. Interdisciplinary relation between Palliative care and other specialties

CONCLUSIONS

Many aspects of oncological care are under development, but the high variability on access to high quality palliative care and substantial regional disparities demand a structured approach to improve patient care.

Deciding who to serve and restricting services only to these people is perhaps the biggest challenge for palliative care services in Romania in the coming years.

Integrating palliative care in oncology would increase the quality of life of patients, would relieve the other sections or hospitals by patients who need palliative care, would decrease hospitalization costs and would avoid performing aggressive maneuvers at the end of life.

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NEWS

TUMOR NECROSIS FACTOR-α AND INTRA-ABDOMINAL CANDIDA INFECTION IN HIGH-RISK SURGICAL ICU PATIENTS

A recent study conducted by Wójtowicz and collaborators evaluated the influence of genetic polymorphisms on the susceptibility to Candida colonization and intra-abdominal candidiasis, a blood culture-negative life-threatening infection in high-risk surgical ICU patients. They conducted a prospective observational cohort study including 89 patients at high risk for intra-abdominal candidiasis (68 with recurrent gastrointestinal perforation and 21 with acute necrotizing pancreatitis). were analysed eighteen single-nucleotide polymorphisms in 16 genes previously associated with development of fungal infections from patient's DNA by using an Illumina Veracode genotyping platform. Single-nucleotide polymorphisms in three innate immune genes were associated with development of a Candida corrected colonization index greater than or equal to 0.4 (Toll-like receptor rs4986790, hazard ratio = 3.39; 95% CI, 1.45-7.93; p = 0.005) or occurrence of intra-abdominal candidiasis (tumor necrosis factor-α rs1800629, hazard ratio = 4.31; 95% CI, 1.85-10.1; p = 0.0007; β-defensin 1 rs1800972, hazard ratio = 3.21; 95% CI, 1.36-7.59; p = 0.008). The authors established a strong association between the promoter rs1800629 single-nucleotide polymorphism in tumor necrosis factor-α and an increased susceptibility to intra-abdominal candidiasis in a homogenous prospective cohort of high-risk surgical ICU patients and concluded that their finding highlights the relevance of the tumor necrosis factor-α functional polymorphism in immune response to fungal pathogens. Immunogenetic profiling in patients at clinical high risk followed by targeted antifungal interventions may improve the prevention or preemptive management of this life-threatening infection. (Wójtowicz A, Tissot F, Lamoth F et. al. Polymorphisms in Tumor Necrosis Factor-α Increase Susceptibility to Intra-Abdominal Candida Infection in High-Risk Surgical ICU Patients. *Crit Care Med*. 2014 Feb 19. [Epub ahead of print] PMID: 24557424).

Simona Tucaliuc

HYPONATREMIA AT AUTOPSY: CAUSE OF DEATH OR SIMPLY A MARKER OF SEVERITY OF THE UNDERLYING DISEASE

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HYPONATREMIA AT AUTOPSY: CAUSE OF DEATH OR SIMPLY A MARKER OF SEVERITY OF THE UNDERLYING DISEASE (Abstract) **Aim**: To analyze the possible interpretations of hyponatremia detected at autopsy in vitreous humor in relation to the cause of death and/or associated pathology (cause of death or a simple marker of the severity of associated diseases). **Material and methods:** Hyponatremia is the most common electrolytic disorder in hospital practice, considered as a risk factor for hospital mortality. We conducted a 3-year forensic casework analysis in view ofestablishing the relationship of hyponatremia with mortality: hyponatremia as a direct cause of death or hyponatremia caused by the severity of the underlying disease which is the cause of death. **Results**: The forensic cases are not fully reliable. Although the target question has not been answered, our findings plead for the association of hyponatremia with severe diseases (sick cell syndrome) and not for hyponatremia as a cause of death, but this does not seems to be proven either, the percentage of cases with significant hyponatremia increasing from zero in cases of trauma and sudden violent death to 50% in cases of severe chronic diseases. **Conclusions**: The values obtained by us are in agreement with the data in the literature. Our findings plead for the scenario hyponatremia as a marker of the severity of the underlying disease. **Keywords**: HYPONATREMIA, POSTMORTEM BIOCHEMISTRY, CAUSE OF DEATH, SICK CELL SYNDROME

Hyponatremia is the most common electrolyte disorder in hospital practice, a risk factor for hospital mortality (1, 2). It is defined as serum sodium concentration of less than 135 mEq/L, and considered severe when below 125 mEq/L; a decrease in Na^+ level below 120 mmol/L causes mental confusion and a drop below 110 mmol/L causes coma (1, 2, 3). There are some differences in the reported limits, depending on the used laboratory methods. Jetter reported a postmortem stability of Na^+ in the blood within the first 12 hours but Coe showed that there is a decrease by an average rate of 0.9 to 1.0 mEq/L/hour; Na^+ appears stable postmortem in the vitreous humor and in relation with antemortem serum levels (6).

Clinically, hyponatremia is associated with increased mortality and it is stated that this could be an indicator of disease severity, improper management or inadequately performed investigations; some studies present it as associated with severe diseases and not as a cause (3), or associated with diseases with increased mortality (4).

Hyponatremia is often due to hyperglycemia and/or hyperlipidemia in some pathological conditions (ketoacidosis in diabetes) or to increases in plasma proteins (multiple myeloma). It is necessary, therefore, to differentiate between a true hyponatremia and pseudohyponatremia (8). Plasma sodium is different from plasma water sodium; the physicochemical and physiological effects of Na^+ depend on the concentration present in water. The serum contains 93% water and 7% minerals, proteins and lipids. Increases in plasma lipids that occur in primary dyslipidemia type I, IV or V, or in secondary dyslipidemia (nephrotic syndrome, biliary cirrhosis, decompensated diabetes, acute pancreatitis), and increases in plasma proteins (multiple myeloma) can reduce significantly the amount of water per liter of plasma, to 800 -850 ml, which will increase the difference between measured and actual Na^+.

The artifactual increase of Na^+ can be calculated knowing the value of plasma triglycerides: increase of Na^+ (%) = (2.1 x g / dL triglycerides) - 0.6. Measuring the plasma lipids after death is quite difficult with relatively unsatisfactory results, with difficulties in interpreting the values obtained. The influence of stomach contents on postmortem determinations is not clear either. The situation is similar for the postmortem determination of plasma proteins.

The diagnosis of pseudohyponatremia can be confirmed when plasma Na^+ is low (correlation between antemortem and postmortem values, between vitreous humor and blood), natriuria is normal, and triglycerides are higher than 500 mg/dL (serum lactescent). If plasma osmolarity is normal the causes of pseudohyponatremia (hyperlipidemia or hyperproteinemia) are excluded. Measuring osmolarity after death is also difficult, the sample pretreatment methods influencing the results; in clinic serum ultraspinning is used. The concentrations in pre-analytic factors are determined in the postmortem investigations of vitreous humor (8, 9); they increase especially the reproducibility of determinations.

MATERIAL AND METHODS

The electrolyte concentrations in samples of vitreous humors from 567 cadavers were biochemically determined. The sampling of vitreous humor was performed using a sterile syringe by puncturing the sclera in the external angle using a 21G x 1.5" needle. The analyses were performed on samples completely transparent, crystalline, without fragments of tissue vacuumed. The samples of vitreous humor were kept in glass tubes with a rubber stopper, without additives. Some of the samples were analyzed immediately, while others were frozen and analyzed at most on the third day (the cases of autopsies performed at the end of the week). The determination of the concentration of ions Na^+, K^+ and Cl^- in the vitreous humor was performed with the semi-automatic Easylyte analyzer, using the ion-selective electrode technique. Quality control was performed daily using the Tri-level EasyQC control kit.

Tanatochemical picture of 6 cases of hyponatremia (Forensic Service - Vaslui)

Case 1: 75-year-old female found dead

at home. Signs of vomiting. Postmortem examination shows intestinal occlusion complicated by inguinal hernia, as well as a lung cancer complicated by acute bronchopneumonia. Tanatochemical picture showed: urea = 535.33 mg / dL, creatinine 2.68 mg / dL, Na^+ = 108.6 mmol / L, K^+ = 15.56 mmol / L, Cl^- = 79.5 mmol / L. There was no question about the cause of death, but the mechanism of death was unclear.

Case 2: 53-year-old male 53 found dead; the cause of death was acute ethylic poisoning in a person with chronic alcoholism. Tanatochemical picture showed: urea = 19.7 mg / dL, creatinine = 0.35 mg / dL, Na^+ = 106.4 mmol / L, K^+ = 5.93 mmol / L, Cl^- = 88 mmol / L, HbA1c = 6.84% (picture of alcohol abuse). Was ethylic coma or hyponatremia the cause the death? Postmortem exam also revealed dilated cardiomyopathy, hepatic steatosis, and pancreatic sclerolipomatosis.

Case 3: A case similar to the previous one. Biochemical values: urea = 42.8 mg / dL, creatinine = 0.41 mg / dL, Na^+ = 95.2 mmol / L, K^+ = 11.06 mmol / L, Cl^- = 81, 8 mmol / L, HbA1c = 6.28%.

Case 4: 40-year-old male found dead at home. The autopsy established the cause of death as being a hemorrhagic enterocolitis. Postmortem biochemistry: urea = 23.19 mg / dL, creatinine = 0.8 mg / dL, Na^+ = 111 mmol / L, K^+ = 12.14 mmol / L, Cl^- = 97.2 mmol / L. The data do not support the diagnosis of hypovolemic shock.

Case 5: 3-month-old child with chronic pulmonary heart, congestive heart failure due to a congenital cardiac malformation. Postmortem chemical tests revealed: urea = 43.33 mg / dL creatinine = 0.36 mg / dL, Na^+ = 107.8 mmol / L, K^+ = 7.71 mmol / L, Cl^- = 68.9 mmol / L.

Case 6: 67-year-old male with cardiovascular disease who followed treatment with diuretics. Tanatochemistry revealed: urea = 21.49 mg / dL, creatinine = 0.41 mg/ dL, Na^+ = 86mmol / L, K^+ = 6.5 mmol / L, Cl^- = 66.4 mmol / L.

Our study aimed at analyzing the possible interpretations of hyponatremia detected postmortem in vitreous humor as a cause of death or a simple marker of the severity of associated diseases).

RESULTS AND DISCUSSION

Out of 567 selected cases, 207 (36.5%) had a Na^+ level below 135 mEq/L. We grouped the cases according to the immediate cause of death, for example a head trauma patient who died from septic complications, was introduced in the sepsis group and not in the head trauma group (tab. I).

In the cases of deaths from cirrhosis and severe liver diseases (neoplasm), hyponatremia always occurred, two cases presenting hyponatremic dehydration; plasma proteins or triglycerides were not determined postmortem. We identified 22 cases presenting hypertonic dehydration (Na^+ > 155 mEq / L). We also identified in this group 2 cases who died from alcohol withdrawal psychosis, but with constants that did not allow the enlisting in any of the patterns, low Na^+, normal urea, increased K^+, Cl^-> 105 mEq / L.

In the cases of death by drowning hyponatremia was common. All drowning took place in freshwater (possible criterion to be used to differentiate from drowning in salty water). The possibility of exchanging fluids in the alveoli is mentioned in the literature, as well as the possibility of postmortem early diffusion. Hyponatremia was reported in case of water birth. Some authors share the idea of using hypo-

natremia as a marker of submersion in fresh water in both the diagnosis and mechanism of death, asphyxia or hydroelectrolyte disorders (7, 10). In some groups, careful assessment is required to differentiate it from a pseudohyponatremia (see the group with pancreatitis, diabetes, cirrhosis). Our group seemed to be problematic or challenging due to the consistence in forensic cases. The problem with the groups for the study of hyponatremia related to mortality is obvious in case of critical analysis, as shown by some authors (2): retrospective studies on small groups without controls; the control studies show a higher mortality in hyponatremia cases (18).

TABLE I

The relationship between hyponatremia and various causes of deaths

	Average	DS	Max	Min	Hyponatraemia(%)	Significance (%)	Hyponatraemia /total	Significtion /total
Strokes	137.44	4.29	143.8	130.2	36	0	4/11	0/11
Spinal Cord Injury	142.11	1.74	145.6	140.6	0	0	0/7	0/7
Renal failure	141.34	8.44	155.8	133.3	14.28	0	1/7	0/7
Shock	147.2	12.11	173	136	0	0	0/7	0/7
Pulmonary embolism	139.1	8.03	150.2	127.6	28,5	0	2/7	0/7
Head trauma	139.26	5.4	147.8	132.4	25	0	3/12	0/12
Dehydration hypertonic / hypotonic	167.11	11.21	192	156.2	0	0	0/19	6/58
Bronchopneumonia	133.83	9.94	151.5	106.3	43.1	10.3	25/58	21/37
Sepsis	131.72	8.02	144.9	108.6	64.15	11.32	34/53	6/53
Intoxication	137.42	8.01	15.01	114.1	28	11.6	12/43	5/43
Drowning	130.46	6.27	140.1	117.2	75	18.7	12/16	3/16
Hypothermia	136.6	7.43	152.9	120	33	12.1	11/33	4/33
Acute pyelonephritis	135.3	7.73	143.8	124.6	50	16	3/6	1/6
Asphyxia	139.2	7.06	151.1	121.1	23.2	6.8	17/73	5/73
Bleeding	138.3	7.96	147.3	123.8	25	12.5	4/16	2/16
Cirrhosis	127,1	5.75	133.6	121.5	100	50	4/4	2/4
Chronic heart failure	134.37	8.77	144.4	118.8	28.5	4.76	6/21	1/21
Cardiopathy	136.29	9.46	152.1	86	30.3	7.4	41/135	10/135

The cases with "low salt pattern" showed postmortem evidence of associated pathology (patients with multiple and severe chronic organic disorders). The osmolarity of external fluids was mostly determined by electrolytes that can spread into the cell but the osmolarity of cellular fluids was determined mostly by „solutes that are constrained within the cell by virtue of their size and physical characteristics, and by the permeability limits of cell membrans" (adenosine triphosphate, creatine

phosphate, amino-acids, metabolic intermediates including the hexose and trinose phosphates -Conway, 1957 quoted by 11). The compensation of negative charges of the intracellular solutions was performed by cations (especially K^+, but also Na^+).

The amount of non-spreading solutions is normally constant; the balance is achieved between the metabolic activity, usage and the slow migration within the cell. An important factor seems to be the pH that alters the dissociation of some solutions. Conway, 1945 -1960, Brown and Stein, quoted by Flear and Singh showed that "the osmolarity of ECF and its Na^+ concentration (Na^+ and its accompanying anions are the major solutes) is determined by the intracellular osmolality" "Disturbance at this level can cause hyponatremia, and because it has arisen as a result of illness or trauma, affected cells can be regarded as << sick cells>>". We made a new analysis and there were still 128 cases with Na^+ below 130 mEq/L, and excluding the cases of drowning and those with an elevated K^+ level, 75 cases remained; these were cases of severe congestive heart failure, pulmonary tuberculosis, cirrhosis and hepatic steatosis, peritonitis, sepsis and severe respiratory failure.

We found a correlation between the severity and the possible chronicity of diseases and the level of hyponatremia. No correlation between age and hyponatremia was found. Hyponatremia in old people seems rather related to the associated pathology and severity of disorders, with a reduced functional reserve. The cases with heart failure revealed by chronic pulmonary stasis presented reduced hyponatremia; those with anasarca with severe, congestive heart failure showed significant hyponatremia; the cases with pulmonary tuberculosis, even with rapid death through massive hemoptysis showed important hyponatremia according to the data in the literature (Westwater, Stiven and Garry 1939, quoted by 11, found hyponatremia in patients with pulmonary tuberculosis, in degrees according to the severity of the disease; they also found correlations between hyponatremia and prognosis). Cases with significant hyponatremia were included in the groups with cellular distress related to the cause of death (bronchopneumonia, severe sepsis, cirrhosis, congestive heart failure), but there were also hyponatremias in the groups with cellular distress without any unrelated to the cause of death (obvious cause of sudden death and violent death in sick patients).

There is very low percentage of hyponatremia-related mortality. In the literature (1, 2) it is stated that the risk of mortality increases with the severity of hyponatremia, but this has not demonstrated. However, "accepted wisdom is that hyponatremia is a marker of poor prognosis" (2). A critical analysis of the studies regarding hyponatremia shows multiple issues (studies on small groups and without controls). In our group, significant hyponatremia occurred only in cases with of cardiac, respiratory, liver, and kidney decompensation. It was noticed that the estimation of hyponatremia depends on the studied population, (higher prevalence in ICU). Although Flear and Singh's hypothesis of "sick cell syndrome" recently gained scientific support, there are still multiple questions related to this issue, being even called a "Cinderella of metabolic abnormalities"; similar studies on the connection between hyperglycemia and the risk of mortality have been performed lately (2, 11, 13).

We noted that in cases with macroscop-

ic evidence of decomposition where we expected Cl^- levels to be below 105 mEq/L, the levels were higher, around 110 mEq/L (we associated these situations with possible hypoproteinemia with hyponatremia consequences of some anionic deficits – the anionic deficit could induce a decrease in plasma Na^+ and an increase in chlorine); studies are needed on this subject.

An important theme related to hyponatremia with possible forensic implications is that of the neurologic complications and of hyponatremia correction (13) to establish an optimal rebalancing rate with the risk to develop a central or an extrapontine myelinolysis, and of the incidence of brain injury in postoperative hyponatremia with a higher risk in menstruating women (12, 13, 15, 17).

CONCLUSIONS

Given the complexity of every case, our studies show that the determination of hyponatremia is not specific in postmortem examinations; we have cases with the same immediate cause of death presenting hypernatremia or hyponatremia, hypertonic and hypotonic dehydration; there are many factors related to the body, associated pathology, postmortem interval (K^+ increased in decomposition, with accelerated growth in the renal failure), postmortem changes. The values obtained by us are in agreement with the data in the specific literature.

Our findings plead for the scenario hyponatremia as a marker of the severity of the underlying disease, an index of the functional reserve, but the deductive connection to the cause of death cannot be made. We recommend a full postmortem examination and routine biochemical tests, and based on all findings the results must be interpreted critically. The affected cells causing a hyponatremia with hemodynamic and cerebral effects may generate a vicious circle making the interpretation of the rate of the influence of hyponatremia in causing death very difficult.

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NEWS

GLUTEN INTRODUCTION AND CELIAC DISEASE

Celiac disease (CD) is a gluten-sensitive enteropathy with genetic predisposition. The dose and timing of gluten introduction's in the child's diet influence the risk of CD. A prospective study conducted by Ketil Størdal show that the risk of CD in childhood depends on age of gluten introduction and duration of breastfeeding. The proportion of cases of CD was increased in children who received gluten before 4 months age (4.24/1000) and after 6 months of age (4.15/1000) than in those with gluten introduction between 4 and 6 months of age (3.68/1000). Also, the same study showed that the risk of CD is great for kids at that breastfeeding was prolonged after the age of 1 year. These results are useful for developing guidelines for infant feeding (Størdal K, White RA, Eggesbø M. Early feeding and risk of celiac disease in a prospective birth cohort. *Pediatrics*. 2013; 132(5):1-8).

Cătălina Luncă

VARIABLES OF JOB SATISFACTION IN MEDICAL ASSISTANT PROFESSION

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VARIABLES OF JOB SATISFACTION IN MEDICAL ASSISTANT PROFESSION (Abstract). **Aim:** To identify the key favorable issues, showing a high degree of job satisfaction, and also the adverse issues that may affect the work performance among medical assistants. **Material and methods:** This research is a type of inquiry-based opinion survey carried out by administering a self-managed, anonymous questionnaire, consisting of five sections with 25 items. The study group included 175 medical assistants from all specialties, working in public hospitals in the city of Iaşi, who answered the questionnaires. A number of 167 subjects have responded, the return rate being of 95.4%. The respondents were asked to indicate the amount of agreement or disagreement on a typical five-level Likert scale. **Results:** The study has identified some positive aspects: positive perception of the medical assistant profession (76.6%); concern about personal growth and career development (86.3%); good relationships established with other colleagues (71.2%), and some negative aspects: inappropriate work conditions and equipments (70%); the income compared to the volume of work was perceived by majority as an important source of dissatisfaction (80.8%); willingness to work abroad (53.9%). **Conclusions:** The findings of the present research focused on the variables of job satisfaction in the medical assistant profession and should be a real concern for managers, because the job dissatisfaction may affect the employee's productivity. **Keywords:** JOB SATISFACTION, MEDICAL ASSISTANT, QUESTIONNAIRE.

Job satisfaction is a major concern for managers because it is generally accepted that a satisfied employee would have more professional achievements (1, 2). It has been demonstrated a direct link between lack of performance and job dissatisfaction (3). The state of satisfaction/ dissatisfaction is a consequence of the degree of motivation to undertake a certain activity; one feels satisfied when one has reached the proposed target and dissatisfied when that did not happen.

Zamfir(4) estimates that job satisfaction encompasses the following dimensions: economic satisfaction, based on salary and obtained material benefits; satisfaction related to the work itself (working conditions, equipments and the actual work content); psycho-social satisfaction, provided by: the relationship with colleagues and the

head of the department, the opportunities for career development, the recognition of work's results and the adopted organizational policy.

A medical assistant is an important member of the health care delivery team by performing clinical and administrative tasks, and supporting the work of physicians and other health professionals. He also represents the link between the medical doctor and the patient, being of a vital importance to the success of the medical practice.

This research is a type of inquiry-based opinion survey carried out by administering a self-managed, anonymous questionnaire, consisting of five sections with 25 items. The aim was to identify the key favorable issues, showing a high degree of job satisfaction, and also the opposite issues that may affect the work performance among medical assistants. The employees have been explained the importance of the study and that the purpose is to improve their activity and not to introduce punitive measures.

MATERIAL AND METHODS

In the design of the questionnaire there were taken into account the following criteria for the assessing the employee satisfaction: the career decision, the work itself, seen as a set of activities; the concern of the employee for the career development; the working conditions and environment; the relationship with the colleagues, the head of the department and other high levels of authority in the hierarchy of the health organization; the recognition of the work's results and performance; the remuneration system and the income.

The questionnaire pre-testing was performed on a total of 50 subjects. The study group included 175 medical assistants working in public hospitals in the city of Iași, who answered the questionnaires. A number of 167 subjects responded, the return rate being of 95.4%.

The respondents were asked to indicate the amount of agreement or disagreement (from strongly agree to strongly disagree) on a typical five-level Likert scale (1= strongly disagree; 5= strongly agree; 6= don't know). After the questionnaire was completed, each item has been analyzed separately. Responses were processed by counting the grades of the same type. The database and statistics were performed in *Epi Info*™ *7*.

RESULTS

The sample included medical assistants from all specialties, with the following structure: medical specialties 81 (48.5%), surgery 70 (41.9%), radiology and clinical laboratory 16 (9.6%). The average age was 38.2 ± 7.3 years, with limits ranging between 24 and 59 years (tab. I).

Gender distribution shows a high percentage of female, 157 female nurses (94%), compared to 10 male (6%). The report male/female was approximately 1/16. The choice of a medical profession was perceived by about three quarters of respondents (76.6%) as a source of high or very high satisfaction. Around two thirds of medical assistants taken in the study declared that they were satisfied (31.7%) and very satisfied (33.5%) of their current job. The proportion of the totally dissatisfied and very little contented was 14.4%. In this respect, the activity effectively performed by the studied subjects is considered to be: dynamic and stimulating in 130 cases (77.8%); routine in 28 cases (16.7%). Nine medical assistants did not give a conclusive answer ("do not know", 5.4%).

TABLE I
Distribution by age groups

Age group (years)	No.	%
< 30	16	9.6
30-39	79	47.3
40-49	60	35.9
50-59	12	7.2
Total	**167**	**100.0**

The degree of concern for self-improvement and career development was declared as being very high by 55.7% of respondents, respectively high (30.6%). The working conditions and environment represented another variable for the employee satisfaction. The general atmosphere in the department/ hospital was assessed positively by half (49.1%) of subjects and the concrete working conditions, equipments were considered to be satisfactory by only 28.1% (tab. II).

TABLE II
Assessment of satisfaction in relation with the working environment

In what measure are you satisfied of the:		Score					
		1	**2**	**3**	**4**	**5**	**6**
General atmosphere from the care unit/ hospital?	No.	15	20	48	50	32	32
	%	8.9	11.9	28.7	29.9	19.2	19.2
Work conditions and existing equipments?	No.	31	32	54	27	20	3
	%	18.5	19.2	32.3	16.2	11.9	1.9

Two types of relationships at the workplace may influence the degree of job satisfaction: those with other colleagues and with various levels of authority from the health organization. In the studied sample, a proportion of 71.2% declared themselves satisfied about the relationship with other colleagues, indicating scores of 4 (agree - 33.5%) and 5 (strongly agree - 37.7%) for this item.

The recorded answers for the item "Did your colleagues, when appropriate, recognize and appreciate your effort/ merits?", were positive in half of cases (56.3%). A proportion of 7.2% answered "don't know". Other scores were awarded as follows: 1 - strongly disagree, 9 subjects (4.8%); 2 - disagree, 18 subjects (10.8%); 3 - neither agree, nor disagree, 35 subjects (20.9%).

The interpersonal relationships at the workplace have been also analyzed in terms of their involvement and support provided in difficult situations. Approximately two thirds (64.7%) of the medical assistants consider that their colleagues would strongly help them in critical moments. In the same time, the relationship with the boss/ supervisor was considered to be satisfactory in 64.1% of cases, this contributing to an increased efficiency of the performed activity (tab. III).

The activity of the hospital management

team was favorably assessed by half of the surveyed medical assistants (54.5%), the share of strong or quite dissatisfied representing about one quarter of total (23.9%).

TABLE III
Perception of the relationship of subordination

In what measure:		Score					
		1	2	3	4	5	6
Your boss recognized and appreciated your effort/ merits?	Nr.	13	17	24	47	55	11
	%	7.8	10.2	14.4	28.1	55	6.7
Your boss supported you in professional and personal problems?	Nr.	8	21	23	36	71	8
	%	3.6	12.6	13.7	21.5	42.5	6.1
In your care unit is it encouraged the freely expression of your own points of view?	Nr.	26	25	37	35	39	5
	%	15.6	14.9	22.2	21	23.3	3

Another variable for the job satisfaction was the income. A proportion of 11.4% of subjects were satisfied with the income in connection to the performed work, and only 5.4% declared themselves very satisfied. The totally dissatisfaction due to an inappropriate income was found in a percentage of 29.9%, meaning that the medical assistants gave the minimum score. The perception "quite dissatisfied" was recorded in almost half of cases (50.9%).

The willingness to work abroad was manifested in a proportion of 53.9%. Approximately one third (37.7%) do not want it, the rest of answers being included in the category "do not know".

DISCUSSION

Nurses are working in all structures and levels of the health system, being by far the largest group of healthcare workers. Due to its specificity (providing health care), the health sector absorbs predominantly female workforce, especially in case of the medical assistants (5). This fact is outlined by the report male/female, of approximately 1/16, found in this research. The distribution by age showed a higher weight in the age group 30-39 years, when the professional performance is high.

A challenging work that gives positive satisfaction, arising from intrinsic conditions of the job itself, represented a key motivator for performance and a reason of job satisfaction in more than three quarters of the sample. In the same time, the findings emphasized the psychological need to achieve and grow, fulfilled by training activities (courses, workshops, peer experience exchange). Thus, a permanent concern for self-improvement and career development was recorded in 86.3% of subjects.

People who get along with coworkers will develop positive interpersonal relationships at workplace, which will fuel the work achievement and the success in the career (1). The findings of the study revealed that the relationships with the colleagues were assessed as a source of: satisfaction (71.2%); recognition of the person-

al efforts/merits (56.3%); support provided in critical moments (64.7%).

Abraham Maslow developed a general theory of human motivation, on the premise that human motivations are based on different needs (6). These were grouped into five categories, ordered under the form of a pyramid, those of the basic level being most pressing (fig. 1). From the perspective of Maslow pyramid, the basic needs could be met by a minimum wage and working conditions that do not endanger health (7).

Positive issues are: the choice of the medical assistant profession and of the subsequent job represent a source of satisfaction; work itself was assessed as being dynamic and stimulating; subjects were highly concerned about self-improvement, personal growth, achievement and career development; good relationships established with other colleagues. *Negative issues* could be: inappropriate work conditions, equipment; willingness to work abroad recorded in more than half of cases; the income compared to the volume of work was perceived by majority as an important source of dissatisfaction.

The existence of good working conditions may not always increase the degree of motivation, but certainly, bad conditions will lead to its reduction (8). In the studied sample the perception of the work itself was significant more favorable compared to the working environment and working conditions.

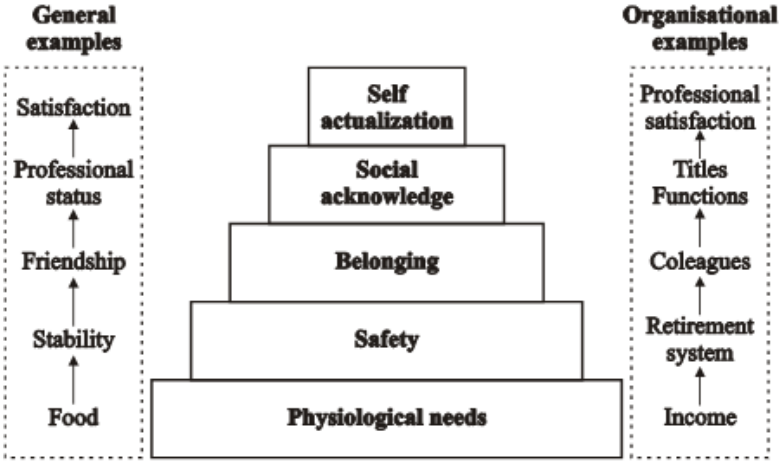

Fig. 1. Maslow pyramid of needs

The research focused, also, on the relation between salary and the work satisfaction, as a premise for the quality of health care provided by the medical staff. In any country, the income was and continues to be considered a strong predictor for job satisfaction (9).

A warning signal is represented by the medical assistants who answered the questionnaire and declared that they were totally or quite dissatisfied in terms of income (80.8%). In the same time, the lack of satisfaction in various degrees was also, founded in relation with the work conditions (71.9%).

The manager of a health organization

must ensure that each employee is enough motivated to be productive, in other words, to provide quality health services. The employees have various needs and different motivations to be met, and the concern for the income is no less honorable than any other motivation from Maslow's pyramid (based on need of safety, need of belonging, companionship or friendship, and need for esteem and social recognition), thus we may conclude that actually all motivations matter in the employee's productivity.

As a consequence, half of the surveyed staff (53.9%) would work in other countries. Thus, the already recorded human capital injection from Central and Eastern Europe to other regions, may lead to a worsening shortage of health human resources (10).

CONCLUSIONS

The study identified some positive aspects, which showed a high degree of job satisfaction, and some negative aspects (both recorded in at least half of cases).

Positive issues are related to good experiences, and these are due to `motivators' - the work itself, achievement, recognition, challenging work, responsibility. Motivators that give positive satisfaction, arise from the intrinsic conditions of the job itself.

Dissatisfaction is rather due to bad experiences and results from the lack of specific factors (good working conditions, appropriate salary and benefits). These are extrinsic to the work itself, and include aspects such as company policies, supervisory practices, or wages/salary.

In order to perform an effective management it is necessary to find a balance between the work satisfaction of the employee and the fulfillment of the organizational proposed goals (providing health care and services).

The findings of the present research focused on the variables of job satisfaction in the medical assistant profession and should be a real concern for managers, because the job dissatisfaction may affect the employee's productivity.

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VIRAL MENINGITES ADMITTED TO AN INFECTIOUS DISEASES HOSPITAL: A RETROSPECTIVE CASE SERIES

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VIRAL MENINGITES ADMITTED TO AN INFECTIOUS DISEASES HOSPITAL: A RETROSPECTIVE CASE SERIES (Abstract):Given its epidemic potential and development of severe forms of disease, viral meningitis (VM) is a serious public health problem. **Aim**: to characterize the main clinical, epidemiologic features, the etiology and treatment of VM cases admitted to the Iasi Infectious Diseases Hospital, in 2012. **Material and methods**: We retrospectively analyzed the medical records of the patients admitted for viral meningitis at the Iasi "St. Parascheva" Infectious Diseases Hospital in the interval January 1- December 31, 2012 (98 cases). The etiologic diagnosis was made by determining the IgM/IgG antibodies against Coxsackie virus and/or West Nile virus in blood/CSF. **Results:** There was a four-fold increase in the number of cases as compared to the average for the years 2009-2011. Most cases (73.5%) were children aged 1 to 14 years. 61.8% of patients were males, 51.7% from urban areas. The most common symptom was headache (85.7%), followed by fever (77.6%), and vomiting (66.3%). Neck stiffness was absent in 28.6% cases. In43.5% of the 39 patients serologically investigated a Coxsackie virus infection was confirmed and 1/20 was positive for West Nile virus; three varicella-zoster virus infections and one mumps infection were diagnosed clinically. 68.3% of the patients received first-line antibiotic treatment. **Conclusions:** The illness mainly affected children, fever and neck stiffness being sometimes absent. The etiology was known in 22.4% of cases; enter viruses being the most frequent causative agent. Most patients received antibiotic therapy. The course was favorable in all cases. **Keywords:** FEVER, HEADACHE, EPIDEMIC.

Viruses are an important cause of acute meningeal infections and sometimes may cause a dramatic clinical picture for which an etiologically focused therapy is not available (1).

Involvement of viruses easily transmitted between humans (digestive or respiratory route) gives them an epidemic potential, especially during the warmer months of the year.

The impact of immunization campaigns (mumps virus), spreading of some arboviroses (West Nile virus) to new areas, climatic and social factors have determined a change in the viral meningitis (VM) profile.

The aim of this study was to characterize the main clinical, epidemiologic fea-

tures, the etiology and treatment of VM cases admitted to the Iasi Infectious Diseases Hospital, in Northeastern Romania, in 2012.

MATERIAL AND METHODS

We conducted a retrospective study based on the medical records of patients diagnosed with VM at "St. Parascheva" Infectious Diseases Hospital of Iaşi in the interval January 1 - December 31, 2012.

Patients of all ages, immunocompetent, with confirmed VM following lumbar puncture and cerebrospinal fluid (CSF) cytological and biochemical analysis were included in the study. Bacterial (culture and latex-agglutination), tuberculous (culture), and non-infectious causes were excluded.

The epidemiological, clinical, hematological and therapeutic variables were also studied. The etiologic diagnosis was made by determining serum IgM and IgG antibodies against Coxsackie virus (VirionSerion kit) and West Nile virus in blood and CSF in collaboration with the Iasi Regional Public Health Institute and Bucharest Cantacuzino Institute.

The collected data were analyzed using dedicated software (Microsoft Excel + Analyze-it) and various statistical tests (mean, Chi^2, 95% confidence intervals). A $p < 0.05$ was considered significant.

RESULTS

A total of 98 cases met the inclusion criteria.

Compared with the interval 2009-2011, when the annual average of diagnosed cases was 24.3, in 2012 a four-fold increase was recorded (fig. 1).

Patients were aged 1 to 36 years, mean age 12.5 years (95% CI: 10.8 - 14.2 years). 73.5% of the cases were children under 14 years of age, with 52.8% of them belonging to the 7-14 years age group (fig. 2).

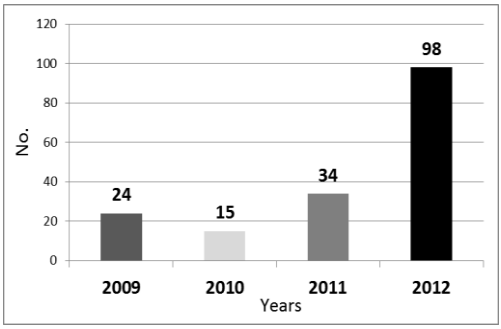

Fig. 1. Yearly distribution of VM cases admitted to the Iasi Infectious Diseases Hospital (2009-2012)

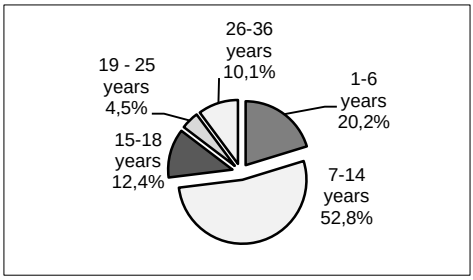

Fig. 2. Age-group distribution of the study patients

The male sex was prevalently affected - 61.8% of the cases, the percentage being slightly higher among patients over 14 years of age (66.7%), but lacking statistical significance ($x^2=0.04$, $p=0.84$). Patients were from both urban (51.7%) and rural areas (48.3%).

The vast majority of VM cases - 74.1%, occurred during the warmer months of the year, between June 15 and September 15 (fig. 3). The highest number of cases was recorded in August - 36 (40.4%).

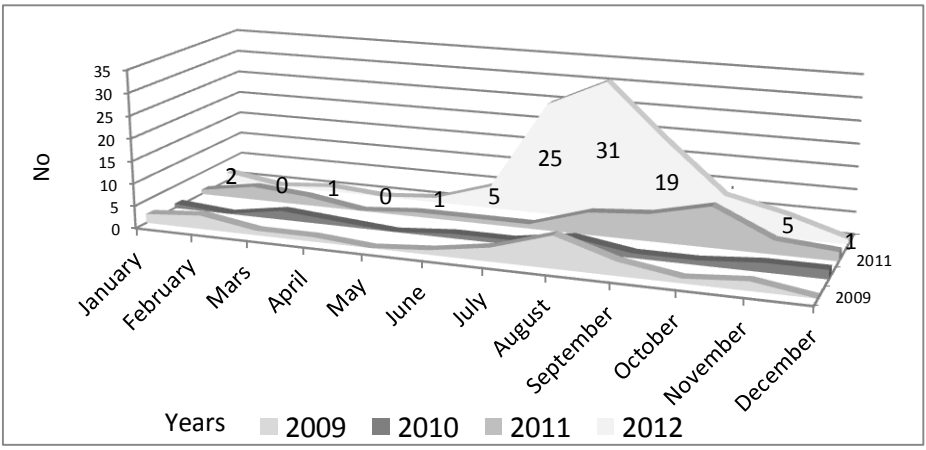

Fig. 3. Monthly distribution of VM cases diagnosed in the interval 2009-2012

The most common symptom of VM was headache present in 85.7% of the patients since the onset. Increases in body temperature (above 37.5^0C) were found in only 77.6% of the patients. Of these, in most cases (79.7%) it did not exceed 38.5^0C. Fever lasted 1- 4 days, average 2.45 days (95% CI: 1.93 - 2.96 days). Two thirds of the patients (66.3%) experienced central-type vomiting (most often before admission - 70.1%). The number of vomiting episodes ranged from 1 to 9 (mean 2.31 - 95% CI: 1.7 - 3.1). Other digestive manifestations were reported in 20.2% of cases: odynophagia - 6.7%, abdominal pain - 11.2% and diarrhea - 2.24%. Other common symptoms were photophobia - seen in approximately one third of cases (35.7%), nonspecific maculopapular exanthema - 19.4%, generalized myalgia - 15.3%, and impaired consciousness (dizziness or drowsiness) - 15.3% of the cases. Few cases presented generalized seizures - 4.1% or lower limb paresis - 1% (fig. 4).

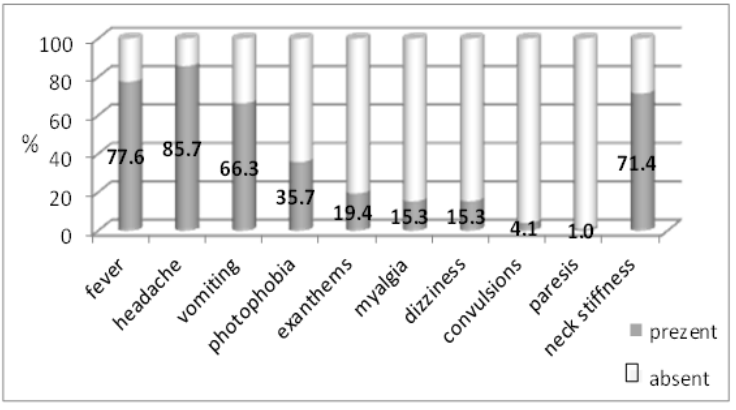

Fig. 4. Signs and symptoms of VM

In nearly one third of the patients (28.6%) neck stiffness and other meningeal signs were mild or absent, possibly due to patients' age.

Blood constants have contributed to the initial etiological orientation (tab. I). Baseline fibrinogen was determined in only 43.8% of the patients, being above 4 g/l in 41% of them.

Thirty-nine patients (39.8%) were investigated serologically for the detection of Coxsackie virus infection. The infection was confirmed in 43.5% of them: 33.3% had IgM and IgG antibodies, and the remaining 10.2% only IgM antibodies. Twenty patients (20.4%) were investigated for West Nile virus infection. The infection was confirmed in one case. Three patients were diagnosed clinically with varicella-zoster virus meningo-encephalitis (2 immune compromised patients with a recent history of herpes zoster and one with chickenpox). One case of mumps meningitis was diagnosed clinically.

TABLE I

Baseline laboratory findings

		Mean	**95% CI**	**Lowest**	**Highest**
Peripheral blood	WBC (/mm^3)	10,911.9	9,454.4 – 12,369.3	4,500	20,400
	PMN (%)	73.2	68.61 - 77.7	46	91
	ESR (mm/h)	27.6	19.9 - 35.2	5	50
CSF	No. leukocytes (/mm^3)	212.5	135.1 - 289.9	32	640
	Lymphocytes (%)	75.2	65.7 - 84.7	39	100
	CSF albumin(g/l)	0.69	0.56 - 0.81	0.24	1.4
	Glycorrhachia (g/l)	0.65	0.57 - 0.70	0.2	1.1
	Glycorrhachia/glycemia	0.63	0.54 - 0.72	0.26	1.07
	Chlorurorrhachia	7.35	7.25 - 7.43	6.7	7.7

Most patients (68.3%) received first-line antibiotic treatment for a period of 48 hours to 10 days, average 4.5 days (95% CI: 3.49 - 5.46). Dexamethasone was administered in 57.3% of the patients for 2 to 10 days (average 6.9 days, 95% CI: 5.43 - 8.43).

The disease had a favorable course in all cases. No death was recorded. The average length of hospital stay was 7.9 days (95% CI: 6.1 - 10.6).

DISCUSSION

Viruses are considered to be the most frequent cause of meningeal infections. The incidence of VM in the US or UK varies between5 and 20 cases per 100,000 inhabitants per year (2, 3). However, given the difficulties in making an etiological diagnosis and the different reporting methods it is likely that the true incidence is underestimated.

In 2012, at the Iasi Infectious Diseases Hospital, the number of VM cases (98) exceeded that of bacterial (42c), tuberculous (12c) or fungal meningitis (3) and was four times higher than the average for the interval 2009-2011. Other counties (Botoşani, Suceava) from Northeastern Moldova reported outbreaks of viral meningitis that started in June 2012 and affected mainly kindergarten children. The highest number of cases was reported in Suceava County, where the incidence of

VM was more than two standard deviations above the regional average (41.78 cases/100,000 inhabitants for the interval May 28-August 12, 2012). Although in the Iasi County the number of cases reported in 2012 has seriously increased as compared to the previous period, the incidence of VM was below the regional average ($8.54\%_{ooo}$).

Both enteroviral and mumps meningitis are more common in children (1), most likely due to the absence of previously acquired immunity (primary infection). A similar situation was found in our study patients, 73.5% of the cases being under 14 years of age.

Sex ratio in VM varies with the involved virus and is in favor of women for herpes viruses (4) and of men for enteroviruses (5) and mumps virus (3); the slight predominance of male patients in our study group may suggest the more frequent involvement of the latter.

Both enteroviruses and West Nile virus commonly cause disease during the warm season. Our group of patients is no exception, 74.1% of the cases being recorded in the interval June 15 – September 15. Compared with 2009-2011, the number of cases began to rise in June, with a peak in August (40.4%) and a significant number of cases reported in September and October (fig. 3).

The association of fever, headache, vomiting, photophobia, and neck stiffness is common in the infectious inflammation of the meninges. However, some authors have shown that sometimes one or more of these symptoms may be absent, making the diagnosis more difficult. In our study, fever was present during the disease course in only 77.6% of the patients, percentage similar to that reported by Gulnar (5) for enteroviral meningitis and Nowak (6) in a series of 43 cases of clear-fluid meningitis (85%). Headache was the most common clinical manifestation in our group of patients, being present in 85.7% of the cases compared with 60-100% in the literature (5, 7). Jet vomiting was described in only 66.3% of our patients, compared to 80% in Turkey (5). Occurrence of transient rash or other digestive phenomena (odynophagia, diarrhea) is common in enteroviral infection, these symptoms often preceding the onset of meningitis (3). These were present in 19.4% of our patients, more often than in other studies (5), and could suggest the involvement of echovirus 9 (3). Neck stiffness was present in 70.8% of the study patients, more frequently than in other similar studies (3, 5, 6).

Baseline WBC count was not very useful in the diagnosis of VM, the average being at the upper normal limit, as reported by other authors (3, 5, 8). Our study showed an increased proportion of polymorphonuclear leukocytes in 72.4% of the patients upon admission. An average ESR of 27.6 mm/h is comparable with the data in the literature (5). The mean levels of CSF parameters were characteristic of viral meningitis and significantly contributed to the diagnosis in most cases.

VM etiological diagnosis rate is relatively low due to its cost and required technical equipment. Recent prospective studies reported the identification of the causative virus in 14.3 - 57.1% of the cases (9, 10). Simion and colleagues found the etiology in only 16.5% of a series of 103 VM cases (11) admitted to the "Dr. Victor Babes" Hospital of Infectious and Tropical Diseases at Bucharest. Under these circumstances, we believe that the identification of the causative agent in 22.4% of VMs in our study (17 cases with Coxsackie virus, 3

cases with varicella-zoster virus, 1 case with mumps virus and 1 case with West Nile virus) is satisfactory, especially if we take into account that not all patients underwent the necessary viro-immunological investigations.

Non-polio enteroviruses, and especially echoviruses and Coxsackie B viruses, are the most common cause of VM (70-85% of the cases) (3, 4, 10). The confirmation of Coxsackie virus B2 as the causative agent in 31 VM cases in Suceava County made us focus the serological investigation in our patients on this viral subtype. Besides the presence of acute phase antibodies against Coxsackie virus in 17 of our cases, the affected age group, the relatively frequent occurrence of skin rash and involvement of the digestive system (odynophagia, diarrhea) as well as seasonality, all plead for a greater involvement of this type of virus in the etiology of VM cases at the Iasi Infectious Diseases Hospital in 2012.

The incidence of mumps meningitis has decreased significantly (4) in areas where routine vaccination of children is practiced, Romania included. In 2012, in our clinic only one case (1.02% of all cases) of mumps meningitis was diagnosed clinically; since some authors estimate that only 50-60% of mumps meningitis is preceded by parotid swelling (1), and immunological tests have not been performed in all our cases, the actual number could be higher.

Arboviruses are an important cause of viral meningo-encephalitis especially in certain geographical areas. An extensive outbreak of neuromeningeal West Nile virus infection occurred in 1996 (12) prevalently affecting adults living in South Romania (morbidity 12.4/100,000 inhabitants in Bucharest). In 2012, only one such case was confirmed in the Iasi County.

Herpes simplex viruses 1 and 2 and varicella-zoster virus can sometimes cause meningitis, especially in women after primary infection or if immunosuppressed (5, 9). In our series one case of varicella meningo-encephalitis in an immunocompetent child and 2 cases of zoster meningitis in immune compromised adults (chemotherapy and therapy with antibody to TNF-alpha) were found.

Differentiation between viral and bacterial infection upon admission based solely on clinical and CSF characteristics is sometimes very difficult. Persistence of uncertainty about the true cause of the disease is leading to the administration (sometimes unnecessary) of antibiotic treatment, which contributes to higher costs, risk for undesirable side effects, development of bacterial resistance, and disruption of endogenous ecosystem (13). This was also highlighted by our research, in which more than half of the patients were treated with antibiotics for at least a few days. Antibiotics were discontinued as soon as a bacterial etiology was excluded by additional laboratory investigations (hematology, microbiology, immunology and virology).

Based on clinical findings and controlled studies, the administration of dexamethasone as adjunctive therapy is recommended in bacterial meningitis, especially in severe forms with altered consciousness (14). In our series, more than half of patients (57.3%) received dexamethasone for an average of 6.9 days.

It is estimated that 98% of VM patients fully recover within 3 months from the disease onset. Rarely, mild cognitive impairment or unusual fatigue may persist over longer periods of time (1). Although no psycho-cognitive tests were performed, our patients were clinically assessed at the

end of the acute phase and one month after discharge, no persistent abnormalities being recorded.

CONCLUSIONS

During the year 2012 there was a significant increase in the number of viral meningitis cases as compared to the average for the previous years, but still below the regional average.

Children were mainly affected. The peak incidence was recorded in August. Fever and neck stiffness were sometimes absent.

Etiological confirmation was possible in 22.4% of cases. The most frequent causative agents were Coxsackie viruses; West Nile, mumps and varicella-zoster viruses were also involved.

Differentiation from other types of meningitis was often difficult at onset prompting the administration of first-line antibiotics in a significant proportion of the cases.

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GASTROESOPHAGEAL REFLUX DISEASE: EPIDEMIOLOGICAL DATA, SYMPTOMATOLOGY AND RISK FACTORS

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GASTROESOPHAGEAL REFLUX DISEASE: EPIDEMIOLOGICAL DATA, SYMPTOMATOLOGY AND RISK FACTORS (Abstract): **Background:** Patients with gastroesophageal reflux disease (GERD), present a typical symptomatology with heartburn and regurgitations in about 80%. **Objective:** This study will assess the prevalence of gastroesophageal reflux disease and some demographic features of the study group. It will also be assessed the sensitivity and specificity of GERD Questionnaire and the risk factors of the disease. **Material and methods:** For the current study 426 patients were selected who were admitted to a tertiary gastroenterology-hepatology hospital in Athens, Greece, complaining about having the common symptoms of GERD (heartburn and regurgitations). The study period was January 2008–March 2011. Positive diagnosis has been based on personal history, clinical examination and by upper gastrointestinal endoscopy. **Results:** Of 426 patients, 20 were excluded from the study. 150 (36.9%) were women and 256 (63.1%) men. The mean patient age was 48.77 years with SD 14.32 years. The age group with the highest frequency of GERD was the 40-59 years including 196 (48.3%) patients and also the group between 20-39 years including 111 (27.3%) patients. 60 (14.8%) patients were not diagnosed with GERD after following an upper gastrointestinal endoscopy. **Conclusions:** The M:F ratio for patients with Barrett esophagus is 5:1. The sensitivity for the diagnosis of GERD using questions about the typical symptoms (regurgitation, heartburn) is about 72.6%. Although, the prevalence of GERD is higher in males than females, the prevalence of esophagitis and GEFV grade between sexes in not statistically significant. On the other hand, there is a strong statistical significance between sex and grade of HH presence ($p < 0.05$). **Keywords:** GASTROESOPHAGEAL REFLUX DISEASE (GERD), UPPER GASTROINTESTINAL ENDOSCOPY, HIATAL HERNIA (HH), ESOPHAGITIS, GASTRITIS.

Heartburn and acid regurgitation, typical symptoms of gastroesophageal reflux disease (GERD), are considered to be reasonably specific for diagnosis (1,2). The dominant mechanism of GERD is contact of the esophageal mucosa with acid, and GERD can also produce symptoms or signs of tissue injury within the oropharynx, larynx and respiratory tract. Recent studies have suggested GERD causes a wide spectrum of non-esophageal conditions including asthma, non-obstructive dysphagia, and non-cardiac chest pain (3, 4). Moreover, it has been reported that acid-suppressive therapy resolves heartburn and epigastric pain in GERD patients (5, 6). Therefore,

epigastric pain may be a clinical symptom of GERD.

GERD is a common condition and its prevalence varies in different parts of the world. Several population-based studies have reported its prevalence is more frequent in Western (7-10) than in Eastern countries (11,12).

MATERIAL AND METHODS

Subjects

From January 2008, to March 2011, 426 consecutive potentially GERD positive patients visiting a Gastroenterology department received an upper gastro-intestinal endoscopy and completed the GERDQ questionnaire (13, 14) regarding GERD symptoms.

Symptom evaluation

Prior to the upper gastrointestinal endoscopy, patients filled out the GERDQ questionnaire regarding the presence and frequency of typical symptoms of GERD (heartburn and acid regurgitation). They also filled out a form about atypical symptoms and extraesophageal symptoms (15).

GERDQ has been created from three different validated questionnaires evaluated in the DIAMOND study (16) and is made up of six questions (four on symptoms, two on disease impact) completed by the patient and overseen by the physician. Answers are compiled according to a scale of options («never», «1 day», «2-3 days», and «4-7 days») and are scored accordingly. Each option equates to a 0-3 score.

Upper gastrointestinal endoscopy and classification of esophageal mucosal injury

Flexible video endoscopy was performed in each patient under premedication with midazolame and topical anesthesia of the pharynx with xylocaine gel. The lower esophagus, squamocolumnar junction, gastroesophageal junction and diaphragmatic hiatus were thoroughly inspected under adequate endoscopic inflation to maintain optimal visualization without over inflation or masking due to tight tonic contraction of the lower esophageal sphincter (LES) (17,18). The presence and degree of esophageal mucosal injury were graded according to the Los Angeles classification (19). 20 patients were excluded from the study. Patients were classified into 6 groups on the basis of upper endoscopic findings: esophagitis, gastritis, esophagitis and gastritis, other than esophagitis and gastritis finding, negative, Barrett esophagus. Hiatal hernia (HH) was defined as a circular extension of the gastric mucosa above the diaphragmatic hiatus and was categorized in three groups as small (< 3 cm), medium (3-5 cm) and big (> 5 cm).

Classification of the gastroesophageal flap valve

The gastroesophageal flap valve (GEFV) was inspected with a retroflexed endoscope and graded I to IV according to the Hill classification as follows (20): grade I, prominent fold of tissue along the lesser curvature closely opposed to the endoscope. Grade II, fold present, but slightly less well defined than in grade I and it opens rarely with respiration and closes promptly. Grade III, fold not prominent. The endoscope was not gripped tightly by the tissues and often failed to close around the endoscope. Grade IV, fold absent with the lumen of the esophagus gaping open, allowing squamous epithelium to be viewed from below. Grades I and II were classified as normal valves and grades III and IV as abnormal valves.

Statistical analysis

The prevalence rates of esophagitis, gastritis, Barrett esophagus, hiatal hernia

and the frequency of abnormal GEFV were calculated. Age, male to female ratio, presence of hiatal hernia, grade of mucosal injury using the Los Angeles classification and symptom category were compared between groups with normal and abnormal grades of GEFV, using unpaired *t*-tests. The χ^2*test* was performed to evaluate normal and abnormal grades of GEFV. Multiple comparative tests were performed among the groups with esophageal mucosal injury or symptoms. A p value <0.05 was considered to be statistically significant. The statistical software used for this purpose is *SPSS version 20.0* for MAC.

RESULTS

In this study the total number of patients were 406 of both sexes. 256 male patients (63.1%) and 150 female patients (36.9%). The mean age for both sexes was 48.7±14.3. Maximum and minimum age was 88 and 15 years respectively. We categorized the patients in 5 age groups as follows: '<20', '20-39', '40-59', '60-79' and '>80' (tab. I).

TABLE I

Age groups of patients with typical symptoms of GERD

Age groups (years)	Frequency	Percent
<20	1	0.2%
20-39	111	27.3%
40-59	196	48.3%
60-79	89	21.9%
>80	9	2.2%

The age group with the highest frequency is the 40-59 with 196 patients (48.3%). There is also a statistical significance between sex and the age groups ($p < 0.001$).

The upper gastrointestinal endoscopy showed the following data about the findings: esophagitis was present in 101 patients (24.9%), gastritis in 92 patients (22.7%), patients that presented both esophagitis and gastritis were 82 (20.2%), Barrett esophagus was present in 20 patients (4.9%), the endoscopy was negative for any pathological findings in 54 patients (13.3%) and 57 patients (14.0%) had some other findings not related to GERD (tab. II).

In tables III, IV and V are presented the GEFV, mucosal injury and HH presence per gender.

TABLE II

Endoscopic findings

	Frequency	Percent (%)
Esophagitis	101	24.9
Gastritis	92	22.7
Esophagitis and gastritis	82	20.2
Barrett esophagus	20	4.9
Negative endoscopy	54	13.3
Not GERD – related	57	14.0

TABLE III

GEFV categories between female and male patients

Hill's scale	Sex	
	Female	**Male**
I	57 (14.0%)	104 (25.6%)
II	67 (16.5%)	97 (23.9%)
III	13 (3.2%)	30 (7.4%)
IV	13 (3.2%)	25 (6.2%)
I + II	124 (30,5%)	201 (49.5%)
III + IV	26 (6.4%)	55 (13.6%)

Data are expressed as *n* (%).
There is not any statistical significance between sex and GEFV.

TABLE IV

Grade of esophagitis between female and male patients

Grade of esophagitis*	Sex	
	Female	**Male**
No esophagitis	91 (40,1%)	136 (59.9%)
A	23 (37.1%)	39 (62.9%)
B	13 (29.5%)	31 (70.5%)
C	15 (34.1%)	29 (65.9%)
D	8 (27.6%)	21 (72.4%)

*According to the Los Angeles classification criteria. Data are expressed an *n* (%).
There is not statistical significance between sex and grade of esophagitis.

TABLE V

Hiatal hernia presence between female and male patients

Hiatal Hernia	Sex	
	Female	**Male**
Without	99 (44.6%)	123 (55.4%)
Small (<3cm)	32 (26.0%)	91 (74.0%)*
Medium (3-5cm)	17 (30.9%)	38 (69.1%)*
Big (>5cm)	2 (33.3%)	4 (66.7%)

*p <0.05. Data are expressed as *n* (%).

TABLE VI

Prevalence of mucosal injury in patients with different grades of gastroesophageal flap valve (Hill's scale)

Hill's scale		Grade of esophagitis*			
	No esophagitis	**A**	**B**	**C**	**D**
I	108	27	22	4	0
II	117	31	11	3	2
III	2	3	6	19	13
IV	0	1	5	18	14

*According to the Los Angeles classification criteria. Data are expressed as *n.* Strong statistical significance (p< 0.01), Correlation r = 0.65.

In Figure 1, there is a better view of the relationship between the grade of esophagitis and the gastroesophageal flap valve grade. When the GEFV is normal (grade I and II), there is no, grade A or grade B esophagitis. When the GEFV is abnormal (grade III and IV), grade C and D esophagitis is more prevalent.

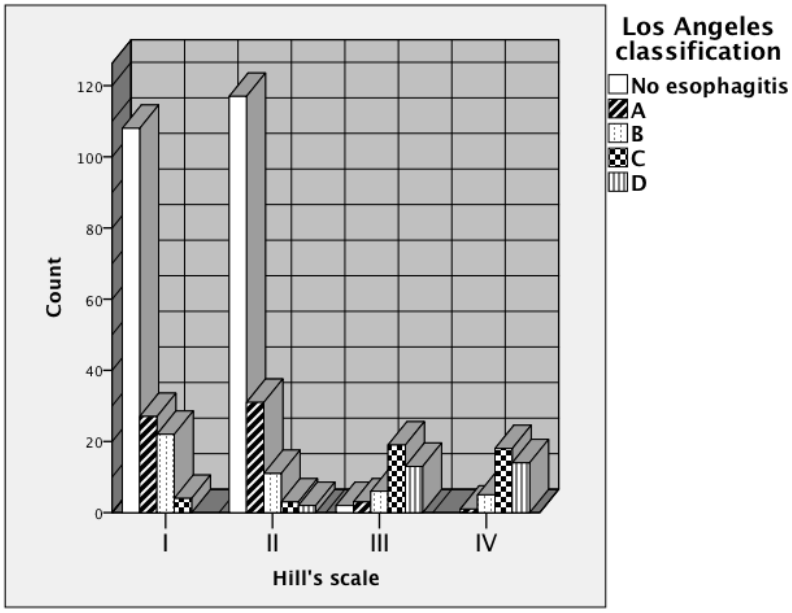

Fig. 1. Bar diagram showing the correlation between the grade of the esophagitis (using the Los Angeles classification) and the gastro-esophageal flap valve grade (using Hill's scale).

CONCLUSIONS

The present study demonstrates a close association between increasing deformation of the GEFV and endoscopically demonstrated GERD. Although, the prevalence of GERD is higher in males than females, the prevalence of esophagitis and GEFV grade between sexes in not statistically significant. On the other hand, there is a strong statistical significance between sex and grade of HH presence. The Male to female ratio for Barrett esophagus is 5:1. Analyzing the GERDQ and the upper gastrointestinal endoscopies of the patients examined, GERDQ in this study had a specificity of 85.9% and a sensitivity of 72.6%.

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LYME DISEASE - UNUSUAL MEDICAL ENCOUNTER FOR AN UROLOGIST

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LYME DISEASE–UNUSUAL MEDICAL ENCOUNTER FOR AN UROLOGIST (Abstract): Lyme disease also called „the illness with a thousand faces" ("The Great Imitator") because it may mimic very well the symptoms and signs of other diseases, is an unusual medical encounter for the urologist. Every patient with Lyme disease has his own clinical feature, while the superposition over an unknown but easy to discover urological disease may lead to a misdiagnosis. **Case presentation**: Male patient A. P. was an emergency transfer in our clinic with multiple system organ failure. The mirage of first imaging finding, bilateral obstructive ureteral calculi was obviated after the serological confirmation of Lyme disease suspected after the thorough history obtained from his family. The intensive care treatment, broad-spectrum antibiotics and hemodialysis sessions, together with external urinary drainage, lead to the improvement of the patient status, and subsequent proper urological treatment to urolithiasis cure. **Conclusions**: This case identifies several challenges faced by practitioners, challenges which involve the diagnosis and the treatment of Lyme disease associated with urolithiasis. Although Lyme disease remains a controversial clinical entity, its diagnosis is based on a history of possible exposure to ticks, the appearance of specific clinical symptoms, whether or not combined with serological tests. **Keywords**: LYME DISEASE, *BORRELIA BURGDORFERI*, UROLITHIASIS.

Lyme disease, also called „the illness with a thousand faces" ("The Great Imitator") because it may mimic very well the symptoms and signs of other diseases, is an unusual medical encounter for the urologist. Every patient with Lyme disease has his own clinical feature, while the superposition over an unknown urological disease but easy detectable through imaging may lead to a misdiagnosis.

CASE PRESENTATION

The patient AP, age 46 (Hx 9546) was hospitalized in the Urology and Renal Transplantation Clinic Iasi being an emergency transfer from a county urological medical unit. The patient with urogical history (left ureterolithotomy for pelvic ureteral calculi during childhood) and known for therapeutically neglected nephrolithiasis was hospitalized in severe condition, 3rd degree coma, GCS=3, fixed mydriasis, respiratory acidosis, hemodynamic instability (also present during transport), TA=40/20 mmHg, heart rate 36/minute, idioventricular rhythm. The biological examination reveals severe

nitrogen retention, severe electrolyte imbalances, inflammatory syndrome and other metabolic changes (tab. I).

The emergency imaging assessment (ultrasonic and radiologic) reveals bilateral obstructing voluminous urolithiasis with 2nd -3rd degree bilateral uretero-hydronefrosis with correct transonicity (fig. 1).

Immediately after admission to the intensive care the patient receives device to aid breathing and is inserted central venous catheter, an inotropic support being set up. Despite the hemodynamic instability a hemodialysis session is performed in order to regulate the severe hydro-electrolytic and acido-basic imbalances.

TABLE I
Initial biological assessment

Test	Result	Normal values		Test	Result	Normal values
Urea	**666**	10 – 50 mg%		Na	**117**	135 – 148 mmol/l
Creatinine	**29,31**	0,7 – 1,3 mg%		K	**9,5**	3,5 – 5,1 mmol/l
Blood sugar	**224**	70 – 115 mg%		Cl	**82**	98 – 107 mmol/l
GOT	18	1 – 38 u/l		pH	**6,8**	7, 31- 7, 41
GPT	14	1 – 41 u/l		White blood cells	**23.650**	4000 – 9000/mm³
TGG	33	10 – 71 u/l		Red blood cell no.	**2,95**	4,63 – 6,1 mil/ mm³
Amylasemia	**1066**	28 – 100 u/l		Hemoglobin	**8,5**	13,7 – 17,5 g/dl
T Proteins	69	66 – 87 g/l		Packed cell volume	**23,9**	40,1 – 51,0 %
CK	**549**	26 – 195 u/l		EST	**112**	2 – 10 mm/h
CK-MB	**51**	0 – 25 u/l		PDF	++++	-
LDH	**351**	135 – 225 u/l		Fibrinogen	**541**	200 – 400 mg%

Fig. 1. Abdominal simple x-ray – bilateral obstructing voluminous urolithiasis

The appearance just hours after admission of the patient's next of kin allow the obtainment of the first historical data and this leads to the idea of a possible infection with *Borrelia burgdorferi*. Thus the onset of the symptoms is related to an insect (tick) bite in the scrotum two weeks ago, followed by the appearance of a polymorphous symptomatology that included erythema at the site of the bite, fever, sweating, headache, myalgia, reason why the patient referred to the territorial ER Department, being recommended a urologic assessment for the scrotal erythema. The examination performed by the territorial urologist 5 days before confirmed the scro-

tal lesion, but was limited to the indication of thorough local washing and subsequent reassessment for the lithiasis associated pathology. Progressively in the next few days the patient's general condition became affected, culminating in the abolition of the consciousness the day he came back to the emergency territorial urological unit.

All these anamnesis completions indicate the possibility to associate the bilateral obstructing voluminous urolithiasis with the Lyme disease. To confirm the suspicion of infection with *Borrelia burgdorferi* we dosed the antibodies to Borrelia by the immunochemical method with chemiluminescence detection (CLIA), whose values were high (IgG 55 AU / ml, IgM 9.2 AU/ml). Western blot confirmatory test was not possible.

But until serological results came out the cardiovascular recovery was a priority being obtained with inotropic support, acid-basic and electrolytic regulation, empirical broad-spectrum antibiotic therapy. 16 hours after admission due to the persistent anuria and due to the presence of the bilateral ureterohydronefrosis, the patient was subject to a urologic intervention being performed a bilateral percutaneous nephrostomy with the aspiration of clear urine from both pyelocaliceal systems.

The subsequent development was sow, no diuresis on nephrostomies, with repeated hemodialysis sessions but the patient experienced gradual improvement of the general condition, he regained consciousness, became cooperative and resumed his spontaneous respiration being extubated 48 hours after admission. Blood pressure values stabilized and gradually the hemodynamic support doses were reduced until their complete interruption. 5 days after admission he gradually resumed diuresis, while maintaining nitrogen retention (serum creatinine between 10.6 and 15.3 mg%, CrCl = 5 ml / min) requiring chronic hemodialysis, which was subsequently performed within the dialysis territorial unit.

The reassessment performed 3 weeks after discharge revealed stationary moderate nitrogen retention (urea = 66 mg%, creatinine = 2.2 mg%, CrCl = 46 ml / min). Given the global diuresis (2000 ml / day) the attending physician decided to suspend hemodialysis and assess the patient subsequently in order to decide the urological behavior.

The patient came back after 3 weeks more in the Urology Clinic being reassessed in order to solve the bilateral ureterolithiasis problem. The biological investigations reveals minimum nitrogen retention (urea = 55 mg %, creatinine = 1.57 mg %). The imaging assessment, ultrasound and pyelography assessment confirm the 1 cm right lumbar ureteral calculus and the 4-5 cm left pelvis one, the elimination of the contrast substance and residual distension of the bilateral pyelocaliceal system (fig. 2).

First a push-back of the lumbar ureteral calculus and its percutaneous extraction were performed and subsequently left pelvic ureterolithotomy which was difficult considering the antecedent intervention and the changes of the subjacent ureter. The postoperative evolution was simple with primary wound healing and suppression of bilateral nephrostomy cathether.

More than 6 months after the intervention the patient who had been monitored all along (creatinine=1.5-1.6 mg %), is clinically and imagistically reassessed, the success of the interventions performed being thus revealed (fig. 3).

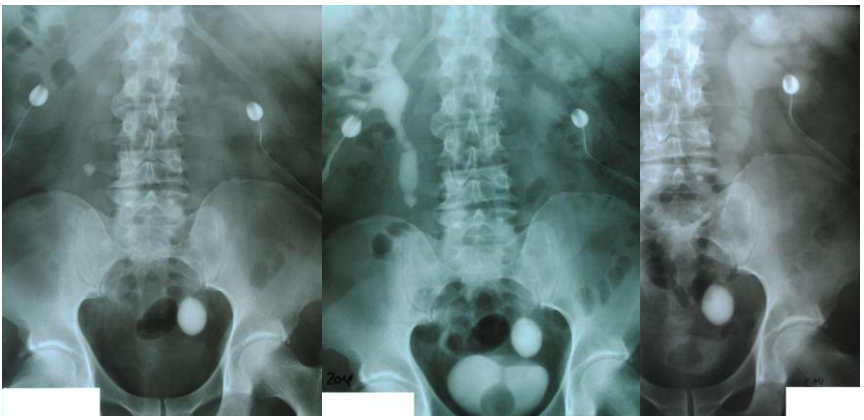

Fig. 2. Intravenous pyelography – bilateral urolithiasis

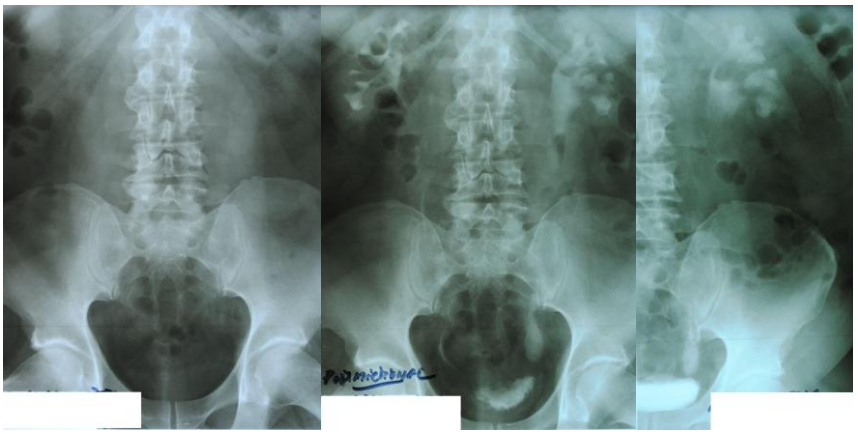

Fig. 3. Intravenous pyelography – control

DISCUSSION

Urologist's coping with Lyme disease is really unusual, a rarity of the ambulatory or emergency medical work; nonetheless, in the case presented herein, it reminds the necessity of a deep and thorough analysis of each clinical case. The challenge for physicians and patients consists of the rapid recognition and appropriate treatment of infection, especially in patients with non-specific clinical manifestations (1, 2).

Analyzing retrospectively the succession of events, we could say that the examination of the scrotum at a first evaluation correlated with a thorough anamnesis should raise the suspicion of a Lyme disease. The rapid evolution of symptoms and the impairment of the general condition made the patient to present for evaluation at the territorial emergency department; this department, based on the evaluation protocol, emphasized organ and system multiple failure, considered a consequence of the evolution of the sepsis with urinary starting point (infected lithiasic bilateral ureterohydronefrosis). The invalidation of these assumptions about the cause of the organ and system multiple failure (MSOF) is based on several arguments: first

of all, the sequence of events reported by the patient's next of kin and subsequently by the patient (the event that triggered the suffering being the tick bite in apparent health status), then the absence of any urinary-type symptomatology (absence of changes in the appearance of urine, absence of back pain symptoms) and. Finally, aspiration of clear urine from the bilateral pyelocaliceal system in the moment of the external urinary derivation in postponed emergency and microbiological confirmation of its sterility at that time.

The morpho-pathological substrate of the renal impairment in Lyme disease is represented by the membrano-proliferative glomerulonephritis with or without interstitial nephritis (3). This post-infectious event seems to be based on a mechanism using circulating immune complexes, fact evidenced in dogs and in some cases in humans (3, 4, 5).

The clinical testing for Lyme disease remains a matter of controversy (6). The Centre for Disease Control and Prevention in the United States recommends using a dual algorithm: screening by indirect immunofluorescence or enzyme immunoassay test, followed by confirmation through Western blot. Although this approach has a high specificity, the sensitivity of the dual algorithm of diagnosis for patients with Lyme disease at 4-6 weeks at least after infection is of only 44-56%, making it unsuitable for a clinical diagnosis test. Therefore, according to the Centre for Disease Control and Prevention in the United States, the diagnosis of Lyme disease is based primarily on the clinical data, on the exposure to the bite in an endemic area, as well as on the high level of anti-Borrelia antibodies (7). In the presented case, lacks from organizational reasons the serological confirmation by Western blot, this test being unavailable in our center.

CONCLUSIONS

The case presented herein identifies several challenges encountered by the urologist in terms of diagnosis and treatment of Lyme disease associated with urolithiasis. Although Lyme disease remains a controversial entity, its diagnosis is based on the history of the exposure to tick bites, as well as on the clinical examination, combined or not with serological tests.

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PATHOLOGICAL COMPARATIVE ASSESSMENT OF TWO CASES OF THYMIC CYST AND CYSTIC THYMOMA AND REVIEW OF THE LITERATURE

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PATHOLOGICAL COMPARATIVE ASSESSMENT OF TWO CASES OF THYMIC CYST AND CYSTIC THYMOMA AND REVIEW OF THE LITERATURE (Abstract): Cystic changes of the thymus are rare lesions. In addition to their appearance in non-neoplastic congenital and acquired conditions, they have been seen in association with certain malignancies of the thymus. Our aim is to highlight the possibility of confusing between benign and malignant thymus cysts having different cure approach. We report two thymic cyst cases, one congenital ectopic condition, and the other one, a cystic thymoma. Investigations included blood counts, echograms, and computed tomography. The cysts were excised by mediastinal route and examined pathologically. The final diagnosis was made only after histopathological examination of the surgery biopsy revealing two types of cystic thymic lesions: congenital and tumoral. Because thymic cysts may present malignant transformation, they represent a diagnostic challenge that is resolved only by surgical excision and histological examination. Due to cystic changes masking tumoral features in these cases, thorough sampling is required to ensure that a malignancy is not overlooked. **Key words:** THYMIC CYST, CYSTIC THYMOMA, MESOTHELIAL CYST

Thymic cysts are regarded as rare anomalies (1); they may occur at any anatomic level, from the base of the neck to the diaphragm. There is some controversy receiving the causes of thymic cysts. According with Speers (2) thymic cysts represents different entities: congenital, inflammatory and neoplastic. Congenital cysts can be found along the anatomical course of the third pharyngeal pouch, inflammatory cysts may be due to an acquired inflammatory process (3, 4, 5), and neoplastic cysts (6, 7) are more frequently in larger tumors than in smaller ones, resulting by cystic degeneration of the tumors. Suster and Rosai (8) reported 18 cases of multilocular thymic cysts, in addition to unilocular cysts, most of the cases represented by congenital or acquired inflammatory cysts, and some of them were related to neoplasms. In any case, local disruption of thymic tissue can induce the formation or growth of cysts. In the current paper, we make a comparative pathological discussion between the two thymic cysts: the first, a thymic cyst of congenital origin,

and the second, a cystic mass of neoplastic origin, represented by a cystic thymoma.

CASE PRESENTATIONS

Case 1.

An asymptomatic 23-year-old man was referred to our hospital with a suspicion of "pericardial cyst" based on chest echocardiography made few months ago. The family history was non-contributory and the past personal history contained no episodes of pneumonia, pleurisy or chest pain. Laboratory data and ECG were normal. Chest X ray demonstrated a mass located in the anterosuperior mediastinum. Echocardiography exam showed a cyst structure located adjacent to the right atrium, measuring about 7 x 4 x 6 cm. There was no pericardial fluid, intracavitary thrombus and pulmonary hypertension. The results of computed tomography (CT) of the chest consisted in a cystic mediastinal mass with a definite, thick, fibrous capsule and fluid content. Cyst resection was recommended because of the cyst's large size and the high possibility of compression to the right atrium.

Following surgical operation, macroscopical examination revealed a well-encapsulated bilocular (fig. 1) thick-walled cystic mass with serous fluid content. Histopathological examination of the cyst revealed that its inner surface was lined with columnar epithelium, with thymic elements in the fibrous wall (fig. 2) and small foci of normal thymus (fig. 3) outside the cyst. From these histological findings, the patient was morphologically diagnosed with congenital thymic cyst. The patient left the hospital in good conditions and the postoperative evolution was favorable.

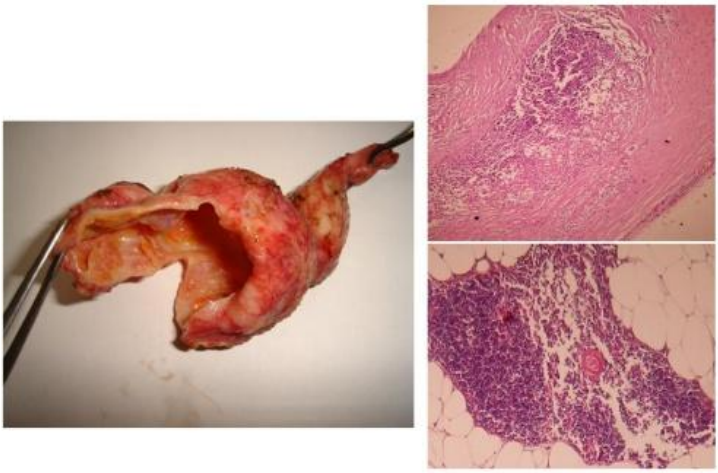

Fig.1- Encapsulated cystic mass; Fig. 2 -Thymic elements in the fibrous wall (HE, x20); Fig. 3 - Small external foci of normal thymus (HE, x10).

Case 2.

A 41 year old man with no significant past history was diagnosed, in 2002, on a routine chest X-ray with a left pleuro-pericardial mass. Subsequently, a CT scan showed a normal thymic region and a well defined antero-inferior mediastinal mass of 50/58/39 mm with areas of attenuation suggestive of cystic lesion, which was considered at that time, a pleuro-pericardial

cyst. After seven years, in January 2009, the patient was diagnosed in Neurological Hospital with myasthenia gravis, Osserman clinical stage IIB. A repeat thorax CT scan (fig.4) showed a bigger mass of 60/60/53 mm, that was inhomogeneous and well encapsulated situated between the pericardium and left inferior mediastinal pleura. Surgical exploration revealed a large cyst measuring approximately 6 cm in greatest dimension (fig.5). On cut section, it was a large cystic mass with less solid areas. The cut surface revealed grayish pink irregular nodules on the inner surface and almost the entire tumor was cystic and filled incompletely with soft yellow gelatinous material (fig. 6).

Pathologically, we found a large cystic tumor and a normal involuted thymic tissue was present outside of the wall (fig. 7). The cyst wall was composed of hyaline fibrous tissue and was lined inside by tumoral areas and eosinophilic debris, representing extensive areas of necrosis.

Fig.4. Mediastinal cystic mass; Fig. 5. Mediastinal tumoral mass; Fig. 6. Cystic mass with solid areas; Fig.7. Normal involuted thymic tissue (HE, x20).

Microscopical examination of the solid areas showed tumoral lobules (fig. 8) separated by fibrous bands and perivascular spaces (fig**.** 9). The lobules were composed of a dual population of cells typical of thymoma, including small lymphocytes and larger epithelioid cells with round to oval nuclei surrounded by abundant slightly eosinophilic cytoplasm. There was no evidence of nuclear pleomorphism or mitotic activity of the epithelioid cells

The capsule (fig. 10) was thickened and fibrotic, presenting focally, adjacent cholesterol clefts and foamy cells. Vestiges of lymphoid tissue were present in the wall. Examination of multiple sections did not reveal any evidence of invasion through the capsule, except a point of incomplete infiltration (fig. 11) of it, where the tumor point was too small to assess capsular invasion. Indeed, there was no evidence of capsular invasion or microscopic foci in the medias-

tinal fat. There were no signs of the tumor infiltration in the lung or any other adjacent structures, as well.

Pathology was consistent with type AB thymoma according to World Health Organization Classification or mixt thymoma according to traditional schema. On the basis of these histological findings, the cystic mass was identified as an encapsulated, well differentiated thymoma, stage I Masaoka, with secondary necrotic and cystic changes.

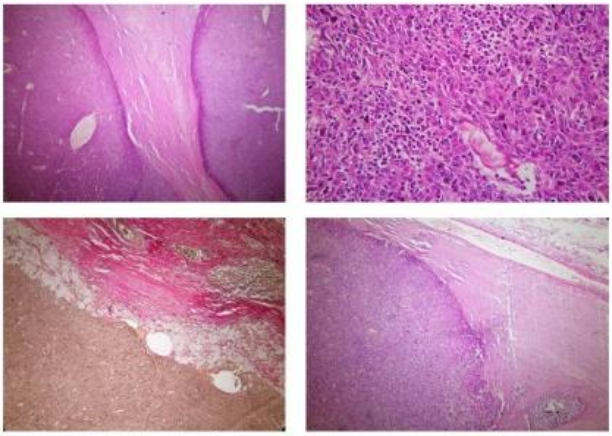

Fig.8. Tumoral lobules separated by fibrous bands (HE, x10); Fig. 9. Perivascular space and dual population of cells typical in thymoma (HE, x20); Fig.10. Thickened fibrotic capsule and subjacent foamy cells (EVG, x10); Fig.11. Point of incomplete capsular infiltration (HE, x10).

DISCUSSION AND CONCLUSIONS

Thymic cysts are relatively uncommon, but their numbers are increasing with the advent of new imagistic modalities of diagnosis, such as CT and MRI. They are most commonly classified into congenital or acquired, including neoplastic cysts (1-4). An important issue is the differentiation between thymic cysts and cystic thymomas.

Thymic cysts are believed to represent different entities, congenital, inflammatory and neoplastic cysts (9). Congenital, most of the thymic cysts can be explained as sequestrated remnants of the thymopharyngeal duct system (3). Suster and Rosai consider that the origin of thymic cysts seems to be: congenital, due to the induction of cystic transformation of ductal epithelium of the thymopharyngeal duct system or acquired, being related by an acquired inflammatory process (10). These cystic spaces are also interpreted as degenerative, probably resulting from liquefactive necrosis or fluid accumulation secondary to hemorrhage. The inflammation is a reactive process.

The same authors consider that diagnosis of inflammatory cysts may be difficult due to cystic changes and degenerative nature of these tumors. Degeneration of thymic cysts may reduce the identifiable thymic tissue, therefore extensive microscopic sampling is important.

Thymic cysts are rare and the etiopathogeny of these benign cysts is still controversial. But, their removal is often easy and the diagnosis is confirmed by the presence of Hassall's corpuscles in the cyst wall.

Cystic changes in thymoma may be the result of two pathogenetic mechanisms: (a) confluence and dilatation of perivascular spaces with creation of large cystic cavities devoid of an epithelial lining or inflammation, and (b) cystic dilatation of Hassall's corpuscles secondary to underlying inflammatory changes of residual non neoplastic thymic epithelium (11). Our case illustrates a secondary necrotic event that may supervene in thymoma that can obscure the main underlying pathological process.

Sometimes, it may be impossible to determine whether the cystic changes supervened in a preexisting thymoma (cystic transformation of the thymoma) or whether the thymoma, developed secondarily, represents an incidental finding in a thymic cyst (12). In our case, thymoma was found as mural nodules attached to the wall of the thymic cyst, pointing an initial thymoma with secondary cystic transformation.

In thymomas, more important than tumoral subtyping is to make a correct diagnosis of malignancy for avoiding confusion with other types of anterior mediastinal cystic lesions. Moreover, the most important prognostic factor of the thymomas, remain the status of capsular integrity. Adequate inking of the outer surface of the specimen and extensive sampling of the capsule are therefore of primary importance for the evaluation of these tumors.

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INDICAȚIILE OSTEOSINTEZEI MINIM INVAZIVE CU ŞURUBURI PERCUTANE ÎN FRACTURILE PILONULUI TIBIAL

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MINIMALLY INVASIVE PERCUTANEOUS SCREWS OSTEOSYNTHESIS INDICATIONS IN TIBIAL PILON FRACTURES (Abstract): Tibial pilon fractures usually result from high energy trauma. Management of these fractures is challenging because of the complexity of the injury pattern and the risk of significant complications. Various treatment methods are available, depending not only on the fracture type but mostly on the extent of the soft tissue injury. In tibial pilon fractures AO type A and B, without intensive comminution, we can tempt orthopedic reduction and percutaneous screw fixation. This protects the soft tissues, an important factor in postoperative care of these fractures. Material and methods: We present a case of a 23 years old woman treated with closed reduction and internal fixation with percutaneous screws. Conclusions: By percutaneous osteosynthesis we avoided possible complications of extensive skin wound and skin-plate conflict. The recovery was much easier, avoiding joint stiffness and therefore osteoporosis at this level. **Key words**: TIBIAL PILON FRACTURE, CLOSED REDUCTION, INTERNAL FIXATION, PERCUTANEOUS SCREWS

Termenul de pilon tibial a fost introdus în literatura ortopedică în 1911 de către Étienne Destot (1). Aceste fracturi se produc de obicei în urma traumatismelor de energie înaltă. Tratamentul acestora este dificil nu numai prin complexitatea traiectelor de fractură, cât şi prin riscul crescut al complicațiilor.

În 1959 Jergesen considera că reducerea sângerândă şi fixarea internă a acestor fracturi este imposibilă (2). De-a lungul timpului însă lucrurile s-au schimbat datorită unei mai bune înțelegeri a importanței părților moi înconjurătoare şi diferențierii între fracturile prin energie joasă şi înaltă, ceea ce a dus la apariția unor noi concepte terapeutice (3,4,5).

MATERIAL ŞI METODE

Pacientă LI, în vârstă de 23 de ani, victimă unui accident rutier, se prezintă în urgență la camera de gardă acuzând dureri şi impotență funcțională la nivelul gleznei stângi şi coloanei dorso-lombare.

Se efectuează radiografie de gleznă în incidență antero-posterioară şi latero-laterală (fig. 1) cu evidențierea unei fracturi de pilon tibial şi maleolă internă, radiografie de coloană dorso-lombară ce evidențiază prezența unei fracturi-tasare de corp

vertebral L4 şi examen CT cranio-cerebral cu decelarea unui edem cerebral moderat.

Fig. 1. Radiografie preoperatorie de gleznă (A - faţă, B - profil) – fractura maleolei interne fără deplasare şi fractura pilonului tibial cu înfundare articulară în jumătatea anterioară.

Se imobilizează membrul inferior stâng pe atelă ghipsată gambo-podală şi fractura de coloană în lombostat, pacienta fiind dirijată spre serviciul de Neurochirurgie cu recomandarea de internare în Secția de Ortopedie după rezolvarea problemelor neurochirurgicale.

După 7 zile, pacienta revine în Secția de Ortopedie cu recomandările de consult neurologic la nevoie şi menținerea lombostatului până la 45 de zile posttraumatism.

Se efectuează examen CT de gleznă cu reconstrucție 3D ce evidențiază prezența unei fracturi de maleolă internă fără deplasare şi a unei fracturi de pilon tibial **AO 43 B2** (fig. 2).

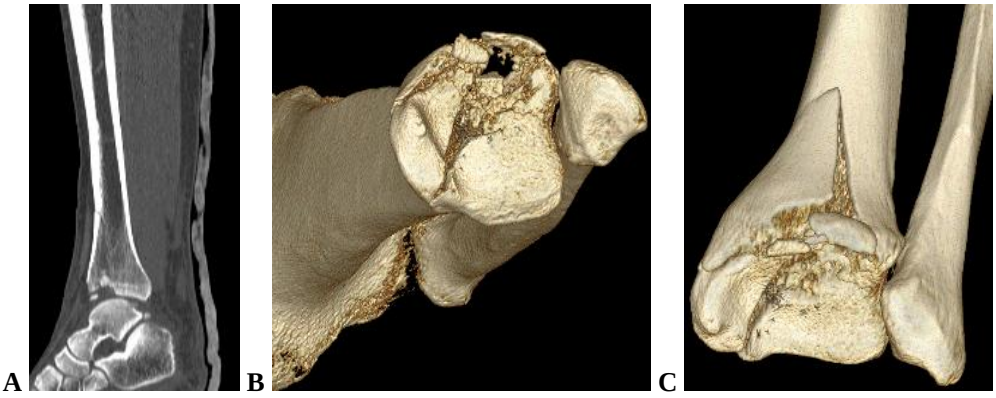

Fig. 2. **A –** CT nativ gleznă; B, C – CT cu reconstrucție 3D gleznă – fractură cominutivă pilon tibial cu înfundare (treaptă articulară de ~ 3 mm) AO 43 B2, fractură maleolă internă cu minimă deplasare.

Se intervine chirurgical, sub Rx-TV, practicându-se reducerea ortopedică a focarului de fractură şi osteosinteză percutană cu 2 şuruburi maleolare a fracturii de pilon tibial şi 1 şurub maleolar a fracturii maleolei interne (fig. 3).

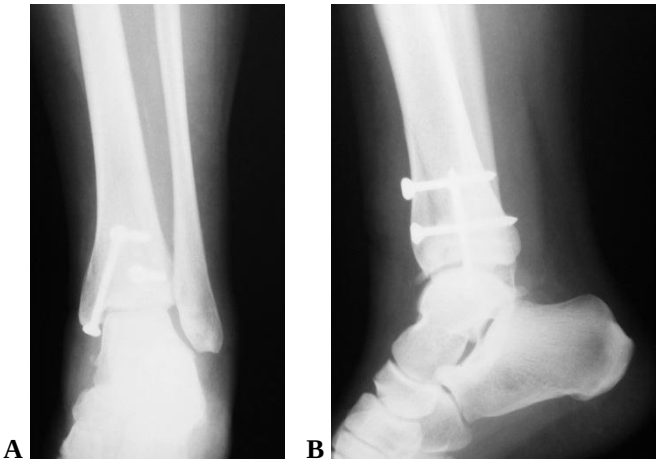

Fig. 3. Radiografie postoperatorie de gleznă (A - faţă, B - profil) – reducerea focarelor de fractură maleolar şi de pilon tibial, refacerea continuităţii suprafeţei articulare, prezenţa materialului de osteosinteză (2 şuruburi maleolare antero-posterioare pe pilonul tibial şi a unui şurub maleolar pe maleola internă).

Starea generală şi locală fiind favorabilă pacienta se externează cu recomandarea de imobilizare pe atelă gambo-podală timp de 30 de zile, timp în care evită sprijinul pe membrul inferior operat, după care revine pentru evaluare clinică şi radiologică.

DISCUŢII

Fracturile pilonului tibial rezultă dintr-o combinaţie de compresii axiale şi forţe de rotaţie. Analizarea critică a condiţiei ţesuturilor moi reprezintă un aspect crucial în managementul acestor fracturi. Poate cea mai mare realizare a tratamentului chirurgical al fracturilor pilonului tibial a fost recunoaşterea necesităţii de a amâna intervenţia chirurgicală primară.

Trebuie să facem mai întâi o distincţie între fracturile extra şi intra-articulare, cele din urmă fiind complet sau parţial articulare. Fracturile extraarticulare şi cele parţial articulare pot fi tratate prin tehnici minim invazive fie cu şuruburi canulate introduse percutan, fie cu plăci anatomice stabile angular (6,7).

Primele studii cu şuruburi canulate introduse percutan au fost publicate în 2004, pe un lot de 7 pacienţi urmăriţi 30.6 luni. Rezultatele au fost foarte bune, obţinându-se un scor de 90,8/100 (8).

Evoluţia şi prognosticul acestor fracturi depinde de severitatea traumatismului şi de acurateţea reducerii la nivel articular. Reducerea şi refacerea lungimii se poate face prin ligamentotaxis, folosind fixatorul extern, dar principiul de bază rămâne reducerea anatomică sângerândă (9,10,11).

Refacerea integrităţii mortezei este un factor determinant în succesul tratamentului acestor fracturi.

Unii autori (10,11) susțin că rata complicațiilor este mai mică când fixarea externă este folosită înaintea reducerii sângerânde, dar alți autori (12) spun că fractura de pilon tibial tratată cu fixator extern are o rată mai mare (p=0.03) de consolidări vicioase decât tratată prin reducere sângerândă şi fixare internă.

CONCLUZII

Avantajul oferit de fixarea percutană este reprezentat de prezervarea țesuturilor moi din jurul sediului fracturii; se menține astfel vascularizația din această zonă şi se reduce trauma chirurgicală, pacientul recuperându-se mult mai rapid (13).

În continuare fractura de pilon tibial reprezintă o provocare pentru chirurgul ortoped. Un plan pre-operator excelent, o atenție sporită față de țesuturile moi şi o intervenție chirurgicală bine realizată încă pot avea un rezultat defectuos datorită deteriorării cartilajului articular la momentul producerii leziunii.

Prin osteosinteza percutană am evitat posibilele complicații ale plăgilor tegumentare extensive şi a conflictului tegument-plăci. În acelaşi timp, recuperarea a fost mai facilă, evitând astfel redoarea articulară şi implicit osteoporoza de la acest nivel.

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THE ROLE OF THE MOTIVATIONAL INTERVIEW IN TREATMENT ACCEPTANCE. CASE REPORT

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THE ROLE OF THE MOTIVATIONAL INTERVIEW IN TREATMENT ACCEPTANCE. CASE REPORT (Abstract): Treatment acceptance and subsequently treatment adherence represent the most difficult aspects of medical treatment. This is the reason why it requires the use of tools from psychotherapy and communication sciences, but also a new approach to the relationship per se. Thus, the use of the motivational interview together with the change in the perspective of the therapeutic relationship, namely the patient-centered approach from the psychosocial perspective, proved to be effective ways to overcome this obstacle. This article focuses on the case of a 45-year-old patient suffering from psoriasis since he was 30. He followed all the therapeutic steps indicated by the therapeutic protocols, reaching the critical moment of disease evolution, when the only effective therapeutic indication was biological agent therapy. Considering the adverse effects of this class of drugs, the patient exhibits a period of ambivalence before accepting therapy. **Keywords**: MOTIVATIONAL INTERVIEW, THERAPEUTIC ACCEPTANCE, PATIENT-CENTERED APPROACH.

One of the most difficult aspects of medical treatment is acceptance of diagnosis, followed by adherence to therapy and prevention of self-medication, as they are the result of the degree of information, therapeutic advice and education of the population (1, 2). At the conceptual level, accepting the diagnosis is the acceptance of a change that sometimes occurs unexpectedly and brutally in a patient's life. In the case of chronic conditions, adherence to therapy necessarily requires the existence of an important motivation. The motivational interview is a useful tool in cases when the patient essentially exhibits resistance to change.

We started from the idea that an optimal and effective therapeutic relationship is based on the concept that, starting from the individual needs of the patient, the therapist (the physician) creates a therapy plan and establishes a therapeutic relationship, placing himself/herself and the patient on the same level. This concept has the advantage of empowering the patient, who will be aware of his/her attributions in the context of the therapeutic relationship and will therefore assume his/her part (3, 4, 5, 6, 7).

Identifying the intrinsic motivation of

the patient is a difficult and long process that requires special communication skills from the physician. There are also studies that report that a wanting and difficult doctor-patient relationship can lead to non-adherence and even self-neglect, refusing medication or medical care and personal safety measures (8, 9, 10).

It is extremely challenging to establish a good medical approach, especially in severely ill patients and in elderly with multiple dietary recommendations and numerous drug regimens as one should try to reconcile the medical benefits of the treatment with the possibilities of each patient to fulfill it. Elaborating a personal, realistic therapy is the main key for providing a good compliance, an excellent evolution of the disease and quality of life at least as good as the patient's expectations. The current approach to medicine is one centered on the patient from a bio-psycho-social perspective (11).

CASE REPORT

The here presented clinical case details the adherence to therapy of a patient with psoriasis vulgaris, who was recommended to undergo biological therapy.

The 45-year-old man, with obesity, was diagnosed at the age of 15 with psoriasis vulgaris. He underwent all stages of therapy, and due to the unfavorable course and the occurrence of psoriatic arthritis at the age of 35, he was given the indication of initiation for biological therapy. Although he had accepted the diagnosis 15 years before and followed the prescribed treatments, the patient is in a state of ambivalence when recommended this last stage of therapy, invoking various reasons for postponing it (the need to quit smoking because of the possibility of infectious complications pulmonary tuberculosis).

As a result, he repeatedly performed imaging investigations and laboratory analyses included in the protocol for the inclusion of patients in the therapy program. Moreover, based on information filtered from the perspective of personal beliefs, corroborated with information assimilated by the reading of articles posted on the internet, the patient formulates diagnostic assumptions that emphasize his ambivalence (12, 13, 14).

The patient presented a state of marked anxiety during that period, which he tried to dissimulate by an exuberant attitude, in disagreement with the symptom he accuses: exhaustion, insomnia, irritability, intrusive thoughts gravitating around the same leitmotif: the need for the recommended therapy.

We considered it useful to apply the motivational interview in the context of the re-conceptualization of the disease state and the necessity of establishing the biological therapy, to obtain the situational reframing. We started from initiating the change process by trying to facilitate it (15).

The patient's profile was: increased importance given to change, but low confidence in the chances of success. We applied the technique of decisional balance that consists in producing a list of status quo (status) and benefits of change. On the other side of the balance we have positioned the costs of change and the benefits of the status quo (16, 17, 18).

Obviously, the patient was aware of the benefits of change, but we got into the *avoidance-acceptance* situation in which he was equally attracted by and scared of the benefits of treatment, the adverse effects and the need to change habits (smoking).

The situation was potentially dangerous for the patient, as psoriasis, a psychosomatic condition with an important emotional component, could be aggravated because of blocking the state of internal conflict (19).

Considering that ambivalence is a process on a continuum of change, we considered it necessary to have a meeting with the patient, explaining that ambivalence is a normal phase in the process of change and he should not be worried.

During the second session with the patient, we triggered an increase in the discrepancy between the present behavior and the perceived significance of that behavior. Thus, at the conceptual level, the patient understood that the significance he had previously attributed to the initiation of treatment could not be reduced to the treatment itself (adverse reactions or benefits) but had to be assimilated to self-destructive behavior with profound family implications, also, of social insertion and especially emotional relationships with his son. In this context, the patient admitted that, because of the extended plaques to about 90% of the body surface, he was forced to not appear in public, which caused deep suffering to his son, who was deprived of his father's presence in certain situations (feasts, visits).

The re-conceptualization of the situation was also favored by the explanation that, if the treatment is discontinued, the patient will be in a state of permanent uncertainty about the benefits of biological agents' therapy.

After four meetings, the patient realized that making personal change is a choice that belongs to him and that ambivalence is a normal state of change. Consequently, he accepted biological therapy, assuming implicitly the potential side effects.

The evolution of the patient under treatment was favorable, and he is still following the therapy with biological agents. A difficult moment was BK infection and the development of a latent form of pulmonary tuberculosis, which required additional isoniazid therapy. Thus, in this situation, the need to maintain change, as evidenced by the continuation of biological therapy, was realized by the patient.

DISCUSSION

The presentation of this case makes obvious the need for effective communication with the patient, but also the importance and benefits of using the motivational interview in increasing the adherence of the patient to treatment (20, 21). For this reason, several programs to increase the communication skills of physicians would be particularly useful. Medical tele surveillance and complex telemonitoring are considered as a promising solution for a better patient-physician communication, especially for chronic and senior patients (22, 23).

In situations of diagnostic and therapeutic uncertainty, the motivational interview and the re-conceptualization and reframing techniques used proved to be useful tools for obtaining maximum therapeutic benefits (24, 25, 26).

Specialty literature confirms the need for medical staff training on the motivational interviewing (MI)-based approach to patients with chronic diseases, particularly with psoriasis. The need for this approach lies in the importance of the physician's ability to influence the attitude of patients with psoriasis towards health in general. The Pso Well study (27) provides evidence to that effect.

Another study reveals the benefits of using MI in patients with psoriasis. It refers

to the cost-utility balance, showing that using MI "showed significant cost saving, the MI approach is cost-effective in addition to its positive effects on clinical outcomes" (28).

In the same way, the counseling of the patient with psoriasis also includes the study on motivational counseling by telephone. It puts to the forefront the lifestyle of patients with psoriasis (29, 30). A study in the USA found that MI primarily by telephone helped to improve patient activation, self-efficacy, perceived health status and promoted lifestyle changes (31). Some evidence even suggests that telephone support may be as successful as face-to-face supported self-management (32, 33).

To conclude, the literature of recent years confirms the necessity of MI implementation as a necessary tool for dealing with patients with chronic diseases, especially patients with psoriasis. The main reason is the emotional factor present in patients with chronic diseases and especially in those with affectation of aesthetic appearance, as is the case in patients with psoriasis (34).

CONCLUSIONS

This case particularly discusses the association of comorbidities (obesity, smoking) in a patient with psoriasis whose evolution had worsened over time. The peculiarity of this case resides in the fact that emotional distress was not present in a significant way at the onset of the disease, during adolescence, which is recognized as a period of psycho-biological crisis. On the contrary, the emotional impact has been determined especially by the need to give up a type of addiction, namely smoking, food addiction, issues that are being discussed more and more frequently by the medical community.

Consequently, the present study is consistent with the current concerns of the scientific community related to the impact of psoriasis, emphasizing in the same time the importance of addictions in adherence to specific therapy.

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NEWS

NATURAL TREATMENT IN BREAST CANCER: BENEFIC OR NOT

Breast cancer is one of the most significant health problem all over the world, despite of the high performance diagnostic procedures and the increased methods of prevention and treatment. Searching articles about herbal treatment in breast cancer on PubMed and ISI Web of Scince it was discovered that the most important plants and natural extracts used to prevent and treat breast cancer are *Echinacea sp*., *Camelia sinensis (*green tea), *Allium sativum*, *Ginseng*, vitamin A, vitamin E, omega 3. Although there are not enough in vivo studies, most studies are performed on breast cancer cell lines, many herbal products are being researched for their pharmacological activity in monotherapy or in addition to conventional cancer treatment. It is not fully known if the effects on the therapy are negative because of the numerous active ingredients and their high risk of adverse effects or positive because of their pharmacodinamyc activity: activation of metabolizing enzymes, supression of reactive oxygen species production. For example *Echinacea sp* will not affect the activity of the chemotheraputic agent, but it may cause liver damage when administrated for a long period of time. În *Allium sativum* the most effective compounds are the oil-soluble ones. Regular consumpsion of green tea seem to have also an protectiv effect against breast cancer. (C. M. Lopes, A. Dourado, and R. Oliveira. Phytotherapy and nutritional supplements on breast cancer. *Biomed Res Int*, 2017).

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THE ROLE OF EEG BRAIN MAPPING IN QUANTIFICATION OF BRAIN DAMAGE IN HYPERTENSIVE DISEASE ASSOCIATED WITH PREGNANCY

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THE ROLE OF EEG BRAIN MAPPING IN QUANTIFICATION OF BRAIN DAMAGE IN HYPERTENSIVE DISEASE ASSOCIATED WITH PREGNANCY (Abstract): The existence of a high blood pressure during the pregnancy represents a real danger for the mother and child, responsible for damage of the kidney, brain, liver, and placenta, cardiovascular and hormonal system. Nervous system changes can include blurred vision, seeing spots, severe headaches, convulsions, brain hemorrhage, and even occasionally blindness. **Aim:** To evaluate the spatial distribution of electroneuro-pathological aspects of brain activity at pregnant women with hypertensive disease by BEAM (Brain Electrical Activity Mapping). **Material and methods:** Twenty pregnant women (week 20 to 39) with hypertensive disease were recruited. Healthy pregnant women were also recruited control group. Maternal monitoring included spectral multichannel EEG analysis (brain mapping), repeated clinical examinations and laboratory investigations. **Results:** The major EEG changes observed were focal slowing of the background activity (14 patients) - occipital lobes especially, and intermittent spike and sharp wave transients (5 patients). **Conclusions:** Our findings indicate that EEG brain mapping are probably sensitive in detecting the extent of the pathology in the brain in women with in hypertensive disease associated with pregnancy. **Key words**: PREGNANCY, BRAIN MAPPING, HYPERTENSIVE DISEASE.

High blood pressure (hypertension) during pregnancy - whether is developed before or after conception - requires a special care. Hypertensive disorders occur in 6 to 12 % of pregnancies and contribute significantly to stillbirths and neonatal morbidity and mortality, also representing the second leading cause, after embolism, of maternal mortality. There are four distinct types of high blood pressure that can complicate pregnancy: 1) chronic hypertension; 2) preeclampsia-eclampsia; 3) preeclampsia superimposed upon chronic hypertension; 4) gestational hypertension (1, 2, 3).

Expectant mothers with hypertension are predisposed to the development of potentially lethal complications, notably *abruptio placentae*, disseminated intravascular coagulation, cerebral hemorrhage, hepatic failure, and acute renal failure. The etiology of most cases of hypertension during pregnancy, particularly preeclamp-

sia, remains mostly unknown (1, 3, 4).

The central nervous system manifestations include headache and visual disturbances including blurred vision, scotomata, and rarely, cortical blindness. Occasionally, focal neurologic signs may develop, which should prompt radiologic investigation. Eclampsia, the convulsive phase of preeclampsia, remains a significant cause of maternal mortality. Hemorrhage and edema have also been documented, and has been attributed both to coagulopathy and fibrin deposition, as well as hypertensive encephalopathy (1, 4, 5).

The aim of this study is to evaluate the spatial distribution of electroneuropathological pattern of brain activity at pregnant women with hypertensive disease by quantitative EEG - also known by the acronym BEAM (Brain Electrical Activity Mapping). Topographic brain mapping is an extension of conventional electroencephalography. It involves the computerized analysis and topographic display of EEG rhythms and evoked potential data on a color video screen. A wide assortment of maps can be created to represent different measurement patterns. The purpose of this technique is to identify patterns that distinguish pathological groups from normal ones (6, 7).

MATERIAL AND METHODS

Twenty pregnant women (week 20 to 39) with hypertensive disease (gestational hypertension or preeclampsia) were recruited from Cuza-Vodă Hospital, Iasi. The patients with cerebrovascular disease, epilepsy, mass lesions, toxic exposures, head injuries, and more were excluded from our study. The control group was formed by pregnant women without hypertensive disease associated with pregnancy. Maternal monitoring included multichannel EEG recording (fig. 1), spectral analysis (fig. 2) and brain mapping (fig. 3) - also blood pressure, weight curve, diuresis, subjective complaints or blood/urine chemistries changes.

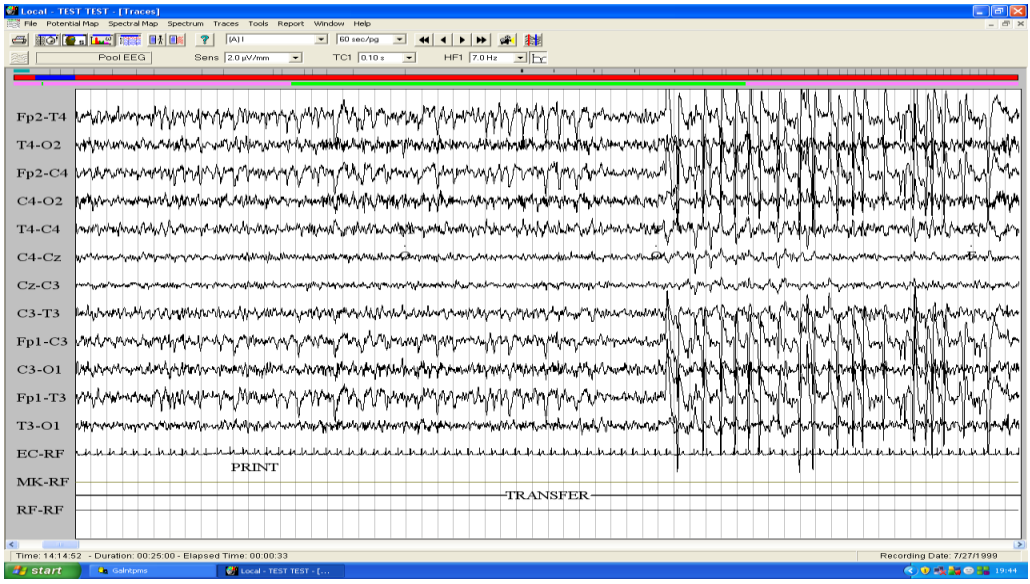

Fig. 1. Multichannel EEG recording

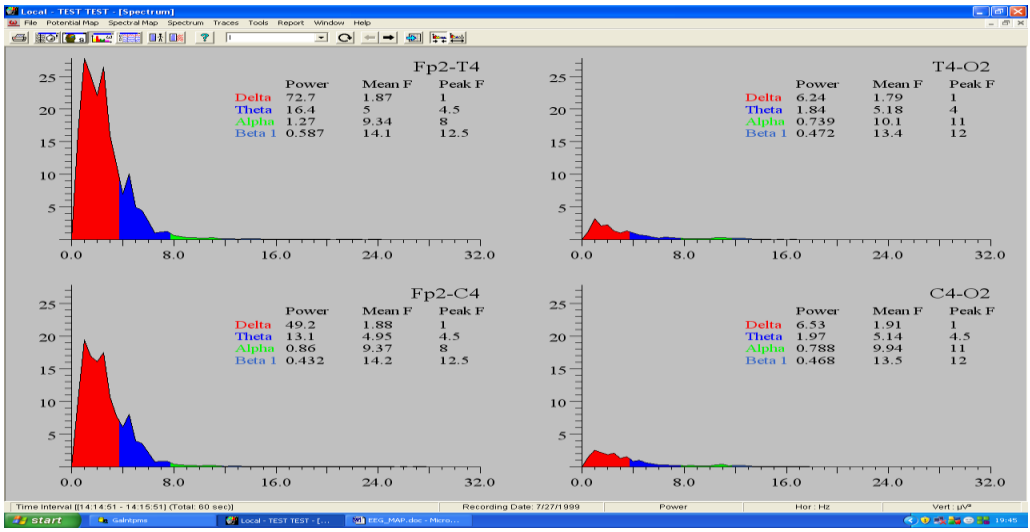

Fig. 2. Spectral analysis of EEG waves

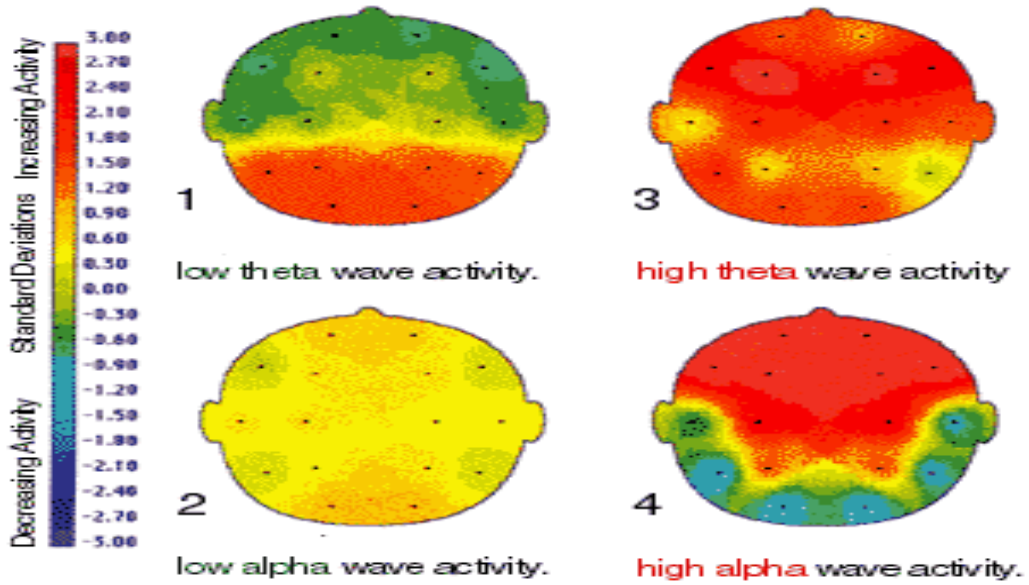

Fig. 3. EEG brain mapping

Fetal monitoring was performed by repeated clinical examinations, as well as by ultrasound assessment of fetal wellbeing, including placental maturity degree and Doppler velocimetry of umbilical /middle cerebral fetal artery- resistance index (U/C RI).

RESULTS

EEG brain mapping showed abnormalities in 14 patients compared to women in control group (fig. 4).

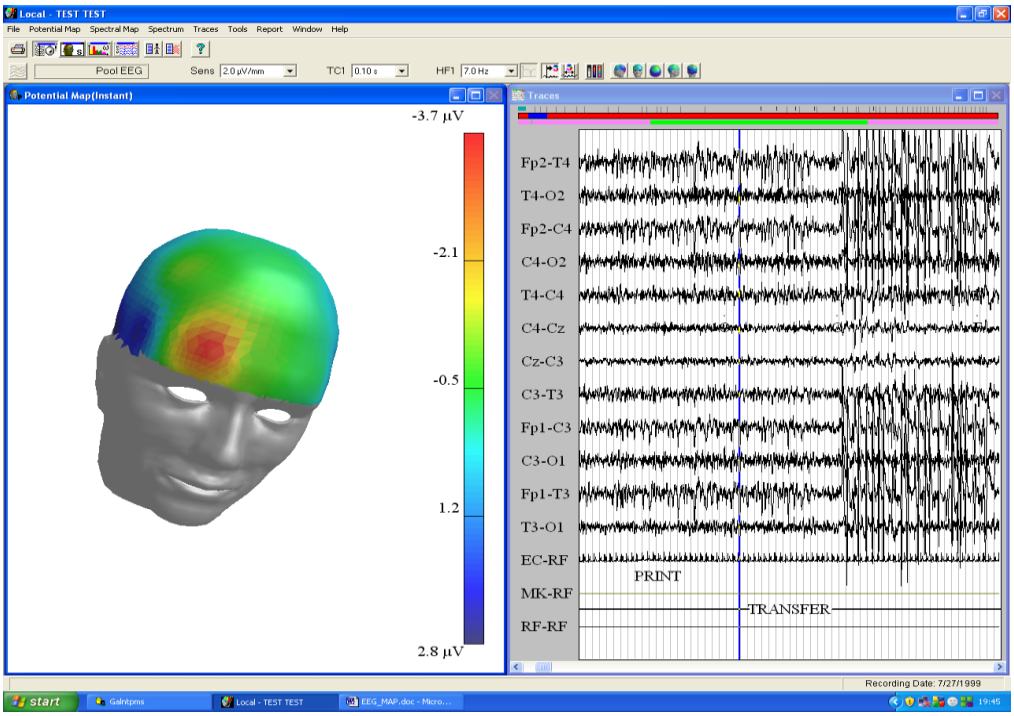

Fig. 4. Transposition of the brain electrical activity in brain mapping mode

The major EEG changes observed were focal (12 patients) or global (2 patients) slowing of the background activity-slowing of normal alpha (α) rhythms, and with the appearance of theta (θ) and continuous or non-continuous delta (δ) activities (fig. 5).

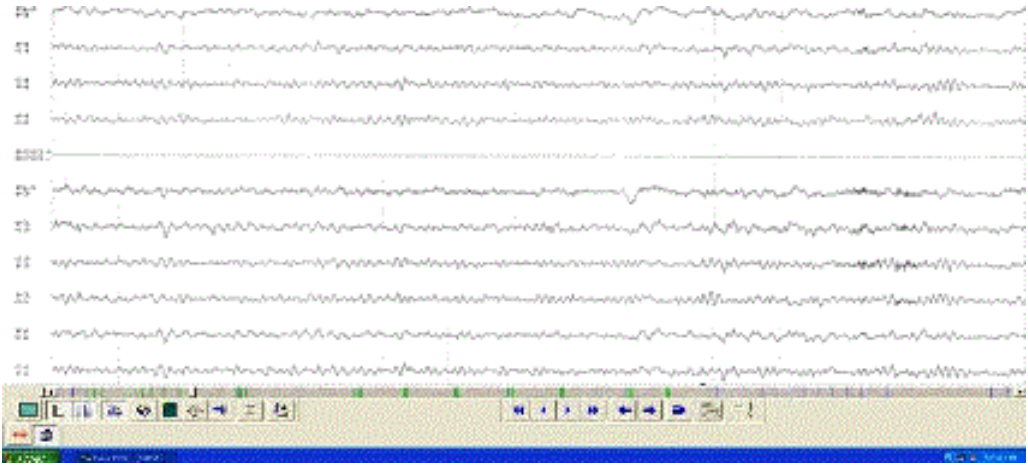

Fig. 5. Slowing of the background activity

Focal slow activity with paroxysmal or intermittent spike and sharp wave transients was also observed (5 patients) (fig. 6).

Topographic brain mapping showed these abnormalities in occipital lobes especially. Also 12 patients with EEG brain mapping changes have fetal ultrasound abnormalities: umbilical /middle cerebral fetal artery - resistance index (U/C RI) modified or abnormal placental maturity degree.

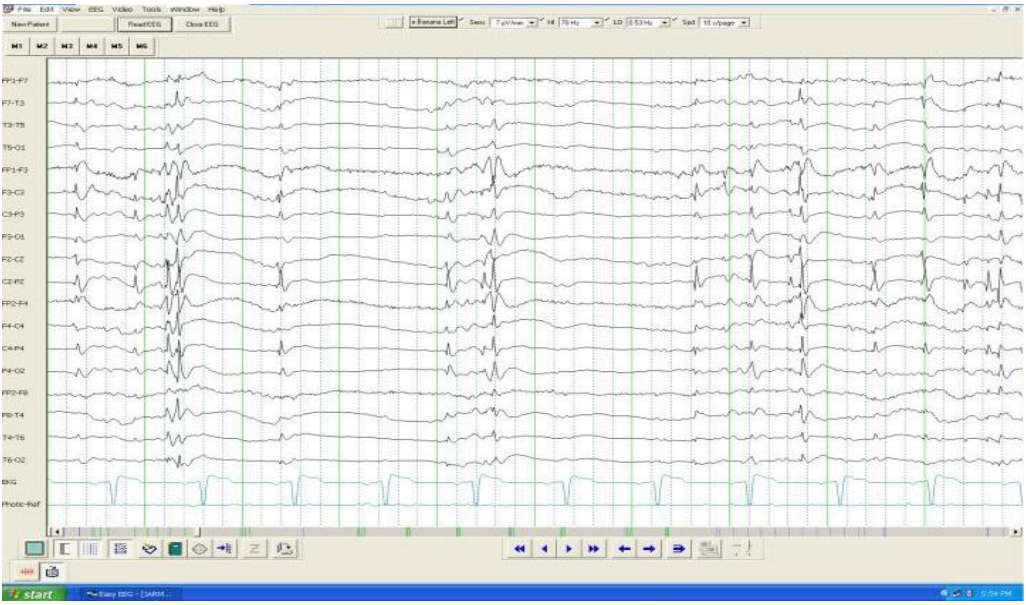

Fig. 6. Discontinuous electrical activity - high voltage electrical activity interspersed with areas of low voltage

DISCUSSION

In our study, abnormal EEG brain mapping findings were observed in the majority of the patients with hypertensive disorders, consisting of slow waves most frequently localized in the occipital lobe, as well as spike discharges. Our results are similar to the few studies on this topic so far, but little has been published on how these QEEG tests could affect the diagnosis or treatment of this patients. These changes are associated to hypertensive encephalopaty with thrombosis, fibrinoid necrosis of cerebral arterioles, diffuse microinfarcts and petechial haemorrhages (1, 2). Large changes in blood pressure (rather than the absolute blood pressure) or local metabolism can result in an imbalance of the capillary and cellular perfusion pressures, leading to vasogenic edema (1, 2, 3). Digital spike detection can identify candidate events that might develop seizures (eclampsia). Predominance of posterior lesions in our study may explain the increased incidence in preeclampsia-eclampsia of visual disturbances in the form of photophobia, diplopia, scotomata, amaurosis or blurred vision. Initially attributed to retinal arteriolar vasospasm or trombosis, these visual disturbances are now recognized to be associated with cerebral edema (1, 4, 5). When used by skilled

professionals experienced in quantitative EEG interpretation, sensitivity and specificity are high for detection of ischemia (6, 7). Ultrasound abnormalities observed at some of the patients with brain mapping changes-umbilical/cerebral flow patterns in combination with chronic placental dysfunction - are associated with fetal risk in preeclampsia. Further studies are required to determine the clinical implications of these abnormal findings in the absence of overt clinical symptoms, or possible correlations between blood pressure and aspects highlighted. A larger study is ongoing and is part of the PhD project, and the objectives are: 1) highlighting the role of EEG brain mapping in quantification of brain damage in hypertensive disease associated with pregnancy; 2) revealing statistical correlations between EEG abnormalities evidenced by mapping and pathological aspects during application maternal/fetal monitoring protocols, and if this changes can be a predictive factor for maternal-fetal risk.

CONCLUSIONS

Little has been published on how these QEEG tests could affect the diagnosis or treatment of individual patients. In summary, our findings indicate that EEG brain mapping are probably sensitive in detecting the extent of the pathology in the brain in women with in hypertensive disease associated with pregnancy, requiring more detailed studies on large groups of patients in order to obtain a clear conclusion.

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ÎNGRIJIRILE PRENATALE ŞI CREŞTEREA PONDERALĂ ÎN SARCINĂ

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PRENATAL CARE AND WEIGHT GAIN DURING PREGNANCY (Abstract): **Aim** To study the relationships between prepregancy body index mass (BMI), women's gestational weight gain and nutritional education during pregnancy. **Material and methods:** We carried a cross-sectional study on 400 women hospitalized in the maternity. Initial data included records on age, nutritional education during pregnancy and prepregnancy body mass index (BMI). The gestational weight gain was determined by using the weight registered in the obstetrics department before delivery . **Results:** In the group of overweight and obese women, 53.1% and respectively 66.7% registered a weight gain above the Institute of Medicine's recommendations, compared with 29.8% of normal weight women ($p<0.001$). Among the women with low prepregnancy BMI, 26.7% registered a weight gain below the guidelines ($p<0.001$). The nutritional education was associated with an appropriate weight gain in 44.3% women, compared with 40.7% who didn't receive any advice. The number of visits to the general practitioner and the obstetrician were associated with achieving recommended weight gain: 3.7% (no control), 38.3% (1-4 visits), 58% (more than 4 visits), and respectively 12.3% (no control), 24.7% (1 to 4 visits), 63% (more than 4 visits) ($p<0.001$). **Conclusion**s: The number of checks carried out during pregnancy plays an important role in achieving a weight gain within the guidelines. The individualized nutritional education has a positive impact on maintaining the recommended weight gain during pregnancy. **Key words**: PREPREGNANCY BMI, WEIGHT GAIN PREGANCY, PRENATAL CARE, NUTRITIONAL EDUCATION

Perioada perinatală este importantă din punct de vedere nutriţional, alimentaţia mamei înainte şi în timpul sarcinii afectând sănătatea produsului de concepţie la naştere şi pe tot parcursul vieţii (1). Creşterea ponderală în sarcină se evaluează după starea de nutriţie a gravidei, vârsta, paritatea, rasa, obiceiurile alimentare, comorbidităţile, numărul fetilor. Creşterea ponderală maternă în sarcină reprezintă unul din elementele esenţiale care influenţează greutatea nou născutului, durata sarcinii (2) şi riscul malformaţiilor congenitale (3). Metaanaliza efectuată de World Health Organization Collaborative Study (4) a relevat că indicele de masă corporală pregestaţional reprezintă un predictor al greutăţii la naştere a copilului. Baker (5) a demonstrat că întârzierea în creşterea intrauterină programează fătul spre mărirea riscului afecţiunilor cardiovasculare, a diabetului zaharat şi a sindromului metabolic.

Obezitatea maternă determină alterarea parametrilor neonatali, un scor Apgar scă-

zut, creşterea frecvenţei nou născuţilor cu greutate mare pentru vârsta gestaţională şi implicit a complicaţiilor asociate macrosomiei (distocia de umăr, traumatisme la naştere, creşterea mortalităţii perinatale). Pentru mamă, creşterea excesivă în greutate sporeşte riscul diabetului gestaţional, a hipertensiunii arteriale induse de sarcină, a bolii tromboembolice şi a infecţiilor urinare (6).

Studiile desfăşurate în România au evidenţiat o serie de deficienţe nutriţionale ale femeilor tinere, a gravidelor şi copiilor. Constatările au stat la baza elaborării unor intervenţii cuprinse în Programul Naţional de Sănătate a Femeii şi Copilului (7).

Scopul studiului a fost evaluarea indicatorilor antropometrici materni şi a efectului supravegherii prenatale şi educaţiei nutriţionale asupra creşterii ponderale în sarcină.

MATERIAL ŞI METODE

Studiul a inclus 400 de lehuze internate în septembrie 2010 în Spitalul Clinic de Obstetrică şi Ginecologie „Cuza-Vodă" Iaşi. Pentru obţinerea informaţiilor de la mame s-a utilizat un chestionar în care s-au consemnat date demografice, informaţii referitoare la utilizarea consultaţiei prenatale şi aspecte privind educaţia nutriţională în cursul sarcinii actuale.

Parametrii antropometrici materni evaluaţi au fost:

– indicele de masă corporală pregestaţional – determinat pe baza datelor consemnate de către medicul de familie în Carnetul de Sănătate al Gravidei sau prin utilizarea greutăţii autoraportate;

– înălţimea şi greutatea gravidei la sfârşitul sarcinii – determinate în maternitate înaintea naşterii;

– creşterea ponderală în cursul sarcinii – diferenţa între greutatea înregistrată la prima vizită la medicul de familie şi cea determinată înaintea naşterii în maternitate.

Pentru evaluarea stării de nutriţie înaintea sarcinii s-a utilizat clasificarea Organizaţiei Mondiale a Sănătăţii (8). În funcţie de indicele de masă corporală (IMC), femeile din lotul studiat au fost împărţite în: subponderale (IMC<18,5 kg/m^2), normoponderale (IMC 18,5-24,9 kg/m^2), supraponderale (IMC 25-29,9 kg/m^2) şi obeze (IMC>30 kg/m^2). Pentru evaluarea creşterii ponderale în relaţie cu IMC pregestaţional s-au utilizat recomandările din 2009 ale Institute of Medicine (IOM) din Washington (8) (tab. I).

TABELUL I

Creşterea ponderală recomandată în funcţie de IMC pregestaţional

IMC (Kg/m^2)	Creşterea ponderală recomandată (kg)
< 18,5	12,5- 18
18,5- 24,9	11,5 - 16
25- 29,9	7- 11,5
≥30	5 - 9

Analiza statistică s-a realizat cu ajutorul programului *SPSS 13.0*. Testul Shapiro-Wilk şi testul Kolmogorov-Smirnov s-au utilizat pentru evaluarea distribuţiei gaussiene a datelor analizate. Variabilele continue au fost exprimate ca medie, mediană, minim, maxim, deviaţie standard. Testul *Chi-Square* s-a folosit pentru evidenţierea existenţei unor diferenţe semnificative între eşantioanele formate din datele de frecvenţă.

REZULTATE

Vârsta medie a gravidelor în lotul studiat a fost de 27,53 ani, ponderea cea mai mare având-o cele cu vârsta între 19 şi 30 de ani (63,8%). Majoritatea gravidelor

(96%) au beneficiat de îngrijiri primare de sănătate, 81,4% dintre acestea prezentându-se la medicul de familie în cursul primului trimestru de sarcină, iar 83,5% au fost consultate în timpul sarcinii de medicul obstetrician. Numărul de controale la medicul de familie a variat între 0 şi 17, media fiind de 4,82, iar la medicul obstetrician a fost între 0 şi 12 vizite medicale, în medie 4,76 pe toată perioada sarcinii.

IMC pregestaţional a fost determinat la 389 persoane (97,3%), constatându-se o valoare medie de 22,31 kg/m^2, cuprinsă între 14,69 şi 44,98 kg/m^2. Creşterea ponderală în sarcină, măsurată la 95,5% dintre femeile din eşantionul studiat (382 gravide), a variat între 0 si 30 kg, având o valoare medie de 14,24 kg.

Femeile subponderale au avut o creştere a greutăţii în medie de 15,8 kg, cele normoponderale de 14,34 kg, pentru ca la cele supraponderale şi obeze să fie mai redusă (13,41 kg şi respectiv 13,11 kg), dar mai mare decât cea recomandată de IOM (tab. II).

TABELUL II

Creşterea ponderală în sarcină şi IMC pregestaţional

IMC (kg/m^2)	Creştere în greutate în sarcină						
	Nr.	%	Minim	Maxim	Media	Mediana	Deviaţia standard
< 18.5	45	11,8	4,0	27,0	15,289	15	4,9480
18.5 - 24.9	255	66,8	4.0	28,0	14,341	14	4,7742
25 - 29.9	64	16,8	5,0	30,0	13,414	12.	5.6122
≥30	18	4,7	0,0	25,0	13,111	14,5	7.5216

În grupul femeilor subponderale, 26,7% au avut o creştere în greutate sub cea recomandată, iar 51,1% în limitele indicate de IOM. Femeile normoponderale au avut o creştere adecvată a greutăţii în 42,7% din cazuri. În grupul femeilor obeze, numai 16,7% au avut o creştere ponderală optimă, iar 66,7% un câştig ponderal mai mare decât cel recomandat (Chi-Square Tests p< 0,001) (tab. III).

TABELUL III

IMC pregestaţional şi creşterea ponderală în funcţie de recomandările IOM

IMC pregestaţional	Creştere ponderală		
	sub recomandare %	normal – recomandare %	peste recomandare %
< 18,5	26,7	51,1	22,2
18,5 - 24,9	27,5	42,7	29,8
25 - 29,9	6,3	40,6	53,1
≥30	16,7	16,7	66,7

Numărul de controale la medicul de familie a fost în relație cu creşterea ponderală. Creştere optimă în greutate a fost mai frecventă la femeile cu mai mult de 4 controale (58%). Gravidele care au subutilizat consultația prenatală (56,2%) au avut mai frecvent o creştere ponderală mai mică decât cea indicată (Chi-Square Tests p=0,001) (tab. IV).

Dintre gravidele cu creştere ponderală optimă în sarcină, frecvența cea mai mare au avut-o gravidele cu mai mult de 4 controale de specialitate (63%), urmate de cele cu 1-4 vizite (24,7%) şi de cele care nu au beneficiat de consult obstetrical în sarcină (12,3%) (Chi-Square Tests p<0.001) (tab. V).

TABELUL IV

Creşterea ponderală în sarcină şi controlul la medicul de familie

		Creştere ponderală		
		sub recomandare %	**recomandată %**	**peste recomandare %**
Nr controale medic familie	fără control	3,4	3,7	1,5
	1-4 controale	56,2	38,3	29,5
	> 4 controale	40,4	58,0	68,9

TABELUL V

Creşterea ponderală în sarcină şi controlul la medicul obstetrician

Crestere ponderală	**Nr controale medic specialist**		
	fără control %	**1-4 Controale %**	**peste 4 controale %**
Sub recomandare	28,1	34,8	37,1
Normal recomandare	12,3	24,7	63,0
Peste recomandare	6,1	22,0	72,0

Gravidele care au beneficiat de educație nutrițională, au avut mai frecvent o creştere în greutate în limitele celei recomandate (44,3% față de 40,7%), fără ca asocierea întâlnită să fie semnificativă din punct de vedere statistic. Dintre femeile care au beneficiat de sfaturi legate de dietă în sarcină 69,2% au avut un număr mai mare de 4 controale, 29% au subutilizat consultația prenatală, iar 1,8% nu s-au prezentat la medicul de familie (p<0.001).

DISCUȚII

În cursul primului trimestru de sarcină, 81,4% dintre femeile din eşantionul studiat s-au prezentat la medicul de familie, în al II-lea trimestru 16,8%, iar în al treilea 1,8% din gravide. Aceste date arata o îmbunătățire a adresabilității în primul trimestru a gravidelor la medicul de familie față de studiile anterioare. În studiul desfăşurat de Institutul Ocrotirii Mamei şi Copilului "Statusul nutrițional al femeii gravide, al copiilor cu vârsta

sub 5 ani, al şcolarilor în vârstă de 6–7 ani" aproape 6% dintre femei nu au beneficiat de nici o consultaţie prenatală în cursul sarcinii, iar 68,8% s-au prezentat la prima consultaţie prenatală în cursul primului trimestru de sarcină. În „Studiului Sănătăţii Reproducerii" din 2004, 74% dintre femei au beneficiat de consultaţii prenatale în primul trimestru de sarcină (7).

Un număr mai mic de 4 consultaţii prenatale este considerat ca subutilizare, în condiţiile în care în România se recomandă minim 8 consultaţii pe durata sarcinii (7). În eşantionul nostru subutilizarea serviciilor prenatale a fost raportată de 44,3% dintre participante (3,8% fără control la medicul de familie, 40,5% cu 1-4 controale), evidenţiindu-se o deteriorare faţă de rezultatele din 2005 ale studiului „Statusul nutritional al femeii gravide", în care ponderea celor care au beneficiat de 1–4 consultaţii s-a ridicat la 33,3%, restul beneficiind de peste 4 consultaţii. În Studiul Sănătăţii Reproducerii din 2004 s-a constatat că numărul femeilor care au beneficiat de peste 4 consultaţii prenatale a crescut de la 59% în 1999 la 76% (7).

Studiile efectuate în SUA au arătat că 30-40% dintre gravide au o creştere ponderală în sarcină în afara limitelor recomandate de Institute of Medicine (9). Datele proiectului WISH (Women and Infants Starting Healthy) au arătat că 24,1% dintre femeile supraponderale şi obeze au avut o creştere ponderală mai mare decât cea recomandată, iar 51,2% dintre femeile subponderale au avut un câştig ponderal mai mic decât cel indicat. Apartenenţa la anumite grupuri etnice (hispanici), nivelul de educaţie, IMC-ul pregestaţional scăzut, lipsa sfaturilor nutriţionale au reprezentat predictori ai unei creşteri ponderale mai mici decât cea recomandată, iar supraponderea, multiparitatea şi vârsta tânără ai unei creşteri în greutate mai mari decât cea corespunzătoare IOM (10). Femeile supraponderale şi obeze din lotul studiat, au avut mai frecvent o creştere în greutate mai mare decât cea recomandată, în timp ce gravidele subponderale şi normoponderale au înregistrat mai des o creştere ponderală mai mică. Categoriile cu creştere ponderală în limitele recomandate de IOM pentru IMC cel mai frecvent întîlnite, îl reprezintă grupa femeilor normo şi subponderale (Chi- Square Tests $p< 0.001$).

Limitările studiului sunt reprezentate de:

- utilizarea greutăţii pregestaţionale autoraportate în cazul femeilor care nu au beneficiat de îngrijire prenatală constituie o sursă de eroare în cazul femeilor supraponderale, care tind să declare o greutate mai mică decât cea reală. Rezultatele studiului pot subestima relaţia dintre IMC pregestaţional şi creşterea ponderală în sarcină, dacă o parte din femeile supraponderale au fost încadrate ca normoponderale (11). OMS acceptă întrebuinţarea greutăţii pregestaţionale autoraportate în studiile epidemiologice pentru că reprezintă un indicator valoros în aprecierea creşterii ponderale şi un predictor al greutăţii nou născutului (12).;
- utilizarea informaţiilor de la gravide privind numărul de controale şi sfaturile privind dieta, determinată de lipsa documentelor medicale la toate femeile, ar putea conduce la o apreciere eronată a calităţii îngrijirilor prenatale.

CONCLUZII

Studiul a evidenţiat rolul major al IMC-ul pregestaţional asupra creşterii în greutate în sarcină, precum şi importanţa numărului de consultaţii prenatale în menţinerea unei creşteri ponderale în limitele celor reco-

mandate de ghiduri. În urma analizei efectuate, am constatat efectul pozitiv al educației nutriționale în menținerea unei creşteri în greutate în limitele indicate, dar eficiența acesteia este influențată de numărul de consultații prenatale. Femeile supraponderale şi obeze, precum şi cele subponderale, trebuie mai atent consiliate în ceea ce priveşte recomandările privind creşterea ponderală în sarcină.

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TUMORILE OVARIENE CU POTENȚIAL MALIGN SCĂZUT ŞI RADIAȚIA GLOBALĂ

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BORDERLINE OVARIAN TUMORS AND GLOBAL RADIATION IN RELATION WITH CLIMATE CHANGE IMPACT **(Abstract)**: There is limited information on borderline ovarian tumor detected intra-surgically and its most favorable treatment in relation with global radiation and climate changes. **Aim:** To study the pre-surgery and intra-surgery differentiation criteria of borderline ovarian tumors from invasive carcinoma, and to make a very complex analysis of the frequency, distribution, and variation in time of global radiation, temperature, and precipitation in North-East Romania. **Material and method:** The 54 patients (age range 20-78 years, mean age 46 years) included in this study had borderline ovarian tumors surgically treated during the last 22 years (January 1988 - December 2009) at the 4th Gynecological Clinic at Iaşi, and representing 4.87% of the total 1107 ovarian tumors detected and treated during this interval. The histological types were: serous (18 cases), mucinous (27 cases), mixed (8 cases), and Brenner tumor (1 case). In order to analyze the impact of climate changes on borderline ovarian tumors a thorough study on the frequency of global radiation in relation with climate changes based on data recorded in the last 55 years was also carried out. **Results:** The distribution of these cases depending on when surgery was performed was analyzed. In our study the frequency of ovarian borderline tumors (4.87%) is lower than in similar reports in the literature being due, in our opinion, to the influence of global radiation in relation with climate changes. **Conclusions:** In our study global radiation is probably responsible for a progression to invasive carcinoma in 0.7% of the borderline ovarian tumors. **Key words:** BORDERLINE OVARIAN TUMOR, GLOBAL RADIATION, CLIMATE CHANGE

Tumorile maligne ale ovarului se găsesc pe poziția a IV-a ca frecvență a cancerului la femei, reprezentând 15% din totalitatea cancerelor feminine. Tumorile cu Potențial Malign Scăzut (TPMS) de ovar reprezintă o categorie ce numără doar între 10% şi 15%, maxim 20% dintre toate tumorile epiteliale de ovar şi sunt de multe ori diagnosticate în stadiu timpuriu. În acest caz, supraviețuirea mai mult de 5 ani este de 100% pentru pacientele diagnosticate în stadiul I şi variază între 92% şi 86% cu stadii avansate în momentul stabilirii diagnosticului (7).

Considerăm că TPMS pot fi uşor subevaluate sau supraevaluate pe latura leziunilor invazive sau non-invazive. În literatura de specialitate sunt date limitate referitor la aspectul de identificare intraoperator a tipului borderline al tumorii (2). La fel de limitată este şi baza de date despre trata-

mentul optim planificat pentru TPMS (8), cât şi referitor la impactul radiaţiei globale în evoluţia unei tumori ovariene borderline spre carcinom invaziv (9).

MATERIAL ŞI METODE

Am realizat un studiu retrospectiv pe un număr total de paciente de 1107, diagnosticate cu tumori ovariene în perioada 1 ianuarie 1988 – 31 decembrie 2009 la Spitalul Universitar "Cuza-Vodă", Clinica a –IV-a Iaşi. Studiul a fost realizat pe mai multe loturi, astfel:

- Lot A - 54 paciente diagnosticate cu tumori ovariene cu potenţial malign scăzut.
- Lot B - 575 paciente internate şi diagnosticate cu tumori ovariene benigne.
- Lot C - 478 paciente internate şi diagnosticate cu tumori ovariene maligne.

Am urmărit 5 direcţii de studiu: **1)** evidenţa din punct de vedere statistic pe loturi; **2)** asocierea tipului de tratament chirurgical aplicat cu păstrarea fertilităţii şi monitorizarea pacientei pe termen lung; **3)** urmărirea unei stadializări corecte cu scopul de a depista eventualitatea transformării unei tumori ovariene cu potenţial malign scăzut într-un carcinom invaziv; **4)** urmărirea evoluţiei post-operatorii a pacientelor pentru a depista prezenţa implantelor invazive peritoneale care reprezintă factorii majori de risc asociaţi cu recurenţa şi un prognostic mai scăzut de supravieţuire; **5)** cercetarea influenţei radiaţiei globale asupra tumorilor ovariene cu potenţial malign scăzut. Am constatat o evoluţie a TPMS spre carcinomul invaziv la un procent de 0,7% dintre paciente, ceea ce considerăm că ar avea o principală cauză în influenţa radiaţiei globale şi a impactului schimbărilor climatice asupra metabolismului pacientei.

Ca metode de lucru am folosit: Studiul foilor de observaţie, studiul registrelor de laborator, studiul imagistic în evaluarea preoperatorie şi metode statistice aplicate: a) testul $ђ^2$ cu abaterea Δ şi eroarea absolută şi relativă ε; b) testul t-Student; c) coeficient de corelaţie "Pearson"; d) testul ANOVA; e) curba de supravieţuire Kaplan-Mayer.

REZULTATE

Am considerat loturile de paciente luate în studiu focalizând pe lotul A ce conţine tumorile ovariene borderline şi am reprezentat distribuţia cazurilor pe cele 3 loturi în funcţie de caracteristicile tumorale (tab. I), cu prelucrarea statistică (fig.1, fig. 2).

Am identificat proprietăţile TPMS din lotul A şi pattern-ul intraoperator (tab. II, fig. 3) şi am realizat distribuţia pacientelor ca număr şi procent în funcţie de tipul procedurii chirurgicale (tab. III, fig. 4).

TABELUL I

Distribuţia cazurilor de tumori ovariene în funcţie de caracteristicile tumorale

Nr. Crt.	Distribuţia cazurilor pe loturi	Nr. Cazuri (n=1107)	Frecvenţa %	Caracteristici tumorale
1.	Lot A	54	4,87	TPMS
2.	Lot B	575	51,95	Benigne
3.	Lot C	478	43,18	Maligne

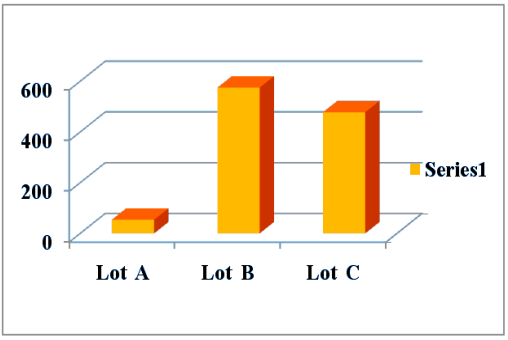

Fig. 1. Evaluarea cazurilor pe loturi A, B, C

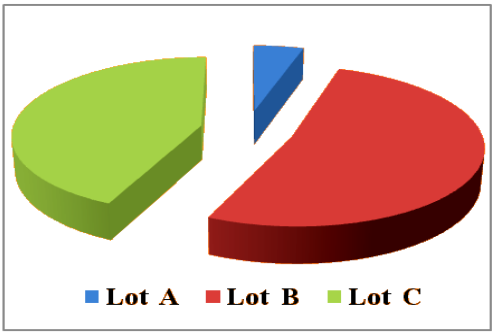

Fig. 2. Distribuția tumorilor pe loturi

TABELUL II

Distribuția TPMS după rezultatele obținute intraoperator în funcție de proprietăți şi tipuri la Clinica a-IV-a Iaşi în perioada 1988-2009.

Nr. crt.	Proprietățile şi tipurile de TPMS intraoperator	Nr. cazuri (n=54)	Frecvența %
1	Unilateral (ovarul drept)	15	27,57
2	Unilateral (ovarul stâng)	6	11,76
3	Bilateral în TPMS	33	61,12
4	Implante non-invazive	11	20,37
5	Implante invazive	6	11,76
6	TPMS mucinoasă	27	50,00
7	TPMS seroasă	18	33,33
8	Încărcare maximă tumorală în câmpul B1	42	82,35
9	Încărcare maximă tumorală în câmpul B1 şi B2	4	7,80
10	Încărcare maximă tumorală în câmpul B3	1	1,96
11	Încărcare maximă tumorală în câmpul A3	1	1,96

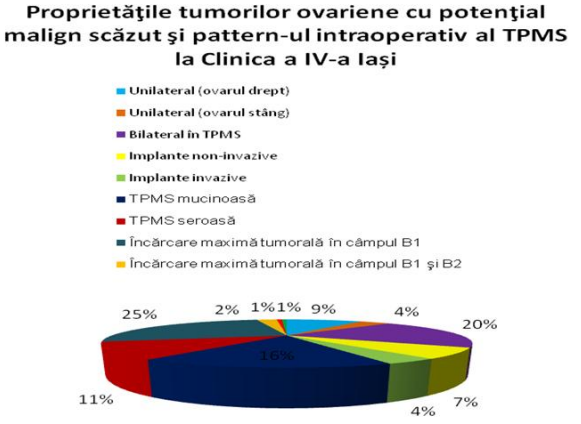

Fig. 3. Distribuția procentuală a cazurilor TPMS în funcție de proprietăți şi tipuri intraoperator.

TABELUL III

Distribuția statistică a cazurilor în funcție de tipul procedurii chirurgicale efectuate la Clinica a-IV-a Iaşi în perioada 1988-2009.

Nr. Crt.	Tipul procedurii chirurgicale efectuate	Nr. cazuri (n=54)	Frecvența %	Rezultate histo-patologic pozitive (nr. cazuri)	Rezultate histo-patologic pozitive %
1	Lavaj peritoneal	28	51.85	7	12.96
2	Omentectomie	19	35.18	9	16.66
3	Histerectomie	25	46.3	4	7.4
4	Apendicectomie	7	12.96	1	1.85
5	Rezecția nodulilor pelvici limfatici	2	3.7	4	7.4
6	Decolarea peritoneului în reducție tumorală prin impli-care macroscopică peritoneală	13	25.5	12	23.53
7	Decolarea peritoneului în reducție tumorală prin biopsie peritoneală pentru stadializare	33	64.7	--	--
8	Rezecția nodulilor para-aortici limfatici	2	3.7	0	0%
9	Mică rezecție intestinală	4	7.84	2	3.95
10	Rezecție de sigmoid/rect	4	7.84	4	7.84
11	Coagulare în infraroşu	7	13.72	--	--
12	Rezecție parțială de diafragmă	2	3.9	2	3.9

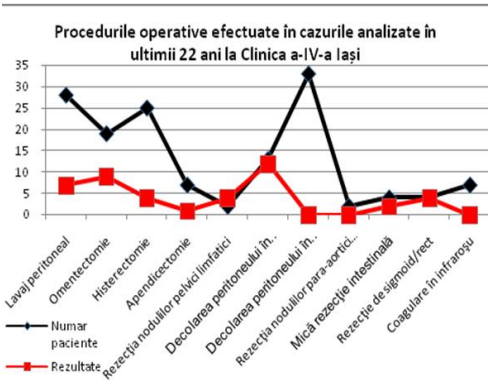

Fig. 4. Prelucrarea statistică a cazurilor de proceduri operatorii efectuate în perioada 1988-2009 la Clinica a-IV-a Iaşi.

Am realizat, de asemenea un studiu al frecvenței radiației globale şi al corelării valorilor radiației globale cu durata pentru a completa tabloul distribuției în timp şi spațiu a schimbărilor climatice în domeniul radiației globale în zona Iaşi (fig. 5).

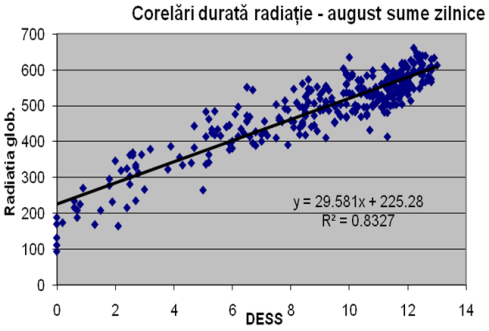

Fig. 5. Distribuția pe praguri de valori medii de radiații la Iaşi în perioada 1960-2009

Prezentăm din cazuistica personală cele mai reprezentative imagini ecografice de tumori borderline de ovar (fig. 6, 7, 8, 9) obținute prin ultrasonografie transabdominală (UTA) şi ultrasonografie transvaginală (UTV) considerată ca având o fiabilitate de 75%, iar sensibilitatea şi specificitatea 100 % (9).

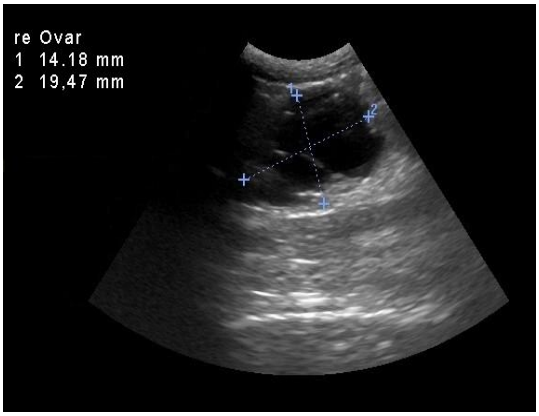

Fig. 6. UTA: ovar drept cu tumoră chistică(colecția personală)

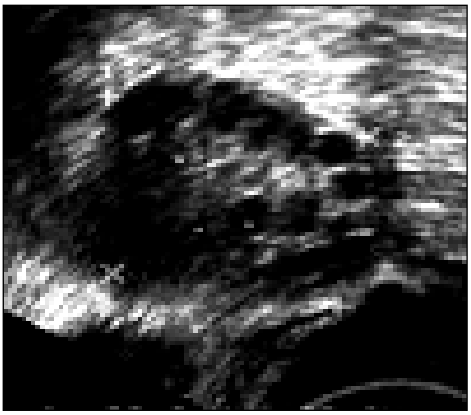

Fig. 7. UTV: ovar drept cu nuclee tumorale (colecția personală)

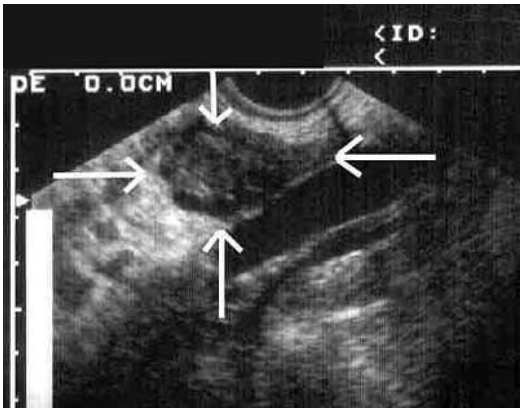

Fig. 8. UTA: ovar drept cu tumoră borderline (colecția personală)

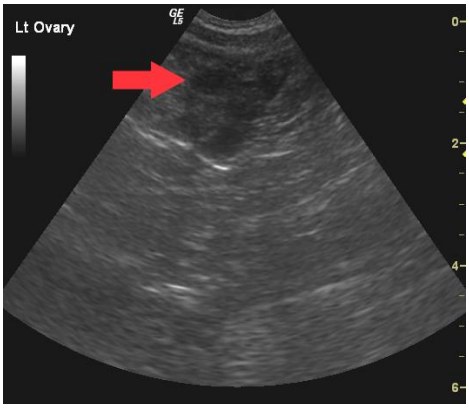

Fig. 9. UTA: ovar stâng tumoral (TPMS) (colecția personală)

În subpopulația cu TPMS recidivate, toate cele 5 paciente au prezentat implanturi epiteliale peritoneale pozitive. Analiza imuno-histologică a arătat că recurența a apărut din nou ca tumoare periferică la 2 paciente (23,53%), în timp ce la 3 paciente (60%) a apărut o conversie la tumoare infiltrantă. Metastazele extrapelvice au fost prezente la 12 paciente (23,53%).

Raportat la procentul de 0,7% calculat de noi pe baza rezultatelor studiului de cercetare, el se referă la pacientele cu tumori ovariene borderline care, din cauze necunoscute (8), trec în carcinom invaziv cu un prognostic rezervat, deşi au urmat aceeaşi schemă de tratament chirurgical şi adjuvant ca cele 99,3% paciente la care nu a apărut această evoluție a TPMS. Conside-

răm că acest procent de 0,7% poate fi explicat prin impactul radiației globale şi al schimbărilor climatice asupra unui număr mic de paciente care vor da procentul de 0,7%.

CONCLUZII

Din analiza distribuțiilor statistice ale radiației globale am constatat că acestea sunt liniare şi crescătoare pentru fiecare lună reprezentativă. Aceasta demonstrează că, în ultimii 50 ani, în perioada 1960-2009, valorile radiației globale au crescut constant şi mult, ceea ce demonstrează faptul că pot influența evoluția tumorilor în general, respectiv a tumorilor ovariene în special de la tumori borderline până la carcinoame invazive, ceea ce ar putea constitui o explicație pentru acel procent de 0,7% TPMS de ovar ce au o evoluție imprevizibilă spre carcinom invaziv.

Seriile lungi de timp cu valori numerice ale radiației globale sunt necesare ca suport pentru un studiu variat. Totuşi, algoritmul considerării datelor observaționale limitează scalele rangului determinărilor ce pot fi raportate ca fiind cu acuratețe, adică fără erori.

Impactul schimbărilor climatice şi al radiației globale asupra numărului de paciente cu TPMS se manifestă prin acel procent de 4,87% TPMS a căror evoluție postoperatorie este imprecis precizată ştiințific. Dintre ele, un procent de 0,7% TPMS vor evolua spre carcinomul invaziv, una dintre cauze fiind radiația globală şi schimbările climatice.

Considerăm că există o legătură importantă între evoluția tumorilor ovariene borderline şi radiația globală ca şi între TPMS de ovar şi impactul schimbărilor climatice asupra pacientelor, ceea ce ar putea explica evoluția într-un procent de 0,7% a TPMS spre carcinomul invaziv, dar ar putea conduce şi spre o mai bună înțelegere a unor feedback-uri între procesele atmosferice legate de cele terestre la o scală spațială şi temporală diferită şi tumorile ovariene borderline, impactul schimbărilor climatice neputând fiind neglijat.

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NOUTĂȚI

CIMENTAREA LUCRĂRILOR PROTETICE PE BAZĂ DE ZIRCONIU: DIFICULTĂȚI ŞI SOLUȚII

Ceramicile fără siliciu, în special zirconiul au devenit un subiect de mare interes în protetica şi implantologia dentară datorită combinației inedite dintre calitățile estetice şi mecanice. Totuşi, acest tip de materiale prezintă o problemă deocamdată nerezolvată legată de dificultatea realizării unei adeziuni adecvate atât cu materialul sintetic asociat cât şi cu țesuturile dentare. Tehnicile tradiționale adezive utilizate cu ceramicile pe bază de siliciu nu funcționează la fel de eficient cu zirconiul. Recent au fost propuse noi tehnologii pentru soluționarea acestei probleme care urmăresc fie modificarea suprafeței ceramicilor, fie funcționalizarea chimică a suprafeței de zirconiu pentru a o face mai reactivă şi aptă pentru adeziune. Cimenturile care par să prezinte adeziune satisfăcătoare sunt materiale pe bază de rășini care care conțin monomeri fosfatați (Panavia Ex - Kuraray Medical Inc; RelyX Unicem - 3M ESPE), sau cele care conțin 4META/TBB/PMMA (Sun Medical, Moriyama City) care pare să se lege de zirconiu prin gruparea anhidridă din 4-META. În cazul cimenturilor care conțin monomeri fosfatați, adeziunea poate fi ameliorată prin adăugarea unor agenți de cuplare silanici. Mecanismul ce stă la baza acestui efect este legat de natura acidă a acestor monomeri care hidrolizează rapid agenții de cuplare, producând legăturile polisiloxanice necesare pentru adeziune. Deocamdată, cimenturile care conțin monomeri fosfatați par să reprezinte soluția preferată de practicieni pentru fixarea lucrărilor protetice din zirconiu datorită ratei de eşec reduse prin pierderea retenției (Thompson JY, Stoner BR, Piascik JR, Smith R. Adhesion/cementation to zirconia and other non-silicate ceramics: Where are we now? *Dental Materials* 2011; 27(1): 71-82).

Gianina Iovan

REZECȚIA ANTERIOARĂ JOASĂ DE RECT CU EXCIZIE TOTALĂ DE MEZORECT. REZULTATE IMEDIATE

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LOW ANTERIOR RESECTION OF THE RECTUM WITH TOTAL MESORECTAL EXCISION – IMMEDIATE RESULTS (Abstract): **Aim:** this study was aimed at analyzing the immediate postoperative course in rectal cancer patients who underwent a low anterior resection of the rectum with total mesorectal excision. **Material and methods:** A retrospective study was carried out on a series of 75 patients operated between January, 1 2004 and December, 31 2010 at the IIIrd Surgical Unit of the Iasi "St. Spiridon" Hospital,. Low anterior resection of the rectum with total mesorectal excision was performed in all the patients. Data from medical files regarding the immediate postoperative course were analyzed. **Results:** Neoadjuvant therapy was instituted in 32 patients. There were 28 mechanical colorectal anastomoses and 47 manual anastomoses. Protective ileostomy was performed in 46 cases, including 11 manual anastomosis and 35 mechanical anastomosis cases. Anastomotic fistulas occured in 11 patients (6 with manual suture and 5 with stapler). Wound complications were identified in 5 cases, while retraction of ileostomy in 2. Two patients died from anastomotic fistula causing abdominal sepsis and multiple organ failure. In both cases ileostomy was performed at the reintervention, concomitantly with drainage of the abdominal abscesses. **Conclusions:** Healing of the colorectal anastomosis remains the major problem with low anterior resection of the rectum. Protective ileostomy reduces the risk of serious complications in the event of anastomotic fistula. **Key words:** RECTAL CANCER, TOTAL MESORECTAL EXCISION, IMMEDIATE POSTOPERATIVE RESULTS

În urmă cu 30 de ani, recurența locală după intervențiile chirurgicale pentru neoplasmul rectal varia între 20-45%. Tratamentul chirurgical în cancerul rectal are un rol primordial în vindecarea pacienților care suferă de această boală. Un studiu multicentric german publicat în 1994 arăta că rata recurenței locale variază în funcție de chirurg, de la 10 la 50%, astfel, pacienții chirurgilor cu rata de recurență de 10% au o şansă de vindecare de 80% pe când pacienții chirurgilor cu rata recurenței de 50% au şanse de vindecare doar de 40%. În acelaşi studiu s-a demonstrat că terapia adjuvantă (radio şi chimioterapia) îmbunătățeşte rezultatele, în cele mai optimiste situații, cu 20% pe când o tehnică chirurgicală corectă poate îmbunătăți rezultatele cu până la 80% (1).

În 1982, R. J. Heald publica articolul „The mesorectum in rectal cancer - the clue to pelvic recurrence?" prin care ridica problema exciziei complete a mezorectului pentru cancerele rectale. Heald susține că,

inițial, mezorectul conferă protecție contra diseminării tumorale iar extirparea sa în bloc cu rectul minimalizează riscul recidivei tumorale (2).

Infiltrația tumorală în peretele rectului este la majoritatea pacienților cu cancere diferențiate de până la un cm (3) pe când infiltrația limfatică distală în mezorect se poate detecta până la 5 cm de tumoră (2). Din acest motiv şi importanța exciziei distale a mezorectului, în cazul cancerelor rectale ampulare inferioare şi mijlocii fiind necesară excizie totală de mezorect. Recidivele la nivelul anastomozelor, sunt de fapt, recurențe în afara lumenului care invadează ulterior anastomoza.

Un alt element important în obținerea unor rezultate favorabile este reprezentat de marginile circumferențiale de rezecție. Dacă secționarea rectului se face, în marea majoritate a cazurilor în țesut neinfiltrat neoplazic, marginile circumferențiale de rezecție fără infiltrație tumorală se realizează printr-o disecție atentă îngreunată uneori de stadiul avansat al bolii neoplazice. Pentru un rezultat favorabil, marginea de rezecție trebuie să fie la peste 1mm față de frontul de invazie tumoral, fie că este vorba de adenopatie neoplazică, depozite tumorale din mezorect sau trombi tumorali intravasculari.

Rata recidivei locale este şi mai mult scăzută prin tratamentele neoadjuvante: radioterapie respectiv radiochimioterapie (4). Beneficiile potențiale ale terapiei neoadjuvante sunt reprezentate de diminuarea dimensiunilor tumorale şi sterilizarea metastazelor ganglionare din aria iradiată (down-sized, down-staged) (3).

MATERIAL ŞI METODĂ

În perioada 1 ianuarie 2004 – 31 decembrie 2010 au fost operați 566 pacienți cu neoplasm rectal şi joncțiune rectosigmoidiană. În perioada menționată au fost efectuate 75 rezecții anterioare de rect cu excizie totală de mezorect. Repartiția pe sexe a fost astfel: 45 bărbați şi 30 femei (fig. 1).

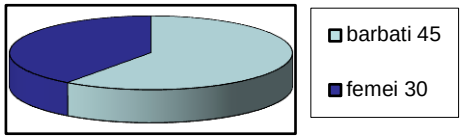

Fig.1.Repartiția pe sexe

Din cele 75 cazuri, în anul 2004 au fost 3 cazuri, în 2005 – 11 cazuri, în 2006 şi 2007 au fost câte 9 cazuri, în 2008 au fost înregistrate 15 cazuri, în 2009, 18 cazuri iar în 2010 au fost efectuate 10 rezecții anterioare joase de rect cu excizie totală de mezorect (fig.3).

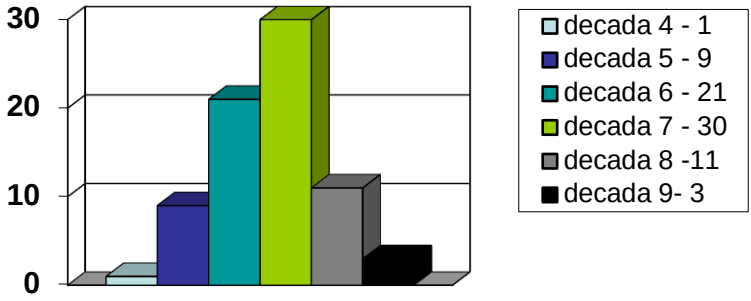

Fig. 2 Vârsta pacienților a fost situată între 39 şi 83 ani, cu o vârstă medie de 64 ani.

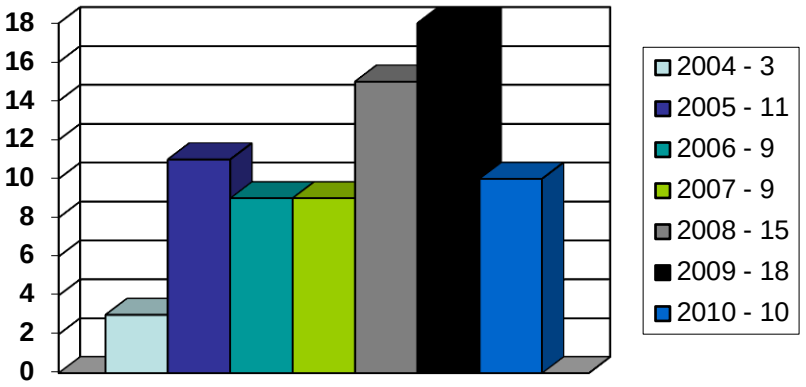

Fig. 3. Repartiția pe ani

Din punct de vedere al localizării, tumorile au fost împărțite astfel:

- neoplasm rectal superior şi joncțiune rectosigmoidiană – 27 cazuri
- neoplasm rectal ampular mijlociu – 42 cazuri (în două situații au fost identificate neoplazii sincrone de sigmoid şi de cec)
- neoplasm rectal ampular inferior – 6 cazuri

Distanța tumorii rectale față de marginea anală s-a situat între 4 şi 15 cm .

Pentru o stadializare corectă a cazurilor, pe lângă tehnicile imagistice uzuale (ecografie abdominală, radiografie toracică), 39 pacienți au beneficiat de explorare RMN, CT şi/sau ecografie endorectală:

- RMN -17
- CT - 13
- Ecografie endorectală - 13
- RMN + CT - 2

Terapia neoadjuvantă a fost aplicată la 32 cazuri, 19 pacienți beneficiind de radioterapie şi 13 de radiochimioterapie. La pacienții care au beneficiat de terapie neoadjuvantă intervenția chirurgicală a fost efectuată la aprox. 6 săptămâni de la terminarea radioterapiei/radiochimioterapiei. Doi pacienți prezentau o colostomie laterală înaintea rezecției rectale, colostomiile fiind efectuate în urgență pentru leziuni rectale stenozante.

Având în vedere vârsta de apariție a neoplasmului rectal, pacienții au o patologie asociată destul de bogată. Afecțiunile asociate întâlnite au fost:

- insuficiență cardiacă Clasa II NYHA 3 cazuri, clasa a III-a NYHA un caz
- fibrilație atrială cronică 6 cazuri
- angină pectorală de efort un caz
- hipertensiune arterială 12 cazuri
- prolaps de valvă mitrală un caz
- cardiopatie ischemică cronică 4 cazuri
- tulburări de conducere cardiacă: bloc major de ramură 2 cazuri
- hepatopatie cronică 7 cazuri (3 virus C, 2 virus B, 2 toxică)
- ciroză hepatică toxică un caz
- litiază biliară 4 cazuri (un caz cu litiază coledociană)
- astm bronşic un caz
- tuberculoză fibronodulară 3 cazuri
- bronşită cronică 2 cazuri
- emfizem pulmonar 8 cazuri

- litiază renală 9 cazuri
- diabet zaharat tip 2 , 3 cazuri
- accident ischemic vascular sechelar un caz
- boală Parkinson un caz
- fibrom uterin 2 cazuri
- ulcer duodenal cronic 3 cazuri
- infecție tract urinar 5 cazuri
- dislipidemie 3 cazuri
- hernie inghinală bilaterală 1 caz
- polip vezică urinară 1 caz
- adenom de prostată 8 cazuri
- lobectomie pulmonară pentru bronşiectazie un caz
- anemie secundară 34 cazuri

La momentul intervenției, 5 pacienți prezentau metastaze hepatice.

REZULTATE

Din cele 75 rezecții anterioare de rect cu excizie totală de mezorect, 28 au fost cu anastomoză colorectală mecanică, celelalte anastomoze 47 fiind efectuate manual, cu fire separate. Ileostomia de protecție a fost efectuată în 46 cazuri (11 ileostomii în grupul anastomozelor mecanice şi 35 în grupul pacienților cu anastomoză manuală). Ca şi incident intraoperator, a fost necesară o sutură de arteră iliacă primitivă ca urmare a unei plăgi accidentale. Datorită stricturilor uretrale, unui pacient nu i s-a putut introduce sonda urinară pe cale uretrală, inserându-se un cateter suprapubian.

Intervențiile chirurgicale asociate rezecției rectale au fost:

- hemicolectomie dreaptă un caz
- apendicectomie 2 cazuri
- rezecție cec şi ultimă ansă ileală un caz
- metastazectomie hepatică 1 caz
- histerectomie totală cu anexectomie bilaterală
- 2 cazuri – fibrom uterin
- un caz – invazie uterină
- cistectomie parțială 2 cazuri
- anexectomie bilaterală un caz
- colecistectomie, coledocolitotomie, anastomoză biliodigestivă un caz
- cura herniei inghinale un caz

Durata medie a intervenției chirurgicale a fost 162 minute. Rezecțiile cu anastomoză mecanică au avut o durată medie mai mică (145 minute față de aprox 168 minute) prin faptul că durata de efectuare a anastomozei mecanice este mai redusă, numărul ileostomiilor a fost mai mic, cât şi prin intervențiile asociate care au fost mai numeroase în grupul rezecțiilor în care anastomozele au fost efectuate manual.

Stadializarea anatomopatologică a pieselor de rezecție a adus următoarele informații:

- T0 - 1 obs N0 31 obs
- T1 - 4 obs N1 17 obs
- T2 - 15 obs N2 14 obs
- T3 - 38 obs Nx 13 obs
- T4 - 7 obs

La un pacient cu radioterapie neodajuvantă, a fost înregistrată o remisiune completă cu dispariția tumorii. Ceilalți pacienți au fost împărțiți, în funcție de clasificarea Dukes, astfel:

- Dukes A – 3 obs.
- Dukes B – 28 obs.
- Dukes C – 38 obs.
- Dukes D – 5 obs.

Numărul de ganglioni examinați a fost între 2 si 42 cu un număr mediu de 15,1. Pentru a formula un stadiu N la examenul anatomopatologic, trebuiesc examinați minim 12 ganglioni.

La 12 pacienți au fost descoperiți pe piesele de rezecție polipi adenomatoşi/ adenoviloşi.

Doar la 31 pacienți au fost descrise marginile de rezecție, la cei descrişi distanța fiind de peste 1 cm la un pacient, peste 1,4 cm la 2 pacienți şi peste 1,5 cm la 2 pacienți. La restul a fost specificat că marginea circumferențială de rezecție este „la distanță" de frontul de invazie tumorală.

Durata de spitalizare (postoperatorie) a variat între 7 şi 44 zile, durata medie fiind de 13,64 zile.

În perioada imediată după rezecție (până la externarea pacienților) au fost înregistrate 11 fistule anastomotice evidente clinic – 14,67% (6 fistule în rândul pacienților cu anastomoze efectuate manual iar 5 la cei cu anastomoză mecanică). Au fost întâlnite şi complicații ale plăgii postoperatorii – 5 obs. - 2 supurații, 2 seroame ale plăgii şi o infecție necrozantă a plăgii postoperatorii.

Ileusul postoperator prelungit (necesitatea menținerii sondei nasogastrice peste 5 zile postoperator) a fost întâlnit la 5 pacienți. Disfuncțiile urinare au fost prezente la 7 pacienți, dintre aceştia un singur pacient a fost externat cu sonda urinară revenind ulterior pentru suprimarea sondei vezicale.

La 11 pacienți s-a reintervenit, ziua şi motivul reintervenției fiind:

- ziua a 2-a remodelarea ileostomiei
- ziua a 3-a drenajul fistulei anastomotice şi ileostomie
- ziua a 4-a suspiciune necroză de colon, s-a identificat o infecție necrozantă de perete abdominal + ziua a 17- a sutură secundară
- ziua a 7-a ileostomie pentru fistulă rectovaginală
- ziua a 7-a remodelarea ileostomiei
- ziua a 9-a refacere ileostomă
- ziua a 10-a devolvularea unei anse de intestin subțire
- ziua a 10-a colostomie pentru o fistulă rectovaginală + ziua a 15 a drenajul perineal al unui hematom presacrat infectat
- ziua a 11-a colostomie laterală pe colon transvers pentru fistulă anastomotică
- ziua a 13-a ocluzie mecanoinflamatorie
- ziua a 14-a ileostomie pentru fistulă anastomotică + ziua a 31-a drenajul perineal al unei colecții presacrate

Alte complicații, fără legătură directă cu intervenția chirurgicală, întâlnite în lotul de studiu au fost:

- infecții urinare 3 cazuri
- pleurezie 2 cazuri
- ureterohidronefroză secundară litiazei ureterale un caz
- pneumonie bacteriană un caz

Au fost înregistrate 2 decese, ambii pacienți au fost de sex masculin, fără terapie neoadjuvantă. Un pacient de 69 ani care a dezvoltat o fistulă anastomotică în ziua a 7a, s-a reintervenit chirurgical în ziua a 14 a şi s-a practicat ileostomie de protecție şi drenajul unei colecții presacrate în ziua 31 postoperator, pacientul decedând prin Insuficiență Organică Multiplă. Al doilea deces a survenit la un pacient cu fistulă anastomotică precoce la care s-a reintervenit în ziua a 3 a practicându-se dirijarea fistulei anastomotice şi ileostomie de protecție. Decedează tot prin Insuficiență Multiplă de Organ în ziua a 24 a postoperator.

DISCUȚII

Excizia totală a mezorectului în chirurgia cancerului rectal are ca scop principal scăderea incidenței recidivei locale postoperatorii. Acest articol are drept scop prezentarea complicațiilor din perioada imediat postoperatorie, recidivele locale şi tulburările funcționale vor face subiectul unui

alt articol.

Marea provocare a acestei intervenții rămâne anastomoza colorectală. Bontul rectal de aproximativ 2-4 cm deasupra marginii anale are o vascularizație precară, ceea ce face ca cicatrizarea la nivelul anastomozei să fie grevată de riscul de apariție al unei fistule. Spațiul liber rămas după extirparea rectului în bloc cu mezorectul favorizează apariția hematoamelor care se pot evacua, la nivelul anastomozei, în tractul digestiv – fiind un factor în plus pentru apariția unei fistule.

Rata fistulelor anastomotice după rezecția rectului cu excizie totală de mezorect variază în literatură. Heald, în articolul inițial, prezenta o rată a fistulelor anastomotice de 13%, frecvența crescând până la 18% dacă anastomozele sunt verificate radiologic (2).

CONCLUZII

Din aceste motive, se recomandă efectuarea unei colostomii sau a unei ileostomii pentru a lăsa anastomoza să se cicatrizeze în absența tranzitului intestinal.

Ileostomia poartă şi ea riscul unor complicații (retracții, stenoze) dar riscurile sunt reduse iar rezolvarea acestor complicații nu presupune gesturi chirurgicale de amploare.

În lotul studiat, au fost înregistrate 11 fistule anastomotice (14,67%), procent comparabil cu datele existente în literatură.

Evoluția nefavorabilă a survenit la doi pacienți cu fistulă anastomotică dar fără o ileostomie sau colostomie de protecție ceea ce arată necesitatea efectuării de rutină a ileostomiei / colostomiei.

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ACUTE GASTROENTERITIS IN CHILDREN. CURRENT EPIDEMIOLOGICAL AND ETIOLOGICAL CONSIDERATIONS

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ACUTE GASTROENTERITIS IN CHILDREN. CURRENT EPIDEMIOLOGICAL AND ETIOLOGICAL CONSIDERATIONS (Abstract): Acute diarrheal disease ranks second among the causes of specific morbidity (after respiratory infections) with higher incidence values in economically undeveloped or developing countries (3 to 7 episodes of diarrhea / year / child compared to only 1-2 episodes / year / child in highly industrialized countries). Infection with *Campylobacter* globally was the most frequently reported zooanthroponosis, with 214,268 confirmed human cases in 2012. Almost any pathogen involved in the etiology of acute diarrheal disease may be involved in the occurrence of healthcare-associated infections; among these, the rotavirus and *Clostridium difficile* are most prevalent. General hygiene measures change the epidemiological aspect of acute diarrheal disease by modifying the factors which favor the transmission of pathogens. **Keywords**: ACUTE DIARRHEAL DISEASE, EPIDEMIOLOGY, HEALTHCARE-ASSOCIATED INFECTION.

Acute gastroenteritis (including acute diarrhea, acute diarrheal disease) is commonly the second cause of death worldwide (after cardiovascular diseases) and the second cause of specific morbidity (after respiratory infections) (1, 2, 3). The social and economic impact is difficult to estimate, considered to be very high: only in the United States and only for rotavirus infections the medical and social costs are over a thousand billion dollars yearly (4, 5, 6).

There are major differences between the manifestation forms of the epidemiological process in developed countries as compared to developing countries. There are also differences in terms of etiology, type of clinical forms and evolution of the disease, prognosis, therapeutics and age groups: child *versus* adult. Despite the progress made in understanding the pathogenic mechanisms and the management therapy, the acute gastroenteritis / acute diarrheal disease (AGE / ADD) remains one of the most important causes of infant morbidity and mortality.

In the early 1980s, it was estimated that the world's "biggest child killer" was diarrheal diseases, which were responsible for 4-6 million deaths every year. The latest studies show that, currently, diarrheal diseases are responsible for the death of 2 to 5 million children, mostly in poorly industrialized countries or developing countries. The annual number of diarrheal episodes

occurring in children under the age of 5 is approximated to 1 billion. It is worrying (but not unexpected) that morbidity is still in excess in developing or underdeveloped countries, with 3 to 7 episodes of diarrhea / year / child as compared to only 1-2 episodes / year / child in highly developed countries (7).

In the USA, the studies with wide coverage of the general population showed the presence of 1.05 episodes of acute diarrhea / person / year; considering that gastrointestinal signs are due to respiratory diseases for one in four people, the final figure was lower: 0.79 episodes / person / year. For the United States, the estimates indicated approximately 211 million cases of AGE / ADD for the entire population and 210,000 / year for the hospitalized pediatric population, resulting in approximately 10,000 deaths (3). From a financial point of view, these hospitalizations represent 4% of the total cost of all admissions of any cause. Acute gastroenteritis of viral etiology has a large clinical impact on children younger than 5 years old. In the United States, more than 500,000 consultations / year are reported, with about 55,000 hospitalizations and over 500 deaths / year for AGE / ADD of viral etiology.

In Australia, 15,000-20,000 children under the age of 5 years are hospitalized annually for severe acute gastroenteritis (8).

A case-control study carried out in the Netherlands, based on a "sentinel"- type surveillance system, estimated the impact of this disease on the general / family medicine network: 128,000 people call for annual family doctor consultations for acute gastroenteritis; basically 79.9 in 100,000 people have an episode of AGE / ADD / year; *Campylobacter* is by far the most frequently involved etiological agent (10%), followed by *Giardia lamblia* (5%), Rotavirus (5%), Norwalk-like virus (5%), and *Salmonella* (4%). The data varies widely depending on the territory under analysis (AGE / ADD has a lower incidence in the North) and on the degree of urbanization (a greater incidence in rural areas) (9, 10, 11).

Questions arise regarding the true extend of the spread of infections with *Campylobacter* spp. other than *C. jejuni / C. coli*, requiring more efficient diagnostic methods.

In the Czech Republic, *Salmonella* and *Campylobacter* infections are reported as early as 1951 and 1984, respectively. In 2003, they represented 92% of the etiologic spectrum of acute gastroenteritis (52% - infection with *Salmonella*, most often *Salmonella* Enteritidis and nearly 40% infections with *Campylobacter*); the most affected age group was 0-4 years. The incidence of infections with *Salmonella* in the Czech Republic recorded a steady rise until 1995, followed by a sharp decline since 1998; infections with *Campylobacter* showed an upward trend starting with 1984, with a peak in 2002 and a current slow decline (12).

A communication signed by some authors in Poland (13) states that, among the cases hospitalized in Warsaw, without generalizing to the whole territory, non-typhoid infections with *Salmonella* are common among children aged 0-5 years: 90% of the samples were positive for *Salmonella* Enteritidis. The same authors remarked the emergence of antibiotic-resistant *Salmonella*. The incidence of the antibiotic resistance of these pathogens ranged from 60% in Taiwan to 4% in Greece and 2% in Italy (14, 15).

In Norway, the incidence of AGE / ADD had a value that is like those found by FoodNet in the United States: 1.2 episodes of acute diarrhea / person / year. The risk factor often met among children less than 15 years old was water consumption from private sources; in contrast, chlorinat-

ed water consumption was not associated with AGE / ADD (16).

Ireland (Food Safety Authority of Ireland, Dublin) (17) reports 0.6 episodes of acute diarrhea / person / year; however, only 29.2% cases of the total number of reported cases presented themselves to the family physician / general practitioner and fecal samples were collected for investigation in only 2%. The authors considered that new ways of promoting prevention measures need to be found given the increased spread of AGE / ADD in the population.

A case-control study conducted in Denmark over a period of two years, with 0-5 years old children as the target population, presents in detail data regarding the etiology of AGE / ADD. In 26% of cases, the viral etiology was determined; in 17%, the etiology was bacterial; in 4%, the etiology was parasitic. The most frequent pathogen was rotavirus, followed by *Salmonella*, *Norovirus*, *Campylobacter*, and *Adenovirus*. Rarely, *Giardia lamblia*, *Blastocystis hominis*, *Cryptosporidium*, *Ascaris spp*. were found. Agents such as *Yersinia enterocolitica* (3% of samples), *Shigella*, *E. coli* are highly prevalent in Denmark (18).

Salmonellosis. In 2003, Germany reports an epidemic outbreak of *Salmonella enterica* serotype Agona infection among children with the average age of 13 months. 42 cases were diagnosed; after an epidemiological investigation, the route of transmission was identified: contaminated anise tea imported from Turkey. Rarely isolated, *Salmonella* Agona is involved in human pathology especially in relation to the consumption of powdered milk, snacks and industrially processed cereals. In similar situations, other serotypes were also involved in various food categories (in 2001-2002 there was an outbreak of epidemic scale, impacting several European countries; *Salmonella* Oranienburg was involved, which was isolated from samples of chocolate produced in Germany) (19, 20).

A study conducted in France in 2003 focused on estimating the number of cases hospitalized for AGE / ADD in children less than 5 years old and the costs associated with their hospitalization. The results showed that 51,125 children were hospitalized with this diagnosis; 56% of cases were "probably infectious", 36% had viral etiology (of these, 43% were coded as "rotavirus") and 8% had bacterial etiology (60% of which were coded as "*Salmonella*"). Complications (particularly acute dehydration) followed "viral" gastroenteritis in 21% of cases and "bacterial" gastroenteritis in 17% of cases, followed by 14 deaths. The mean length of stay was 3.2 days and the costs were estimated to reach 62 million EUR (21).

The report of the European Food Safety Authority (EFSA, Parma, Italy) and of the European Centre for Prevention and Control (ECDC, Stockholm, Sweden) published on July 17th, 2014 based on the information presented by 27 EU member states shows that the number of diseases caused by *Salmonella* continued to decrease in 2012. Thus, a total of 92,916 cases diagnosed with salmonellosis were reported by the 27 EU member states, with 91,034 confirmed cases, representing a 4.7% decrease in the confirmed cases compared to 2011. In 2012, the Czech Republic and Slovakia reported the highest number of cases of contracted diseases caused by *Salmonella* ($\geq$85 cases per 100,000), while Portugal, Greece and Romania reported the lowest figures ($\leq$4 in 100,000 cases). The proportion of endemic cases versus imported cases (favored by population movement) varies significantly from one country to another: most cases associated with travels (>70%) were reported by the Nordic countries, Finland, Sweden, and Norway. The

seasonal trend of salmonellosis is confirmed by the 2008-2012 Reports, with most cases reported during the summer months. The downward trend in the number of salmonellosis cases in the EU is significant; this has been noted for several years and continued in 2012 as well ($p < 0.001$ with linear regression), particularly in the 15 member states and in two non-EU countries: Austria, Belgium, Cyprus, Denmark, Estonia, Finland, Germany, Greece, Iceland, Ireland, Lithuania, Norway, Portugal, Slovakia, Slovenia, Sweden, and the United Kingdom (14, 22).

Ten EU Member States have reported data according to which 45% of the hospitalized cases were admitted for *Salmonella* infection: 45.1% of the confirmed cases of salmonellosis were hospitalized; the highest rates of hospitalization have been reported in Greece, Romania, Cyprus, and Portugal (73-91% of cases). It was revealed that three of these countries also reported the lowest rates of notification for infections with *Salmonella*, which indicates that the surveillance systems in these countries capture the most severe cases first. A total of 61 fatal cases have been reported by eight EU member states (23).

Contaminated food that was most often involved in causing *Salmonella* infections are: meat and meat products (intended to be eaten as such or heat-treated), mechanically separated meat, gelatin, collagen, cheese, butter, cream, powdered milk, whey powder, ice cream, eggs and products containing raw eggs, crustaceans, shellfish, sprouted grains, fruit and pre-cut fruit, vegetables, unpasteurized juices, dehydrated diet foods (24) .

Among infectious diseases, including those of the gastrointestinal tract, such as some types of food poisoning, they are currently considered as “emerging” diseases either because they are too frequently encountered or because they are caused by recently discovered pathogens, or because the role of contaminated food or water has recently been determined for the transmission of etiologic agents. For example, there have always been outbreaks of salmonellosis; however, in the last 25-30 years, their incidence has greatly increased in almost all regions of the globe. In Western Europe, *Salmonella* Enteritidis has become the dominant strain (23, 25).

Salmonellosis has stood out over the last decades; it is the first among the pathogens which can contaminate food and the external environment, and which have also been involved in the occurrence of sporadic cases of AGE / ADD as well as in the appearance of foodborne outbreaks. Morbidity and mortality data are inaccurate: there is no system for reporting isolated cases of salmonellosis in humans, animals or foods of animal origin. In addition, regarding the cause of death, the etiology of salmonellosis is not always detected. Only the investigation of foodborne salmonellosis outbreak can provide a clearer picture of this public health issue. Given the wide spread and the possibility of outbreaks, several types of surveillance and control programs for *Salmonella* infections have been proposed, such as those in Denmark, the USA, Australia, etc. Since 2003, a Control Program for these diseases has been implemented for the EU states, with the focus on food quality (especially for poultry and pork) (13, 19, 20).

Campylobacteriosis today tends to rank on the same position as the infections caused by non-typhoid *Salmonella*. The incidence is higher in summer in temperate zones; there are no differences related to seasons in the tropics. The morbidity assessment is made based on bacteriologically-confirmed cases.

The reported data generally come from

laboratories and hospitals but also from epidemiological surveys carried out in foodborne outbreaks; some countries have "sentinel"- type systems of supervision and reporting. CDC reports show values of incidence of 5-6%000 for these infections in the USA. *Campylobacter* infections can be hyperendemic, endemic or epidemic outbreaks (almost characteristic of economically developed countries with high sanitary standards). In Thailand, *Campylobacter* infections seem to dominate bacterial etiology, *Campylobacter jejuni* serotypes 36, 4, and 11 are found quite frequently, rather than *Salmonella* and *Shigella* infections (26).

In 2012, at a worldwide level, campylobacteriosis was the most frequently reported zooanthroponosis, with 214,268 confirmed human cases. In developed countries, *Campylobacter spp* was the most important cause of AGE / ADD; thus, in 2009, in England and Scotland, there were over 64,000 cases but due to the phenomenon of underreporting, the actual number of cases is estimated at 450,000; about 10% of all reported cases are hospitalized; variable percentage of patients experience complications – severe abdominal pain and impaired musculoskeletal, neurological or articular functions, Guillain-Barré syndrome (15% of all cases are due to infection with *Campylobacter spp*) and others (25). Epidemiological studies have shown that 50-80% of diseases in humans are caused by the consumption of contaminated poultry (21, 22, 27, 28).

Young children in rural areas are the category at the highest risk of catching the disease due to their lifestyle that is why prevention should be promoted everywhere the world. In 2006-2007, New Zealand recorded the highest incidence ever reported in the world (12,778 cases); however, after the implementation of the new regulations concerning the marketing and consumption of fresh poultry, the figures fell dramatically in 2008 (6,693 cases) (23).

Among the measures limiting the occurrence AGE / ADD, the following are included: the proper handling and operation of water supply stations and the reduction of bacterial load in the poultry destined for consumption (*E. coli* O 157, *Cryptosporidium*, *Campylobacter spp* and *Salmonella*).

In Iceland, before 1996, the consumption and marketing of refrigerated poultry was banned, and only frozen meat was allowed; the measure was established to control *Salmonella* infections (23), but it also led to fewer cases of *Campylobacter* infection; after 1996, the ban was withdrawn, and refrigerated meat products were allowed for consumption once again. The reports have shown a significant increase in the number of people who caught the diseases caused by both *Salmonella* and *Campylobacter* (29, 30).

The highest rates of hospitalization for infections with *Campylobacter* (74 -78%) were reported by Cyprus, Latvia, Lithuania, Romania and the UK. At the same time, Cyprus, Latvia and Romania have the lowest number of reported cases, revealing that the surveillance in these areas includes only the severe cases. In 2012, the UK reported 20 of the 31 deaths reported by the EU member states (23, 31).

In the United States, the incidence of campylobacteriosis decreased in the late '90s and still remains at low levels due to the establishment and revision of hygiene measures on decontamination of drinking water and on the chlorination of the water used for washing chicken carcasses – the latter is still highly controversial in the European Union (32).

Listeriosis. In the European Union countries, *Listeria monocytogenes* has seldom been detected above the legal safety

limit when the food distributed for consumption was analyzed. However, the number of confirmed cases of human listeriosis increased in 2012 to 1,642 (compared to 1,583 cases in 2006, considered the largest number of cases in the EU up to that moment) (22, 23). Relatively rarely reported, listeriosis is a zooanthroponosis which causes high morbidity, hospitalization and mortality especially for the population at risk; the most vulnerable are fetuses, newborns, immunosuppressed patients of any age, seniors (especially men) over 74 years old. Symptoms range from mild, flu-like, with associated diarrhea, life threatening such as septicemia and meningoencephalitis; in pregnant women, *Listeria* can affect the conception product, usually causing severe diseases of the fetus, premature birth or miscarriage. The incriminated foods were not only milk products (soft varieties of cheese were often involved) but also fish and derivatives (4 samples reported in 2012 by Slovenia, but also by Austria, Belgium, Bulgaria, Cyprus, the Czech Republic, Germany, Hungary, the Netherlands, Latvia, Poland, Romania, Estonia, Spain) (38). Mollusks and crustaceans have been identified as contaminated in Bulgaria, Hungary, Poland, Portugal and Spain (23). There were additional red flags concerning the foods for consumption (pre-cut vegetables and fruit, sandwiches, pasta, salads, dishes based on eggs); however, in the last EFSA / ECDC Report, there are relatively few laboratory investigations in this regard and the results are virtually insignificant (23).

Colibacillosis. Many *Escherichia coli* serotypes are said to produce verotoxin (VTEC), yet those with the ability to cause severe diseases in humans are found in a limited number of serotypes; of these, *E. coli* O157 is the most frequently reported (synonymous terms: VTEC - verotoxin-producing *E. coli* and STEC - *Shiga* toxin-producing *E. coli*). It is normal to see occasional manifestations; in humans, the evolution of the infection includes bloody diarrhea and abdominal pain, usually without being accompanied by fever; the appearance of the hemolytic-uremic syndrome (HUS) aggravates the clinical picture (renal failure, hemolytic anemia and thrombocytopenia); about 10% of cases of infection with *E. coli*-VTEC will develop into SHU. In 2012, a total number of 5,671 confirmed cases of infection with *E. coli* VTEC were reported in the EU (23).

Since 1982 (when it was first described) until today, the infection with *Escherichia coli* serotype O157: H7 is considered a major cause of acute bloody diarrhea and acute renal failure, sometimes progressing to death, especially in children.

In Japan, in 1996, the largest outbreaks of infection with *Escherichia coli* serotype O157: H7 was reported and it involved almost 10,000 people, mostly school children (14 outbreaks). The largest outbreak included 6,300 children. There were 2 deaths (30).

Spain was faced with several outbreaks of infection with VT2 verotoxin-producing *Escherichia coli* O157: H7, first reported in 1986, 1994, 1997, which involved only foreign tourists. In 2000, the infection with *E. coli* O157: H7 affected 158 people (children from 6 schools), and 6 of them had the hemolytic uremic syndrome (23).

Cyclosporidiasis. An emerging pathogen in the United States is *Cyclospora cayetanensis*. Recently, it has been categorized as an enteropathogenic in humans. It causes outbreaks, some of which can be quite extensive. In 1997, in the USA and in 1998, in Canada, *Cyclospora cayetanensis* caused many diseases (over 1,500 cases in the USA); all the cases were related to the consumption of raspberries imported from Guatemala. There were no fatalities; the

infection was self-limiting in immunocompetent patients. In patients with HIV / AIDS, there is the risk of massive dehydration and unfavorable evolution or chronicization of diarrhea. Again, the most vulnerable age group is represented by children aged 0-2 years (33).

In the USA, the prevalence of the infection with *Cyclospora cayetanensis* in the community outside outbreaks is below 1%. In Peru, among the children from disadvantaged environments, the prevalence is estimated to be between 6% and 18%, where most cases are symptomatic (27). Two outbreaks reported in two successive voyages of an Australian cruise ship, affecting 73 passengers (confirmed with the laboratory) of the total number of 241 suspects required investigation using a case-control study, which failed to incriminate a certain food product or a food product available aboard that ship (41). In some areas (Egypt), 10-17% of the immunocompetent child population, with or without clinical signs of AGE / ADD, had fecal samples positive for *Cyclospora cayetanensis* (28, 34, 35).

Cholera, endemic in Asia and Africa and in some South American countries, was no longer an issue for the USA in the twentieth century. *Vibrio cholera* got into the South Coast waters of the United States in 1991, when a cargo unshipped contaminated ballast; basically, the first case of cholera reported in South America after 1900 was recorded in 1991 in Peru; an extensive epidemic broke out and lasted for almost five years; it extended to other countries in South America, Mexico and Central American countries and it resulted in 1,099,882 cases and 10,453 deaths. In 1993, a new serotype was identified, O139 (Bengal), which was involved in outbreaks of cholera in India and Bangladesh. A history of infection with *V. cholerae* O1 does not confer immunity, which explains the endemic manifestation of serotype O139 cholera (36, 37).

Shigellosis manifests endemically worldwide and it is extremely prevalent in undeveloped countries. Shigellosis is a highly contagious disease, especially for children younger than 5 years old, who are very receptive to it and who can die because of it. Even today, the United States still must deal with a prevalence of 6-10%$_{000}$ of the population. The most affected age group is 0-4 years (where there were 10 times more isolations compared to people over the age of 20). In Romania, the incidence of infections with *Shigella* has a downward trend, from 130.6%$_{000}$ in 1975 to 31.8%$_{000}$ in 1990 and 6.7%$_{000}$ in 2004. The most frequent in Romania are *S. flexneri* followed by *S. sonnei* (especially in Banat and Transylvania). It can be noted that, at a worldwide level, the circulation of *S. sonnei* is more intense in the areas that are better developed economically. In Romania, the disease remains endemically manifested; there is a certain summer-autumn seasonality. In the territories where the epidemiological process manifests epidemically, the most affected is the age of childhood. Infants who are exclusively breastfed are rarely affected. Receptivity decreases with age, in parallel with the increase in the number of anti-LPS antibodies and a higher concentration of these antibodies. Epidemiological observations suggest the installation of a serotype-specific immunity (24, 38).

Yersiniosis. *Yersinia enterocolitica* causes gastroenteritis or enterocolitis with self-limiting evolution; it is a frequent cause of acute infectious diarrhea and abdominal pain syndrome, especially in children, who are most receptive to it between 5 and 15 years old; the ratio boys: girls is 1: 3. The emergence of outbreaks in schools or other centers for children is possible. *Yersinia enterocolitica* is univer-

sally widespread (cases have been reported in tropical areas, as well); the prevalence of these infections is higher in Northern European countries, where the climate is cooler, and in North America, in winter. The Report for 2003 on the evolution of zooanthroponosis in the EU includes 9,399 reported cases of AGE / ADD with *Yersinia enterocolitica*. A study on the prevalence of acute gastroenteritis in children, conducted in Iran, places the infections with *Yersinia spp.* right after the infections with the enteropathogenic *E. coli* and *Shigella spp*. There was no reported antibiotic resistance (24).

Enteroviruses. In economically developed countries like the United States, Japan, Great Britain, countries in Northern Europe, the pathogens causing AGE / ADD with sporadic manifestation are caliciviruses (Norwalk, Sapporo) in patients of all ages, except for infants and toddlers in who rotaviruses are the most common. Adenovirus serotypes 40, 41 and astroviruses are also found quite frequently. Coronaviruses, picornaviruses and picotrirnaviruses, pestiviruses, and toroviruses are considered pathogens that cause acute gastroenteritis, being associated with AGE / ADD in animals; however, they have not yet been isolated from the fecal samples collected from the people who had specific symptoms. Caliciviruses are considered responsible for 50% of the foodborne outbreaks (39) and 90% of the outbreaks in the United States, with approximately 23 million cases of AGE / ADD each year; the estimates also based on questionnaires applied to the general population show much higher numbers, nearly 74 million cases / year in the USA. The small inoculum (less than 100 viral particles), the very short incubation period (12-48 hours), the resistance to the environment and to usual decontaminants correlate with the high incidence attack rates (estimated value in practice: 41%; experimentally obtained value: 50-80%). The transmission can take place indirectly through food and contaminated water or directly through the phenomenon of aerosolization during emesis (40, 41).

In the Iasi County, acute gastroenteritis recorded averages values, comparable to other counties from Romania (2.3 - 3% of all cases reported nationwide). The etiologic diagnosis was not possible for most laboratory tests, but the 0-1 age group positivity rate is higher (42).

CONCLUSIONS

Acute diarrheal disease ranks second among the causes of specific morbidity (after respiratory infections). Morbidity has higher values in economically undeveloped or developing countries, with 3 to 7 episodes of diarrhea / year / child compared to only 1-2 episodes / year / child in highly industrialized countries.

There are numerous etiological bacterial agents which cause acute diarrheal disease globally, but the infection with *Campylobacter* was the most frequently reported zooanthroponosis, with 214,268 confirmed human cases in 2012.

Almost any pathogen involved in the etiology of ADD may be involved in the occurrence of healthcare-associated infections; among these, the rotavirus and *Clostridium difficile* are most prevalent.

In our opinion, general hygiene measures could change the epidemiological aspect of ADD by modifying the factors which favor the transmission of pathogens: crowds (number of children in care), the occurrence of a case of disease (or more), the presence of sick children in the community (without isolation), two or more children sharing the same bed, common areas for changing diapers, lack of personnel's compliance.

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THE IMPORTANCE OF EDHF IN ENDOTHELIUM – DEPENDENT RELAXATION INCREASES DISTALLY IN MESENTERIC ARTERIES DEPENDING UPON THE CONTRACTING AGENT

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THE IMPORTANCE OF EDHF IN ENDOTHELIUM - DEPENDENT RELAXATION INCREASES DISTALLY IN MESENTERIC ARTERIES DEPENDING UPON THE CONTRACTING AGENT (Abstract). **Aim** : The importance of endothelium-derived hyperpolarizing factor (EDHF) in endothelium-dependent relaxation (EDR) is influenced by multiple factors, including vascular territory and caliber, pre-existing tone and its determining factors. **Method** : Using isometric myography we noticed that in rat mesenteric resistance arteries (RMA2 ; 2nd order branches) EDHF-mediated relaxation is increased when precontraction is induced by prostaglandin F2 (PGF) compared to phenylephrine (PHE) and we investigated the participation of certain K channels. Here we extend the study on more proximal vascular fragments ; from mesenteric arcade and from 1st order branches. **Results** : The EDHF component of EDR is stronger distally only when precontraction is induced by PHE. Moreover, morphometric analysis shows a strong inverse correlation between the magnitude of EDHF response and arterial caliber. **Conclusions** : Other authors have shown that EDHF increases in relative importance distally, but we show that this change in EDR profile depends upon the contracting agent used, with implications regarding the physiological relevance of accumulated data refering to EDHF and nitric oxide as mediators of EDR in resistance arteries. **Key words :** ENDOTHELIUM-DERIVED HYPERPOLARIZING FACTOR ; ENDOTHELIUM--DEPENDENT RELAXATION ; SMALL MESENTERIC ARTERIES.

The complex picture of EDR shows differences related to species, sex, age, hormonal status, territory and vessel size, mechanical or chemical vasodilators, pre-existing tone and its factors (1, 2, 3, 4). There are few explicit studies of the latter (1, 4). NO may be the main mediator of EDR on the vascular tree wide scale, but EDHF largely mediates the NO-independent EDR which predominates in small arteries. Of the many candidates (1), EDHF in RMA seems to be C-type natriuretic peptide (5), while gap junctions also play a critical role (6), yet another line shows that EDR induced by acetylcholine in RMA is mediated by an S-nitrosothiol (7). We noticed (3) that when NOS and COX are blocked EDHF-mediated EDR induced by carbachol in RMA2 is enhanced with precontraction by PGF vs. PHE. Here we extend the isometric myography study upon RMA1 and mesenteric arcade.

MATERIALS AND METHODS

Proximal jejunal loops were removed from adult male Wistar rats (~200 g body weight, decapitated) and placed in solution (PSS)

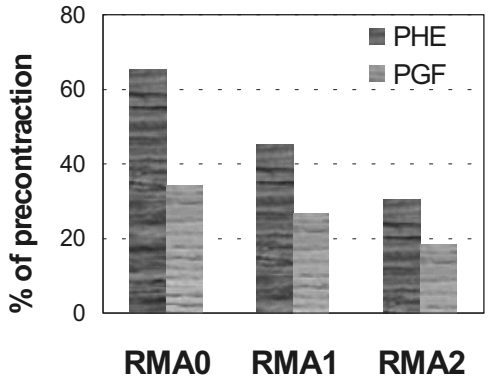

Fig. 1. Effects of 10^{-5} M carbachol upon contraction of rat mesenteric artery (RMA0) and branches (RMA1, RMA2) induced by 10^{-5} M PHE or 10^{-5} M PGF, in presence of 10^{-5} M L-NAME and 10^{-5} M indomethacin (residual active tension; % of precontraction; mean ±SEM; n=6; *p<0.01).

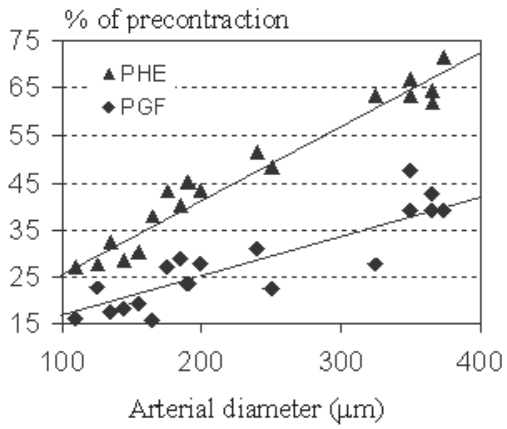

Fig. 2. Effects of 10^{-5} M carbachol upon contraction of rat mesenteric arteries induced by 10^{-5} M PHE or 10^{-5} M PGF, in the presence of 10^{-5} M L-NAME and 10^{-5} M indomethacin (residual active tension; % of precontraction; regression lines for PHE vs. PG; data pooled for all vessel types).

containing (mM): NaCl 118; KCl 4.7; $CaCl_2$ 2.52; $MgSO_4$ 1.64; $NaHCO_3$ 24.88; KH_2PO_4 1.18; glucose 5.55. Rings (Î1 mm) from mesenteric artery (RMA0) and its first and second order branches (RMA1 and RMA2) were mounted in wire myographs, in oxygenated PSS at 37°C (pH 7.2-7.4) and equilibrated for 1 h under 1 g tension. We evaluated the EDHF component of EDR induced by 10^{-5} M carbachol (e.g. in the presence of 10^{-5} M L-NAME and 10^{-5} M indomethacin); results expressed as residual active force, % from precontraction by PHE (10^{-5} M) or PGF (10^{-5} M); all drugs from Sigma. All data are presented as mean ± SEM (n=6) and differences are considered significant if $p<0.01$ (Student's t-test for grouped data). Correlation analysis was performed based on individual values for the EDHF effect and the external diameter of the mounted ring, as measured with the ocular micrometer at zero parietal tension.

Ethical aspects were evaluated and protocols were approved by local Committee on Research Animal Use, in agreement with ECC 609/24.11.1986.

RESULTS

There is a general tendency of increased EDHF towards periphery (fig. 1), but significant differences occur only with PHE; with PGF this is obscured by an already strong EDHF in RMA0. Thus, EDHF participation in EDR in this territory displays an increase with branching (and smaller size; fig. 2), more or less abrupt depending on the contracting agent.

DISCUSSIONS

Our data are in agreement with the few existing studies which use PGF in RMA (8), showing that both NO and EDHF are involved in EDR, and also with the many studies on EDHF in these vessels using a_1-adrenoceptor stimulation (1, 2, 4, 7, 9).

In RMA small and intermediate conductance calcium-dependent K^+ channels do increase in expression and function towards periphery (10), where EDHF is more pronounced (2).

While carbachol-induced global EDR in RMA is complete (6, 11), irrespective of

the artery caliber and of the contracting agent, the EDHF component of EDR varies significantly ($p < 0.01$) with the contracting agent (fig. 1).

CONCLUSIONS

EDHF component of EDR is stronger distally when contraction is induced by PHE but not by PGF. Others have shown that EDHF importance increases towards periphery, but we show that this may depend on the contracting agent.

This finding may influence the interpretation of existing data on EDHF and NO as mediators of EDR in resistance arteries (12), including human small mesenteric arteries, which behave similarly ; EDR is based on NO & EDHF but not on prostaglandins (13).

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PREVALENCE OF NASAL CARRIAGE OF *STAPHYLOCOCCUS AUREUS* WITH SPECIAL REFERENCE TO NUMBER OF METHICILLIN RESISTANCE AND ANTIMICROBIAL EVALUATION AMONG APPARENTLY PEOPLE WITH GOOD HEALTH STATUS

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PREVALENCE OF NASAL CARRIAGE OF STAPHYLOCOCCUS AUREUS WITH SPECIAL REFERENCE TO NUMBER OF METHICILLIN RESISTANCE AND ANTIMICROBIAL EVALUATION AMONGST APPARENTLY PEOPLE WITH GOOD HEALTH STATUS (Abstract): The emergence and spread of *Staphylococcus aureus* (*S. aureus*) strains that are resistant to some first line and supplementary group of antibiotics is a matter of public health importance. *S. aureus* is ubiquitously found in the environment as a commensal organism, but pathogenic strains of the bacterium that are resistant to some commonly available antibiotics puts antimicrobial therapy at risk. **Objectives:** To determine the number of nasal carriers of *S. aureus* among apparently healthy people from Iasi and to assess the antibiogram profile of isolated strains, with special reference to methicillin sensitivity. **Material and methods**: This study was conducted in Biodev Medical Center, Iasi, Romania, during 2014 - 2018, where a total of 757 apparently healthy patients (men, women and children) were tested for *S. aureus* infection. The samples were collected from their anterior nares and incubated on culture media for isolation and antibiotic susceptibility testing in microbiology department. The data was compiled and analyzed for the prevalence of *S. aureus* carriers, the number of MRSA strains and the antibiotic resistance pattern. **Results and discussion:** Our study has shown that from 757 people involved, 229 of them were carriers of *S. aureus* strains and 22 of them were MRSA strain carriers. Additionally, we found that the male / female portability ratio is similar and also the fact that the most carriers are in the group of age over 18, followed by school age people (age 8-18 years old). **Conclusions:** This study will help raise awareness regarding the danger of developing antibiotic resistance and in the same time also reduce or prevent self-medication and antibiotics abuse without medical recommendation. **Keywords:** STAPHYLOCOCCUS AUREUS, METHICILLIN-RESISTANT *S. AUREUS*, STAPHYLOCOCCAL INFECTIONS, ANTIBIOTIC RESISTANCE.

S. aureus is a gram-positive, round-shaped bacterium, member of the Firmicutes phylum and it is a member of the normal flora of the body, frequently found in the nose, respiratory tract and on the skin. *S. aureus* is the most common species of the genus *Staphylococcus* that causes staphylococcal infections in humans (1). The carriage of *S. aureus* is an important source of nosocomial infections, community acquired infections and antibiotic resistant infections due to the organisms including but not limited to methicillin-resistant *S. aureus* (MRSA). Frequently, *S. aureus* can be present on the skin of the host, but a large proportion of its carriage is through the anterior nostrils of the nasal passages. The nose is one of the few openings thorough which bacteria has direct access to the body. Thus, the nose and nasal passages can be perfect environments for some bacteria. *S. aureus* has been recognized as one of the most important bacterial pathogens, significantly contributing to hospital and community-acquired infections all over the world (2). The ability of the nasal passages to host *S. aureus* results from a combination of a weakened or defective host and the ability of bacteria to evade host innate immunity. It was estimated that 20 - 30% of the human population are long-term carriers of *S. aureus* (3, 4). It is the most frequently isolated pathogen causing bloodstream infections, skin and soft tissue infections, and pneumonia. Diseases caused by *S. aureus* include folliculitis, boil, furunculosis, scalded skin syndrome, conjunctivitis and toxic shock syndrome (4, 5, 6, 7, 8). In attempts to control these diseases through the use of antimicrobial agents particularly antibiotics have led to increased prevalence of resistance to these agents. *S. aureus* diseases and their high mortality were greatly reduced after Alexander Fleming discovered the effects of penicillin, but success was short-term because the bacteria immediately gained resistance to penicillin by secreting an enzyme called beta-lactamase, that breaks the antibiotic beta-lactam ring thus inactivating antimicrobial action. A group of narrow spectrum antibiotics (Nafcillin, Oxacillin, Methicillin), which are resistant to beta-lactamase activity secreted by *S. aureus*, offered new treatment options for staphylococcal infections, but the occurrence of methicillin-resistant *S. aureus*, determined its resistance to all beta lactam antibiotics.

MRSA (**M**ethicillin-**r**esistant ***S***. ***a****ureus*) strains have gained resistance not only to beta-lactam antibiotics, but also to several other classes of antibiotics (9) MRSA strains play a crucial role in disease acquisition from the community and there has been a global increase in the number of infections caused by MRSA (9, 10). However, data concerning the frequency of MRSA nasal carriage in Iasi community is unknown. The aim of this study was to determine the prevalence of MRSA in *S. aureus* carrier individuals, from Iasi area and to determine the pattern of antibiotic resistance.

MATERIAL AND METHODS

This study was conducted in Biodev Medical Center, Iasi, Romania and was planned in the year 2014. The studied subjects were people with apparently good health status, from Iasi city and nearby villages. With their agreement, a total of 757 of people were included in this study, 348 males and 409 females, from which 508 persons were under the age of 18 and 249 over this age.

Microbiology

All 757 Specimens were collected during January 2014-June 2018 from anterior nostrils with sterile cotton swabs (the same swab being used for both nostrils of the patient). Swabs were cultured on 5% blood agar and Mannitol-salt agar, incubated at +37°C (±1°C) and examined for the growth of characteristic colonies after 24-48 hours of aerobic incubation. The organism was identified by microscopy, using Gram staining technique and colony characteristics: colonies are large (2-4mm in diameter), circular, convex smooth, and show hemolysis on blood agar. After colony smear and Gram staining of bacterial colonies, the biochemical identification was carried out by following tests: catalase test, slide coagulase test and tube coagulase test, Mannitol salt agar for fermentation and latex agglutination kit (Prolex^{TM} Staph Latex Kit).

Antibiotic susceptibility test

Antibiotic susceptibility of the isolates was performed on Muller Hinton agar plates and interpretation was done according to Clinical and Laboratory Standard Institute (CLSI) testing guidelines. We used Kirby-Bauer Disc diffusion technique, using following antibiotics: Penicillin G (10 units/disc), Oxacillin (OX 30mcg/disc), Cefoxitin (CX 30 mcg/disc), Ciprofloxacin (CIP 5mcg/disc), Clindamycin (CD 2 mcg/disc), Erythromycin (E 15mcg/disc), Doxycycline Hydrochloride (DO 30mcg/disc), Tetracycline (TE 30mcg/disc), Kanamycin (K 30 mcg/disc), Chloramphenicol (C 30 mcg/disc), Vancomycin (10 mcg/disc), all produced by HiMedia.

Screening for methicillin resistance was done using Oxacillin (OX 30 mcg/disc) antibiotic or Cefoxitin (CX 30 mcg/disc, surrogate for Oxacillin). Inducible resistance to Clindamycin in *S. aureus* was tested by 'D test' also as per CLSI guidelines. For the preparation of the bacterial suspension, the scale of 0.5 on McFarland was used. The entire surface of the plate medium was inoculated using sterile cotton swab soaked in inoculum and antibiotic discs (HiMedia) were applied, then the plates were inverted and incubated at +37°C (±1°C) for 24 hours. Quality control of the test was done by Standard Strain (Staphylococcus aureus ATCC 25923). The Zone diameter around the disk is measured using a measuring scale and the results were interpreted as susceptible, moderately susceptible or resistant as per recommendations.

Analysis: All data was compiled using MS Excel and presented in tabular form. A Visual Basic script was used to process data.

Ethical consideration: Data collection permission was obtained from the subjects before results were used in the study.

RESULTS AND DISCUSSION

The prevalence of *Staphylococcus aureus* (carrier state) was 30.25% (fig. 1) observed in apparently healthy people (in line with specialty literature), with a percent of 90.39% MSSA (**M**ethicillin-**s**usceptible ***S***. ***a****ureus*) strains and 9.60% MRSA (**M**ethicillin-**r**esistant ***S***. ***a****ureus*) strains (fig. 2).

The incidence of MSSA strains carriers of female (fig. 3) compared to male (fig. 4) individuals were similar: we found 106 (46.28%) female and 101 (44.10%) male respectively, compared to MRSA strain carriers, where the female carrier rate was lower (fig. 3), with a number of 9 infec-

tions (3.93%), versus 13 male (fig. 4) infections (5.67%).

Regarding age incidence, we noticed that the highest rate was found in adult patients over 18 years (31.87%), followed by school age people between 8 - 18 years with 26.63% (tab I, II). Although the number of patients from rural environment was not equal to those in the urban area, as a percentage of the number of individuals tested, we found 29 carriers (38.66%) of *S. aureus*, from which 25 had MSSA strains (86.30%) and 4 had MRSA strains (13.70%). In comparison, among urban residents 200 persons where we found carrying *S. aureus* (29.32%), of which 182 MSSA strains (91%) and 18 with MRSA strains (9%) (fig. 5).

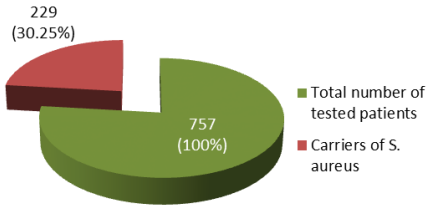

Fig. 1. The prevalence of *Staphylococcus aureus*

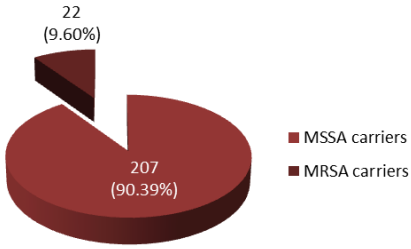

Fig. 2. MSSA and MRSA strains distribution

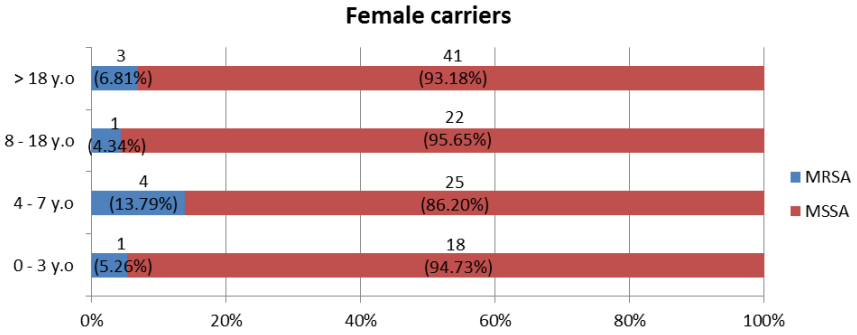

Fig. 3. The incidence of MSSA and MRSA strains carriers of female

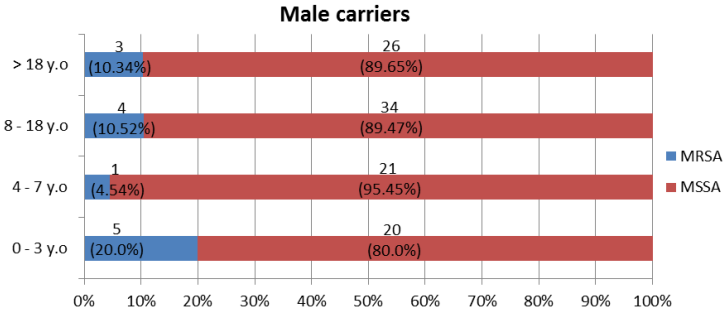

Fig. 4. The incidence of MSSA and MRSA strains carriers of male

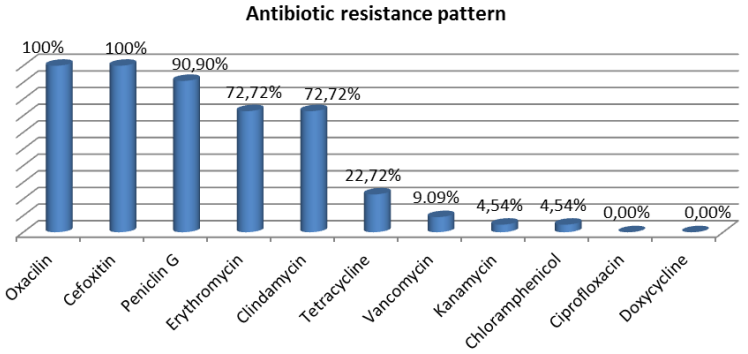

Fig. 4. Antibiotic resistance pattern

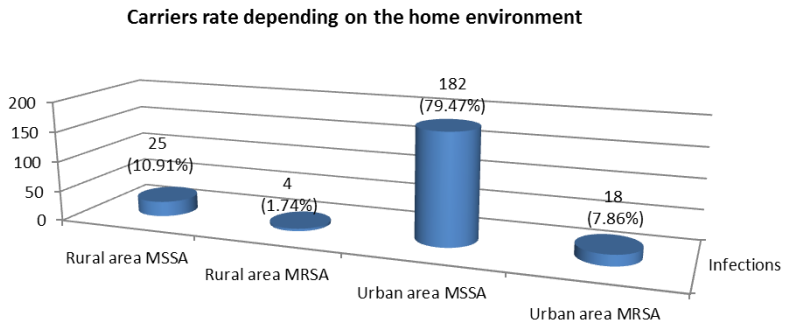

Fig. 5. Carriers rate depending on the home environment

TABLE I

Female carriers' ratio between MSSA and MRSA strains, according to age

Age group (Years)	Female tested	S. aureus carriers	Methicillin susceptibility (%)	Methicillin Resistance (%)
0 - 3	94	19 (4.64%)	18 (94.73%)	1 (5.26%)
4 - 7	85	29 (7.09%)	25 (86.20%)	4 (13.79%)
8 - 18	75	23 (5.62%)	22 (95.65%)	1 (4.34%)
> 18	155	44 (10.75%)	41(93.18%)	3 (6.81%)
Total	409	115 (28.11%)	106 (92.17%)	9 (7.82)

TABLE II

Male carriers' ratio between MSSA and MRSA strains, according to age

Age group (Years)	Male tested	S. aureus carriers	Methicillin susceptibility (%)	Methicillin Resistance (%)
0 – 3	108	25 (7.18%)	20 (80.0%)	5 (20.0%)
4 - 7	69	22 (6.32%)	21 (95.45%)	1 (4.54%)
8 - 18	81	38 (10.91%)	34 (89.47%)	4 (10.52%)
> 18	90	29 (8.33%)	26 (89.65%)	3 (10.34%)
Total	348	114 (32.75%)	101 (88.59%)	13 (11.40%)

DISCUSSION

Development of resistance to antimicrobial agents by staphylococci is a major concern primarily because they are still frequently associated with hospital and community - acquired infections (11). The organisms exhibit remarkable versatility in their behavior towards antibiotics, with some strains having become resistant to most commonly used antibiotics (12). The higher percentage of people with antibiotic resistance in rural areas can be attributed to self-medicine due in principle to the lack of medical education (people do not know the risks and mechanisms of acquiring antibiotic resistance, e.g. incomplete treatments or the prescribing of broad-spectrum antibiotics as a preventive measure).

MRSA is a common pathogen in many healthcare facilities and communities worldwide. It causes mild to severe infections which is sometimes difficult to treat due to its resistance to multiple antibiotics and carriage of multiple virulence factors. The highest percentages of resistance of MRSA strains were shown to Oxacillin and Cefoxitin (100%), followed by Penicillin (90.90 %), Erythromycin and Clindamycin with 72.72%, Vancomycin (9.09%), Kanamycin and Chloramphenicol (4.54%) (Fig.4). This finding is in accord with other studies that show the predominance of MRSA strains resistant to Oxacillin and Cefoxitin (13,14,15). The most susceptible antibiotic for treating MRSA strains was Ciprofloxacin and Doxycycline with a percentage of 100%. Regarding the inducible resistance to Clindamycin, we found that it appeared in 52 of the MSSA strains and in 16 of the MRSA strains.

Clinical infections with *S. aureus* will likely remain both common and serious. Not only have there been waves of increasing antimicrobial resistance (16,17,18,19) but the spectrum of clinical diseases also continues to change (20).

CONCLUSIONS

There is no doubt that there will continue to be a shifting landscape in the interactions between host and pathogen in the decades to come. We have the belief that this study, will help to raise awareness regarding the danger of developing antibiotic resistance and at the same time to reduce and eventually prevent self-medication and abuse of antibiotics without medical recommendation.

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TAMOXIFENUL ŞI MODIFICĂRILE ENDOMETRIALE

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TAMOXIFEN AND ENDOMETRIAL PATHOLOGY (Abstract): Tamoxifen is a non-steroid antiestrogen, used as adjuvant therapy for carcinoma breast in women with positive estrogen receptor (ER+). In this report we evaluated endometrial histological changes in the women treated with Tamoxifen for breast carcinoma ER+. **Material and method**: We performed transvaginal ultrasonography for the endometrial thickness and biopsy of endometrium on a group of 125 symptomatic women with Tamoxifen therapy for breast carcinoma ER+. The endometrial specimens were histologically processed for histological diagnosis. **Results**: Of all patients, 49.6% had endometrial pathology (women with functional hemorrhage), and 32% had atrophic endometrium. Endometrial pathology was represented by endometrial simple hyperplasia 17.6%, and endometrial complex hyperplasia 8.8%. Endometrial complex hyperplasia with atypia was found in 5.6%, endometrial polyps in 11.2%, endometrial carcinoma in 4.8% and müllerian mixt tumor in 1.6%. **Conclusions**: Endometrial pathology was more likely to be diagnosed in gynecological symptomatic postmenopausal and premenopausal women with breast carcinoma treated with Tamoxifen. Therefore, it is necessary that these patients to be supervise, first, with transvaginal ultrasonography for endometrium, and after, with biopsy of endometrium. All pacients on long term Tamoxifen therapy should be annually screened by endometrial biopsy for endometrial pathology. **Key words**: BREAST CARCINOMA, TAMOXIFEN, ENDOMETRIAL PATHOLOGY

Incidenţa adenocarcinomului endometrial este în creştere, determinat de obezitate, de înaintarea în vârstă şi mai recent de terapia adjuvantă cu Tamoxifen.

Tamoxifenul, un antiestrogen, nonsteroidian de sinteză a fost utilizat cu succes ca terapie adjuvantă, efectuată după tratamentul primar, la femeile în postmenopauză cu cancer de sân, cu receptor estrogen + (ER+). Efectul său benefic în reducerea recurenţei şi creşterea supravieţuirii a fost confirmat de mari trialuri clinice (1).

Actualmente este evaluat ca agent chemoprotector pentru tratamentul femeilor sănătoase, care prezintă un risc crescut de a dezvolta cancer de sân (2).

De-a lungul timpului, în literatura de specialitate, au apărut numeroase rapoarte care au indicat, prin cazuistica studiată, apariţia de modificări endometriale în urma tratamentului de lungă durată cu Tamoxifen (3). Una dintre cele mai semnificative complicaţii ale folosirii Tamoxifenului pe termen lung este dezvoltarea cancerului de endometru, evidenţiindu-se o creştere a riscului cu 7,5% faţă de populaţia generală (4).

Studiului nostru a încercat să evidenţieze modificările histopatologice la nivelul endometrului, apărute la femeile diagnosticate cu cancer de sân şi aflate sub tratament hormonal cu Tamoxifen, concomitent cu analiza leziunilor histopatologice endome-

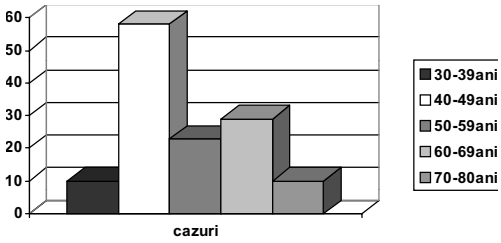

Fig. 1. Distribuţia cazurilor pe grupe de vârstă

triale curente în raport cu durata tratamentului hormonal.

MATERIAL ŞI METODĂ

S-a realizat un studiu în perioada 1995-2009, care a cuprins 125 de paciente prezentate în acest interval de timp, în cadrul Clinicii de Obstetrică şi Ginecologie „Cuza Vodă" din Iaşi, pentru hemoragie disfuncţională apărută sub terapie cu Tamoxifen.

Pacientele au fost diagnosticate anterior cu cancer mamar in stadii diferite, dar cu receptor ER+, pentru care s-a instituit terapie hormonală cu Tamoxifen, post intervenţie chirurgicală în doză de 20-40 mg/zi.

Tehnicile de investigaţie au constat din echografie endovaginală, urmată de recoltare de biopsie endometrială. Produsele de chiuretaj au fost fixate în formaldehidă 10% tamponată şi prelucrate prin includere în parafină. Secţiunile histologice de 4-5μ au fost colorate prin metode uzuale (colorare hematoxilin-eozină şi van Gieson) pentru interpretare şi diagnostic histopatologic.

REZULTATE

Dintre cele125 paciente cu cancer de sân tratate cu Tamoxifen, 104 (87,2%) erau la postmenopauză şi 16 (12,8%) la premenopauză. Grupul de vârstă a fost cuprins între 36-83 de ani, iar media de vârstă a fost de 56 de ani (fig. 1).

Diagnosticul clinic, la toate pacientele din studiu, a fost în momentul recoltării biopsiei endometriale, de hemoragie disfuncţională. Ecografic, s-a observat o creştere în grosime a endometrului peste 8mm

TABELUL I

Modificările histopatologice

Modificări histopatologice	Nr. paciente	Procent (%)
Endometru normal	23	18,4%
Endometru atrofic	40	32%
Polipi endometriali	14	11,2%
Hiperplazie simplă	22	17,6%
Hiperplazie complexă	11	8,8%
Hiperplazie complexă cu atipii	7	5,6%
Adenocarcinom de endometru	6	4,8%
Tumoră mixtă mezodermică	2	1,6%

(cu excepţia cazurilor de atrofie endometrială) cu aspect eterogen şi lacune anecogene difuze în grosimea endometrului, iar uterul a fost mărit de volum.

Durata tratamentului până la apariţia simptomatologiei a fost cuprinsă între 8 şi 72 de luni.

La 23 de paciente (18,4%) nu s-au constatat modificări endometriale. Hiperplazia simplă s-a observat la 22 paciente (17,6%) cu aspect tipic cu numeroase glande cu epiteliu de tip proliferativ pseudostratificat (tab. I).

Histologic, s-a evidenţiat endometru atrofic la 40 de paciente în postmenopauză. În cursul tratamentului cu Tamoxifen, 14 paciente au prezentat polipi endometriali, care histologic prezentau glande, în general neregulate cu epiteliu atrofic sau cu metaplazie eozinofilă. Stroma a fost fibroasă, cu condensări periglandulare (strat cambium).

Cea mai frecventă leziune a fost hiperplazia endometrială (40 de cazuri), din care hiperplazie simplă (22 de cazuri), hiperplazie complexă (11 cazuri) şi hiperplazie atipică (7 cazuri). În hiperplazia complexă fără atipii, glandele prezentau o arhitectură neregulată cu muguri intraglandulari şi în stromă (fig. 2), dar cu epiteliu pseudostratificat cu nuclei uniformi, alungiţi şi polarizaţi. Stroma a fost uşor redusă, mai

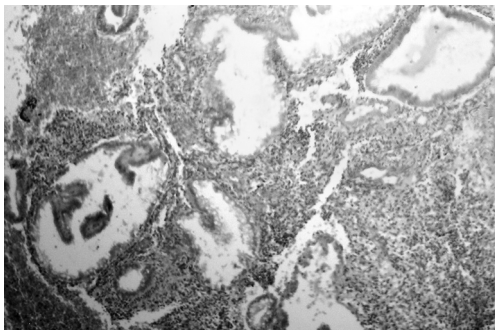

Fig. 2. Hiperplazie endometrială complexă cu muguri epiteliali intraglandulari şi în stromă

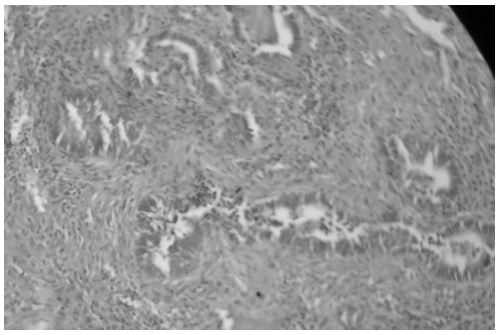

Fig. 3. Hiperplazie endometrială atipică

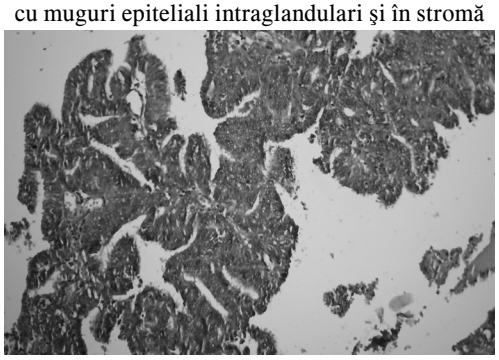

Fig. 4. Adenocarcinom endometrioid papilar

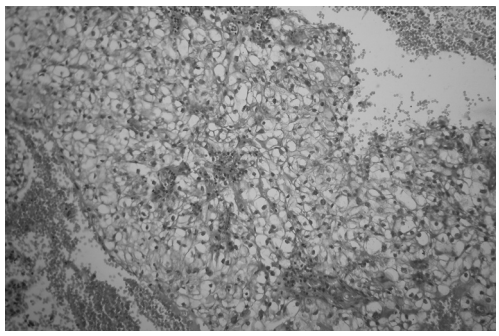

Fig. 5. Adenocarcinom endometrial de tip seros cu tablou histologic solid

ales în focarele cele mai bogate glandular. În hiperplazia atipică (fig. 3), diagnosticul histopatologic s-a bazat pe atipia citologică a epiteliului glandular, ce prezenta pierderea polarităţii nucleare axiale, anizocorie, nucleoli proeminenţi şi cromatină clară sau densă. Aceste aspecte atipice s-au observat în focar, iar stroma a fost redusă, dar prezentă. În cele 6 cazuri de carcinom endometrial, histologic s-au diagnosticat 4 cazuri de adenocarcinom endometrioid de tip I şi II , de tip papilar (fig. 4) şi adenocarcinom de tip seros cu tablou histologic solid (fig. 5).

În două cazuri, transformarea malignă a apărut sub formă de tumoră mixtă mezodermică de tip heterolog cu aspecte carcinomatoase – carcinom seros-papilar cu focare de carcinom scuamocelular keratinizat, asociate cu sarcom stromal, leiomiosarcom, fibrosarcom, condrosarcom şi celule multinucleate, posibil rabdiomioblaste.

DISCUŢII

În literatura de specialitate există numeroase argumente documentate pentru asocierea terapiei cu Tamoxifen şi dezvoltarea de numeroase aspecte patologice endometriale (5).

O astfel de asociere a fost bazată pe observaţia că ratele de incidenţă a endometrului proliferativ, hiperplaziilor endometriale, polipilor endometriali şi carcinomului endometrial au fost mai mari la pacientele cu carcinom de sîn tratate cu Tamoxifen decât la pacientele similare netratate, deşi diferenţa nu a fost statistic semnificativă (5,6,7,8,9,10,11).

În grupul de 125 paciente vârsta medie a fost 56 de ani, cu un maximum grup de vârstă 40-49. Toate pacientele au prezentat sângerări uterine în momentul recoltării,

iar în produsul de chiuretaj s-au constatat 81,6% leziuni endometriale - din care 32 % atrofie, 11,2% polipi, 26,4% hiperplazie simplă şi complexă, 5,6% hiperplazie atipică, 4,8% carcinom şi 1,6% tumoră mixtă mezodermică. Cele mai multe leziuni au fost decelate în grupul 40-49 ani, iar chiuretajele au fost efectuate după 24-36 luni de la începutul tratamentului cu Tamoxifen.

În studiul nostru incidenţa de 49,6 % a patologiei endometriale detectată la pacientele simptomatice tratate cu Tamoxifen este mai redusă decât cea din studiile lui Cohen et al., care a utilizat doza de 40mg/zi (11).

Cel mai mare risc cumulativ al dezvoltării leziunilor endometriale a survenit la 36 luni de utilizare a Tamoxifenului, iar leziunile frecvente au fost polipi endometriali, hiperplaziile simple, complexe şi cu atipii. În cazuistica noastră cazurile de apariţie a carcinomului endometrial a fost după 5 ani de administrare zilnică a 40 mg de Tamoxifen.

Studiul prezent confirmă datele din literatură legate de efectul inductiv lezional al terapiei cu Tamoxifen, administrat pe termen lung, asupra endometrului.

CONCLUZII

După maxim 36 luni terapie cu Tamoxifen, toate pacientele ar trebui supravegheate prin sonografie transvaginală, urmată de chiuretaj biopsic, dacă aspectele sonografice sunt patologice. Beneficiile utilizării tratamentului cu Tamoxifen sunt deosebit de importante, deoarece reduce recurenţa şi apariţia de noi carcinoame în sânul controlateral.

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EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *CRATAEGUS MACRACANTHA LODD* FLOWERS

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EVALUATION OF FREE RADICAL SCAVENGING ACTIVITY OF *CRATAEGUS MACRACANTHA LODD* FLOWERS (Abstract): The antioxidant activity of biological products has been demonstrated by various "in vitro" and "in vivo" studies. **Aim**: To measure the capacity of an extract obtained from *Crataegus macracantha Lodd* flowers to capture free radicals from chlorpromazine (radical indicators), in order to evaluate the entire antioxidant activity. **Material and methods**: The lyophilisate from *Crataegus macracantha Lodd* flowers were obtained in the Department of Pharmacognosy of the Iaşi "Grigore T. Popa" University of Medicine and Pharmacy. The radical indicators were obtained through oxidation of chlorpromazine with potassium persulphate in acid environment. The diminution of radical solution color was determined by means of spectrophotometry (maximum absorbance at ƛ=525 nm), according to the amount of lyophilisate. Ascorbic acid was used as a standard antioxidant. **Results**: The antioxidant activity was evaluated by using the calibration curve, obtained by plotting the radical solution absorbance variation according to ascorbic acid concentration. Due to the fact that antioxidant activity is simultaneously influenced both by the antioxidant concentration and response time, a variant of the method using the calibration curve obtained by plotting the area under the curve (AUC), against ascorbic acid concentration. AUC was calculated for each ascorbic acid concentration by graphic representation of the absorbance inhibition percentage at every two predefined time frames. The antioxidant activity of the sample is calculated by using the regression line equation (r^2=0.999) and was expressed in micromolar equivalents ascorbic acid/g product (59,70 µM/g). **Conclusions**: The obtained results confirm the capacity to capture free radicals and, implicitly, the antioxidant activity of *Crataegus macracantha Lodd* flowers. **Keywords:** FREE RADICAL SCAVENGING, SPECTROPHOTOMETRIC ANALYSIS, CHLOR-PROMAZINE RADICAL CATION.

The reactive oxygen and nitrogen species (free radicals or non-radical compounds) interfere in various oxidative processes, which are produced both in the human body and other systems: food, medicine, cosmetics, petroleum products etc.

The interference of antioxidant substances ensures a balance between the prooxidative processes initiated and catalyzed by the reactive species and normal metabolic processes.

Exogenous antioxidants supplied

through foods or drug intake are extensively studied, of which herbal products play an essential role.

Finding new sources of natural antioxidant substances represents a challenge for researchers (1-10).

At the same time, methods of analyzing the antioxidant activity of biological products have expanded and diversified (11-15).

The aim of this study was to determine the ability of lyophilisate obtained from *Crataegus macracantha Lodd* flowers to capture free radicals resulting from the oxidation of chlorpromazine with potassium persulfate and to evaluate their antioxidant action by means of spectrophotometry.

Crataegus macracantha Lodd, fam. *Rosaceae* is a widespread species in North America (south of Canada and USA), but a rare species in Europe.

Unlike *Crataegus monogyna* (leaves and flowers), extensively studied and with a demonstrated antiarrhythmic and antioxidant action, *Crataegus macracantha* was less analyzed due to its reduced distribution area. The indicator radical cation chromophore of chlorpromazine has a maximum absorbance at 525 nm. In the presence of the antioxidant, the color of the solution diminishes proportionally with the amount of lyophilisate. Ascorbic acid was used as a standard antioxidant and the antioxidant activity of the sample was expressed in molar equivalents (micromolars) of ascorbic acid per gram of dried product.

MATERIAL AND METHODS

The lyophilisate was obtained in the Department of Pharmacognosy, Faculty of Pharmacy, Iasi "Grigore T. Popa" University of Medicine and Pharmacy, as it follows: 10 g of plant product (flowers) was treated with 100 mL of 70% methanol (v/v); the mixture was stirred for one hour (FALC F-30 ST magnetic stirrer), filtered, concentrated (Rotavapor, BUCHI R-210) and lyophilized (UNICRYO TFD 5505 freeze-dryer).

Of the 10g of plant product, 2.66g of crude extract were obtained.

Radical preparation. The preformed radical of chlorpromazine obtained by oxidation of chlorpromazine by potassium persulfate in an acid environment was used for measurements (16). The optimum molar ratio between chlorpromazine and potassium persulfate, where the radical absorbance remains constant, was established (fig. 1).

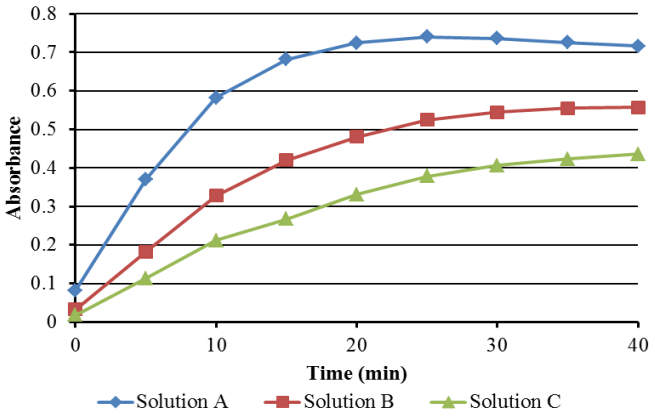

Fig. 1. Stability over time of the $CP^{\bullet+}$ radical

It was found that solution A (molar ratio chlorpromazine/persulfate of 20:1) reaches its maximum absorbance 20 minutes after the addition of persulfate and remains constant for approximately 20 minutes.

Thus, the optimal time frame to carry out the measurements is 20 to 40 min after adding the potassium persulfate. An excess of chlorpromazine ensures radical stability.

Sample preparation. Approximately 0.05 g lyophilisate are dissolved in 10 mL of distilled water. After 30 minutes, the obtained suspension is filtered, and the resulting solution is diluted to 25 mL (V).

Procedure. To 2 mL radical solution a certain amount of lyophilisate solution diluted to 1 mL with distilled water was added. The solution absorbance was measured 10 and 20 minutes after the initiation of the reaction, at λ=525 nm, in 1-cm cuvette, with distilled water as a control.

Statistical analysis

The micromolar equivalents of ascorbic acid/g product values were calculated by linear regression on six different gross extract and the results were expressed as average, standard deviation (SD) and relative standard deviation (RSD%).

RESULTS AND DISCUSSION

In order to express the antioxidant activity in molar equivalents (micromolars) of ascorbic acid, we used a calibration curve obtained by plotting the radical solution absorbance according to the concentration of the standard antioxidant, namely, ascorbic acid. The method was validated for a concentration of ascorbic acid in the range of 10-100 µM/L. The linearity of response function, linearity of results, detection limit (LOD=3.13 µM/L), quantification limit (LOQ=9,49 µM/L), system and method accuracy (16) were determined.

Using the equation of the calibration curve (Absorbance = –0.001944 × calculated concentration (µM/L) + 0.5192514), we calculated the concentration of the samples taking into account the dilutions made.

Micromolar equivalents ascorbic acid/g product = a × V × C calculated /v × b × 10^4

a = 2.66 g lyophilisate

b = g lyophilisate involved in the working process (~0.05)

V = 25 mL

v = volume of the analyzed lyophilisate solution

The results refer to 1 g of dried plant product.

The measurements were carried out 10 minutes (tab. I) and 20 minutes after the reaction was triggered (tab. II).

The RSD values of measurements at 10 and 20 minutes, respectively, fit the RSD values for method.

It is concluded that radical capture speed by the antioxidants from the plant product depends both on the antioxidant concentration and the set time frame for the determination (fig. 2).

The two factors influencing the measurements were reunited in one parameter – area under the curve (AUC), calculated for each concentration in ascorbic acid (10-100 µM/L) by plotting the variation of percent inhibition at 10 and 20 minutes against ascorbic acid concentration,

% Inh = $A_r - A_s/A_r.100$, *where*

A_r – radical absorbance;

A_s – sample absorbance.

A_r = 0,53103 (17) (tab. III)

Taking into consideration that the lyophilisate obtained from *Crataegus macracantha Lodd* flowers contains substances with different antioxidant activity, hence with a different capacity of capturing the radicals, it is very important to carry out the measurements at a set time.

TABLE I

Micromolar equivalents of ascorbic acid (AAE) / 1 g of *Crataegus macracantha* flowers (10 minutes after the reaction was triggered)

No.	b (g) gross extract	v (mL) lyophilisate	Absorbance	AA (μM/L lyophilisate)	AAE (μM/1 g flowers)
1	0.0498	0.1	0.43621	42.71	57.03
2	0.0498	0.3	0.26725	129.6	57.69
3	0.0348	0.4	0.25688	134.94	64.46
4	0.0348	0.6	0.16555	181.91	57.94
5	0.0221	0.5	0.32447	100.18	60.29
6	0.0221	0.7	0.24425	141.43	60.80
Statistical data				Average	59.70
				SD	2.7761
				RSD	4.6500%

TABLE II

Micromolar equivalents of ascorbic acid (AAE) / 1 g of *Crataegus macracantha* flowers (20 minutes after the reaction was triggered)

No.	b (g) gross extract	v (mL) lyophilisate	Absorbance	AA (μM/L lyophilisate)	AAE (μM/1 g flowers)
1	0.0498	0.1	0.41806	52.04	69.49
2	0.0498	0.3	0.22649	150.57	67.02
3	0.0348	0.4	0.23093	148.28	70.84
4	0.0348	0.6	0.14038	194.85	62.06
5	0.0221	0.5	0.30067	112.42	67.66
6	0.0221	0.7	0.22843	149.57	64.29
Statistical data				Average	66.89
				SD	3.2572
				RSD	4.8692%

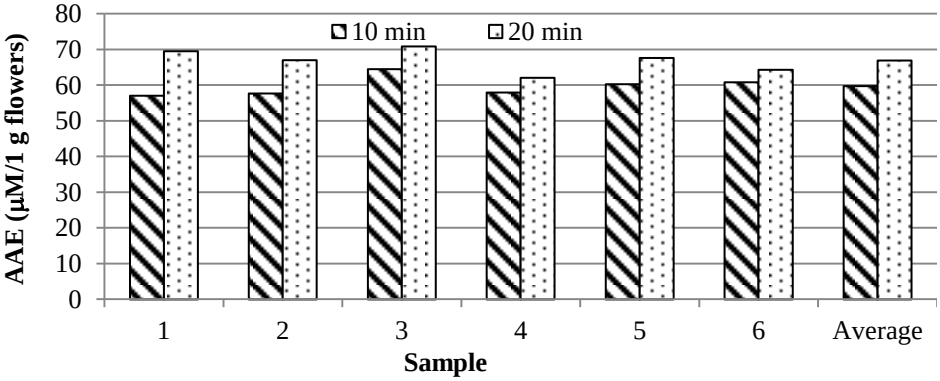

Fig. 2. Micromolecular equivalents of ascorbic acid (AAE) / 1 g of *Crataegus macracantha* flowers (10 and 20 minutes after the reaction was triggered)

TABLE III

Micromolar equivalents of ascorbic acid / 1 g of *Crataegus macracantha* flowers (values calculated from AUC)

No.	Absorbance		Inhibition (%)		AUC	AA	AAE
	10 min.	20 min.	10 min.	20 min.		(μM/L)	μM/1 g flowers)
1	0.43621	0.41806	17.86	21.27	195.65	45.73	61.06
2	0.26725	0.22649	49.67	57.35	535.11	135.57	60.34
3	0.25688	0.23093	51.63	56.51	540.69	137.05	65.47
4	0.16555	0.14038	68.82	73.56	711.95	182.37	58.08
5	0.32447	0.30067	38.90	43.38	411.39	102.83	61.88
6	0.24425	0.22843	54.00	56.98	554.94	140.82	60.53
Statistical data						Average	61.23
						SD	2.4339
						RSD	3.9751%

In order to evaluate the entire antioxidant activity, it is recommended to use the area under the curve as an indicating parameter, since it combines the two variable factors influencing the kinetics of the reaction between radicals and antioxidants: set time for determination and antioxidant concentration.

CONCLUSIONS

In our study, the obtained results show that *Crataegus macracantha Lodd* flowers have a remarkable antioxidant activity. The proposed methods can be used for a qualitative assessment of radical absorption capacity by this plant, and by optimizing these methods a quantitative estimate of total antioxidant activity can also be made.

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NEWS

THE RELATIONSHIP BETWEEN LOW INCOME AND PHYSICAL CAPACITY IN COPD

The capacity to exercise effort depends on the degree of chronic obstructive pulmonary disease (COPD) and on the lung recovery program to which the patient is subject. The prognosis of COPD is associated with the use of health care and quality of life. The 6 minutes' walk test (6MWT) and the cardiopulmonary effort test (CPET) are two methods of measuring effort capacity. Studies suggest that socio-economic status in COPD can also be associated with physical activity effort. For this study, two groups of patients with higher and lower annual incomes of $ 30,000 were formed. Lower-income patients had generally lower outcomes at 6MWT ($p < 0.0001$). An association between income and 6MWT was seen at patients with severe COPD after they were matched by age, gender, pulmonary function, dyspnea and living conditions ($p < 0.0007$). Our research confirms and extends previous hypotheses that conduct to importance of approaching social factors in health, such as income at patients with COPD (Ana Sofia Porta, Nyanjok Lam, Paul Novotny, *et al.* Low income as a determinant of exercise capacity in COPD. https://doi.org/10.1177/14799 72318809491).

SMALL MEDICAL CYCLOTRON PRODUCED RADIOISOTOPES: FROM CLASSIC TO NOVEL RADIOTRACERS

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SMALL MEDICAL CYCLOTRON PRODUCED RADIOISOTOPES: FROM CLASSIC TO NOVEL RADIOTRACERS (Abstract): Positron emission tomography (PET) has become a widespread and indispensable tool for non-invasive oncological, cardiological and neurological diagnosis. With its increase use, there has been a surge in medical cyclotron facilities to answer this demand as well as a continuous research into novel radiopharmaceuticals. Although the high demand for new and efficient radiotracers has spurred the use of new radioisotopes, the classic PET isotopes such as fluorine-18, carbon-11, nitrogen-13 and oxygen-15 have still widespread use, due to their fast ease of synthesis, when compared with other isotopes. The purpose of this mini-review is to present both old and novel radiopharmaceuticals, based on the aforementioned radionuclides, used in clinical diagnosis and biomedical research. **Keywords**: POSITRON EMISSION TOMOGRAPHY, MEDICAL CYCLOTRON, RADIOPHARMACEUTICALS.

Small medical cyclotrons are generally characterized by a small energy range (less than 20 MeV), located in or near hospitals, to answer the daily radiopharmaceutical needs of local PET centers (1). Ease of operation, low cost and minimal preparation of target material is a characteristic of most PET cyclotrons, but these limitations are meant to provide a fast and reliable production of very specific and highly utilized isotopes.

Radioisotopes used in PET need to have specific physical properties, such as a mostly β^+ decay, with adequate energy emission, that will not negatively affect the resolution of the image, a small half-life to ensure a low exposure to the patient and medical personnel but long enough to make the synthesis, quality control and diagnostic procedure feasible (2, 3). From a radiopharmaceutical point of view, the radioisotopes need to be easily labeled, preferably to a variety of molecules that in turn have high specificity, to increase their diagnostic use.

TABLE I
Common isotopes produced by small PET cyclotrons

Radioisotope	Half-life (min)	Max Energy (MeV)
^{18}F	109.8	0.633
^{11}C	20.4	0.980
^{13}N	9.97	1.19
^{15}O	2.06	1.73

As small medical cyclotrons are generally aimed for the production of only the most common radioisotopes, such as the ones presented in first table, the energy output and beam current are relatively small. This, coupled with the target handling system, makes the production of other isotopes difficult if not impossible. In spite of this, we will show that, although the isotopes production by small medical cyclotrons is limited, the radiopharmaceuticals derived from these isotopes can more than satisfy the daily clinical diagnostic needs of local PET centers as well as for biomedical research.

FLOURINE-18

Fluorine-18 (^{18}F) is one of the oldest and most common used PET radioisotopes, especially in oncology, but also in cardiology, neurology and in the diagnostic of infectious diseases (4). ^{18}F is produced by irradiating an ^{18}O enriched water target with a proton beam, with energies from near threshold (3 MeV) up to 20 MeV (5). The $^{18}O(p, n)^{18}F$ reaction yields fluorine in aqueous solution which is then transferred to the synthesis module for extraction, purification and labeling.

The vast majority of PET investigations are conducted using [^{18}F]-Fluorodeoxyglucose ([^{18}F]-FDG), which enables the study of glucose metabolism, especially for the early diagnosis of cancerous cells (6). [^{18}F]-FDG can also be used for myocardial viability studies but due to glucose competition in cardiomyocytes, the specificity is generally low, which may lead to false positive results (7). [^{18}F]-FDG has further limitations when it comes to the diagnosis of malignant cells in tissues with high glucose metabolism which also may cause false positive results, prompting the development and use of other ^{18}F based radiotracers (8).

Neuro-oncological diagnosis also makes use of [^{18}F]-Fluoro-ethyl-L-tyrosine ([^{18}F]-FET), for the detection of brain tumors (9). The higher metabolic activity of malignant cells leads to an abnormal uptake of certain amino acids, such as L-tyrosine, which can be detected. Clinical studies have shown [^{18}F]-FET to have a high sensitivity (87%) in the diagnosis of brain tumors but limited specificity (68%), due to the passive influx and limited uptake in tissues (10).

[^{18}F]-Fluorothymidine ([^{18}F]-FLT) is another PET radiotracer used in oncology, especially in therapy response evaluation and in brain tumors. FLT acts as a cellular proliferation biomarker, that is transported inside the cell in the same way as thymidine, but due to the overexpression of the TK-1 enzyme in tumors, FLT undergoes phosphorylation and it predominantly accumulates inside them (11).

[^{18}F]-Fluorocholine ([^{18}F]-FCH) is used to signal rapid cellular metabolism, as the lipid choline is the precursor for the biosynthesis of phosphatidylcholine (PC) and thus for many PC rich cellular membranes, such as cancerous cells (12). [^{18}F]-FCH has been used extensively to image prostate cancer and to monitor the effects of therapy (13, 14).

Beyond oncological applications, certain ^{18}F based PET radiopharmaceuticals have been developed for the diagnosis of Alzheimer's disease, such as [^{18}F]-Florbetaben and [^{18}F]-Flutemetamol (15, 16). Both of these tracers help visualize the presence of β-amyloid plaques (Aβ) in the brain and have recently received approval for medical use by the FDA and the EMA. As with all radiopharmaceuticals, the drug is only produced when needed, in limited quantity, due to the high cost and time consuming nature of the synthesis and quality control procedures, that reduces the useful ^{18}F radioactivity (17).

The use of positron emission tomography has also found widespread use in medical research, as it enables the *in vivo* examination of cerebral functions, especially concerning the activity and role of certain neural receptors (18).

[^{18}F]-Mefway is 5-HT_{1A} serotonin receptor antagonist, that has shown high stability and selectivity in both animal and human studies (19, 20). These initial results make ^{18}F-Mefway a highly useful tool to quantify the serotonin 5-HT_{1A} receptor distribution, which may assist the diagnosis and evolution of various central nervous system disorders, such as depression, anxiety and Alzheimer's disease (21). Similar to [^{18}F]-Mefway, MPPF is also a serotonin receptor antagonist with increased use in academic and medical centers, for preclinical and clinical studies (22).

[^{18}F]-Nifene is a PET radiotracer that acts as an agonist to α4β2* nicotinic acetylcholine receptors (23). These receptors have been shown to be involved in the evolution of certain brain disorders such as Parkinson's, Alzheimer's and schizophrenia, and a precise and reliable technique to analyze their distribution and activity *in vivo*, could hold the key to early diagnosis and treatment (24-27).

CARBON-11

Due to the natural abundance of carbon in organic matter, ^{11}C can be attached to various organic molecules without disrupting their function within the target organism. There are a number of possible reactions that yield ^{11}C, but the most common and the most relevant for routine production in small medical cyclotrons is the $^{11}N(p, \alpha)^{11}C$ nuclear reaction. ^{11}C decays 98.1% by β^+ and 0.19% by electron capture, with a half-life of 20.3 minutes, making it a useful radionuclide for PET imaging. Using natural nitrogen gas with a small percentage of O_2 (0.1-0.5%) will yield [^{11}C]-CO_2, while using nitrogen gas with a small percentage of hydrogen (5-10%) will yield [^{11}C]-CH_4. Due its short half-life the time available for the labeling and diagnostic procedures is limited, but this also means that the dose received by the patient is lower when compared to ^{18}F based radiotracers (28).

Beyond $^{11}CO_2$ and other simple molecules, like ^{11}CO and $^{11}CH_3I$, ^{11}C labeled organic molecules are widely available for PET investigations, such as amino acids, enzymes, tumor specific receptors and drugs (29). As with ^{18}F, a comprehensive study of all the different ^{11}C based radiotracers would be beyond the scope of this review, thus we will focus instead on the most common and widely used tracers available.

[^{11}C]-Acetate was found to be highly versatile and it has widespread use in many PET centers, for oncological applications, such as for the diagnosis of brain tumors

(30) and prostate cancer (31, 32). The use of [^{11}C]-acetate for hepatocellular carcinoma (HCC) was proven very effective and it exhibited a higher sensitivity when compared to ^{18}F-FDG (33). Outside of oncology, [^{11}C]-acetate has been used in cardiology, for the study of myocardial blood flow (MBF), cardiac input and cardiac efficiency (34, 35).

[^{11}C]-Choline is one of the most important PET imaging radiotracers. The use of choline labeling was stated previously, when we presented [^{18}F]-choline, but the use of ^{11}C is more advantageous, as it offers a lower radiation exposure to the patient. In this regard ^{11}C-Choline has been used to study prostate (32, 36) and breast cancer (37) as well as brain tumors, where it was shown to have a higher specificity than ^{18}F-FDG (38).

[^{11}C]-Methionine ([^{11}C]-MET) is another commonly used PET tracer for the imaging of brain tumors, due to specific upregulated amino acid transporters (39). [^{11}C]-MET has a high specificity and sensitivity in detecting most malignant tumors (40), having a greater ability to outline the region of low-grade glioma when compared to [^{18}F]-FDG (41).

Several other ^{11}C radiotracers are used in biomedical research, on animal or human studies.

[^{11}C]-DASB has been used for PET neuroimaging, for the study and treatment of major depressive disorder (42, 43). Due to its high selectivity for serotonin transporter, imaging during treatment with selective serotonin reuptake inhibitors (SSRIs) allows for the evaluation of their effectiveness on patients (42, 44).

25B-NBOMe is a derivative of the psychedelic drug 2C-B, that acts as an agonist of the $5HT_{2A}$ serotonin receptor, which through radiolabeling with ^{11}C becomes a valid PET radiotracer, named [^{11}C]-Cimbi-36 (45). [^{11}C]-Cimbi-36 may act as a functional marker for the $5HT_{2A}$ receptors in the human brain, involved in the pathophysiology of a variety of brain diseases such as depression, Alzheimer's disease, and schizophrenia (46).

[^{11}C] labeled Flumazenil is a selective $GABA_A$ antagonist used as a radiotracer to assess the distribution and function of these receptors in relation to epilepsy evolution (47).

NITROGEN-13

Nitrogen-13 is generally produced by irradiating a water target with a proton beam, by way of $^{16}O(p, \alpha)^{13}N$ nuclear reaction. The reaction produces nitrate (NO_2^-) and nitrite ions (NO_3^-) which are later converted into ammonia ([^{13}N]-NH_3), either directly in the target by using a reducing agent (48) or a radical inhibitor, or later following a reduction reaction (49).

Due to its short half-life (9.97 min), the use of ^{13}N in nuclear medicine is limited to facilities that have access to a cyclotron, the production and labeling procedures needs to be fast and efficient to ensure minimal loss and the biological half-life of the radiopharmaceutical needs to match the radionuclide's physical half-life. Currently ^{13}N labeled ammonia (NH_3) is the only viable radiotracer used in clinical practice. [^{13}N]-NH_3 is taken up by the myocardium and the brain, where it is used for the imaging and measuring of the myocardial and cerebral blood flow (50) while the use of simple [^{13}N]-N_2 has been applied for nitrogen fixation and ventilation studies (51). Although the use of [^{13}N]-NH_3 has become

more common, ^{13}N has limited use, which is unfortunate when considering the large variety of complex and biologically active molecules that contain nitrogen. Fortunately, several studies have reported the synthesis of more complex molecules.

The synthesis of [^{13}N] labeled amino acids has been reported, such as L-[^{13}N]-asparagine L-[^{13}N] glutamate, L-[^{13}N]-valine, L-[^{13}N]-alanine and L-[^{13}N]-leucine through either chemical or enzymatic techniques but the fast synthesis procedures provided limited purification which made it unacceptable for human use (52).

^{13}N based amines and amides, such as [^{13}N] labeled amphetamine, β-phenethylamine (PEA) and [^{13}N]-adenosine have been produced but none demonstrated any significant use (53-55).

The use of microfluidic systems, which have been employed for other PET radioisotopes, my offer an useful alternative to traditional synthesis methods (56).

Thus, it is important to understand that although ^{13}N is currently much less developed than the previous radioisotope-based tracers, research is being conducted on faster and more reliable labeling and synthesis techniques that could provide more versatility to this PET radionuclide in the near future.

OXYGEN-15

^{15}O was one the first artificial radioisotopes produced using low energy cyclotrons either through the $^{14}N(d, n)^{15}O$ or the $^{15}N(p, n)^{15}O$ nuclear reactions, depending on the cyclotron capabilities (deuteron or proton beam). The ^{15}N production reaction is the only feasible reaction for small energy medical cyclotrons but the expensive enriched nitrogen-15 gas limits the use of ^{15}O based radiotracers. In both scenarios, a nitrogen gas target is used - which yields ^{15}O in gaseous form. Having the shortest half-life (2.06 min) when compared to the previous PET radioisotopes, the processing available is minimal and certain circumstances being carried out inside the cyclotron target system.

^{15}O is mainly used for the synthesis of [^{15}O]-water, either by adding 5% hydrogen directly in the target or by transferring the ^{15}O to a 400°C oven, where it undergoes platinum catalysis in the presence of high grade hydrogen (57). [^{15}O]-water is used for measuring cerebral (58) and myocardial blood flow, undergoing free-diffusion across cellular membranes, and having a 100% extraction rate, which makes it superior to other PET radiotracers (59). But practical limitations, due to its low resolution and low half-life, combined with the previously mentioned high production costs, make the use of [^{15}O]-water generally rare, even though its usage has been approved by both the FDA and EMA.

Although not very common, studies using ^{15}O labeled gases have been reported. ^{15}O-CO_2, ^{15}O-O_2 and ^{15}O-CO_2 have been used for *in vivo* measurements of cerebral oxygen metabolism, cerebral blood flow and cerebral blood volume in animals that may pave the way for their use in human research and clinical practice (60, 61).

CONCLUSIONS

As small medical cyclotrons designed for routine PET isotope production will become more efficient, more automated and accessible, PET imaging for either clinical or research purposes will become even more common and thus it is important to understand the full diagnostic potential

of the most readily available radioisotopes: ^{18}F, ^{11}C, ^{13}N and ^{15}O. The aim of this mini review was to highlight that these nuclides are highly versatile and with the development of novel synthesis techniques their potential is ever expanding.

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NEWS

CANCER: "OFF-THE-SHELF" STEM CELL TREATMENT ENTERS CLINICAL TRIAL

Now, researchers at the University of California (UC) San Diego School of Medicine are running a clinical trial with industrial collaborator Fate Therapeutics to investigate NK cells both alone and in combination with checkpoint inhibitors in people with advanced solid tumors. One particular factor sets this study apart from others using NK cells for similar purposes. This "off-the-shelf" NK immunotherapy trial is the first in the U.S. to use cells that the researchers have derived from induced pluripotent stem (iPS) cells. The use of iPS cells allows researchers to produce a never-ending stream of cells. All that they need is a robust method to turn iPS cells into the particular cell type that they require. A single iPS cell can thus become an "off-the-shelf" source of cells for therapy, which it is easy to produce time and time again. In 2013, Dr. Dan Kaufman - professor of medicine in the Division of Regenerative Medicine and director of cell therapy at UC San Diego School of Medicine - and his team developed a method of expanding large numbers of NK cells from human iPS cells for cancer therapy. They published the method in the journal Stem Cells Translational Medicine. After extensive preclinical testing, the Food and Drug Administration (FDA) gave Dr. Kaufman and Fate Therapeutics the go-ahead last November to set up a phase I clinical trial to test their iPS-derived NK immunotherapy in people with advanced solid tumors. The phase I trial started in February and will include up to 64 people with advanced, untreatable cancer. The main aim of the trial is to assess the safety of the treatment. The other objectives are to determine the extent to which the tumors respond to NK cell therapy and to find out how long the cells stay in the participants' bodies. The team will administer the cells once per week for 3 weeks, either on their own or in combination with one of three checkpoint inhibitors, namely nivolumab, pembrolizumab, or atezolizumab. The study is an open-label trial, which means that all of the study participants will know which treatment they are receiving. "This is a landmark accomplishment for the field of stem cell-based medicine and cancer immunotherapy" explains Dr. Kaufman in a press release. "This clinical trial represents the first use of cells produced from human-induced pluripotent stem cells to better treat and fight cancer". "Together with Fate Therapeutics, we've been able to show in preclinical research that this new strategy to produce pluripotent stem cell-derived natural killer cells can effectively kill cancer cells in cell culture and in mouse models" he continues. The first person to receive treatment as part of the trial was Derek Ruff. After 10 years in remission, Mr. Ruff has stage 4 colon cancer, which is progressing despite aggressive treatment. It will be a while before the results of the trial are available, with expectations being that the study will run into 2022. However, it paves the way not only for a new generation of immunotherapies to treat cancer but also for other iPS-derived cell therapies to follow (https://www.medicalnewstoday.com/articles/324916.php).

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EARLY RECURRENCE IN FAVORABLE STAGE II BREAST CANCER – WHICH APPROACH IS THE BEST ?

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EARLY RECURRENCE IN FAVORABLE STAGE II BREAST CANCER-WHICH APPROACH IS THE BEST ? (Abstract) : **Aim** : Changing the sequence of therapeutic options in stage II breast cancer : first, a core biopsy, followed by the evaluation of the tumoral markers, adaptation of the chemotherapy scheme and finally, surgical approach. Thus would be possible to improve the hope of life in some stage II breast cancer patients, in whom survival is poorer than in some stage III patients. **Material and method** : 144 patients in stage II breast cancer were included in this study, over a period of 5 years (2000-2004). In all these patients the first therapeutic option was surgery (radically modified mastectomy type Madden), followed by systemic chemotherapy-FAC or FEC, 6 cycles, and finally Tamoxifen. **Results** : 34 out of them developed metastases in a period between 6 and 72 months, most of them in the first 26 months ; 25 out of these 34 didn't have metastases in the axillary lymph nodes, and in 18 patients estrogen - and progesterone - receptors were highly positive. HER 2 neu was negative or low expressed in patients with metastases. CD 34 wasn't evaluate in the whole group. **Conclusions** : Early onset of metastases in the studied patients, in whom tumoral aggressiveness markers were not obvious, impose the evaluation of the angiogenesis markers and, when positive, chemotherapy as the first therapeutic option. **Key words** : STAGE II BREAST CANCER, CORE BIOPSY, TUMORAL MARKERS, NEOADJUVANT SYSTEMIC CHEMOTHERAPY

Stage II breast cancer, considered as a curable clinic stage, benefits today of surgery, as the first therapeutic sequence, followed by radiotherapy (for those operated conservative), and chemo-hormono-therapy, monoclonal antibodies, depending on the tumoral markers.

The last WHO classification-2008-, includes in stage II the following categories : St. IIA : T0-1 N1 M0 ; T2 N0 M0, and St.IIB : T2 N1 M0 ; T3 N0 M0 (1). Most of the patients with stage II breast cancer have a better prognostic in comparison with patients in stage III, as well known for many years. Meantime, it is a reality that some patients in stage II have a disease-free survival and overall survival shorter, comparative with patients in stage III. Tumoral markers'analysis should, theoretically, find the explanation of certain more aggressive biological tumors, thus explaining the negative evolution of some stage II breast cancers in the first five years after the beginning of treatment : the presence of tumoral emboli, the presence of different histological types in the same tumor, positive axillary lymph

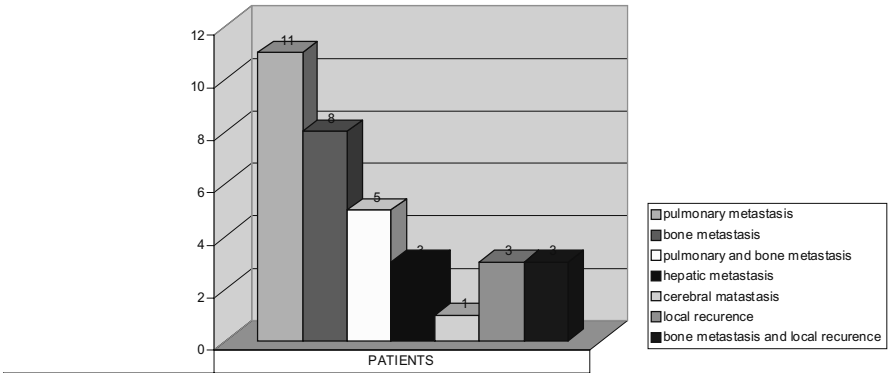
nodes, type III histological gradding, mitotic index greater than 1,2, estrogen receptor (ER) and progesterone receptor (PR) negative, HER-2 neu negative(triple negative). It is true the fact that some patients in stage II with positive tumoral markers have an unexpected prolonged survival, while part of the patients in the same stage II, with negative tumoral markers-axillary lymph nodes without metastases, estrogen and progesterone receptor positive in high percentage- develop in a period of months-years after the onset of adjuvant therapy, metastatic disease and death. This evolution could be explained by the incomplete evaluation of the tumoral aggressiveness with the actual tumoral markers, thus avoiding to use more specific therapeutic options in such cases; another explanation could be the delay in starting the systemic therapy when the first sequence was surgery; on the other hand, surgery itself, when applied first, could be considered as a "whip" for the systemic disease, favoring the installation of the metastatic disease.

MATERIAL AND METHOD

Between January 2000 and December 2004, 455 patients were operated for breast cancer in our unit; 66 were stage I, 144 stage II, and 245 were stage III. Patients in stage II were submitted to surgery as the first therapeutic option, including a radically modified mastectomy type Madden; axillary dissection was performed with an en-bloc removal of the connective tissue, and the three levels of the axillary lymph nodes. Adjuvant therapy was applied after 14 days from the surgical excision in patients with positive axilla it consisted in chemotherapy FAC or FEC (considered standard after 1990), 6 cycles in 6 months, and Tamoxifen for 5 years, in patients with high levels of estrogen and progesterone receptors. Fourteen patients in the group with negative axilla couldn't benefit of standard chemotherapy, due to severe myocardiopathy.

RESULTS

Our study analyses almost exclusively patients with unfavorable evolution. Thirty four patients out of 144 in stage II breast cancer developed metastases in a period between 6 and 72 months postoperatively, most of them in the first 26 months: 11 -pulmonary metastases, 8 –bone metastases,



	PATIENTS
pulmonary metastasis	11
bone metastasis	8
pulmonary and bone metastasis	5
hepatic metastasis	3
cerebral matastasis	1
local recurence	3
bone metastasis and local recurence	3

Fig. 1. Developed metastasis in a period of 6-72 months postoperatively for patients in stage II

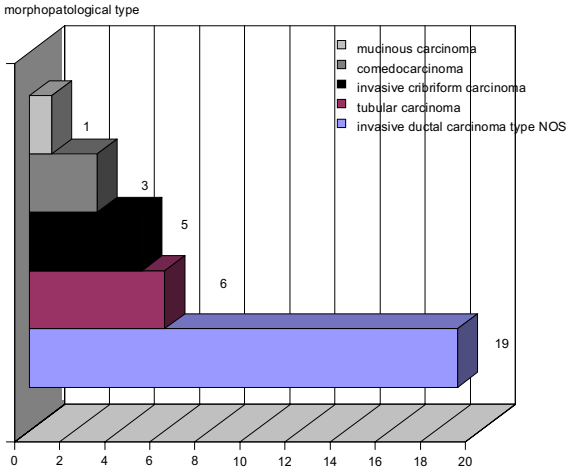


Fig. 2. Morphopatological types for patients in stage II breast cancer with unfavorable evolution

5-pulmonary and bone metastases, 3-hepatic metastases, 1-cerebral metastases, 3-bone metastases and local recurrence and 3 with local recurrence (fig.1).

As from morphopathological point of view, most of the tumors were ductal invasive carcinoma (one third of the patients), followed by tubular carcinoma and invasive cribriform carcinoma (fig.2).

Patients with pulmonary, cerebral and hepatic metastases were not alive at the moment of the evaluation, death occurring between 3 and 14 months from the onset of metastases. Patients who died of metastatic disease were aged between 49 and 81 years, with a median of 61,47 years. Sixteen patients with pulmonary metastases and pulmonary plus other metastases, as well as 2 patients in the group with hepatic metastases didn't have metastases in the axillary lymph nodes; estrogen and progesterone receptors were > 70%, HER- 2neu was negative or poor expressed (fig. 3) Seven patients in the group with bone metastases didn't have invasion in the axillary lymph nodes, and the receptors for estrogen and progesterone were in low percentage.

CD 34 wasn't evaluated in the whole group of the patients included in the study, so that we can't make any comments for the patients that had a good evolution. CD 34 was moderated or over-expressed in 8 patients who developed pulmonary metastases,

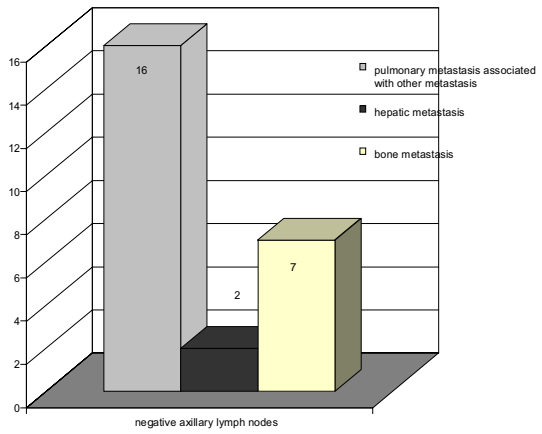


Fig. 3. Patients (stage II) with metastasis and negative axillary lymph nodes

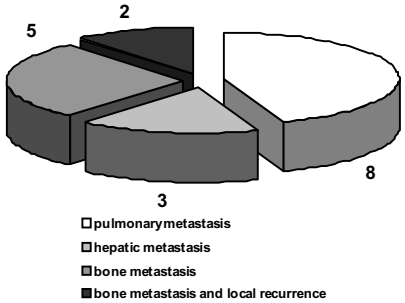


Fig. 4. CD 34 moderated or over-expressed

TABLE I
Disease free survival

pT	Disease free survival(months)
pT2	60-96
pT1,pT2,pT3	60-117

3 patients with hepatic metastases, 5 patients with bone metastases and in 2 patients with bone metastases and local recurrence (fig. 4).

Out of the patients with tumors greater than 2 cm, 39 were alive, without clinical recurrence in the moment of evaluation (between 60 months and 96 months) (tab. I) and the disease – free survival in all patients ranged between 60 and 117 months (tab. I).

DISCUSSIONS

The benefit of adjuvant chemotherapy in breast cancer was established in many prospective randomized trials, started in the last 40 years. A comprehensive meta-analysis of all randomized trials prior to 1985 (Early Breast Cancer Trialist's Collaborative Group) and published in 1992, including 75.000 patients, showed the advantages of poly-chemotherapy in terms of survival, especially in women aged under 50. Before 1990, standard chemotherapy in breast cancer consisted in Cyclophosphamide plus Methotrexate and Fluorouracil. The association of anthracyclines (Doxorubicin or Epirubicin) with Fluorouracil and Cyclophosphamide (FAC or FEC) for a period of 3-6 months, after 1990, was correlated with the improving of the clinical results, thus becoming the standard therapy (2). For patients in stage II with positive axilla, chemotherapy reduces the risk of death with 15%. The association of Paclitaxel to the standard treatment of Doxorubicin and Cyclophosphamide, improved the 5 years disease-free survival to 70%, and the overall survival to 80%, in comparison with 65% and respectively 77%, reached in anterior studies (2). More recently, the association of another Taxane-Docetaxel with Doxorubicin and Cyclophosphamide, improved the efficacy of chemotherapy in comparison with FAC(3).

Another problem is represented by the patients with negative axilla, as one third of them will develop metastatic disease, thus negative axillary lymph nodes cannot preclude the systemic treatment. In this group, patients with tumors greater than 2 cm have a recurrence risk equivalent to those with one or two positive lymph nodes (4,5). Most of the studies consider that the most important, reliable and simpliest indicator in assessing the risk of recurrence in stage II patients with negative axilla is the volume of the tumor. In our database, more than 30% of the patients with tumors greater than 2 cm were alive in the moment of evaluation. Another useful criteria in evaluating the recurrence in patients with negative axilla is represented by a series of histopathological types, considered to have a lower tumoral aggressiveness. Thus, a study conducted in Sloan-Kettering Memorial Center on 767 women with negative axillary lymph nodes have demonstrated that the ductal invasive carcinoma type NOS, found in tumors less than 1cm, had a recurrence of disease of 13% at 21 years of follow-up (6). Despite all these data, many prognostic factors were not identified up to now for clinically curable breast cancers, as showed by Donegan 20 years ago (7).

It is known today that certain fenotypes of cells, structuring the tumor, present some aggressiveness factors, thus offering a lower or higher risk for developing the systemic disease. These prognostic tumoral markers have also the role in adjusting the therapeutic protocol to be followed by the patient: the status of steroid hormones, the expression of HER 2neu, CD 34 or other angiogenesis tumoral markers. Around 15 years ago, some medical centers have proposed the utilization of computerized programs which incorporate the effects of the prog-

nostic factors, thus establishing an individual risk (8).

The first therapeutic option in patients with positive estrogen- and progesterone-receptors is Tamoxifen, which is less toxic comparative with chemotherapy.

The benefit of Tamoxifen in cases with negative axilla is pointed out by the results obtained in large series of patients with positive receptors, treated over a period of 5 years ; thus, recurrence was reduced with 47% per year, and the absolute benefit at 10 years of follow-up was of 5-7% (9). More recently, aromatase inhibitors, such as anastrozole (10), letrozole (11), aromasin (12), have improved the clinical benefit for patients with positive receptors. The value of combined chemo-hormono- therapy in patients with negative axilla isn't yet well established by the clinical trials.

Based on data published in literature by the different trials, our study tries to analyze the rapid unfavorable outcome of some patients with stage II breast cancer, who didn't have lymph node metastases, hormone receptors were positive, Her 2 neu negative or low expressed. This group of patients, who rapidly evoluted unfavorable, included women in late menopause, who presented T 2 tumors (most of the patients--32 cases), and histologically was confirmed the common type of breast cancer (28 cases). The tumoral aggressiveness factors were not presented in these cases (the triple negative character, positive axilla, the association of combined histological types in the same tumor, the presence of emboli in peritumoral vessels), exception making the size of the tumor (T2).

Patients in whom recurrence early occurred and died of metastatic disease were adjuvantly treated with chemotherapy and Tamoxifen, excluding those with myocardiopathy, who couldn't benefit of chemotherapy due to its cardiotoxicity (13,14).

The unfavorable outcome of these patients can be also explained by the heterogenous aspect of tumors in the same stage of disease, which have molecular differences even they are histological similar ; it is known in literature the new molecular classification of breast cancer, based on gene expression profile (15). We consider that the early onset of metastases in our cases could be also favored by the delay in the beginning of chemotherapy, due to surgical treatment (as first therapeutic option), and, on the other hand, surgery acted as a "whip" for the systemic disease, favoring the early onset of the hepatic, pulmonary and bone metastases.

These unfavorable conditions in the evolution of the disease could be counterbalanced by changing the sequence of the therapeutic options ; thus, a core biopsy is to be initially performed, followed by an evaluation of the tumoral markers, adaptation of the therapeutic scheme with systemic target, and finally, the surgical approach. In this manner, we could improve the hope for a prolonged survival for these patients.

The value of this hypothesis will or not be confirmed in the future by studies on larger groups of patients, continuing the efforts of identification of risk factors which will allow a better selection of patients for treatment.

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NOUTĂŢI

PEPTIDUL GALANIN-LIKE : UN FACTOR CHEIE IN REGLAREA HOMEOSTATICĂ A ALIMENTAŢIEI ŞI A METABOLISMULUI ENERGETIC

Hipotalamusul deţine un rol cheie în reglarea comportamentului alimentar, a reproducerii şi a metabolismului energetic. Peptidul galanin-like este un neuropeptid alcătuit din 60 de aminoacizi, produs de o populaţie discretă de neuroni din nucleul arcuat bazomedian şi eminenţa mediană, trimiţând proiecţii spre nucleul anterior paraventricular. În prezent se consideră că acest peptid exprimă receptori pentru leptină, care intervine direct în controlul saţietăţii şi implicit în instalarea obezităţii. Stimularea constantă a producerii peptidului galanin-like acţionează la distanţă asupra altor peptide reglatoare, inclusiv asupra leptinei, întrucât neuronii care îl sintetizează formează reţele care se regăsesc pe spaţii extinse. La animale de laborator, peptidul galanin-like induce o scădere a consumului alimentar, putând reprezenta o soluţie de viitor în terapia obezităţii (Shioda S, Kageyama H, Takenoya F, Shiba K. Galanin-like peptide : a key player in the homeostatic regulation of feeding and energy metabolism. *Int J Obes* 2010 ; 12 : 132-136).

Carmen Zamfir

NOVEL APPROACHES IN THE PHARMACOTHERAPY OF RHEUMATOID ARTHRITIS

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NOVEL APPROACHES IN THE PHARMACOTHERAPY OF RHEUMATOID ARTHRITIS (Abstract): Rheumatoid arthritis is an autoimmune disease, a chronic inflammatory disease that affects more than the joints. There is currently no cure for rheumatoid arthritis, but remarkable progress has been made over the last two decades in therapeutic regimens, with the possibility of long-lasting remission and in particular, prevention of sequelae (joint deformities, ankylosis, reduced mobility and functional capacity). Various drugs are used in the pharmacological treatment: nonsteroidal anti-inflammatory drugs, corticoids, disease-modifying anti-rheumatic drugs, biological agents (acting on the immune system, reducing its erratic activity, with maximum efficacy combined with disease-modifying anti-rheumatic drugs). **Keywords:** RHEUMATOID ARTHRITIS, NON-STEROIDAL ANTI-INFLAMMATORY DRUGS, BIOLOGICAL AGENTS.

Rheumatoid arthritis (RA) represents an inflammatory disorder that particularly concerns extremity joints (hands, legs) causing injuries, swelling, deformities of the regions and pain. Statistical data show that this disease can occur at any age, even in children (juvenile RA), but usually affects individuals aged 25 to 50 years, with a higher incidence in women compared to men (1, 2).

Over time, degenerative manifestations worsen, the erosions and destructions of cartilages, ligaments and bones causes local fibrosis that progressively deforms the extremities, generating from mild to particularly severe disabling disease forms. Moreover, pain, local deformities and functional impotence affect the day-to-day, domestic and occupational activity of patients (2). In parallel, existing autoimmune reactions induce extra- articular manifestations, affecting other systems such as the connective tissue, the cardiovascular system or respiratory system. There are situations in which the pain is particularly intense and limits the joint movements, significantly affecting their functionality and implicitly the motor activity of the person, who will present various degrees of disability.

The mechanisms involved in the etiopathogenesis of this disease have not been fully elucidated yet, but the most viable pathophysiological hypothesis implies the intervention of autoimmune processes, especially in susceptible individu-

als (3). An idea that has been raised is that under the conditions of particular predisposing genetic factors, the action of certain triggers (such as trauma, stressful situations, infections, smoking, alcohol consumption) induces an autoimmune reaction, causing a series of articular changes with chronic inflammation and synovial hypertrophy, as well as extra-articular manifestations (in the pulmonary and circulatory system) (4, 5).

Nearly all compartments of the lung structure are potential sites of injury, common pulmonary manifestations of rheumatoid arthritis being interstitial lung disease (ILD), airways disease (bronchiolitis, bronchiectasis), nodules, pleural and vascular disease (6, 7). ILD is a significant cause of morbidity and mortality which can arise before and throughout lifetime after the diagnosis of RA (6), therefore patients presenting risk factors for ILD or with ILD should be closely monitored for lung function (pulmonary function tests and/or high-resolution computed tomography) (8).

Furthermore, conventional immunosuppressant or biologic drugs (methotrexate, anti-tumor necrosis factor alpha agents, leflunomide, sulfasalazine, rituximab) used in RA therapy are associated with an increased incidence of pulmonary toxicity (7, 8).

CLASSIC PHARMACOTHERAPY

In 2008, recommendations and algorithms were developed for the use of disease-modifying anti-rheumatic drugs, non-biological and biological agents, corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) and analgesics for the management of RA; an updated version of these algorithms was later published in April 2012.

Immediately after diagnosis, the main goals of treatment are to control disease activity and to reduce the rate of joint damage, while minimizing pain, joint stiffness, inflammation, and complications (2).

The disease-modifying anti-rheumatic drugs can be classified into non-biological agents and biological agents. Non-biological agents include hydroxychloroquine, azathioprine, sulfasalazine, methotrexate, leflunomide, cyclosporine, gold salts, D-penicillamine, minocycline (2). Gold-injected salts and penicillamine rarely induce sustained disease remissions, which is why their frequent use in the therapy of this condition has largely been replaced by other more effective anti-rheumatic agents.

In October 2013 the USA Food and Drug Administration (FDA) approved for therapeutic use a single-dose methotrexate with a disposable subcutaneous autoinjector for self-administration of the drug. This therapeutic agent is indicated for use in adults with severe active rheumatoid arthritis who have an inadequate response or cannot tolerate the first line of treatment, as well as for children with juvenile idiopathic rheumatoid arthritis.

Corticosteroids are potent anti-inflammatory drugs habitually used in patients with RA in the first stages of the disease, until treatment with disease-modifying anti-rheumatic drugs is necessary (9). These agents can also be effective adjuvants of therapy with disease-modifying anti-rheumatic drugs (2). Administration of corticosteroids as well as NSAIDs may be advantageous, as it allows reduction of required doses of anti-rheumatic drugs modifying the disease (9). There are a few dozen NSAIDs available on the market, but those commonly used in the treatment of RA include ibuprofen, keto-

profen, naproxen, piroxicam, diclofenac, celecoxib. Treatment with chelating agents in RA can suppress inflammation and arthritis by inhibiting the T cell activity.

MODERN PHARMACOTHERAPY

The debate concerning the involvement of tumor necrosis factor alpha (TNF-α) and interleukin-1 (IL-1) as central pro-inflammatory cytokines has led to the development of biological agents that block or inhibit these cytokines (tab. I). In addition to alleviating disease symptomatology and improving the quality of life, all biological agents significantly delay the progression of joint erosions, action that has been radiologically demonstrated (10). It has been highlighted that biological agents are meaningfully more effective than non-biological treatments in improving the joints physical function in patients with RA (11). It is fundamental not to recommend the use of these agents until the failure to treat the disease with at least one non-biologically-modifying antirheumatic drug, usually methotrexate.

The following rheumatic disease modifying agents are mentioned: adalimumab, certolizumab, etanercept, golimumab and infliximab (2, 12). For patients with incipient RA (less than 3 years of development), who have not previously administered methotrexate, the beginning of monotherapy with methotrexate, adalimumab or etanercept effectively controls the symptoms of the disease (11, 13). Combining a biological agent with methotrexate usually improves better the clinical manifestations than monotherapy (10). Rituximab is the most commonly used anti-rheumatic drug in combination with methotrexate in the treatment of RA (14). On the other hand, for patients with incipient RA, the combination of methotrexate and sulfasalazine does not bring significant benefits compared to the use of any of these drugs alone (13).

There is not sufficient evidence to show that combining two biological agents has superior efficacy to the use of any biological agent alone; in addition, it has been pointed out that concomitant use of two biological agents has been associated with an increased risk of complications (the administration of methotrexate and most biological agents increase the probability of a severe infection), but without a clear benefit (11, 12).

In 1998, *etanercept* was the first TNF inhibitor to receive approval from FDA and the European Medicines Agency (EMA) for the treatment of moderate to severe RA and remains a first-line biologic disease-modifying drug therapy for RA worldwide. Other indications for etanercept are psoriatic arthritis, plaque psoriasis, axial spondylo arthritis, ankylosing spondylitis and polyarticular juvenile idiopathic arthritis (15,16). It was found that combination treatment with methotrexate improves functional capacity, disease control and remission compared with monotherapy with either etanercept or methotrexate; discontinuation and side effects are similar (17).

Tocilizumab represents an inhibitor of the IL-6 receptor, and it is indicated for the treatment of moderate to severe active RA in adults with no response to one or more TNF antagonists (2). It has been approved for intravenous infusion or subcutaneous injection and can be used alone or in combination with methotrexate or other disease-modifying antirheumatic drugs (18, 19). The treatment with tocilizumab produces significant improvements in clinical trials in patients with inadequate response to TNF inhibitors, therefore showing that

the association of methotrexate with tocilizumab is more clinically effective than association of methotrexate with adalimumab in rheumatoid arthritis (20).

Anakinra, a recombinant non-glycosylated form, is derived from the antagonist of the receptor for human IL-1 and can be disposed alone or in combination with other disease-modifying anti-rheumatic drugs other than TNF blockers. It is indicated to reduce the symptomatology and reduce the progression of structural damage in moderate to severe active RA unresponsive to treatment with one or more disease-modifying anti-rheumatic drugs (2, 21).

Abatacept is a selective costimulatory modulator that inhibits T lymphocyte activation by binding to CD80 and CD86 (which are proteins, differentiation clusters), thus blocking their interaction with CD28 (22,23). This agent has been approved for the reduction of clinical manifestations because it produces significant inhibition of structural damage progression and improves physical function in adult patients with moderate to severe RA (22). It can be administered in monotherapy or in combination with disease-modifying anti-rheumatic drugs other than TNF antagonists (23). It has been emphasized that in patients with RA with previous failure of treatment with anti-TNF agents, abatacept administration had increased efficacy and safety over long periods of treatment (6 months to 5 years) (22, 24).

TABLE I

Biological agents used in RA therapy (28,29)

Target	Drug	Mode of action	Starting dose	Usual dosage or Maintenance dose
Soluble TNF	Adalimumab	Immunoneutralization		40mg SC every 2 weeks
	Certolizumab		400 mg SC at weeks 0, 2, 4	200mg every other week
	Etanercept	Neutralization		50mg SC once weekly or 25mg twice weekly
	Golimumab	Immunoneutralization		50mg SC once monthly
	Infliximab		3mg/kg IV at 0, 2, 6 weeks	3-10 mg/kg IV every 4-8 weeks
Soluble IL-1	Anakinra	Neutralization		100mg SC daily
Soluble IL-6	Tocilizumab	Neutralization	4 mg/kg IV q 4 weeks	4-8mg/kg IV every 4 weeks
T cells	Abatacept	Prevents co-stimulation of T cells	IV: <60kg = 500 mg; 60-100kg = 750mg; >100 kg= 1000mg at 0, 2, and 4 weeks or initial IV dose followed by 125 mg SC within 24 h	IV: dose based on weight every 4 weeks SC: 125 mg once weekly
B cells	Rituximab	Causes B cells lysis	1000mg IV infusion given twice, 2 weeks apart	Initial dose may be repeated every 16-24 weeks based on response

Adverse effects associated with biological agents include production of antibodies against these compounds, the emergence of antinuclear antibodies, occasional drug-induced lupus syndromes and infections. Rarely, demyelinating disorders, as well as bone marrow suppression (25) may occur. The presence of acute and chronic infections, demyelinating disorders, NYHA class III and IV cardiac insufficiency, recent malignancies, contraindicates the use of TNF inhibitors (2, 24, 26).

Tofacitinib, an inhibitor of the pathogenic pathway of Janus kinase (a non-receptor tyrosine kinase family that mediates the transmission of cytokine-mediated signals), is indicated as a second treatment line for moderate to severe active RA in patients with inadequate or intolerant methotrexate response. It may be administered as a monotherapy or in combination with methotrexate or other non-biological anti-rheumatic agents but is not used in combination with anti-rheumatic biological agents or with potent immunosuppressive agents (for example, azathioprine and cyclosporine) (2, 27).

EXPERIMENTAL THERAPIES IN RHEUMATOID ARTHRITIS

Despite significant progress over the past decades, RA continues to be a chronic disease. It remains active in many patients whose general condition does not partially, or completely respond to disease-modifying anti-rheumatic drugs. Therefore, efforts are continually being made to discover new effective therapeutic agents.

Experimental research and clinical studies include:

- new biological agents with B cell targeting for CD20, including: atacicept, AMG 623, B3-fcc, Br3-Fc, belimumab, epratuzumab, ofatumumab, ocrelizumab and TRU-015 (30);

- small molecules directed on enzymes, involved in signal transduction of TNF and other pro-inflammatory cytokines are also studied in RA (31);

- a Phase II clinical study reported that, compared with placebo, examatinib, a spleen tyrosine kinase inhibitor, reduced disease activity in patients with RA unresponsive to methotrexate therapy. However, additional investigations are needed to determine the safety and efficacy of this agent in patients with RA (31, 32);

- the administration of agents that produce matrix metalloproteinase inhibition, or agents that inhibit osteoclast activity, although initially failed to be effective, may still be taken into consideration in the treatment of RA (30);

- immunosuppressive drugs in high dosage combined with autologous stem cell transplant have been used in study protocols for patients with resistance to other therapies (2).

CONFLICT OF INTERESTS

There are no conflicts of interest. All authors made equal contribution to the paper, to that of first authors, and thus share main authorship.

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NEWS

TRADITIONAL CHINESE MEDICINE USED ON PATIENTS WITH RHEUMATOID ARTHRITIS: ALTERNATIVE OR COMPLEMENTARY TREATMENT?

The use of traditional Chinese medicine (TCM) on patients with rheumatoid arthritis (RA) had a magnitude of 30% as compared to the general population of Taiwan, according to the results of retrospective cohort study used the Longitudinal Health Insurance Database (LHID) 2000. The authors, Ming Chi-Lu and colleagues, aimed to describe the utilization of TCM and determine the factors related to TCM usage among RA patients. Herbal remedies (49.6%) constituted the most commonly used therapeutic approach, followed by combined therapy (32.7%) and acupuncture or traumatology manipulation alone (17.7%). The profile of the patients who used TCM is female, younger age, higher monthly income and higher Charlson-Deyo co-morbidity index scores, living in urbanized areas, in higher TCM physician density. The top three reasons for seeking TCM services were diseases of the musculoskeletal system and connective tissue, the respiratory system, and the digestive system.(Lu MC, Livneh H, Chiu L M, Lai NS, Yeh CC, Tsai, TY 2019. A survey of traditional Chinese medicine use among rheumatoid arthritis patients: a claims data–based cohort study. *Clinical rheumatology* 2019; 38(5): 1393-1400).

METAPLAZIA MIELOIDĂ AGNOGENICĂ ŞI SARCINA. PREZENTARE DE CAZ

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AGNOGENIC MYELOID METAPLASIA IN PREGNANCY. CASE REPORT (Abstract): Idiopathic myelofibrosis is a rare myeloproliferative disorder characterized by excessive accumulation of connective tissue in the bone marrow in association with anemia, splenomegaly and extramedullary hematopoiesis. A rare case of pregnancy in a patient with agnogenic myeloid metaplasia who carried a term pregnancy is described. In spite of the increased perinatal risks, a favorable outcome was possible with close antepartum surveillance. Conclusion: Idiopathic myelofibrosis was once thought to be a contraindication to pregnancy. With careful management, successful pregnancies can be achieved. **Key words**: AGNOGENIC MYELOID METAPLASIA, PREGNANCY, VENOUS THROMBOEMBOLISM

STUDY ON THE PREVALENCE AND RISK FACTORS FOR CARIES ADJACENT TO POSTERIOR COMPOSITE RESIN RESTORATIONS

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STUDY ON THE PREVALENCE AND RISK FACTORS FOR CARIES ADJACENT TO COMPOSITE RESIN CORONAL RESTORATIONS (Abstract). **Aims** of the study were as follows: to determine the prevalence of caries adjacent to direct posterior composite resin restorations (CARS); to analyze the distribution of caries adjacent to coronal restorations in relation to socio-demographic, individual, and local parameters; to identify significant risk factors associated with caries adjacent to composite resin coronal restorations. **Materials and methods**: The retrospective study included 45 patients (mean age: 23.40 ± 4.027 years; gender: 31 males, 29 females) with 305 direct nanohybrid composite restorations aged 1-5 years. Univariate analysis was used to identify risk factors and odds ratios for the occurrence of CARS. Multivariate analysis was performed to identify combinations of risk factors that significantly increased the probability of CARS. The association between CARS and marginal integrity was assessed by the Spearman test. **Results:** According to FDI criteria, 8.9% of restorations had a score of 12.4 (micro-cavitated CARS extended to dentin), 3.9% had a score of 12.5 (cavitated CARS). Odds ratio for CARS were 2.135 for high caries risk, 2.179 for female gender, and 19.19 for impaired marginal integrity (scores 6.2-6.5) (OR 19.19). **Conclusions:** The prevalence of CARS in micro-cavitated and cavitated stage was 26.6%. Risk factors for CARS in direct posterior composite resins restorations were high cariogenic risk, female gender, and impaired marginal integrity. Risk predictors for caries adjacent to posterior composite resin coronal restorations, identified in multivariate analysis, were female gender and impaired marginal integrity. The risk of developing CARS increases progressively with the degree of marginal integrity impairment. **Keywords:** COMPOSITE RESTORATIONS, CARS, PREVALENCE, RISK FACTORS.

INTRODUCTION

Caries adjacent to restorations are considered the most common biological criteria leading to decisions for repair or replacement, significantly affecting the longevity of the involved teeth and increasing healthcare costs (1). Epidemiological studies report wide variations in the prevalence of caries adjacent to coronal restorations, depending on geographic regions and the demographics of the investigated population groups. A prevalence of 3.6% for caries adjacent to restorations (CARS) was reported in a study involving clinical and radiographic examination of 4,036 restorations in a cohort of 450 patients (2), while another research group found a prevalence of 12% for caries adjacent to coronal restorations made of nanohybrid or micro hybrid composite resins at 15 years post-treatment (3). A system-

atic review reported an average annual failure rate of 1.8% after five years and 2.4% at ten years post-treatment, with most failures attributed to CARS (4). In a population-based study conducted in the North-Eastern region of Romania, prevalence of CARS was between 13.5% (5) and 23.23% (6) of posterior composite-based restorations within 2-5 years post-treatment. Since the primary goal of clinical and therapeutic management of caries adjacent to coronal restorations is to control etiological factors and halt the progression of active lesions through non-operative or minimally invasive preventive methods, understanding the processes involved in the pathogenesis of these carious lesions is essential for selecting and developing the most effective approaches to avoid repeating the restorative cycle. Patient-related risk factors that can significantly contribute to the failure of composite resin coronal restorations include high cariogenic risk, smoking status, and periodontal involvement of teeth with Class II or Class V cavities (7). Material-related factors are critical and can lead to different scenarios of recurrent caries incidence (8). Regarding the influence of composite resin type on the likelihood of marginal defects, hybrid composites, nanofilled composites, and condensable composites exhibit the lowest polymerization shrinkage values (9). The elasticity modulus of composite resins materials can affect the marginal adaptation and stress distribution, resin composite with the highest modulus of elasticity resulting in the highest number of gap-free enamel margins but with an increased incidence of enamel fractures (10, 11). Regarding the clinician's role, their competencies are partially associated with the occurrence of caries adjacent to coronal restorations, influencing significantly the decisions of repair *vs.* replacement (12).

AIM OF STUDY

The objectives of the study were as follows:

- to determine the prevalence of caries adjacent to composite resin coronal restorations;
- to analyze the distribution of caries adjacent to coronal restorations in relation to socio-demographic, individual, and local parameters;
- to identify significant risk factors associated with caries adjacent to composite resin coronal restorations.

MATERIALS AND METHODS

The retrospective study included 45 patients (mean age: 23.40 ± 4.027 years; gender: 31 males, 29 females; p=0.091) who presented for various treatments at the Clinical Base of the Faculty of Dental Medicine, "Grigore T. Popa" U.M.Ph. Iași (Romania). A clinical examination was conducted to evaluate 305 direct nanohybride composite restorations aged between 1 and 5 years. The research was carried out in accordance with the ethical principles of the Declaration of Helsinki and received approval from the Ethics Committee of "Grigore T. Popa" U.M.Ph. Iasi. All patients were informed about the study objectives and provided written informed consent.

Inclusion criteria: occlusal and occluso-proximal posterior composite restorations; cavities classified as ICDAS 05-06; vital molars/premolars; age ≥ 18 years.

Exclusion criteria: non-carious lesions; bruxism; high consumption of cola beverages, coffee, or black tea.

The independent variables evaluated as risk factors for CARS were:

- Black's classification (Class I *vs.* Class II);
- gender (male *vs*. female);
- restoration age (1-2 yrs. *vs*. 3-5 yrs.);

- cariogenic risk (high *vs*. low);
- arch location (maxilla *vs*. mandible);
- dental group (molar *vs*. premolar).

CARS diagnosis and the assessment of CARS stage were performed according to FDI criteria (13). The patient's caries risk was determined by assessing anamnesis and intraoral risk factors, as well as caries disease indicators. The presence of at least two risk factors and one caries disease indicator classified the patient as high cariogenic risk.

Statistical analysis. The statistical analysis was conducted using *SPSS version 29.0*. The analyzed parameters were described using frequency distributions and descriptive statistics. Univariate analysis was performed to identify risk factors and odds ratios for the occurrence of CARS. Multivariate analysis was employed to identify combinations of risk factors that, when present in the same patient, significantly increased the probability of CARS. The identified risk factors were incorporated into a logistic regression model to determine their cumulative effect on risk of CARS. The association between CARS and marginal adaptation was characterized by calculating Spearman's nonlinear correlation coefficients.

RESULTS

Distribution of posterior composite resins direct restorations was as follows (table 1):

- By gender: 47.9% of restorations were evaluated in male patients, while 52.1% were evaluated in female patients.
- By restoration age: 47.5% of restorations were evaluated at 1-2 years post-treatment, while 52.5% were evaluated at 3-5 years post-treatment.
- By arch location: 46.6% of restorations were maxillary, while 53.4% were mandibular.
- By dental group location: 81% of restorations were located in molars, while 19% were in premolars.

TABLE I.

Distribution of direct posterior composite resins restorations related to analyzed parameters

Gender	male	146	47.9
	female	159	52.1
Age	1-2 yrs.	145	47.5
	3-5 yrs.	160	52.5
Arch	MX	142	46.6
	MD	163	53.4
Dental group	PM	58	19.0
	M	247	81.0
Cariogenic risk	low	217	71.1
	high	88	28.9
Black Class	Class I	246	80.7
	Class II	59	19.3
FDI score	12.1	198	64.9
	12.2	26	8.5
	12.3	42	13.8
	12.4	27	8.9
	12.5	12	3.9
Total		305	100.0

• By cavity type: 80.7% were occlusal restorations (Class I), while 19.3% were proximo-occlusal restorations (Class II).

• By patient caries risk: 71.1% of restorations were evaluated in low-caries-risk patients, while 29% were in high-caries-risk patients.

CARS indices distribution (tab. I):

• 64.9% of restorations were classified as score 12.1 (no caries adjacent to restorations)

• 8.5% were classified as score 12.2 (non-cavitated CARS).

• 13.8% were classified as score 12.3 (micro-cavitated CARS clinically limited to enamel, non-intervention or preventive intervention).

• 8.9% were classified as score 12.4 (micro-cavitated CARS clinically extended to dentine, repair needed).

• 3.9% were classified as score 12.5 (cavitated CARS extended to dentin, replacement required).

Study on correlation between CARS and marginal integrity

The Spearman test demonstrated a strong, positive, and statistically significant correlation (r=0.559) between the quality of marginal integrity of composite resin restorations and CARS diagnosis (tab. II). 100% of coronal restorations with score 12.5 were evaluated with marginal integrity score 6.5. 96.5% of coronal restorations that were not diagnosed with CARS (score 12.1) were evaluated with marginal integrity score 6.1 (tab. III).

TABLE II.

Spearman test: correlation between CARS and marginal integrity

	Spearman's rho	p-value	95% CI	Interpretation
Marginal integrity-CARS	0.559	<0.001**	0.474 ÷ 0.633	Positive, strong, statistical significance

TABLE III.

CARS scores distribution related to marginal integrity

		MARGINAL INTEGRITY SCORES										Pearson Chi-squared test
		6.1.		6.2.		6.3.		6.4.		6.5.		
		N	%	N	%	N	%	N	%	N	%	
CARS	12.1.	53	96.4	137	69.5	2	9.5	6	37.5			Chi^2 = 335.920 p <0.001**
	12.2.			20	10.2	6	28.6					
	12.3.	2	3.6	32	16.2	6	28.6	2	12.5			
	12.4.			8	4.1	7	33.3	8	50.0	4	25.0	
	12.5.									12	75.0	
Total		55	100.0	197	100.0	21	100.0	16	100.0	16	100.0	

Univariate and Multivariate Analysis Studies on Risk Factors for CARS

The results of univariate analysis for risk factors of CARS in table IV. Coronal restorations in female patients have a 2.179 times higher risk of CARS occurrence. High caries risk increases the CARS risk by 2.135 times. Restorations with marginal integrity scores of 6.2-6.5 have a 19.19 times higher risk of CARS compared to coronal restorations with unaffected marginal integrity (score 6.1). The results of the multivariate analysis of predictors for caries adjacent to composite resin coronal restorations are presented in table V.

TABLE IV.

Univariate analysis of risk factors for CARS

		CARS				Pearson Chi-squared	OR (95% CI)
		ABSENT		PRESENT			
		N	%	N	%		
Gender	male	108	54.5%	38	35.5%	$Chi^2 = 10.082$	2.179
	female	90	45.5%	69	64.5%	p = 0.001**	(1.342 ÷ 3.538)
Cariogenic risk	low	152	76.8%	65	60.7%	$Chi^2 = 8.684$	2.135
	high	46	23.2%	42	39.3%	p = 0.003**	(1.283 ÷ 3.553)
Black class	Class I	165	83.3%	81	75.7%	$Chi^2 = 2.593$	-
	Class II	33	16.7%	26	24.3%	p = 0.107	
Age	1-2 yrs.	91	46.0%	54	50.5%	$Chi^2 = 0.566$	-
	3-5 yrs.	107	54.0%	53	49.5%	p = 0.452	
Arch location	MX	92	46.5%	50	46.7%	$Chi^2 = 0.002$	-
	MD	106	53.5%	57	53.3%	p = 0.965	
Dental group location	PM	38	19.2%	20	18.7%	$Chi^2 = 0.011$	-
	M	160	80.8%	87	81.3%	p = 0.915	
Marginal integrity	6.1.	53	26.8%	2	1.9%	$Chi^2 = 88.913$	
	6.2.	137	69.2%	60	56.1%	p <0.001**	
	6.3.	2	1.0%	19	17.8%		
	6.4.	6	3.0%	10	9.3%		
	6.5.			16	15.0%		
Marginal integrity	Healthy	53	26.8%	2	1.9%	$Chi^2 = 29.134$	19.190
	Microleakage	145	73.2%	105	98.1%	p <0.001**	(4.574 ÷ 80.507)
Total		198	100.0%	107	100.0%		

Multivariate analysis of CARS risk factors

The Hosmer-Lemeshow goodness-of-fit test indicates that the constructed model is viable. Initially, the prediction accuracy for CARS is 64.9%, while using the model improves the prediction accuracy for CARS to 81.6%. The model explains 44.2% of the variation in CARS (Nagelkerke R coefficient). The predictors included in the model show the following progression:

TABLE V.

Predictors in binary logistic regression model

	Coef. B	p-value	OR	95% C.I. / OR
Age	-0.098	0.025*	0.907	0.832 ÷ 0.987
Gender (1)	0.961	0.004**	2.615	1.368 ÷ 4.996
Cariogenic risk (1)	-0.715	0.070	0.489	0.226 ÷ 1.061
Marginal adaptation		<0.001**		

	Coef. B	p-value	OR	95% C.I. / OR
Marginal adaptation (1)	2.512	<0.001**	12.329	2.853 ÷ 53.276
Marginal adaptation (2)	5.683	<0.001**	293.910	35.499 ÷ 2433.384
Marginal adaptation (3)	4.553	<0.001**	94.938	14.532 ÷ 620.237
Marginal adaptation (4)	25.450	0.998	1.1294E+11	
Constant	-1.474	0.216	0.229	

Equation for binary logistic regression model is as follows: Ln(p/1-p) = -1.474 - 0.098*Age + 0.961*Gender- 0.715* Cariogenic risk + 2.512* Marginal adaptation 6.2. + 5.683* Marginal adaptation 6.3. + 4.553* Marginal adaptation 6.4. + 25.450* Marginal adaptation 6.5.

Based on this equation, the probability of patients developing CARS in the combined presence of the identified risk factors can be calculated, considering the following observations:

- The risk of CARS is 2.615 times higher in women than in men.
- The risk of CARS is 12.329 times higher in patients with marginal adaptation score 6.2 compared to those with marginal adaptation score 6.1.
- The risk of CARS is 293.910 times higher in patients with marginal adaptation score 6.3 compared to those with marginal adaptation score 6.1.
- The risk of CARS is 94.938 times higher in patients with marginal adaptation score 6.4 compared to those with marginal adaptation score 6.1.

DISCUSSION

The identified risk factors in our research included high caries risk, female gender, and marginal adaptation impairment. High caries risk has been classified as a significant risk factor for the occurrence of caries adjacent to composite resin restorations (14). High-risk and non-compliant patients regarding etiological therapy have a significantly higher risk of developing caries adjacent to direct coronal restorations (15). A high caries risk increases the risk of restorative failure by 2-3 times (16). The higher prevalence of caries adjacent to restorations located in molars (*vs*. premolars), as found in our study, is supported by literature data (17). High-caries-risk patients had failure rates of 3.2% at five years and 4.6% at ten years post-treatment, while low-risk patients had significantly lower failure rates of 1.2% at five years and 1.6% at ten years post-treatment (18). Krämmer *et al*. (2015) also reported that direct Class I and II restorations in molars had inferior clinical performance compared to premolar restorations regarding marginal integrity at four years post-treatment (19). The significantly higher prevalence of Class II restorations, noted also in our study, can be explained by the fact that biofilm removal is more difficult at proximal and cervical margins, the enamel in these areas is thin and aprismatic (making acid etching ineffective), access is challenging, and these regions are predominantly subjected to flexural forces (20). A high C-factor can contribute to stress accumulation at the adhesive interface, favoring the occurrence of marginal microleakage (21). In Class II cavities, adapting composite resin to the dentinal margin is difficult, and the formation of microgaps along this margin can, in the medium and long term, lead to post-operative sensitivity and microleakage

associated with caries development adjacent to composite resin coronal restorations (22). We found that the risk of caries adjacent to composite resin restorations progressively increases with the degree of marginal impairment, from OR 12.329 for restorations with marginal adaptation scores of 6.2 to OR 94.938 for restorations with marginal integrity scores of 6.4, compared to coronal restorations with optimal marginal integrity (scores 6.1). The likelihood of caries developing adjacent to posterior composite resin coronal restorations correlates with both the size of marginal gaps at the interface between the cavity walls and the external surfaces of the restoration, as well as with the functional loading specific to occlusal and proximal-occlusal restorations (23). Microgaps may appear over time due to the hydrolytic degradation of the hybrid layer, followed by adhesive failure at the adhesive restorations (24). Polymerization contraction stress can also cause crack formation, marginal microleakage, margin discoloration, and postoperative hypersensitivity (25). Adhesive failure leads to nano-metric-sized microleakages that serve as pathways for bacterial penetration into the hybrid layer, facilitated by occlusal forces and deep-water penetration (26). A limitation of these studies is related to the performance of composite resins in real-world conditions, where dentists are time-constrained and treat populations different from those investigated in dental schools (27).

CONCLUSIONS

The prevalence of CARS in micro-cavitated and cavitated stage was 26.6%. Risk factors of CARS for direct posterior composite resins restorations were high cariogenic risk, female gender, and impaired marginal integrity. Risk predictors for CARS were female gender and impaired marginal integrity. The risk of developing CARS increases progressively with the degree of marginal integrity impairment.

CONFLICT OF INTEREST AND FUNDING

The authors declare that there is no conflict of interest, and they received no specific funding.

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EVOLUTION OF BIOLOGICAL PARAMETERS IN THE METABOLIC SYNDROME

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EVOLUTION OF BIOLOGICAL PARAMETERS IN THE METABOLIC SYNDROME (Abstract)**:** The metabolic syndrome is responsible for the threefold increase of the atherosclerotic cardiovascular diseases and for the increase of the mortality due to these disorders compared to the general population. **Aim:** To evaluate the biochemistry dynamics and the specific medication in patients diagnosed with metabolic syndrome. **Materials and methods**: The study was carried out in the 3rd Medical Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital of Iasi, being a retrospective study and including 250 patients which fulfilled at least three of the five defining criteria of the metabolic syndrome. **Results**: It was obtained the improvement of the inflammatory metabolic syndrome through the decrease of the fibrinogen and of the C-reactive protein (p=0.001). It was observed the fact that, both in men as well as in women, serum value of the uric acid proved to be significant in hypertensive subjects, a statistically significant decrease being observed in the patients treated with Candesartan. **Conclusions**: Obesity and especially the central adiposity is the dominant risk factor for the development of the type 2 diabetes mellitus, which usually is accompanied by the arterial hypertension due to a common physiopathology. Although the pharmacologic therapy is often necessary, a very important element remains the lifestyle change which represents the only full therapeutic approach which can reduce the resistance to insulin and visceral obesity. **Keywords**: METABOLIC SYNDROME, CARDIOVASCULAR DISEASES, BIOLOGICAL PARAMETERS, PHARMACOLOGICAL THERAPY.

The metabolic syndrome (MetS) is defined as being a group of interdependent metabolic factors which together represent a cardiovascular multiple risk. MetS is characterized by the presence of at least three of the following risk factors: central obesity, increased serum triglycerides, the decreased value of the high molecular density lipoproteins (HDLc), increased arterial tension (AT) and hyperglycemia. A variable number of organizations such as the World Health Organization, European

Group for Study of Insulin Resistance, National Cholesterol Education Program, International Diabetes Federation and American Association of Clinical Endocrinologists have defined the criteria for its diagnosis.

The prevalence of obesity and of the MetS has increased in epidemic proportions at the global level. According to the World Health Organization, the obesity rate has doubled since 1980, with 600 million persons, representing 13% of the global population. Metabolic syndrome is responsible for the threefold increase of the atherosclerotic cardiovascular disease (CVD) risk and for the increase in the mortality due to its disorders, compared to the general population (1).

Taking into consideration the relation with the obesity, CVD and the type 2 diabetes mellitus (DM), the prevalence of the MetS varies according to the geographic region at the global level. The general prevalence in adults in the United States of America is of 34,7%**,** compared to the urban India of 33.5%, Malaysia with 27.5%, Brazil with 29.6% and 7.3% in China (2-4). From the point of view of the prevalence on gender, it varies, predominating in men during adolescence (10.9% *vs.* 6.29%), the ratio being inverted during the ages 20-39 (18% *vs.* 20%), reaching the adult age with a ratio predominant in women (42% compared to 51%) (2, 5).

It is known the fact that the major cardiovascular events at the hypertensive patients are preceded by the development of the structural anomalies and the cardiovascular and asymptotic renal functions, of which the majority are recognized as significant independent predictors of the adverse cardiovascular results (6).

According to the appreciations of the European Cardiology Society, the European Atherosclerosis Society and the Hypertension European Society, the plasmatic cholesterol increase, especially of the low molecular density lipoproteins (LDLc) and of the low plasmatic level HDL cholesterol represents the risk factors for the atherosclerotic cardiovascular diseases.

It was shown statistical differences according to sex, in situations of HDLc's decrease, being lower in male patients, and in case of hypertriglyceridemia, the triglycerides level was significantly higher in women (7).

It was proven that the hepatic transaminases, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and, to a lower degree, the gamma-glutamyltransferase (GGT) represents markers of the hepatic disorder due to the infiltration of the fatty acids and of the inflammatory stimulations, these being associated with several components involved in the MetS physiopathology.

The ALT's increased values are associated with the MetS's components (increase of the triglycerides, glycaemia, abdominal circumference, diastolic arterial pressure and low levels of the HDLc) (8). GGT is a sensible indicator, but with a low hepatic disorder specificity, suggesting the possibility of a link between it, the endothelial dysfunction and the cardiovascular risk (9).

The study carried out by Music M. et al (10) has shown that GGT was significantly higher in patients with type 2 DM and MetS than in type 2 DM without, while the ALT and AST did not present significant variations. Monitoring studies are required to determine if the hepatic enzymes can be used as hepatic component of the MetS.

The increased serum levels of the uric acid were observed as being a prediction

factor, independent from the apparition of the coronary arterial disease, thus indicating the involvement of its presence in the development of the vascular inflammation (11, 12).

The hyperuricemia is frequently associated with the arterial hypertension and it is present in 25 % of the patients with non-treated arterial hypertension, 50 % of the patients under treatment with diuretics and more than 75% of the patients which suffer from malignant arterial hypertension (13).

To reach the therapeutic objectives, the European Society of Hypertension / European Society of Cardiology recommends as treatment: four classes of antihypertensive medication: inhibitors of the angiotensin-converting-enzyme inhibitors / angiotensin II receptor blockers, calcium channel blockers, beta blockers and diuretics. It is important the initiation of the MetS treatment for the reduction of the increased cardiovascular risk. The administered medication addresses the individual components of the syndrome. However, although the pharmacology therapy is often necessary, a very important element remains the lifestyle change, which represents the only full therapeutic approach which can reduce the resistance to insulin and the visceral obesity (6).

Robinson *et al.* (14) carried out a meta-analysis which has proven that the HMG-CoA reductase inhibitors medication, such as statins and fibrates, used in the monotherapy, generate an approximate relation of 1:1 between the LDL-c lowering and the reduction of the risk of the coronary disease apparition.

MATERIAL AND METHODS

The aim of the study was to evaluate the biochemical dynamics and the specific medication in patients diagnosed with the metabolic syndrome.

The study was carried out in the 3rd Medical Clinic of the "Sf. Spiridon" County Clinical Emergency Hospital of Iasi, it being a retrospective study. 250 patients were included in the study, patients which fulfilled at least three of the five criteria for defining the metabolic syndrome according to the American Heart Association/National Heart, Lung and Blood Institute and World Health Organization. From the total of patients, 57% returned to the clinic for re-evaluation after 12 months of treatment.

To carry out this study, it was used as working instrument the patient's clinical observation sheet, with the drafting of some special sheets relating to the: clinical, biochemical and treatment aspects. The following inclusion criteria were: age above 18 years, presence of the metabolic syndrome, absence of the chronic renal disease in the terminal stage and absence of the severe hepatic insufficiency.

The exclusion criteria were represented by: pregnancy, patients with neoplasm or patients with major psychic/ cognitive disorders, acute or chronic inflammatory disorders, which could have influenced the results of the laboratory investigations, the hormone treatment.

The study protocol was applied according to the recommendations of the Ethics Committee of the "Sf. Spiridon" County Clinical Emergency Hospital of Iasi, abiding with the ethic norms of the European Community about the investigations carried out on the patients.

Data statistical analysis. The data was centralized and statistically handled using the *SPSS version 22.0* for Windows 10 and the ANOVA method. In the statistical anal-

ysis were used not only descriptive methods, but also the analytical ones at the 95 % confidence interval (CI 95 %). When calculating the significant difference between the two averages it was used the t-student test or the F test (ANOVA) used for the comparison of the average values, in three or more groups with normal distribution. Moreover, it was used the Microsoft Excel program for the generation of graphics using the values obtained in *SPSS version 22.0.*

RESULTS AND DISCUSSION

The clinical proof related to the inflammatory nature of the atherosclerotic process is represented by the increase of the circulatory levels of the inflammatory markers at the patients with cardiovascular diseases. Numerous epidemiologic studies have reported positive associations between the risk of the atherosclerotic and the fibrinogen's plasmatic levels, this parameter's high level being significantly associated with the cardiovascular risk, independent of the lipid profile. Thus, we can observe an improvement of the inflammatory process through the markers' decrease ($p = 0.001$) (tab. I).

It has shown a statistically significant relationship between elevated C-reactive protein and fibrinogen levels and the presence of hypertension, isolated, or in combination with obesity, which confirmed that hypertensive patients presented a proinflammatory and procoagulant status, which implies an increased risk of cardiovascular disease (15).

MetS is spread across the ill persons with chronic renal disorders, being reported that it might have a role in the progression and development of the renal lesions (16), favoring the instalment of the renal disease in its final stage (17). In the sample of the studied patients, it was obtained the improvement of the renal function after the 12 months of treatment and diet regime.

TABLE I

Evolution of the biological markers in patients with metabolic syndrome

Marker	Initial	Re-evaluation	p value for paired T test
Cholesterol	201.25 ± 53.06	186.35 ± 49.35	0.001
LDL	123.03 ± 44.12	112.21 ± 39.15	0.001
Serum urea	52.74 ± 17.20	51.78 ± 15.98	0.050
Creatinine	1.41 ± 0.54	1.35 ± 0.51	0.001
CRP	2.97 ± 3.59	2.61 ± 3.25	0.001
ESR	9.81 ± 7.07	8.54 ± 7.06	0.001
Uric acid	5.91 ± 1.51	5.70 ± 1.42	0.001
Fibrinogen	384.78 ± 114.10	358.22 ± 98.60	0.001
ALT	27.11 ± 22.61	21.57 ± 15.79	0.001
AST	22.92 ± 10.69	17.85 ± 8.07	0.001
GGT	42.45 ± 35.99	30.99 ± 23.84	0.001

The patients with present inflammatory treated with Rosuvastatin had the weakest response to the treatment, in the sense of this syndrome's improvement (fig. 1).

Taking into view the results of the various studies which have proved a direct

relation between the increase of the uric acid's values and of the arterial hypertension at the level of the studied sample, it is observed the fact that not only in men, but also in women, the increased serum value of the uric acid was met significantly more frequently in the hypertensive subjects (fig. 2).

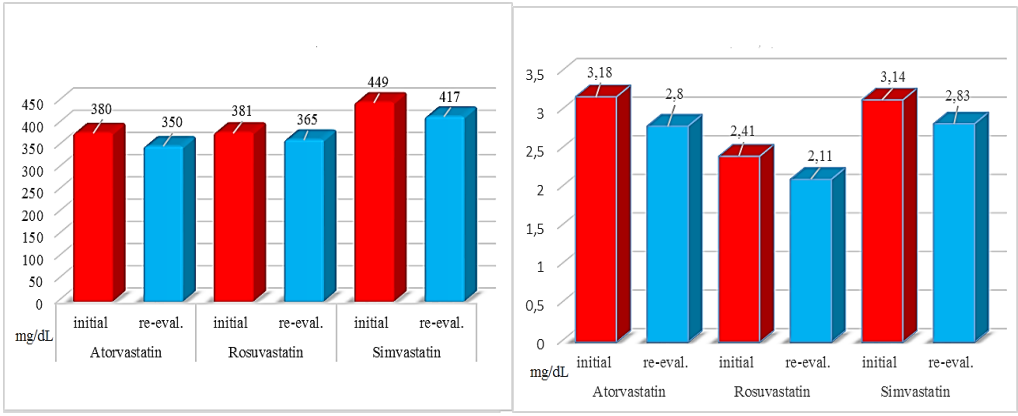

Fig. 1. Initial average distribution and the re-evaluation of the fibrinogen and C-reactive protein according to the type of administered statin

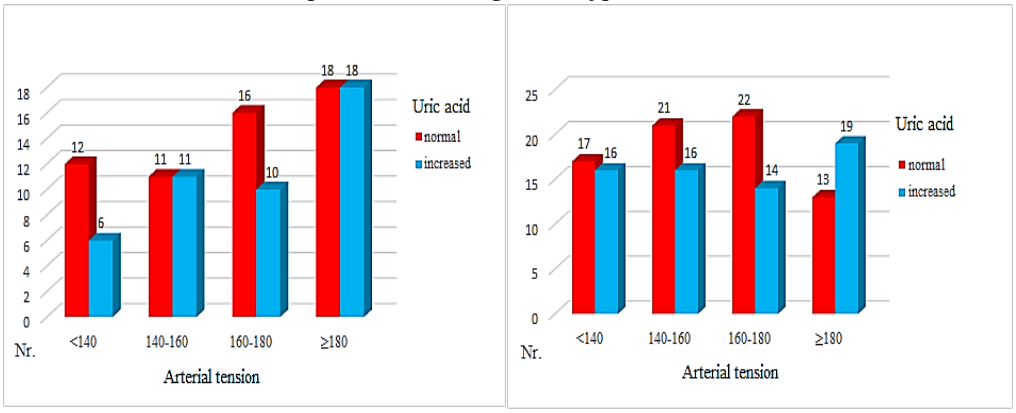

Fig. 2. Distribution of the initial values according to the AT and uric acid for men and women

At patients treated with Candesartan it was possible to observe the decrease of the uric acid's values by 0,36 mg/dl in comparison with Perindopril, where the decrease was not statistically significant (fig. 3).

Establishing the values of the C-reactive protein is a practical modality to evaluate the presence of the inflammatory syndrome. The increase of the CRP values (≥ 3 mg/L) is a risk factor for the later apparition of the CVD. The medicine used for the treatment of the metabolic risk factors were efficient also regarding the reduction of the CRP serum levels (statins, fibrates, ACE, nicotinic acid).

An intervention study which has evaluated the study with Rosuvastatin (*Justification for the Use of Statins in Primary Pre-*

vention: An Intervention Trial Evaluating Rosuvastatin - JUPITER) has emphasized the statins additional benefit for the patients with increased CRP value, even in the presence of an LDL within normal limits (18).

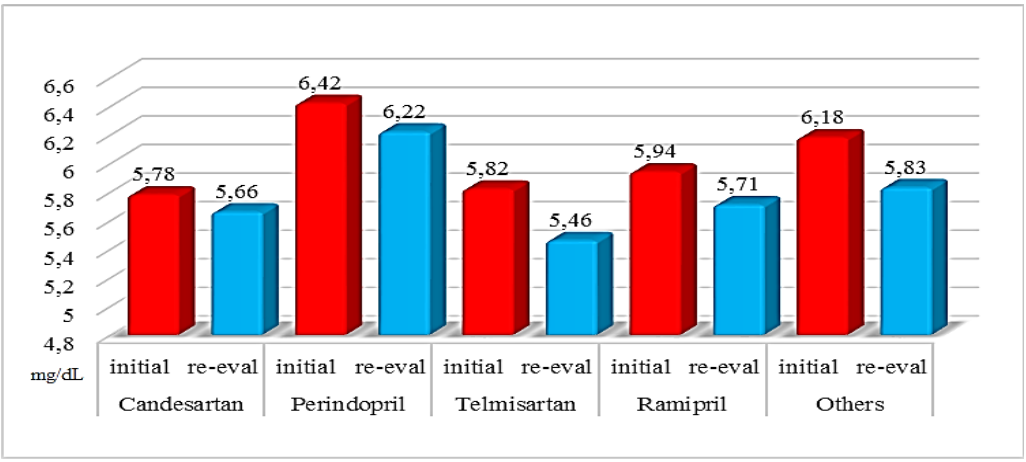

Fig. 3. The distribution of the initial and re-evaluation average values of the uric acid, according to the administered ACE / ARB type

In the studied casuistry, it can be observed a decrease of the CRP values compared to the initial moment, the most important improvement being observed at the patients which have received Perindopril (fig. 4).

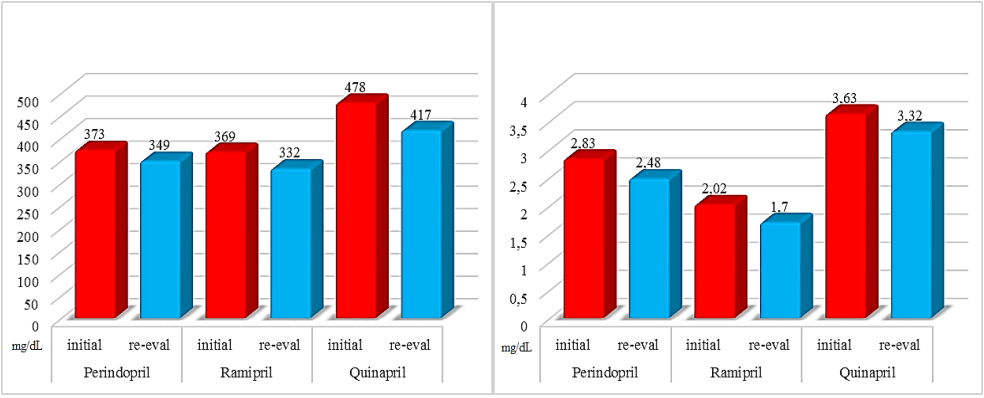

Fig. 4. Initial and re-evaluation average value distribution of the fibrinogen and C-reactive protein according to the administered ACE / ARB type

CONCLUSIONS

In the present study, we demonstrated that inflammation within the metabolic syndrome decreased differently depending on the type of statin administered.

Also, we demonstrated a direct relationship between the increased uric acid's concentrations and arterial hypertension, its values being significantly higher in hypertensive individuals.

Our study revealed that at patients which have received Perindopril was observed a significantly decrease of the CRP values.

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PREVALENCE OF SELF-MEDICATION WITH ANTIMICROBIAL DRUGS IN THE SOUTHEAST REGION OF ROMANIA

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PREVALENCE OF SELF-MEDICATION WITH ANTIMICROBIAL DRUGS IN THE SOUTHEAST REGION OF ROMANIA (Abstract): Self-medication represents a global health issue with an increasing negative impact on the society regarding health evolution and socio-economic progress. There are few studies regarding the self-medication practices with antimicrobial drugs in general population in Romania. **Aim:** to evaluate the prevalence of self-medication with antimicrobial drugs in general population in the counties of the south-east region of Romania. **Material and methods:** a questionnaire-based cross-sectional study which investigated the rate of self-medication with antimicrobial drugs in a population from the counties of the southeast region of Romania during 2016-2017. Data were analyzed using descriptive statistics and the chi-square test, when applicable. **Results** There were 355 persons included in the study, with a predominance of female sex (62.8%) and of persons with urban residence (57%). The most common antimicrobial drugs used for self-medication were amoxicillin (34%), followed by amoxicillin-clavulanate (32%), and the most frequently reported reasons for self-medication were respiratory infections (52%), and genitourinary infections (25%). **Conclusions:** This study reveals a medium prevalence of self-medication with antimicrobial drugs in the population from South-eastern Romania. **Keywords:** SELF-MEDICATION, ANTIMICROBIAL DRUGS, PREVALENCE.

Self-medication represents a global health issue with an increasing negative impact on the society regarding health evolution and socio-economic progress (1). According to World Health Organization (2), self-medication is defined as "the selection and use of medicine by individuals to treat self-recognized illness or symptoms". Self-medication practice involves procuring medicines without a prescription, reusing old prescriptions in order to buy medicines, recommending particular medicines to relatives and acquaintances, consuming leftover medicines kept at home, non-compliance with the professional prescription by interrupting or decreasing the doses (3). Several studies have established the main factors that influence self-medication practice: socio-demographic characteristics (gender, age), socio-cultural aspects (level of education, access to information, accessibility to health care facilities), economic and political factors (3, 4).

Self-medication behavior has a major impact on health promotion of the population. The health system is positively influenced by a responsible self-medication practice which helps in preserving the medical resources and reducing costs. An inappropriate self-medication practice has mul-

tiple negative effects such as a delay in managing the health problem by a doctor, a failure in identifying the contraindications of the drug and the interactions between the self-administrated drugs, the appearance of adverse effect leading to hospital admissions, risk of abuse and addiction, an inadequate period of treatment and an increasing resistance to pathogens (5, 6).

In Romania, antibiotics are released only with medical prescription, but leftover medicines stored at home are sources for self-medication in the case of antibiotics. Few studies (7) regarding self-medication practices with antimicrobial drugs in the general population in Romania were published. Therefore, a questionnaire-based cross-sectional study was conducted with the aim to evaluate the prevalence of self-medication with antimicrobial drugs in the general population in the counties of the south-east region of Romania.

MATERIAL AND METHODS

The present study represents a questionnaire-based cross-sectional study which investigated the rate of self-medication with antimicrobial drugs in a population from the counties of the southeast region of Romania during November-December 2016. The Ethics Committee of the University of Medicine and Pharmacy Grigore T. Popa approved the study. Verbal consent was obtained from all participants after a brief introduction by explaining the aim and the significance of the study. To collect data, we cooperated with pharmacies from Galati, Braila, Buzau and Vrancea, counties of the south-east region of Romania. In the study were included only adults (>18 years). Before filling the questionnaires, each potential participant was informed about the purposes of the study and the confidentiality of the information. We excluded from the study the individuals who presented at the pharmacy with a medical prescription for a disorder that required antimicrobial drug treatment or people buying cosmetics or medical equipment. A self-administrated questionnaire, organized in two parts, was prepared after reviewing the literature for similar studies and validated as previously described in the study of Damian et al., in 2014 (7). The first part of questionnaire contained information regarding demographic characteristics of the population (gender, age, employment status) and information related to general self-medication practiced during the last 6 months. The second part of the questionnaire was focused on the use of the antimicrobial drugs (name of antimicrobial drug, treatment period, person who recommended the treatment and the illness that required the use of antibiotic, in case of confirming the use of antibiotic in the last 6 months). Means ± standard deviation (SD) were used for quantitative variables like age, while frequencies and percentages were used for qualitative variables. Data were analyzed using SigmaPlot 11 descriptive statistics and the chi-square test was used to test the differences between proportions. A limit of p value less than 0.05 was considered significant.

RESULTS

There were 355 persons included in the study, with a predominance of female sex (62.8%) and of people with urban residence (57%). Participants' age in the study ranged from 18 to 90 years, with a mean of 42±9. The unemployed group included students and retired individuals (tab. I).

The results showed that the practice of self-medication is differently correlated to age. In the group of >60 years old, the rate of self-medication is the highest (51%) in comparison with the 51-60 years group, where the practice of self-medication was

the lowest (26%). The irrational use of the antimicrobial drugs among the respondents was applied based on self-recommended medicine, the pharmacist recommendation, the advice of a relative or a friend and a low percentage using internet information. The most common antimicrobial drugs used for self-medication were amoxicillin (43%), followed by amoxicillin-clavulanate (32%), and the most frequently reported reasons for self-medication were respiratory infections (52%), and genitourinary infections (25%).

TABLE I

Socio-demographic characteristics of the study population (n=355)

Characteristics	Age (years)						Total
	≤20 (n=37)	21-30 (n=123)	31-40 (n=56)	41-50 (n=69)	51-60 (n=38)	> 61 (n=32)	
Gender							
Male	16	43	19	25	12	17	132
Female	21	80	37	44	26	15	223
Place of residence							
Urban	13	23	53	61	32	20	202
Rural	24	100	3	8	6	12	153
Employment status							
Employed	2	60	49	61	26	2	200
Unemployed	35	63	7	8	12	30	155

DISCUSSION

The irrational use of antimicrobial drugs represents a much-explored area by the researchers. Many studies (8, 9, 10) focused on establishing the prevalence of antibiotic self-medication.

A study (8) conducted in Central Saudi Arabia in community pharmacies showed that a group of medicines with the highest rate of dispensing without a medical prescription is represented by antibiotics in a percentage of 22% (8). Another study (9) organized in Saudi Arabia after 3 years, in 2014, recorded a very high rate of irrational use of antibiotics. The 78.7% percentage showed that from a total of 681 responders to the study, 536 were antibiotic self-medicated. Another data showed by the study was the probability of the prevalence of self-administrated antibacterial drugs, which was higher among the older respondents compared to the younger participants from the age group 18 to 30 years.

In 2014, Banerjee et al. (10) evaluated the rate of irrational use of medication in a community of Nepal medical students. The results showed a self-medication rate of 81.35% with the antibiotics on the second place among the most common self-administrated drugs. The students' community, the young and informed section of the population, represents an important target of determining the self-medication practices and the prevalence of irrational use of drugs. A study designed to evaluate the self-medication behavior among medical and nonmedical students from the northeast region of Romania in 2014, showed that the irrational antibiotic self-medication had a percentage of 34%. The results clearly defined the difference among the medical group where the antibiotic self-administration rate was 38% and the non-medical group where was obtained a rate of 9% antibiotic self-administration (7).

The importance of demographic and socio-economic particularities is proven also by a study (11) conducted in 2013 in Guatemala City where self-medication behavior of peo-

ple from general population was compared the in suburban pharmacies versus city center pharmacies. The rate of self-medication was 79% in the suburban pharmacies and 77% in the city center pharmacies. Another study (12) that assessed the impact of demographic features was conducted in 2014 in the rural areas of Sindh, a province of Pakistan, where the prevalence of antibiotic self-administration was 81.25%.

A study (13) conducted in Lithuania in 2014 reported an antibiotic self-medication rate of 31% in the general population stated the significance of the demographic features in the evolution of antibiotic self-behavior (age, gender, residence, level of education, parenthood).

CONCLUSIONS

This study reveals a medium prevalence of self-medication with antimicrobial drugs in the population from South-eastern Romania. Self-medication with antimicrobial drugs is a relatively common practice, but the prevalence is reduced compared with other studies from previous years or other populations, maybe because of the effect of national education programs regarding the risks of irrational self-medication with antimicrobial drugs. Educating the population about the consequences of irrational use of antibiotics is urgently needed.

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RARE TYPES OF HERNIA

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RARE TYPES OF HERNIA (Abstract): Depending on their origin hernias are classified into congenital and acquired, the latter occurring in people in which the respective hernia area is fully developed but is weak or weakens under the complex action of etiological factors. The development of hernia is the result of the imbalance between two constantly acting forces: positive intra-abdominal pressure and contraction of abdominal muscles which by their tonicity keep the viscera in place. The correct diagnosis and early treatment are keys to successful repair. **Material and methods**: This study analyses the clinical and laboratory findings and therapy response in 13 patients with hernia. The sample is representative for this condition, and the cases have been selected based on the acutization of inflammatory phenomena. **Results and discussion:** In the treatment and management of these rare hernias the fact that they are prevalent in the elderly with significant weight loss and associated chronic conditions has to be considered. **Conclusions:** These types of hernia have a precise anatomic identity; their onset is favored either by an inherited abdominal wall defect, or acquired by multiparity and physical strain. **Keywords**: STRANGULATION, PAIN, HERNIAL SUOCCLUSION, SURGICAL TREATMENT.

Hernias have been known since ancient times when simple methods were used for their repair (1, 2). Reducible hernias were supported with the help of a strap or bandage, whereas strangulated hernias were treated with palliative means (diet, rest, purgatives, astringent compresses/poultices, bleeding, massage) (1, 3).

A more rational treatment was used by Phoenicians, 900 years B.C. The first man to perform surgeries for hernia repair was Celsus (100 AD.); surgery consisted in the ligature and excision of the hernial sac without opening it. Heliodorus (second century A.D.) was the first to isolate the inguinal hernia sac from the cord, while Oribasius (fourth century) performed the ligature and excision of the freed sac followed by cautery of surrounding tissues. (1). In Middle Ages, the Arab surgeons Haly Abbas (930-994), and Avicenna (980-1037) frequently practiced surgery to treat hernias (1).

The 19th century marks the beginning of modern hernia surgery, famous monographs, such as those by Cooper and Scarpa, being published (1).

Despite the fact that people have started to lose confidence in surgery due to the abusive practice of castration, suppurations and relapses, in order to rehabilitate the surgical art, numerous authors strived to

find better treatment procedures (3).

The breakthrough in hernia therapy was the discovery of antiseptics, which resulted in less postoperative infections and thus encouraged more surgery.

MATERIAL AND METHODS

The study included 13 patients, study group representative for this condition. The cases were selected based on the acutization of inflammatory phenomena, and were admitted in the interval 2005-2010 to the General Surgery Department of the Iasi Railroad Hospital. The data collection process was followed by data processing and interpretation, using 95% confidence intervals; for differences χ^2 and t-Student tests were used. In the end, the data were centralized in the epidemiologic systems *Excel* and *SPSS* 13.0.

RESULTS

Rare hernias include Spigelian, obturator, lumbar, ischiatic, and perineal hernias.

Our study included: 5 (38.46%) cases of Spigelian hernia, 4 (3.76%) cases of obturator hernia, 3 (23.07%) cases of perineal hernia, 1 (7.69%) case of lumbar hernia, and no case of ischiatic hernia (fig.1).

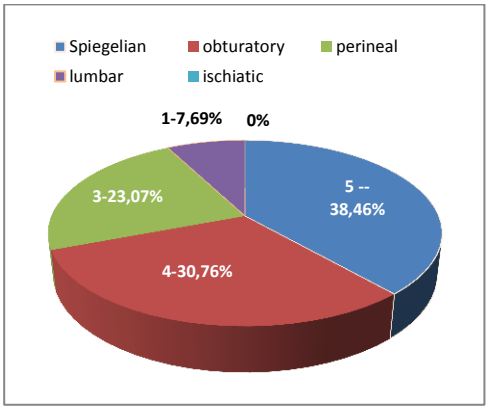

Fig.1. Case distribution by hernia type

Spigelian hernia (or lateral ventral hernia) is a rare abdominal hernia through a congenital or acquired defect in the aponeurosis of the transverse abdominal muscle limited by the semilunar line (or Spigelian line) laterally and the lateral edge of the rectus muscle medially. The semilunar line is in fact an aponeurotic line located between the muscle mass of transverse abdominal muscle and the flashy mass of the large abdominal muscles. In its upper part it is narrow and solid, but in the lower part it progressively widens, being visible (fig. 2).

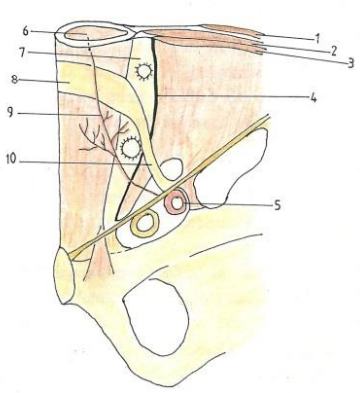

Fig. 2. Deep orifice of Spigelian hernia (collection of the clinic)
1. External oblique; 2. Internal oblique; 3. Transverse abdominis; 4. Spiegel's line; 5. External iliac artery; 6. Rectus capitis posterior major; 7. Lateral flank of the abdomen; 8. Douglas' line; 9. Epigastric artery; 10. Hesselbach's triangle.

The weak point of the aponeurotic fascia is located at the intersection of the spinal-umbilical line with the lateral margin of the rectus abdominis and it corresponds to the arcuate line of Douglas, where the peritoneum contacts the interstitial space of the aponeurosis of the transverse muscle, or even the space between the internal oblique

and the transverse muscle; here, the fibers of the two deep major muscles are parallel, they do not meet in order to strengthen the wall. There are high Spigelian hernias, near Douglas' arcuate line, and Spigelian hernias which can be mistaken for inguinal hernias. This type of hernia is acquired, usually unilateral, and may contain omentum, intestine or colon.

Clinical examination detects the parietal character of the formation presenting the features of a hernia: pain, irreducibility, expansion, impulsion on cough. The diagnosis is based on clinical and laboratory findings; once diagnosed, the surgical treatment is necessary; because strangulations are frequent, the removal of hernia sac and closure of the inguinal orifice are required. The surgical approach may be classical, direct, or laparoscopic.

Of the 13 studied cases 5 (38.46%) presented Spigelian hernia located at the lateral margin of the rectus abdominis muscle, underneath and outside the umbilicus on the line that unites the anterior superior iliac spine and the umbilicus. The deep orifice where the hernia sac is engaged is located in the anterolateral fascia of the abdomen under Hesselbach triangle and above the epigastric artery, which ascends obliquely inwards, behind the transverse fascia and then in front of Douglas' arcuate line. The content of hernia sac is a thin loop of sigmoid, colon, cecum, and vermiform appendix. Due to the fact that the hernia orifice was usually small and had fibrous wall in 4 (30.76%) out of 5 cases, occlusive phenomena occurred, with paroxysmal abdominal pain, vomiting, Koenig's sign, absence of bowel movements, and poor general health status.

Hernias were repaired in all 5 cases via indirect paraumbilical medial approach, repairing the abdominal wall (1 case - 7.69%); the classic, direct, hernial approach was used in 3 cases (23.07%); the medial and paraumbilical approach was used in 1 case (7.69%).

A special mention deserves the case of the patient admitted one year earlier for a mass in the left iliac fossa the size of an orange and increasing in size during physical exercise; the mass was more visible in orthostatism and smaller in supine position. Personal history included multiparity and a uterine fibroma, surgically removed when the patient was 34 years old.

During local examination in orthostatism, we noticed that the patient presented on the bispinal line an approximately 2/3 cm mass expanding during physical exercise. The clinical examination did not reveal anything special.

The intraoperative diagnosis was of left Spigelian hernia; we used the Bauthelemy technique; it revealed a hernia duct the size of an orange, containing a fragment of sigmoid colon attached to the bottom of the sac. The neck of the hernia sac crossed the muscle part of the transverse abdominis muscle. The hernia sac was resected together with the serrated sigma, then the wall was repaired with catgut. We closed the hernia gap by fastening the Douglas arcuate line at the external margin of the left rectus abdominis muscle. The anatomic plans were remade. Above, we fastened the aponeurosis of the oblique muscle in the vest. The patient was discharged from hospital clinically cured.

Obturator hernia occurs through the obturator groove situated between the obturator membrane and the horizontal branch of the pubis, a groove through which the obturator package passes from pelvis towards the thigh. The hernia sac is engaged

in this groove, and, depending on its progress, there are three anatomical-clinical forms: - interstitial hernia, in which the sac and the groove do not extend the obturator groove; recto-pectineal hernia, in which the cul-de-sac extended the groove and reached the thigh under the pectineus muscle; and the pre-pectineal hernia, in which the sac superficially reaches the cribriform fascia. The sac is thin and it may contain intestine, omentum, tube, or ovary. Obturator hernia is more frequent in frail elderly women with changes of the pelvis (figs.2,3).

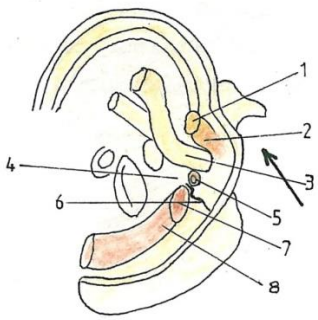

Fig. 2. Palpation of strangulated obturator hernia in vagina (apud Wakeley) (1).
1. Pubic symphysis; 2. Pectineus muscle; 3. Hernia sac; 4. Vagina; 5. External obturator muscle; 6. Obturator membrane; 7. Obturator muscle, 8. Levator ani.

Clinically, obturator hernia is characterized by pain at the inner side of the thigh and the knee due to the compression of the obturator nerve. When it exceeds the obturator groove, we can palpate a tumoral formation at the root of the thigh, which is irreducible, unpainful, located near the obturator groove. Most commonly the diagnosis is made intraoperatively, when the surgeon repairs a mechanical bowel occlusion of unknown causes. A strangulated obturator hernia is a surgical emergency. The approach can be transperitoneal in complicated forms of occlusion, femoral or pro-peritoneal inguinal. The repair of hernia consists in exposing the sac, opening it, reducing the viable contents or resection in case of occlusion with necrosis and closure of the defect, which can be done with mesh.

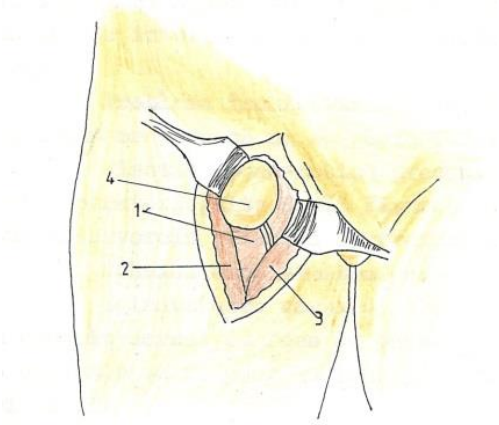

Fig. 3. Crural approach of obturator hernia (collection of the clinic).
1. external obturator; 2. pectineus; 3. middle adductor muscle; 4. sac.

Of the 13 study cases, 4 (30.76%) presented obturator hernia; the hernia sac passed through the obturator canal and established intimate relations with the vascular-nerve package, especially with the obturator artery and its anastomosis with epigastric artery. The sac was thin and small, containing small intestine. The tumor was not felt at palpation, but we noticed a painful spot while pressing the obturator area, under the genitofemoral groove, in the cranial medial part of the Scarpa triangle. During medial subumbilical exploratory laparotomy, we discovered a loop engaged in the deep orifice of the obturator groove. The orifice was removed from accessing the gullet, masked by dilated loop (fig.4).

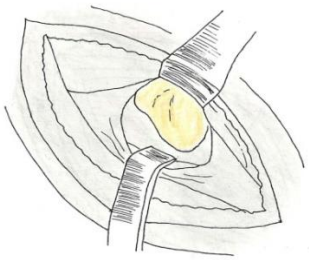

Fig.4. Revealing the strangulated loop, engaged in the obturator groove. Reduction. (Collection of the clinic).

Special Note related to patient S. S., 60 years old, hospitalized for abdominal cramps, nausea, repeated episodes of vomiting, absence of bowel movements and flatulence.

The patient presented several years earlier pain at the root of the right thigh, irradiating towards the knee, more intense in physical strain and which involved a certain degree of functional discomfort. Due to the fact that the pain exacerbated, also the general state of the patient worsened. We chose subumbilical medial laparotomy. Parts of the intestinal loops were dilated, and during exploration we noticed the fastening of a terminal loop on the right lateral wall of the pelvis. In Trendelenburg position, it is easy to reveal a "barrel gun" loop in the subpubic groove. It was possible to reduce it only after the digital enlargement of the abdominal orifice. Only few centimeters of the intestine were involved and it was not compromised. The orifice was closed using the peritoneal fold technique. The patient was discharged healed.

Perineal hernias are rare, being caused by parietal defects of the pelvic floor; they are more frequent in women, and can be medial and lateral. Medial hernias are muscular-aponeurotic in women, favoring the externalization of the pouch of Douglas through the posterior vaginal wall. The vaginal and rectal examinations reveal a soft, reducible mass, larger on cough. Bilateral perineal hernias are located in the anterior perineum in women, and in the posterior ones in both genders. Anterior lateral hernia crosses the pelvic floor in women and exists through labia, while the posterior lateral hernias cross the levator ani and reach beneath the gluteus like ischiatic hernias. The diagnosis is clinically suspected and confirmed by tomodensitometry. The treatment is surgical and it consists of identification and treatment of the sac, repair of the wall by transperitoneal approach for medial hernias, as well as perineal and mixed approaches (fig.5).

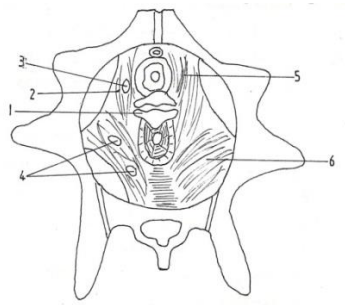

Fig. 5. Perineal hernias (collection of the clinic).
1. Medial perineal hernia - hydrocele;
2. Medial perineal hernia - elytrocele;
3. Lateral ventral pudental or labial perineal hernia; 4. Dorsal lateral perineal hernia.
5. Levator ani; 6.- Ischiococcygeus muscle.

Perineal hernia incorporates in its structure 3 cases (23.07%) out of the 13 cases included in this study. The hernia orifice is found in the soft parts of the pelvic floor.

Special note concerning patient P.S. 39-year-old, hospitalized for a mass the size of an apple, painful, with limited consistency, reducible, accompanied by intestinal gurgling, pulsating on cough. The patient complained of a feeling of intense perineal pressure, with discomfort during walking, sitting

or defecation, pain or cramps. The hernia contained vesical structures, with particular symptoms (dysuria, pollakyuria, miction) or frequent urination. The treatment is surgical, with perineal approach. The patients is cured and released from hospital.

Another mention refers to patient M. M., 34 years old, hospitalized for an ovoid, unpainful, irreducible mass located on the posterior vaginal wall. The patient was a multiparous woman, the last childbirth being laborious. After the last childbirth, the patients felt permanent pelvic pain. After a few months, she noticed the occurrence during exertion of a small, soft, and reducible mass at the level of the pudental cleft; in time, the mass increased in size.

During the local orthostatic examination, we noticed a prominence of the posterior wall of the vagina, forming a reducible tumor of the size of a pigeon's egg. In gynecologic position, we noticed that the vulvar orifice is half opened, with a posterior vaginal hernia on the posterior wall of her vagina, a smooth, stretched and shiny mucosa; at palpation, it felt soft and reducible; during vaginal examination we noticed a lax posterior vaginal wall and free vaginal cul de sac; during mixed examinations, we noticed that the fingers are separated by the thickness of the tumor. After reduction of the tumor, we noticed that the fingers are separated only by the rectovaginal septum, with deficient levator ani.

We applied a surgical perineal intervention; we split the posterior vaginal wall and we reached to a cystic formation, partially reducible, prolonging upwards to a diverticulum between the rectum and vagina. This pedicle is sectioned after the ligature. We performed a colpoperineorrhaphy. There was a good postoperative evolution.

The cystic formation contained a whitish fluid. The histologic examination showed that its internal facet presented a serous epithelium that looked like the peritoneal serous membrane, doubled by a lamellar coating of connective tissue.

Lumbar hernia can be congenital, post traumatic or due to lumbar, lumbosacral and iliac crest abscesses, areas in which there are two weak points of the inferior abdominal-lumbar wall.

One variety of hernia is produced by lumbar-abdominal denervation or relaxation, because of parietal paralysis of oblique muscles after urologic interventions affecting the last two intercostal nerves. The clinical examination in orthostatic position revealed a painful and reducible mass in the lumbar area, pulsating on cough. It naturally grows, with a high risk for strangulation. The differential diagnosis includes lipoma or other tumors of the area. The clinical diagnosis can be confirmed by imaging investigations, ultrasound, CT, etc. The treatment is surgical and consists in an incision over the mass site, detection and reduction of the sac, replacing its contents and repairing the abdominal wall (fig.6).

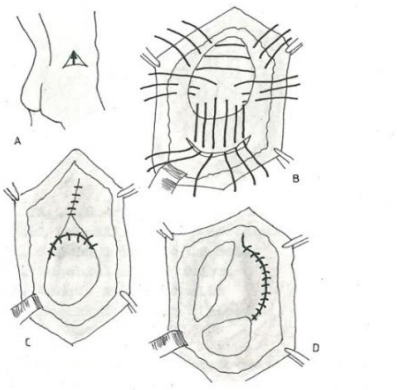

Fig. 6. Treatment of lumbar hernia. Dowd procedure: A. vertical incision; B. bringing the muscle fascicles closer; C. suture of margins; D. creating the second flap. (Collection of the clinic).

In small hernia, the plain muscular-aponeurotic suture can close the parietal defect. In large hernias, the preperitoneal placement of a prosthetic mesh is required.

In our study, only 1 (7.69%) of the 13 cases had lumbar hernia.

Ischiatic hernias are exceptionally rare and they are produced by parietal defects located at the level of the ischiatic notches, between the muscles and ligament formations. There are three anatomoclinical forms of ischiatic hernias: suprapyramidal, the most common, subpyramidal, both supraspinous and infraspinous, developing between the greater and lesser sacrosciatic ligament. The diagnosis is difficult, and it is usually made due to strangulation, when the patient experiences pain along the course of the sciatic nerve and signs of bowel obstruction. The treatment is surgical and the transperitoneal or gluteal approaches are used (4, 8).

DISCUSSION

Our study supports that the main cause of rare hernias is strain, rarely sudden and violent, usually less intense, but repeated, especially in a certain position of the body.

Under the influence of strain, the pressure in peritoneal cavity increases, with visible effects. The peritoneum forms initially a small diverticulum, in an area of the wall with a limited resistance and then, under the influence of repeated increases of pressure, the diverticulum widens the parietal orifice, like a "ram head" (5).

All the presented cases of the clinic point to the reduced incidence of these types of hernia. In all studied cases, hernia was highlighted only in middle-aged women (3 cases in women under 50 years), which made or make physical strain, with hypotonic abdominal wall, lax, altered by many pregnancies. These rare types of hernia may be accompanied by obvious weight loss or obesity. Cooper (6) reports a vascular hernia that shows that the hernia crosses over along a ram of the inferior epigastric artery, deep circumflex iliac artery or of greater or lesser abdominal-genital nerves. Mayer-Wildisen and Quervain (8) ascribe the spontaneous break of lateral abdominal wall to a muscular, neurogenic insufficiency and paralysis.

Related to **Spigelian hernias**, we could individualize two clinical pictures: a) spontaneous or provoked pain, intense or dull, accentuated by coughing while strain, sub- and paraumbilical, at the external margin of the straight muscle (2 cases); b) in three cases, the reason for hospitalization was a progressive tumor mass, of variable size, located in the right iliac fossa in 2 cases and in the left iliac fossa in 1 case, at the lateral margin of the rectus muscle, the masses being irreducible (13,15).

In all cases, the mass was unilateral and matte. In one case each, Spigelian hernia was interpreted as postoperative eventration after operated umbilical hernia and as inguinal hernia, and in three cases the correct diagnosis was made postoperatively.

In all our five cases, the clinical picture of Spigelian hernia was not accompanied by occlusive or subocclusive phenomena. Due to the fact that the hernia orifice was deep, at the external margin of the rectus muscle, in the proximity of the penetration point of the epigastric artery in the right abdominal muscle, we chose to use the direct hernial approach. We performed a McBurney incision and we dissociated the fibers of the greater oblique muscle. The hernia sac contained omentum in 2 cases, cecum in one case, sigmoid in one case, and a small sac without contents in another

case. The postoperative outcome was favorable in all cases, as mentioned by other authors as well (3, 8).

In cases of ***obturator hernias***, the diagnosis was made intraoperatively, the clinical picture being characterized by signs of occlusion or subocclusion. The intervention consisted in subumbilical midline laparotomy, during which we noticed an intestinal loop engaged in the obturator groove.

Due to the lesions that arose by strangulation of the intestinal loop in the obturator groove, in all 4 cases segmental enterectomy with termino-terminal suture was performed. In 2 cases the engaged intestinal loop was perforated. The serous citrine fluid was present in the abdomen in 2 cases, and mixed with feces in 2 cases. Fredet, Bonnet, Cottalorda, Lortat-Jacob et al. (11) studied the clinical and therapeutic aspects of obturator hernias.

Due to the complications and the fact that the obturator hernia was diagnosed late in strangulation phase the postoperative outcome was not favorable. Of the 4 cases, 2 patients died within the first 3 postoperative days due to cardiopulmonary arrest, and 2 had a good postoperative outcome. The deceased patients were 60 and 86 years old, respectively. The severity of these hernias was also due to their late detection (occlusion), as well as the complexity of the surgical intervention.

Perineal hernia is the only type of hernia that was diagnosed in our study in relatively younger women, 39 years old. One of the two multiparous women experienced a laborious delivery which affected her perineal muscles. The other woman underwent surgery for umbilical hernia 6 years before she developed perineal hernia. Obviously, this patient was prone to hernias.

In all the 3 cases of perineal hernia the diagnosis was made postoperatively, the outcome being favorable.

Lumbar hernia is the less common of the rare hernias. In Grance's statistics (quoted by 8), 22 hernias formed via Petit triangle, 8 via Grynfeltt quadrangle, 2 crossed the greater oblique muscle, and 3 were caused by congenital abnormalities in this area.

The presented case was a multiparous woman with intraoperative diagnosis and favorable postoperative outcome.

CONCLUSIONS

Our study highlighted the fact that rare hernias have a precise anatomic identity, while their onset is favored by the relationship between the intra-abdominal pressure and abdominal wall strength.

Rare hernias are an actual concern of emergency surgery, although due to their frequency they are not regarded as priorities.

Strangulated hernia, by the brutal, tight and permanent constriction of the contents inside the hernia sac makes the reduction maneuver impossible and very painful.

We pointed out the basics of clinical practice, crucial for making the diagnosis and an optimal surgical indication.

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NEWS

THE ROLE OF OMICS TECHNOLOGIES IN STAPHYLOCOCCAL INFECTION CONTROL

According to a study by Holtfreter *et al*, omics technologies (such as genomics, transcriptomics, proteomics, metabolomics and immunoproteomics) are powerful tools that can be used to draw a comprehensive picture of the pathophysiological processes that occur within tissues, organisms and populations and improve the control of some infectious diseases. Thus, omics technologies may contribute to the understanding of the interaction between *Staphylococcus aureus* and the human host and the adaptive immune response. *S. aureus* is an important cause of infection both in hospitals and in the community and many circulating strains have multiple resistance to antibiotics. The humoral immune response was described in detail using immunoproteomics, but T-cell mediated immunity was not yet fully analyzed by omics technologies. Increasing the knowledge of the protection mechanisms can prove useful for the development of new strategies for successful prevention or treatment of *S. aureus* infections and these technologies can speed up vaccine development by enabling reverse vaccinology approaches (Holtfreter S, Kolata J, Stentzel S, Bauerfeind S, Schmidt F, Sundaramoorthy N, et al. Omics approaches for the study of adaptive immunity to *Staphylococcus aureus* and the selection of vaccine candidates. *Proteomes* 2016: 4(1) pii: E11).

Teodora Vremera

IMPORTANCE OF PSYCHOLOGICAL INTERVENTION IN THE MANAGEMENT OF A PATIENT WITH NON-HODGKIN LYMPHOMA AND STAGE C3 AIDS DISEASE

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IMPORTANCE OF PSYCHOLOGICAL INTERVENTION IN THE MANAGEMENT OF A PATIENT WITH NON-HODGKIN LYMPHOMA AND STAGE C3 AIDS DISEASE (Abstract): Long-term evolution of HIV because of noncompliance and nonadherence to antiretroviral therapy favors the occurrence of difficult to treat HIV-related malignancies. We present the case of a female patient in "the pediatric cohort" registered at the Iaşi Regional HIV/AIDS Center since year 2000, now with stage C3 AIDS. In 2014, a Burkitt lymphoma was pathologically confirmed, and chemotherapy was initiated as recommended by the hematologist. The clinical course was characterized by multiple complications: hematologic and hepatic toxicities, opportunistic infections and depressive episodes. Highly active antiretroviral therapy associated with sustained psychological support resulted in stabilization of the patient's clinical course (lower HIV viral load and higher CD4 lymphocyte cell counts), anticancer therapy being better tolerated. Currently, patient's clinical-biological status is quasi-normal. The depressive episodes in this HIV-positive cancer patient undergoing chemotherapy contributed to her non-adherence and non-compliance to treatment, with serious consequences both on clinical and viroimmunological status. Therapeutic strategy in this patient with AIDS and Burkitt lymphoma raised management difficulties as both the drug interactions and cumulative adverse effects had to be considered. Multidisciplinary collaboration and especially psychological intervention are essential for creating a functional team, effective communication being key to achieving long-term adherence to treatment and diagnosis acceptance. **Keywords:** HIV/AIDS PSYCHOLOGICAL COUNSELING, NONCOMPLIANCE, NONADHERENCE, NEOPLASIA.

In Romania, the late 80s and early 90s marked the onset of a HIV epidemic characterized by perinatal transmission of the virus to many children (1). Changes over time in the guidelines for antiretroviral therapy in the patients included in the *"pediatric cohort"* were determinants of non-adherence and non-compliance. Maintaining low CD_4 cell counts for a long period in advanced stage HIV-positive patients predisposes to opportunistic infections, particularly tuberculosis, and HIV-related neoplasms (2, 3).

From a statistical point of view, there

was an increase in the number of non-Hodgkin lymphoma at the same time with the number of AIDS cases (4). For most lymphomas, a specific cause was not identified, however, some distinct non-Hodgkin lymphomas are associated with a viral infection (4, 5). According to some studies, patients infected with HIV are 59 to 104 times more likely to develop lymphomas, particularly the aggressive type (Burkitt or Burkitt-like lymphoma) with central nervous system damages (20-30%), and have a poor prognosis (6).

Non-Hodgkin lymphoma in patients with AIDS is often characterized by extra nodal involvement and severe course. Patients are treated with chemotherapy specific for non-Hodgkin lymphoma, though there are several potential risks: overgrowth of opportunistic pathogens and discontinuation of treatment in the context of the commonly associated depressive episodes (6, 7). The disease burden experienced by patients determines changes in self-perception with significant decline in self-esteem and motivation for a good adherence and compliance to antiretroviral and antineoplastic therapy, thus the role of the psychologist in the of HIV/AIDS department being essential (8).

CASE REPORT

We present the case of a 27-years-old woman registered to the Iaşi Regional HIV/AIDS Center since year 2000, currently with stage C3 AIDS. During patient monitoring a progressive decrease in CD4 count was found and the viral immunology tests showed moderate to severe immunosuppression (fig.1, fig.2) because of non-compliance and non-adhesion to treatment. In 2013 the patient was referred to the gynecology department for irregular periods and lower abdominal pain. Continuing the investigations, an abdominal-pelvic CT scan revealed an expansive tumor mass of 7/3/2.5 cm, located on the right ovary. A salpingo-oophorectomy was performed and pathological examination and immunohistochemical tests made the diagnosis of Burkitt lymphoma. Due to a depressive episode, the patient decided to delay the investigations for malignant non-Hodgkin lymphoma (NHL), during which time she received counseling and antiretroviral therapy consisting in combinations of Darunavir/Ritonavir, Etravirine and Raltegravir. Compliance and adherence to HAART were influenced by psychological support, the CD4 cell counts increasing from 33 cells/mm^3 (fig. 1), value recorded at the time of neoplasia diagnosis, to 165 cells/mm^3 (fig. 1) and the viral load decreasing to VL = 10,730 copies/ml (fig. 2). In January 2014, after repeated discussions with the infectious disease doctor, while benefiting of sustained psychological counseling, the patient was referred to the hematology department. Physical examination at the admission to the Regional Cancer Institute confirmed the presence of bilateral painless submandibular lymph nodes of about 2 cm in diameter. Bone marrow biopsy and CT scan of the thorax, abdomen and pelvis confirmed the diagnosis of stage III A Burkitt lymphoma, per ANN ARBOR staging. Hyper-CVAD (Cyclophosphamide, Adriamycin, Vincristine, and Dexamethasone) chemotherapy protocol was initiated, being subsequently associated with Rituximab. Secondary hematologic toxicity required granulocyte growth factor support and prophylactic antimicrobial therapy (acyclovir and cotrimoxazole). The patient received psychological support throughout the hospital stay, resulting in

improved adherence to antiretroviral and antineoplastic therapy. In November 2014, she had and emergency admission to our clinic for: vertigo, nystagmus, abnormal gait, with onset in the context of fever and marked anxiety. Biological exams revealed thrombocytopenia (Tr=84,000/mm^3), hepatic cytolysis syndrome (TGO=108 IU/L; ALT = 84 UI/L) and cholestasis syndrome (FA =134 IU/L; GGT =72 IU/L). Laboratory exams were supplemented by lumbar puncture, which ruled out lymphomatous meningitis and other causes, and cranio-cerebral MRI, which both invalidated the presence of tumor masses and brain infiltrates. Biological changes were considered secondary to oncologic therapy and to HIV infection. Following psychiatric and psychological evaluation the patient was diagnosed with generalized anxiety disorder for which antidepressant and anxiolytic therapy was initiated. Daily psychology sessions were resumed. In December, the same year, CD_4 cell count increased to 466 cells/mm^3 (fig. 1) and the viral load was undetectable (fig. 2).

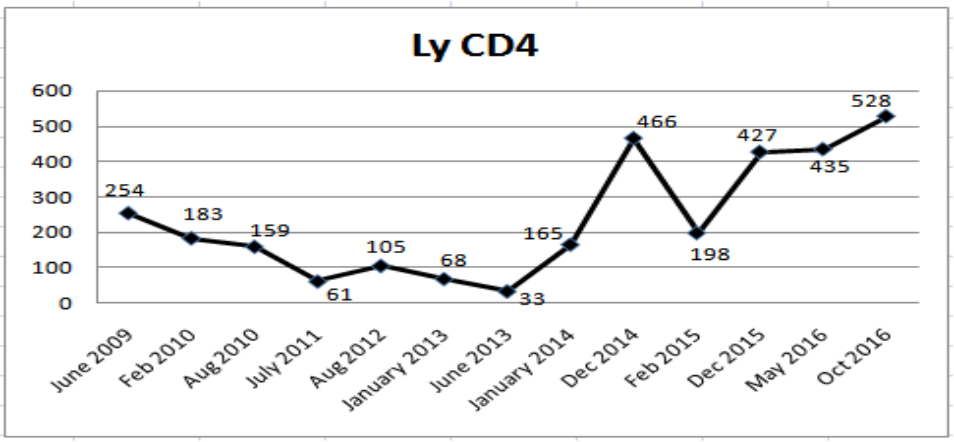

Fig.**1**. CD_4 cell count

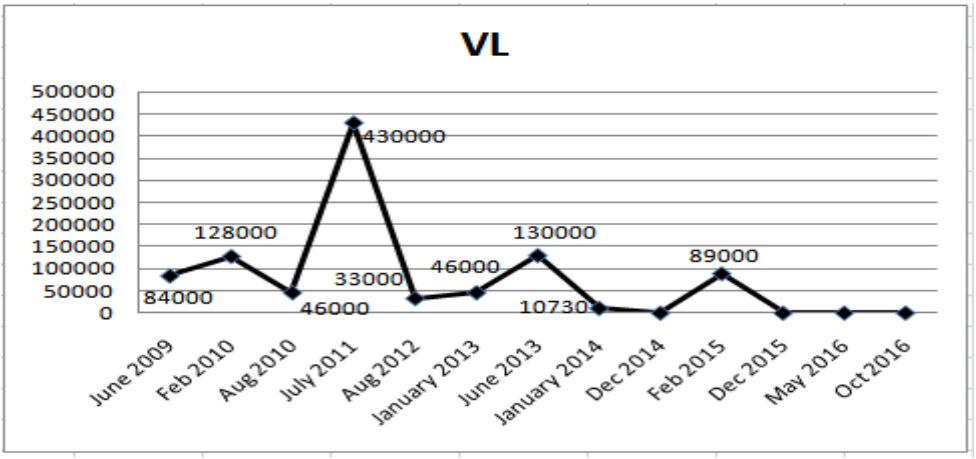

Fig. **2**. Viral load

Subsequently, in February 2015, the patient was reassessed for cough, chest pain and fever. Laboratory investigations have shown a decrease in CD_4 count to 198 cells/mm^3 (fig. 1) and a viral load of 89,000 copies/ml (fig.2). A chest X-ray was performed and it described a characteristic appearance of bronchopneumonia. But the etiology of infection could not be determined. Empirical antibiotic therapy was initiated with moxifloxacin and imipenem the clinical course being slowly favorable. Following discussions with the infectious disease doctor and psychologist the patient admitted that after her general status had improved she started missing doses of HAART, initially 3-4 days a week to complete discontinuation for one month. Remission of lung phenomena allowed patient's transfer to the hematology department. CT and PET-CT examination confirmed the recurrence of an abdominal lymphadenopathy and thus the relapse of the disease, and therefore antineoplastic therapy was reinitiated. In May 2016, the patient achieved partial remission of the disease, supported by imaging investigations, undetectable viral load (fig.2), and CD_4 count of 435 cells/mm^3. The viral immunology assessment in October 2016 showed that viral load remained undetectable, and CD_4 count increased to 528 cells/mm^3 (fig. 1).

DISCUSSION

Given the therapeutic advances in the management of HIV/AIDS the prognosis and the quality of life of patients with HIV have improved. However, the association between the diagnosis of HIV infection and a neoplastic disease has a strong psychological impact, causing recurrent depressive episodes that are a favorable factor of therapeutic abandonment. Under these circumstances the psychologist plays an important role in the multidisciplinary team, namely to guide the patient through acceptance of the diagnosis and adapting to challenges of everyday life. Counselling and psychological support, aimed at providing support in managing negative emotions was based on an honest and comfortable exchange of information (9).

Depression is a natural step in accepting the diseases with serious prognosis, characterized by apathy, insomnia, loss of appetite, constant anxiety, and fear of death (10). The recurrent depressive episodes were initially relieved and subsequently overcome by talks with the physician, psychological support, and antidepressant medication. However, the factors that contribute to the degree of patient adherence to therapy are myriad and include the socioeconomic status, level of education, family support, and finally motivation. In the here reported case the patient's high level of education and her desire to start a family were all decisive factors for accepting the psychological counseling.

CONCLUSIONS

Our case emphasizes the fact that the association of AIDS with a malignant disease predisposes to recurrent depressive episodes leading to nonadherence and noncompliance to antiretroviral therapy. We believe that the role of the psychologist tends to be minimized in favor of drug therapy, but the presented case highlights the important contribution of appropriate psychological support in the multidisciplinary management plan of the HIV-positive patient.

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NEWS

HYDROGEN PEROXIDE VASORELAXATION INVOLVES S-GLUTATHIONYLATION OF VOLTAGE-DEPENDENT K CHANNELS

H_2O_2 is an endothelium-derived hyperpolarizing factor (EDHF), but H_2O_2 effects vary with the vascular bed and experimental conditions. If H_2O_2 relaxes rat mesenteric artery, what is the mechanism? Methods: myography of isolated resistance arteries, patch clamp of mesenteric arterial smooth muscle cells (MASMCs), streptavidin pull-down assays with biotinylated glutathione ethyl ester. Relaxation of precontracted arteries by H_2O_2 was reversed by dithiothreitol, reduced by 4-aminopyridine (4-AP), blocker of voltage-dependent K channels (K_v) channels, but not by blockers of big-conductance Ca^{2+}-activated K^+ channels or of inward rectifier K^+ channels. H_2O_2 increased K_v currents; this was prevented by glutathione reductase; K_v incorporates glutathione (GSH) in the presence of H_2O_2. Thus, H_2O_2 activates Kv channels (maybe via S-glutathionylation) with artery relaxation, while the basal redox status of MASMCs determines K_v response to H_2O_2 (Park SW, Noh HJ, Sung DJ et al. Hydrogen peroxide induces vasorelaxation by enhancing 4-aminopyridine-sensitive Kv currents through S-glutathionylation. *Pflugers Arch* 2015; 467(2), 285-297).

Gianina Georgiana Lalău

SPONTANEOUS PREGNANCY IN SUBSEQUENT CYCLE AFTER LAPAROSCOPIC CYSTECTOMY. CASE REPORT

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SPONTANEOUS PREGNANCY IN SUBSEQUENT CYCLE AFTER LAPAROSCOPIC CYSTECTOMY. CASE REPORT (Abstract): Most women will experience a cyst on the ovaries at least once, and most are painless, cause no symptoms, and are discovered during a routine pelvic exam. Large cysts that can cause symptomatology or infertility problems occur in about 8% among women of reproductive age. The current case report comes to show that laparoscopic surgery is the key for persistent organic tumors of the ovary, after a complete diagnostic of the cyst; not the expectant management or hormonal therapy, but laparoscopic cystectomy after transvaginal punction and drainage of the fluid is minimal requiring as recovery timing, medical care period, low costs, and not the least, ovarian functionality, after suppressing ovulatory function. **Keywords**: CYSTECTOMY, PREGNANCY, OVARIAN CYST.

Abdominal cysts are sacs or lumps surrounded by a membrane, encapsulating fluid or semi-fluid material. While most cysts are benign, the presence of a mass may indicate an under-laying disease therefore, further investigation should be done. One of the most common abdominal cysts is the ovarian cyst which is formed on the ovarian follicles (1). In the last years, the number of diagnosed ovarian cysts has increased once the regular physical examination has been completed by more complex investigations such as imagistic technology and specific ovarian tumor markers. Nevertheless, the discovery of an ovarian mass may cause anxiety for the patient since future fertility is one of the major concerns in women of reproductive age.

CASE REPORT

A 28 years-old Romanian woman, normal conformation (65 kg body weight and 175 cm height), with no history of pregnancy or gynecological pathology, presented in our clinic with pelvic pain irradiated in the left inferior member accentuated during sexual intercourse and physical activity. The patient also experienced polakiuria, lumbar pain, low abdominal distension, bloating and perturbation of the intestinal transit and defecation, starting 6-8 months before but became acute in the last weeks. A non-investigated 2-year primary infertility was also declared by the patient.

According to the anamnesis, menarche commenced at the age of 15 years with subsequent irregular cycles and moderate dysmenorrhea. Deep abdominal palpation revealed sensitivity in the left flank, which was irradiating towards the hypo gastric region and sigmoid colon. The bimanual vaginal examination showed a mobile retroverted uterus, with normal dimensions. On the left adnexal topography, a voluminous mass, with imprecise limits was observed. The tumoral mass, whose appurtenance was established at the ovary, presented tenderness. The endovaginal echography showed a highly deviated uterus towards the right side, although the dimensions and aspect were normal. The left ovary was enlarged in volume, presenting a well-defined mass, 113 mm / 110 mm/100 mm, with thick membrane, clear content, without any proliferations or intracystic septae (fig.1, 2).

Fig. 1. Ultrasound image of anechoic cyst. No septations were observed; thin walls and a nodule without flow on Doppler was present on the posterior wall

Fig. 2. Normal ultrasound of right ovary**.** A part of left ovarian mass, compressing the right adnexa and the uterus

Doppler signal was reduced and the resistance index was normal. The adnexal torsion was excluded, additionally, no intracystic hemorrhage was observed. According to the biochemical exams, the ovarian tumor risk markers were in normal limits ranging: CA 125 15,59 U/ml (normal value < 35), CA 19-9: 4,8 U/ml (normal range < 27), CAE: 0,497 ng/ml (normal range < 4,3) results which are supported by (2).

The MRI evaluation revealed an extremely voluminous cystic formation (134 mm / 116 mm / 110 mm) (cranio-caudal/ transversal / AP) located in the pelvis and in the hypogastrium area. At MR imaging, the well-defined formation presented hyper-intensity in T2 and T2 iso-signal with moderate restriction, inhomogeneous diffusion and a discreet outlet contrast at the level of the thin wall (2 mm). The lesion, which originated in the left ovary, showed significant mass effect on the urinary bladder, uterus, right ovary, sigmoid colon and intestinal loops, as well as strong compression syndrome on the common and external iliac veins. In the upper pole of ovarian cyst formation, a wide oval area of 30/10 mm, hyper-signaling in T1, T2, with diffusion restriction and nonspecific appearance was observed plated on the right anterolateral wall. The uterus, which was shifted

posteriorly, presented normal size and structure. The dimensions of the right ovary were normal, although few small follicular cysts were present. Based on the ultrasound report, MRI evaluation and biochemical markers we decided to evacuate the cystic fluid throughout a minimal invasive procedure. Thereby, 1.3 liters of fluid were evacuated by transvaginal puncture and aspiration, using echo-guidance (fig. 3, 4).

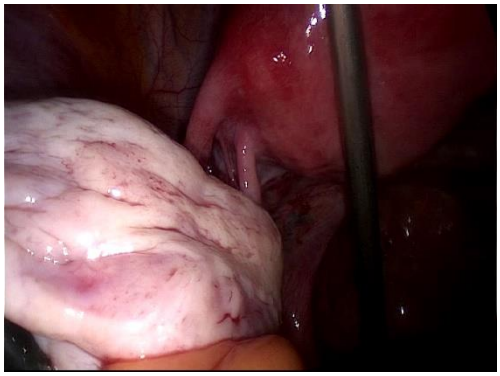

Fig. 1. Intraoperatory image of the tumor

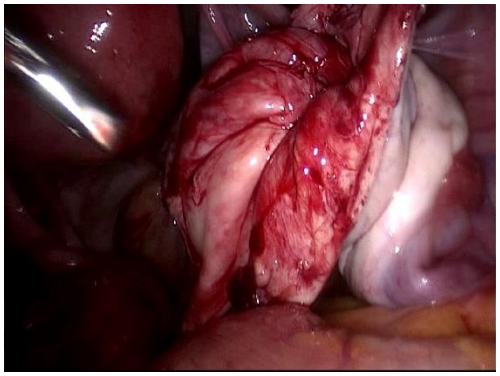

Fig. 4. Intraoperatory image of remaining ovary

The histological exam revealed smooth cystic walls, serous fluid and no vegetation inside. After the evacuation of the fluid, the cystic membrane was removed by laparoscopic excision. It was established that the tumor mass was a mature cystic teratoma, which presented epidermal cells and thyroid tissue. One-month post-operatory intervention, the patient presented with a positive pregnancy test, the single intrauterine gestational sac being confirmed by ultrasound scan. The luteal corpus was observed on the left ovary, which was previously affected by the tumor.

DISCUSSION

Mature cystic teratoma (MCT), also called dermoid cyst, is the most common ovarian benign germ cell tumor in women of reproductive age (from teens to forties) (3). Future fertility is one of the major concerns among these women therefore, the management of the tumor must focus on preserving ovarian tissue and minimizing adhesion formation. The particularity of this case resides in the fact that the left ovary, whose ovulatory and gametogenesis function has been inhibited by presence of the tumoral mass, regained its activity in the first month after the laparoscopic removal of the cyst. Conform to literature data regarding the surgical treatment of Stein-Leventhal syndrome, this mechanism is similar to the one observed in PCOS patients, which were submitted to cuneiform resection of the ovary.

Laparoscopic management offers many advantages for the patient: accurate diagnosis, minimal bleeding, reduced need for analgesia, less adhesion formation, fast recovery, better cosmetic results, shorter hospital stays (one-day surgery in selected cases), and reduced costs for the patient and hospital (4-7).

CONCLUSIONS

Laparoscopic cystectomy after transvaginal punction and drainage of the fluid

is minimal requiring as recovery timing, medical care period, low costs, and not the least, ovarian functionality, after suppressing ovulatory function.

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NEWS

CHRONIC ORAL MG LOWERS ARTERIAL PRESSURE INCREASED BY CHRONIC INHIBITION OF NITRIC OXIDE SYNTHASE (NOS)

Male rats received N-nitro-l-arginine methyl ester (l-NAME, 25 mg/kg/day in drinking water) for 6 weeks, alone or together with magnesium-oxide (0.8% in diet). Systolic arterial blood pressure (ABP) was measured weekly (tail-cuff). Constriction and relaxation was studied in rings isolated from thoracic aorta and third order mesenteric artery. Gradual hypertension induced by l-NAME was attenuated after five weeks of oral Mg. Chronic l-NAME increased phenylephrine induced constriction and decreased acetylcholine (ACh) induced dilation, both restored by Mg. Nitric oxide (NO) contribution to ACh relaxation of mesenteric arteries was suppressed by l-NAME, corrected by Mg. Flow-mediated dilation (FMD) of mesenteric arteries was blunted by chronic l-NAME; not corrected by chronic Mg. Vascular eNOS and Mg were not changed but plasma nitrite was reduced in hypertensive rats. Restoring arterial relaxation, chronic Mg decreases ABP increased by chronic l-NAME. (Basralı F, Koçer G, Ülker Karadamar P et al. Effect of magnesium supplementation on blood pressure and vascular reactivity in nitric oxide synthase inhibition-induced hypertension model. *Clin Exp Hypertens* 2016; 37(8): 633-642.)

Mihai Constantin

COMBINED APPROACH FOR LARGE TUMORS OF THE NOSE AND PARANASAL SINUSES - CASE REPORT

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COMBINED APPROACH FOR LARGE MALIGNANT TUMORS OF THE NOSE AND PARANASAL SINUSES – CASE REPORT (Abstract): Due to the insidious onset and common symptoms, the diagnostic of sinonasal tumors is late in the evolution. Usually, at that time, the preferred endoscopic approach alone is not recommended, therefore a mixed open-endoscopic approach appears to be the best choice in terms of exposure of the tumor and a clear margin resection. The purpose of this case report study is to demonstrate the necessity of careful preoperative assessment and the advantages of combined approach for a complete resection and favorable long-term results. **Keywords**: COMBINED APPROACH, NASAL ENDOSCOPY, AUTOLOGOUS GRAFT, SINONASAL TUMORS

Sinonasal malignancies are quite rare (5:1000000 rate in USA – 1% of all cancers) affecting women and men with equal rates, usually in the 6th decade of their life. The diagnostic is made late in the evolution due to the insidious onset and common symptoms (nasal obstruction, mucous rhinorrhea, sometimes recurrent minor nasal bleeding), therefore surgery – if still possible – needs open approaches to allow a wide exposure of the tumor. The endoscopic approach is rarely used alone, usually completing the open approach, allowing in the same time a better control of the excision margins.

We are reporting a case of a male patient diagnosed with a large tumor occupying the nose, paranasal sinuses and operated using a double approach (open and endoscopic) allowing a good control of the excision. The importance of preoperative assessment of the extent of the tumor and the best approach chosen are discussed.

CASE REPORT

D.D. a 53 years old male patient was admitted in ENT Department of the "Sf. Spiridon" Clinical Emergency Hospital Iasi, for bilateral nasal obstruction, mucous and purulent rhinorrhea, minor recurrent nasal bleeding on the left side, frontal headache, complete loss of smell, with an

onset 18 months before. 12 months before he was operated in a different ENT Department for nasal polyposis but no histopathological evidence was present at the time of actual admittance in the hospital.

The anterior rhinoscopy revealed a large purple, irregular, covered with puss, easily bleeding when touched mass, that could suggest some confluent polyps, and is blocking completely the left nasal fossa in its posterior 2/3 and almost completely in the anterior 1/3. The posterior rhinoscopy showed the complete blockage of the left choana and the protrusion of the mass to the nasopharynx.

The CT scanner revealed the presence of a large solid mass involving the left nasal fossa, ethmoid and frontal sinuses (fig. 1) with some small areas of bone erosion on the anterior skull base and the posterior wall of the left frontal sinus (fig. 2).

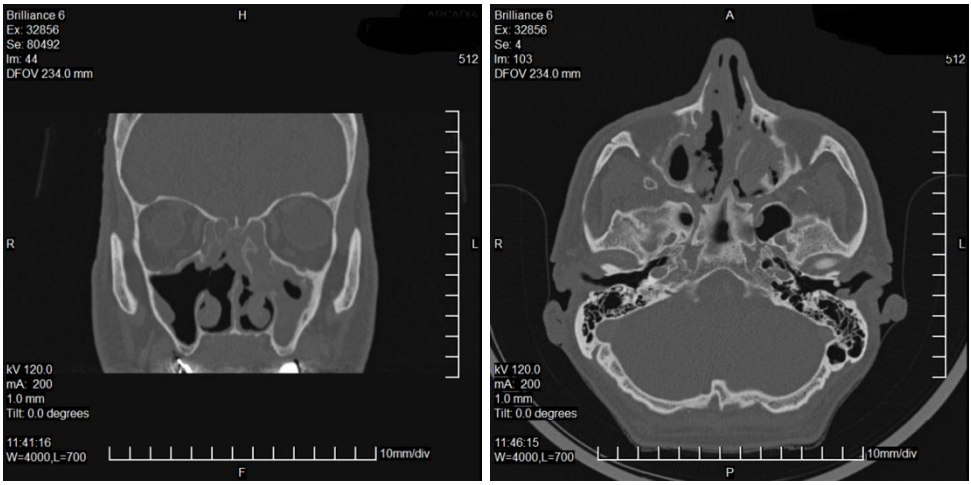

Fig. 1. CT scanner showing the tumor

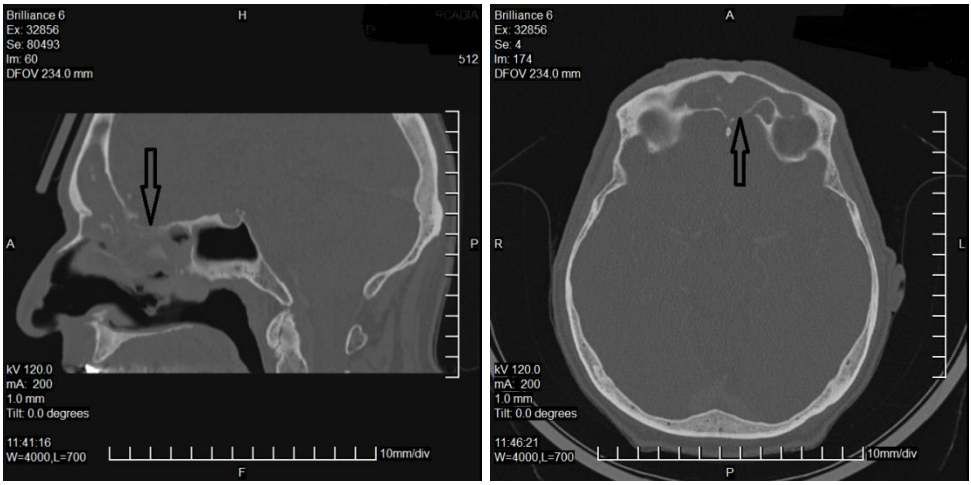

Fig. 2. CT scanner showing bone erosion areas

The complete removal of the tumor was performed using a combined approach – both open subcranial and endoscopic approach. The subcranial approach required the removal of a segment from the frontal bone (fig. 3) and due to the erosions of the posterior wall of the frontal sinus and the exposure of the duramater, the frontal sinus cavity was filled with an autologous abdominal fatty tissue graft (fig. 4).

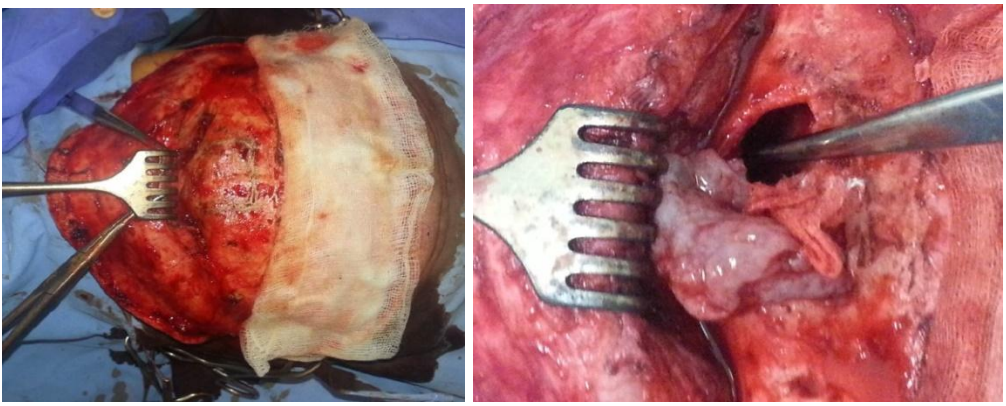

Fig. 3. External subcranial approach exposing the tumor; probe in the right frontal sinus

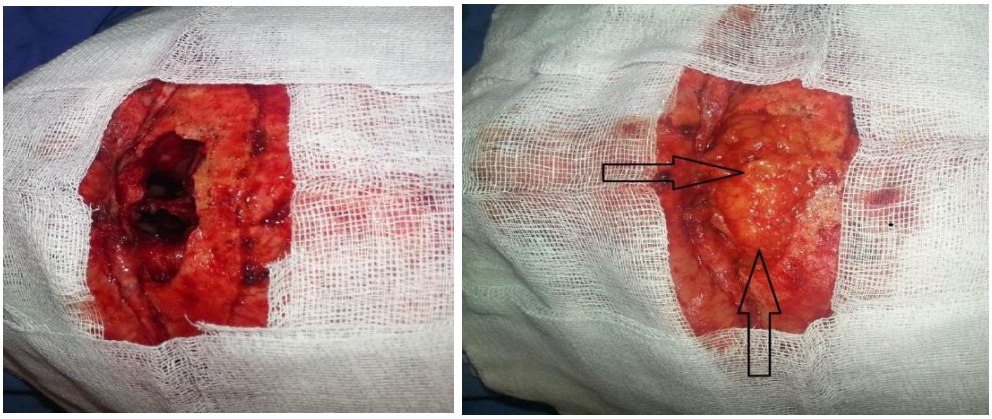

Fig. 4. The cavity left after resection; autologous abdominal fatty tissue graft

The histopathological examination revealed an inverted Schneiderian type papilloma. The postoperative evolution was good allowing the patient to be discharged after 4 days. The periodic follow-up showed no recurrence after 2 years.

DISCUSSION

The surgical approach of sinonasal tumours is not standardized and it has to be individualized for each patient, considering the extension. Due to the patient's late visit to the otolaryngologist and the large extensions of the disease, endoscopic surgery alone rarely solves the case. However, it can very often be a reliable resource in ensuring an accurate excision. This happens when the extensions are towards the orbit, sphenoidal sinus, pterygomaxillary

cavity, which is difficult to reach and control through external approach (1,3).

The frontal sinus in its inferior side as well as the frontal recess can be accessible through an endoscopic approach (using angular optics of 40 or 70) but in invasions superior to these areas, external approach is more favourable (2,4).

The invasion of the lamina papyraceea, the floor of the orbit, the anterior wall of the maxillary sinus and particularly of the covering skin ends with large excisions and important esthetical problems, especially if the resection is made for a malignancy, can be a relative contraindication to postoperative radiotherapy, due to lack of bone support of the pedicle flap harvested for reconstruction.

Sometimes the endoscopic approach is not satisfactory for a good exposure of the tumor and in the same time, the open approach alone can result in large cosmetic defects difficult to be accepted by the patient. In these cases, endoscopy due to the given magnification can complete and guide the excision of the tumoral tissue left in place, with lesser facial scars.

CONCLUSIONS

In the past, large tumours of the nose and paranasal sinuses were approached classically, using different facial incisions for a good exposure and leaving unesthetic scars. Lately, some authors are the advocates of endoscopic surgery that allows a good visualisation of the otherwise inaccessible areas. In our opinion, the approach must be individualized for every patient (considering general status, comorbidities), for every tumoral extension and for every histologic type.

The presented case demonstrated that, in this specific localization, the endoscopic approach alone would have been allowed the complete excision in the middle and lower part, but not in the frontal sinuses, while the external approach alone, in order to completely remove the tumour, would have been created some inesthetic facial scars. Combining these approaches gave us a complete control of the tumour and a very good esthetic result.

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HEALTH CARE ECONOMICS IN ROMANIA – DYNAMICS AND EVOLUTION

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HEALTH CARE ECONOMICS IN ROMANIA – DYNAMICS AND EVOLUTION (Abstract): Health economics refers to the analysis of medical institutions considering their economic and social efficacy, but also the regularity and the relationships that govern the phenomena and the processes from the field of health with the final purpose of achieving better results with the minimum of resources; it represents the study of health price in its complexity. The economics of the population's health needs and in particular the health needs in case of the poor groups of the population, consider health to be the main component of global human vulnerability. Health economics tries to change the simple interpretation of health price and disease cost into a wider consideration of a system administration similar to educational and social economics and the study of health in the context of the multiple specializations of the macro economy of the national group, as it is an instrument in the country's great economics symphony. **Keywords:** MANAGEMENT, HEALTH ECONOMICS, HOSPITAL SERVICES.

Health economics appeared as a result of the interest the economists showed in all social life's fields, starting with industry and agriculture and finishing with education and health.

In general, it is considered that the population's state of health deteriorates and the mortality increases when the macroeconomic situation gets worse. Nonetheless, nowadays, there are more and more data indicating the fact that physical health and not psychical health, actually improves in this type of periods (1).

In the middle of an age of interdisciplinarity, economists see health as an economic good. It is determined by biological factors (heredity, sex, and age), environmental factors and life style factors. But since prevention and early identification of diseases can be accomplished with huge financial investments and because health is quantified in terms of efficiency and efficacy, it becomes on abject of economic study.

This is only one of the arguments that place health in its position of segment of the national economy. Consequently, the economists wander to what extent it answers to the supply and demand relationship, what the society wants, what it is offered to it and what it gives in return. The answers are difficult and relative because health, just like education, provides long-term services. But precisely this type of

observations confirms the efficacy and social utility of the health care system. Naturally invaded by numerous and different diseases, in its need of preservation, the society formulates requests that cannot be postponed.

Medicine represents a dynamic field if we consider the technology it works with, but completely inert if we refer to its financial situation, as the reform of the health care system requires time and the answers to macroeconomic shocks take numerous years, even decades. Even if the population presents a better state of health which generates higher incomes and vice-versa, the improvement of life standards leads to a better state of health in people, the relationships between the state of health and economics remain difficult to quantify and even study (2).

As an answer, the health care system comes with a diverse offer of services. The national level and its economic fund generate the value a society invests in health. Human value health is a right of man protected by medical science but also by economic resources and means.

ETHICS AND HEALTH CARE

Decades ago the differences between health economic analyses and clinical practice were significant, with the physicians and patients who defended Hippocrates' Oath on one side and policy makers and health economists on the other side. It was the policy makers the first ones to have to solve the difficult allocation problem: the health care budget was growing faster than any other public spending. Later, the economists came in trying to make the optimal decisions in limited financial situations. Then, health policy makers decentralized their budget, which was an intelligent political move, as the responsibility for the healthcare budgetary allocations was now shared. However, clinical quality concerns started to appear: waiting lists and insufficient care, these rapidly becoming hot subjects in the public eye, with patients claiming their right for health care by law or by public indignation. It became clear that budget policy had to be justified by more arguments than just financial constraints.

In this context, the health's base results to be economic, and its management consist in obtaining maximum performances with the minimum (often limited) investments. This is how health economics was born, a science that can be mastered only by those with a dynamic, flexible thinking manner and endowed by the ethics of the health care system (5).

Ethical rationale for publicly financed health insurance rely on four logically related issues: (1) the ultimate purpose of a human life or human society; (2) the role of health and its distribution in society in advancing this ultimate purpose; (3) the role of access to or utilization of health care in maintaining or improving the desired level and distribution of health among members of society (4) the role of public financing in ensuring the ethically justified access to and utilization of health care by members of society.

It is true that economics must not prevail over the medical act, but it is equally true that the physician cannot ignore the efficacy of his actions (maximum results with minimum of resources) and their efficiency (health days, recoveries, years of life regained, lives saved). Deriving from political economics, health economics is part of a triad, along with education economics (medical education, in particular) and science economics (medical scientific research).

All the three entities work together un-

der the auspices of economics, according to the law based on maximum efficacy with the minimum of effort. The collaboration between physicians and economists results a necessity that can only be fulfilled through interdisciplinarity. Health economics contributes to economic health (6). C. Gastmans (cit. by 6) pleads for economists and ethicists to try and approach each other so that the tool of cost-effectiveness analysis is further developed to combine the two most important goals of health care systems: health maximization and the reduction of health inequalities.

In Romania, because the allocation of resources does not yet represent a purpose in itself, this necessity (for health economists) is less present. Nonetheless, there is hope for a rational perspective that might, at least support, if not impose, a decision and a smooth regulation of the decisional process.

HEALTH CARE ECONOMICS AND HTA

Romania spends approximately 6 percent of GDP (*gross domestic product*) on health (public and private) vs. the EU average (all EU countries) of 9.8 percent. Among current challenges are lagged health outcomes, patient dissatisfaction, lack of access to quality care by the poor and other vulnerable groups, and poor financial performance.

Romania had the highest infant mortality rate in the EU (3). Furthermore, the life expectancy gap between Romania and EU15 (EU countries on December 31, 2003) has increased 2 times since 1970, mainly due to the rising incidence of non-communicable diseases (NCDs).

Romania's health infrastructure and its service delivery system have not adjusted to modern technologies and do not meet the current needs of the population, which has suffered both a demographic and an epidemiological transition in last two decades. The hospital network in Romania is large and fragmented, with over 350 public hospitals that have been mostly been decentralized. Except for SMURD (*Mobile Emergency Service for Resuscitation and Extrication*) and several maternities, no other significant investments have been made in the last 20 years in medical infrastructure.

The rhythm of health care sector reform has been slow due to the lack of financial resources required to support the new policies.

Health technology assessment (HTA) is the 'systematic evaluation of medical technologies regarding their effectiveness, appropriateness, efficiency as well as social and ethical aspects and implications'(1). Its main purpose is to inform technology-related policymakers in health care and thus has a decision-support role in pricing and reimbursement. HTA provides the responsible bodies and organizations with timely, accurate, and sound information on new medical technologies (3, 4).

HTA focuses primarily on pharmaceuticals and medical devices (standardized technologies with clear ownership) as these constitute a high financial cost and their social impact and visibility are significant (7).

As pharmaceutical expenditures grew rapidly over the past decade and the 2008 crisis posed a major financial risk, the Romanian government, financed by the World Bank, contracted as consultants NICE International (*The National Institute for Health and Care Excellence*) (UK) to provide recommendations on how to reform the health care system. The legal framework for HTA in Romania was created

through Government Decision 351/2012. The responsibility of the HTA unit is broad: HTA can be applied to all existing medical technologies such as pharmaceuticals, medical devices, health policies and public health.

According to an HTA guideline, the assessment of innovative drugs is made using a 6-item scoring chart where the maximum number of points is 10, and for a positive reimbursement recommendation, the pharmaceutical product has to score at least 6.

The decision on reimbursement is reached by assessing two distinct types of evidence:

1. Reimbursement recommendations given by HAS (Haute Autorité de Santé) France, and 3 HTA bodies in the UK (NICE - *The National Institute for Health and Care Excellence* / SMC - *Scottish Medicines Consortium* / AWMSG - *All Wales Medicines Strategy Group*), and reimbursement status in other EU member states;

2. Clinical profile of the intervention: relative efficacy, relative safety and *patient reported outcome* (PRO).

The family physician, being well informed about the social and demographic dimensions of health care and on the extremes of medical consumption because he deals with patients of all ages, must be aware of the balance between promoting health and treating diseases, the amount of services provided, their cost and efficacy.

Applying the cost-advantage and cost-efficacy (benefit), he obtains the monetary value, the result of the relationship between the value of the resources invested and the value of the program made. Analysing the correlation advantage-unit and time unit, the physician observes that efficacy represents an economic manner of achieving a well-defined purpose.

The role of reason in using health resources eases the difficult action of keeping records of the cost-advantage. For example, the role of personal hygiene in preventing infectious-contagious diseases, especially digestive ones, represents a logical, cultural, scientific and concise resource used rationally as an investment for health. Its absence generates higher costs in treating the inherent diseases. The quantitative decrease of the number of these diseases reduces the number of the ones who get ill, as well as the financial results (costs) due to the logical and positive use of the resource in question.

Of course, if the group it is addressed to is well analysed from multiple points of view: the members' cultural level, receptivity period, logistical and support component of theoretical investment etc.

The high costs in health represent one of the biggest problems. High quality medical services, the production of medical science with sophisticated means, the consumers' request for more and more sophisticated devices and solutions that are mainly logistical, contribute fully to this phenomenon. The cost-efficacy relationship is always relative, depending of the results of other programs or of a general rule.

In order to make an informed choice about if and how much to pay for a medicine, it requires evidence beyond the classical safety and efficacy data from the clinical trials. Compiling huge amounts of economic data, *electronic health records* (EHRs), genomic data, labor statistics etc., business players can better illustrate the value of their products. Two studies showed that half of American consumers are willing to pay more for personalized medical products but at the same time, are divided about paying for the tests that enable these treatments (cit. by 6).

The analysis of the relationship between

cost and utility is measured in precise results: the effect of treatment on the patient's life quality; years of life won depending of the quality of life; number of days without getting ill; number of lives saved, early identification of cancer etc. Considering this multitude of advantages at human level, we understand that the moral aspect of the problem comes first (6, 8).

But providing an etiological treatment, apart from its efficacy, it also induces the advantage of diminishing the financial value of those treatments that are difficult to define, reducing the labour incapacity period, the hospitalization period etc. When referring to maximum efficacy with the minimum of resources, by minimum we must not understand "insufficient" or "cheap", but a scientific, rational, attentive use of resources.

Returning to preventive investment, it proves to be less financially demanding than curative investment, although none of the components can be pointed out in the detriment of the other as they create a logical balance. Prevention interventions are not necessarily more effective than the ones that focus on treating. The prevention and curation % worldwide are almost equal. The idea is that the damage once created brings along a lot of human suffering and medical effort, including material and financial effort.

Considering its efficacy, any treatment program must be subjected first to a keen examination: to what extent it is feasible; if it prevents complications effectively; if it justifies itself from a humanitarian (reduces pain, deaths) and economic (reduces the number of patients) point of view; to what extent it influences life quality. The decrease of the number of deaths, of irretrievable biological losses that are impossible to compare and label depends of preventing and counteracting the avoidable factors of post neonatal mortality with the aid of the managerial conception and intervention of health care services in correlation with social, economic, political and legal factors.

It results that any physician is invested with economic responsibilities even if he only writes down a recipe. Economic assessment is professionally necessary but also out of respect for the patient, as it represents a fundamental part of the decision making process. A good, useful, efficacious and effective use of resources depends of rational, scientific thinking that is the prime self-resource of total management. Resource management taken into custody must be credited with purpose, as an action, always compatible with similar activities.

HEALTH CARE AND INFORMATION TECHNOLOGY

While IT (*information technology*) has the potential to greatly improve the quality of health care, the evidence that IT improves important health related outcomes is limited. Some authors (9, 10) argument that while HIT (*health information technology*) has the potential to lead to a dramatic transformation in healthcare delivery, the empirical research showed limited results supporting HIT benefits. Their recommendations on a successful HIT implementation require a system that is intuitive to use and that requires little training for users; a system that can be modified and developed easily.

The authors also note that although there is an increasing number of a HIT solution designed to be used directly by the patient (for example, internet self-help sites), very little is known in terms of use and outcomes. Also, the main HIT implementation barriers are cost and overcoming misgivings from physicians due to per-

ceived adverse effects on their time.

The Integrated Health Information System of the Social Health Insurances goal is to ensure the computerized management of the economic and medical data necessary for an efficient Romanian health insurance system. Thus, it contributes to the development and improvement of the medical and pharmaceutical services.

System's coverage area: National Health Insurance House, all the County Health Insurance Houses, Physicians, Family Doctors, Medical assistants, Hospitals, Providers of ambulance services, Providers of homecare services, Out-patient recovery, Providers of medical devices, Providers of rehabilitation sanatoria services, Providers of pharmaceutical services.

IHIS (*Integrated Health Information System*) in Romania aims at improving the social health insurance system among others by collecting and managing the economic and medical information, ensuring the transparency regarding the control and management of the budgetary funds, creating and administering the National Registry of Insured Persons and the National Registry of Medical Services Suppliers. The main functionalities of IHIS include the management of the social health insurance funds, the management of the insurant, the management of the medical and pharmaceutical service providers, the evidence of taxpayers and control of the medical services.

However, the drive for more health data and integration comes also from the private sectors, as both consumers and physicians cheer the dramatic expansion of the personal medical kit, with wearable tech, smartphone-linked devices and mobile apps becoming increasingly valuable in health care delivery.

Personal medical kits could help diagnose illness, detect early signs of disease, and facilitate the recovery and rehabilitation closer to home. Clinicians seem more open to using these tools than consumers, according to HRI (*Health Research Institute*) survey findings. One-fifth of consumers said they would use a home urinalysis device. But nearly half of physicians said they would use data from such a device to prescribe medication or decide whether a patient should be seen. Nearly 90% of MDs said that patient devices and apps will be important to their practices in the next five years.

The app market – filled with 50,000 free and nearly-free products – is highly saturated. The Romanian market of smartphones had around 2.5 million units sold in 2014, and the tablets reached 700,000 units with at least as many potential users for health care apps.

PRIVACY AND SECURITY

All that may come to my knowledge in the exercise of my profession or outside of my profession or in daily commerce with men, which ought not to be spread abroad, I will keep secret and will never reveal. (The Hippocratic Oath, 4th Century BC) (11).

The number of websites and social networks providing health care related information and opportunities for patients to both share information and meet other patients is growing fast. For example, Yahoo health or WebMD, have around 20 million unique visits every month while in Romania, romedic.ro has over 1.5 million (1).

Several studies have showed the benefit of using Internet to share health-related information. Patients use the information to self-diagnose symptoms, understand the treatments and the medication devices (12) create or improve relationships (13), and deal with symptoms.

As the volume of personal information captured and digitized continues to grow at exponential rates, the concept of privacy has also been put on the top of the list of concerns for the public opinion. Recent research highlighted the significance of privacy concerns over the internet sharing of health or intimate data or how individuals deal with threats to their electronic privacy. Another author believes that privacy concerns could be the greatest threat to successful rollouts of EHRs.

At the same time with the increased shared and "in the cloud" patient and health data, the proliferation of medical devices in patients' homes, and apps on their phones points to an even more pressing need for strong information security systems.

In summer of 2014 alone, over five million US patients had their personal data compromised in health system privacy breaches. As for the mobile eco-system, the US Department of Homeland Security issued a warning to police officers, firefighters, emergency medical services and security personnel about the security issues in Android. The report says Android accounts for 79 percent of mobile malware, while Apple's iOS accounts for 0.7 percent. According to analysis company Stat Counter, Google's Android platform is the clear leader in Romania, reaching 62.73% market share in September 2014.

Still, the consumers want one-click access to their data and the challenge is to find the right balance between privacy, security and ease of use. Over 65% of respondents in a research said data security was more important to them than convenient access to imaging and test results, doctor's notes, diagnoses and prescriptions. For fitness data, the reverse was true. However, the consumers could agree to share health data if they would see social or increased care value in doing so.

Future cyber security safeguards will have to focus on what consumers look for – health data that is private, secure and one click away.

HEALTH CARE ECONOMICS – FUTURE DIRECTIONS AND CONDITION OF SUSTAINABLE HEALTH

The complete confidence with which a physician establishes the correct diagnosis determines the number of hospitalization days, the cost of the treatment, the number of recovering days, briefly, the efficacy of the medical act which is an important indicator for economic sciences.

Consequently, in spite of the fact that the economic factor is not the one guiding health, its decisive role cannot be ignored. From this point of view, Romania is facing severe problems. This situation will become even worse considering that the health care system confronts itself with three different pressure sources: the justified expectations of the population, which asks for a fundamental right of human beings; the progress of medical technology represented by equipment's, therapies and drugs, research studies in this field, which require important investments; population ageing (the costs with an older patient are 5-10 times higher than with a younger patient) (12, 14).

Seen from the economist's point of view, health, although it does not have a material meaning, is absolutely necessary for social and economic development, the economic activity being, by excellence, a support for protecting and conducting health care activities.

Health must be included in the political, economic, social, environmental and institutional context, the strategic frame being oriented toward accomplishing actions for

improving the state of health and assessing the impact of health care.

The economic principle applies to all the dimensions of the disease (apparition of pathogenic agents; their adjustment to the organism's defence mechanisms, to the artificial antimicrobial means; recombination of viruses; multi-causality and multi-diversity) and of health (preventing complications, premature death, disabilities, infirmities, diseases) (12).

Promoting health endows people with the best ways of ensuring and improving their own state of health. Health is not the purpose of life but one of its resources and it must be promoted through social responsibility, more investments and partnerships and the development of an appropriate infrastructure.

The promotion of health includes flexible models, adjusted to the present and future demographic situations, all of this in the general frame of the principles applicable to all people and all parameters that condition health care.

The health price and the disease cost are established based on an analysis that has at its base the medical and economic principles of efficacy and efficiency. The health price requires a longer period of time, even decades to be accomplishes. It is cheaper if moral investments are made, first of all, common sense in promoting health for the individual's physical, mental and social state of good. The disease cost refers to the disadvantages it brings at human level (13).

The priority for health economics in the process of providing health care services resides in the economy of human lives. Promoting health is an economic segment of the financial – material investment, and on the other hand, heath structure's relies on efficacy and efficiency.

Any attempt of restructuration of the health care system in Romania must begin from the analysis of the way in which the ones financially supporting the health system think and react; the health subsystem, as part of the global social system, relies on market economy, on the contributor's financial effort. The quality and the efficacy of the ones coordinating the professional destinies of the health care system must provide a convincing answer, based on the criteria and the methodology of an economic analysis at the level of health care services and individual practice. It is very important to be aware of the utility, the purpose of the activity, the patient's satisfaction and the contribution to the global health of the two essential factors: ethics and economic under-layer.

In Romania, decentralization and the reform of the financing system were made without a serious analysis of the consequences on the population in terms of territorial inequity and of the availability of the infrastructures and implicitly of the services. Pros and cons (*pro et contra*) for decentralization of the health care system:

- *Pros* (advantages):
  - Subsystems can be organized considering the local values, available infrastructure, diseases, preferences and services;
  - Increase the income in the community (local) level and the efficiency in resources allocation
  - Decentralized units can be used as "laboratories" for national policies.
- *Cons* (disadvantages):
  - Possible additional costs;
  - System fragmentation;
  - Decisional centralism vs. Administrative decentralization;
  - Misappropriation of national programs or institutions redundancy.

NSPHHSMPD (SNSPMS - *National School of Public Health and Management B ucharest*) contributes to improving the performance of health care services and improving the health of the population in Romania by consulting services and technical assistance to decision makers and providers of healthcare services, offered in order to optimize health policies and health services and developing health promotion programs with the purpose of responsibility and increasing health education among population

Main actions include providing scientific evidence to support health decisions and policies; evaluation, application and dissemination of research results to improve health practices; conducting studies and operational research in order to increase health system performance; educating and informing the population on attitudes and behaviors related to health, healthy lifestyle and public awareness about the importance of individual involvement in the maintenance and/or improvement of the health status.

Short and medium-term steps for health care system reform in Romania should focus, among others on:

a. Streamlining hospital services, implementation of regional hospitals networks, and reducing redundant capacity for inpatient services;

b. Enhancing primary health care services at the community level;

c. Implementing specialized secondary ambulatory care;

d. Increasing sector governance and stewardship to: strengthen the implementation of HTAs improve the management control mechanisms; implement health system continuous quality improvement and health system performance assessment; adjust the DRG (*diagnosis-related groups*) payment mechanism; streamline the National Health Programs; create the conditions for increasing the participation of private funds and private services; develop a Human Resource Strategy; strengthen the communications strategy

Examples of Public Health Schools with HTA dedicated departments or programs: University of Alberta, UMIT (University for Health Sciences, Medical Informatics and Technology, Tirol, Austria), Harvard School of Public Health, University of Birmingham, University of Adelaide, Aarhus University, and National University of Singapore.

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NEWS

CLOSER AND CLOSER TO THE IDEAL BIOMARKER IN INFLAMMATORY BOWEL DISEASES

The role of the intestinal microbiota in the pathogenesis of inflammatory bowel disease (IBD) is more and more taken into consideration. Biomarkers obtained from alterations in the gut microbiome may be an alternative marker of disease presence and severity. A recent study showed that Trimethylamine-N-oxide (TMAO) production is dependent on the metabolism of GI tract microbiota. A case-control study carried out for 106 IBD patients and 373 non-IBD controls from southwestern Ontario found plasma TMAO levels to be significantly lower in patients with IBD. With further research, plasma TMAO levels may become a relevant, cheap, noninvasive means of diagnosing IBD (Wilson A, Teft WA, Morse BL, Choi YH, Woolsey S, DeGorter MK, Hegele RA, Tirona RG, Kim RB. Trimethylamine-N-oxide: A Novel Biomarker for the Identification of Inflammatory Bowel Disease. Dig Dis Sci. 2015 Dec;60(12):3620-30. doi: 10.1007/s10620-015-3797-3)

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CONTRIBUTIONS TO RECOGNITION OF SOME PARTICULARITIES OF THE LINGUAL MUCOSA CONDITIONS

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CONTRIBUTIONS TO RECOGNITION OF SOME PARTICULARITIES OF THE LINGUAL MUCOSA CONDITIONS **(Abstract)** Part of the oral cavity structure, the tongue plays an important role, both by its functions, and pathology, which sometimes can seriously damage the state of the entire body. As an important component of the dento-maxillary apparatus, the tongue has particular appreciative and perceptive abilities, also playing a role in articulation. The conditions of the mucosa and profound tissues of the tongue are generically named glossitis. Glossitis encompasses a wide range of conditions, from the common saburral deposits up to various forms of glossitis or the impressive tumoral lesions. **Material and method**: a clinical-statistical study has been conducted on a group of 24 patients, females and males, 55 - 70 years old, in order to monitor the detection of the lingual mucosa lesions. **Results:** the damage of the lingual mucosa of the examined patients was subject to statistical analysis, revealing 24 patients with lingual mucosa lesions. The analysis of the type of lesion highlighted the diversity of the presented forms of lesions. **Conclusions**: for a proper medical conduct, the dentist should know the degree of involvement of the aggravating factors in the onset of oral mucosa lesion, its particularities, and apply them in a customized manner; proper examination can be very important in detecting and treating these conditions. Keywords: PERIODONTAL MANIFESTATIONS, AGGRAVATING FACTORS, GLOSSITIS, CANDIDOSIS.

Tongue is a muscular – membranous organ, located in the proper oral cavity, which takes part to the formation of the floor of the oral cavity, essentially influencing the dynamics and morphology of the lower wall of the proper oral cavity (1).

During oral cavity examination, the dentist constantly meets this organ, therefore he/she must know the pathological states of the tongue. The proper tongue examination can offer very important data for detecting certain conditions, while the importance of knowing the tongue manifestations is also related to dentist's area of expertise (2).

Often, the appearance of the tongue is not only the expression of local manifestations, but can also indicate damage of important organs. This has been known and proved in Hippocrates's era, when the changes of the tongue mirrored the functionality of the digestive apparatus, liver, blood circulation, etc. This is why the patient's examination starts from checking

the appearance of the tongue.

The inflammatory forms of the tongue are called glossitis, which often shows as a symptom of certain diseases (3).

Superficial acute glossitis coexists most of the times with a general inflammation of the mucosa of the buccal cavity.

Lingual enanthema appear in eruptive fever or various forms of stomatitis. Glossitis is the early symptom of the diseases, in Biermer anemia, present in 80% of the cases as Hunter glossitis or as erythematous glossitis in hypochromic (4).

According to Klostermann, red or bluish tongue, with obvious atrophy of papillae and mucosa is frequently met in hepatic cirrhosis.

Glossitis frequently appears in combination with candidosis, especially after using antibiotics (5).

There are several classifications of lingual conditions, on etiologic, anatomic – chemical, evolutionary criteria, etc.

Not all lingual pathologic manifestations are under the competence of the dentist. That is why, in this paper, I will only write about the forms of illness mainly affecting the lingual mucosa, and less the lingual parenchyma. (3, 4).

The main goal of this paper is to identify the most frequent oral lesions of the tongue in elderly patients, and to present the information in an accessible manner, highlighting their variations during diagnosis. The importance is given by the fact that the changes are not only the expression of local disturbances, but they can also indicate severe general conditions.

MATERIAL AND METHODS

The study was conducted on 24 cases with various forms of glossitis. The study group consisted of 14 males (58.33%) and 10 females (41.67%), aged 45-60; 18 cases (75.00%) come from urban areas, while 6 cases (25.00%) live in rural areas.

TABLE I

Demographic parameters in studied cases

Parameter	Study group with glossitis (24)
Gender (n, %)	
F (female)	10 (41.67%)
M (male)	14 (58.33%)
Age (years)	53.07±7.27
Average age of the patient at the onset of disease (years)	52
Area (n, %)	
1 - Rural	6 (25.00%)
2 - Urban	18 (75.00%)

ANOVA test was used for descriptive statistical analysis. T-Student test, which takes into consideration the measurement of variability and the weight of observations, was used for quantitative significance.

The database was managed in Excel, Microsoft Office package, while the statistical analysis was made in statistical programmes MedCalc and Epi Info 2000.

RESULTS

We considered the possibility of the presence of general risk factors, since with advancement into age, the patients suffer from general conditions that, through their

evolution and medication (especially drugs that cause xerostomia), can be the causes of lingual mucosa conditions. Oral mucosa is the place of a great variety of lesions, including ulcerous, vesiculobullous, desquamative, lichenoid, infectious and malignant lesions (7, 8). Both the normal changes associated with old age, and the pathologic factors contribute to oral pathology. As we grow older, the mucosa atrophies, leading to a thinner and less elastic tissue. This change of the cell structure, in combination with the decline of the immunologic responses, facilitate the increased susceptibility to infections and traumas (10, 12). In adults, oral infections with ferments are more and more common as they grow older, especially in denture wearers (5). Other risk factors are: antibiotic therapy, xerostomia, uncontrolled diabetes mellitus, immunosuppressive drugs, corticosteroids (systemic and inhaled corticosteroids), and poor oral hygiene (8).

The analysis of the type of lesion revealed the diversity of the forms of lesions.

Diagnosis was set after ruling out all the possible etiologic factors, based on the patient's history, (6) physical evaluation and lab studies, by a thorough clinical examination, completed in some cases by a mycological examination (table II).

In the presentation of the clinical forms, it is obvious that *mycotic glossitis* prevailed (50%), to which the lab test confirmed the presence of yeast on the lingual mucosa.

Intraoral examination revealed the presence of a white-yellowish creamy deposit on the dorsal surface of the tongue, almost spread on the complete lingual surface. During palpation, the tongue is supple, unpainful. Scraping the deposit is not painful, removing the sticky mucosal deposits that leave behind a slightly bleeding lingual mucosa. This happened in white hairy tongue. In black hairy tongue, the colour of the deposit was brown-black, similar to coffee grounds (fig. 1)

TABLE II

Case distribution in relation with diagnosis

Forms of disease	No of cases	%
White hairy tongue	4	16,66
Black hairy tongue	2	8,33
Lingual candidiasis	2	8,33
Median rhomboid glossitis	2	8,33
Symmetric congenital plicated tongue	4	16,66
Marginal exfoliative glossitis	2	8,33
Erythematous glossitis	4	16,66
Saburral tongue	2	8,33
Glossodynia	2	8,33

Fig. 1. Black hairy tongue (clinical casuistry)

The lesion is mostly spread on the medial area of the tongue.

In another case, a female patient, 56 years old, wearer of upper and lower mobile dentures, showed small whitish deposits on the jugal mucosa and on the tongue, accompanied by an acute sensation of buccal dryness and motility disorders. The lab

examination confirms the presence of digestive and oral candidosis, as well as in cases of white hairy tongue (fig.2).

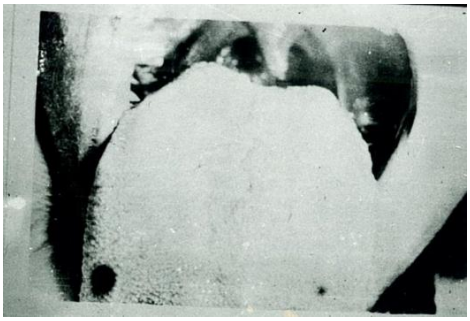

Fig. 2. White hairy tongue (clinical casuistry)

In all the 8 cases of candidosis (33.33%), patients were previously treated with various antibiotics for various infections.

There was one particular case, with *"median rhomboid glossitis"* diagnosis. During the clinical examination, we detected on the dorsum of the tongue, before the lingual V, a rhomboid area, depapillated, plane, neat, well demarcated, slightly recessed. The remaining tongue shows a white-creamy deposit, without any sensation of pain.

Median rhomboid glossitis (central papillary atrophy) is regarded as a congenital anomaly of the tongue, due to the persistence of the tuberculum impar, a depapillated area on the dorsum of the tongue, a rhomboid area, well demarcated in the midline of the tongue, immediately before the circumvallate papillae, with "kissing lesions" on the hard palate.

Recent studies have incriminated oral candidosis, an opportunistic infection, most of the times caused by the overspread of *Candida albicans*, as the etiopathology of the condition. Clinically, there are two forms: a plane form, red, neat, well demarcated, depapillated, slightly recessed comparing with the underlying normal mucosa; a mamelonated, hardened, reddish form, with depapillated, neat areas (fig.3).

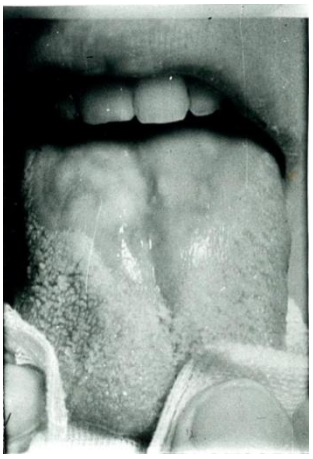

Fig. 3. Median rhomboid glossitis (clinical casuistry)

Median losangic glossitis is asymptomatic, but can be secondary colonized with *Candida Albicans*, provoking an inflammation with subjective signs. It is necessary to perform the differential diagnosis with: luethic glossitis, candidosis glossitis, geographic tongue (benignant migratory glossitis), lymphangioma, cancerous lesions. It is a chronic condition relatively frequent, usually asymptomatic.

The case which we diagnosed with this form of glossitis was a 49 years old woman, to whom the mycological examination showed the lack of levuric infection.

The 4 cases (16.66 %) of symmetric congenital plicated tongue were met in 49 and 60 years old men. In all 4 (16.66%) asymptomatic cases, the patients were not aware of this problem. Clinically, 2 (8.33%) had a scrotal character, while the other 2 (8.33%), a cerebriform character. The sur-

face of the tongue is crossed by numerous sulci, more or less profound, which imitate the appearance of a leaf, or of the brain (typical appearance). It usually appears in gastric conditions or other diseases. Informing the patients in this condition, an appropriate oral hygiene was recommended, avoiding local irritants of any type (fig.4).

Fig. 4. Scrotal tongue (plicated, symmetric congenital) (clinical casuistry)

In other situations, the patients who wore older removable acrylic dentures were diagnosed with erythematous glossitis, and erythematous stomatitis. They complained of burning and itching sensation on the buccal mucosa, and they had a poor oral hygiene. To limit the development of caries, it is important to improve the oral hygiene. (14).

They also complained about a metallic, sour or salty taste. In these kind of conditions, the burning sensation is continuous, but it doesn't affect the eating and sleeping. The Burning Mouth Syndrome is characterized by the constant burning sensation in the oral mucosa and/or the tongue, without other clinical and laboratory data (10). These sensations are caused by physiologic or psychic phenomena, related to menopause, accompanied by the redness or pallor of the skin, sweat, and palpitations.

During the clinical examination, the lingual mucosa was congested, with hypertrophied filiform papillae, appearance of a burned tongue.

Out of the 24 cases we studies, a retired woman had glossodynia. This patient was deeply affected by her suffering tongue.

The patient complained about lingual pain or itching during the contact with food, but also spontaneously, without any objective cause. The patient associated the lingual symptoms with the presence of a thicker, pathologic saliva. The clinical examination of the tongue did not reveal any change. The odontal examination also ruled out any lesion that might explain the lingual complaints.

The treatment consisted of sedative drugs, associated with antiseptic, alkaline therapy of the oral cavity, together with psychotherapy.

DISCUSSIONS

In general, there is no final remedy. To be able to determine the right diagnosis, one should do the biopsy of any lesion of the mucosa that does not fit the expectations on a certain period of time, or which persists despite all attempts to resolve the etiology, as Miriam R. Robbins recommends (Department Oral and Maxillofacial Pathology, New York University) (quoted 6,7).

We must assure patients that they do not suffer from an infectious or malignant condition, but they deal with chronic pain. After 6 – 7 years, approximately 50% of the patient experience an improvement of symptoms, as Sardella et al. states (9). Various topic and systemic treatments were suggested, with various evidence in their favour (10). There is some evidence in favour of cognitive behavioural therapy as adjuvant of pharmacologic therapies (9).

For a proper medical conduct, the den-

tist should know the degree of involvement of the aggravating factors in the onset of the oral mucosa lesion, their particularities and apply them in a customized manner.

CONCLUSIONS

24 cases have been studied, with various forms of damage to the lingual mucosa, with 4 (16.66) cases with erythematous glossitis (more severe symptoms) and 2 cases of glossodynia.

There was one distinct case, consisting of a form of median losangic glossitis (rhomboid glossitis), but this happens rarely.

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INTERACTION BETWEEN ANGIOTENSIN II, HYPERTENSION AND INFLAMMATION IN RAT KIDNEY

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INTERACTIONS BETWEEN ANGIOTENSIN II, HYPERTENSION AND INFLAMMATION IN RAT KIDNEY (Abstract): The renin-angiotensin system is one of the most important regulatory mechanisms of blood pressure homeostasis. Hypertension, a risk factor for chronic kidney disease, is able to induce renal injury by promoting inflammation. Inflammatory cytokines can induce fibrosis in various organs, including the kidney. **Aim:** The study verifies if angiotensin II is implicated in kidney T cells infiltration. **Material and Methods:** The experiment was performed on Wistar male rats, divided in two groups who received either a sham operation (control group, n=9) or continuous angiotensin II (Ang II) infusion (300ng/kgc/min)(Ang II group, n=9) subcutaneously, via minipumps. Blood pressure was measured noninvasively (tail cuff). After 14 days, the animals were sacrificed under anesthesia with xylazine/ketamine. The tissue obtained of each kidney was used for histopathological exam or flow cytometry. Renal inflammation was assessed using a flow cytometric analysis. The Histo Quest program quantified automatically the cellular elements and performed a statistical analysis on the obtained data. **Results**: Inflammation was observed in Ang II Wistar rats group, accompanied by an increase in renal CD45RC+, CD45RA+, CD19+. **Conclusions**: Our research underlines an undefined role for T cells in the genesis of high blood pressure up to that time and sustains the involvement of inflammation in the pathogenesis of this prevalent disease. Thus, T cells might correspond to a novel therapeutic target for the management of hypertension. **Keywords:** ANGIOTENSIN II, HYPERTENSION, KIDNEY, INFLAMMATION

Renin-angiotensin-aldosteron system (RAAS) is a hormonal system that contributes on regulation of arterial pressure (1, 2). The increase of RAAS, especially by Ang II levels, affects target organs and increases the risk of cardio-vascular disease by raising blood pressure and also by a direct effect that Ang II has on vascular endothelium of renal and cardiac tissue (3, 4), promoting inflammatory phenomena and arteriosclerosis. Ang II reduces the renal capacity of sodium excretion and initiates a range of events that leads to increased blood pressure (5, 6). Ang II also mediates key events related to inflammatory processes (7). Ang II leads to the accumulation of inflammatory cells in the tissues by stimulating the production of cytokines / chemokines. RAAS plays an essential role in morphopathologic changes that

produce progression of renal diseases. Renal inflammation represents the early stage of renal injury and is followed by tubulointerstitial fibrosis, tubular atrophy and glomerulosclerosis (8).

MATERIAL AND METHODS

Male Wistar rats weighing about 250g, were divided into two groups: the first group (control) made of animals who received continuously isoosmotic solution using osmotic minipumps Alzet, model 2001-1µl / h (9 rats) and the second group (9 rats) who received continuously Ang II infusion (300ng/kgc/min) (Bachem - 1008593) subcutaneously, via minipumps (Alzet), for 14 days. Systolic blood pressure was measured noninvasively using tail-cuff plethysmography (Biopac), every 3 days. After 14 days, the animals were sacrificed under anesthesia with xylazine/ketamine. Each kidney was divided into 2 equal pieces: a piece was immersed in 4% paraformaldehyde for tissue fixation in order to perform a histopathological exam (Hematoxylin-Eosin or Szekely trichromic methods) and the other was used to perform immunophenotyping (flow cytometry). The Histo Quest analysis program allowed the study of renal periglomerular and tubulointerstitial inflammatory reaction. Renal inflammation was assessed by quantifying CD45RC, CD45RA expression, using a flow cytometric analysis (6). Results were expressed as mean ± SEM. All protocols were approved by the Animal Care and Use Institutional Committee of the University of Medicine and Pharmacy "Gr. T. Popa".

RESULTS

Systolic blood pressure

The values of systolic blood pressure (BP) were similar among the two groups before the treatments. However, systolic BP progressively and significantly increased to 200±7 for Ang II vs. 120±5 mmHg for Sham at day 14 (fig.1).

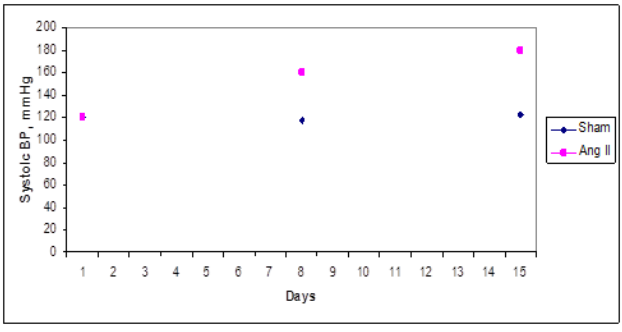


Fig. 1. Blood pressures at baseline and after angiotensin II infusion measured by tail cuff

Kidney inflammatory infiltration area

Histo Quest program allowed the mapping and the separation and quantification of all nuclei related to the periglomerular and tubulointerstitial infiltrate (fig. 3-14). We used as analysis criteria the following parameters: nuclear size and intensity of the nuclei in Hematoxylin-Eosin stain.

Flow cytometric analysis

CD45RA. As demonstrated in figure 16A, B, chronic Ang II infusion significantly ($p<0.005$) increased CD45RA (5.5±0.5% for Ang II vs. 2.7±0.8% for sham). Absolute numbers of T cells (CD45RA) in kidneys

from sham and angiotensin II infused rats were expressed as the means ±the SEM.

CD45RC. As shown in figure 17A, B, chronic Ang II infusion significantly decreased CD45RC ($p<0.05$). Data are expressed as the means ±the SEM.

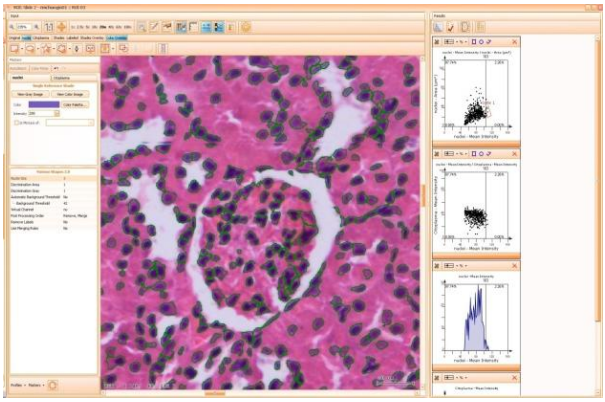


Fig. 3. Periglomerular inflammatory analysis in control group

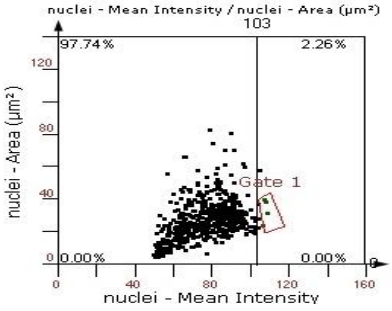


Fig. 4. Scattergram: graphical representation of periglomerular inflammatory infiltrate(2.26%) (control group)

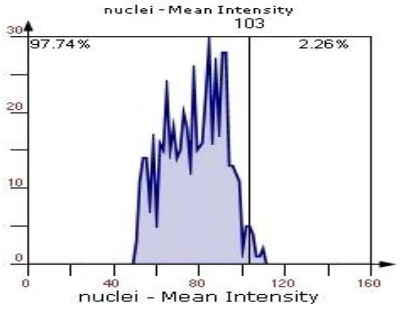


Fig. 5. Histogram: graphical representation of periglomerular inflammatory infiltrate (2.26%) (control group)

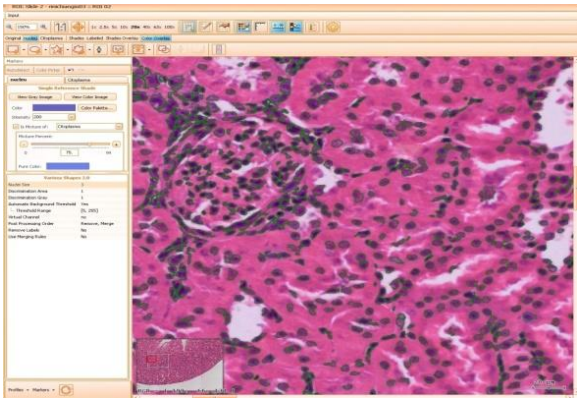


Fig. 6. Periglomerular inflammatory analysis in Ang II group

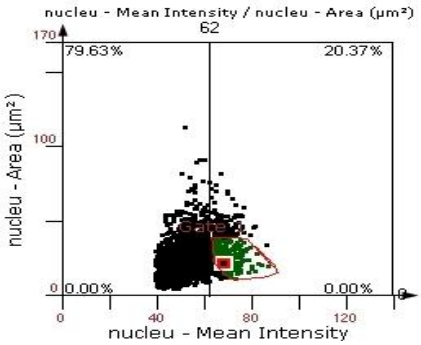


Fig. 7. Scattergram: graphical representation of periglomerular inflammatory infiltrate (20.37%) (Ang II group)

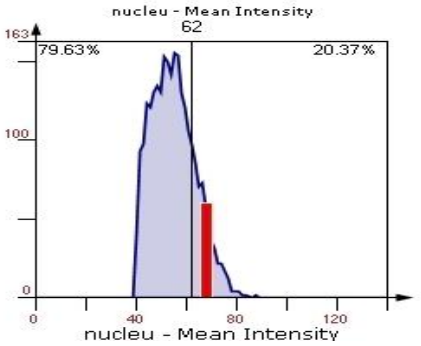


Fig. 8. Histogram: graphical representationof periglomerular inflammatory infiltrate (20.37%) (Ang II group)

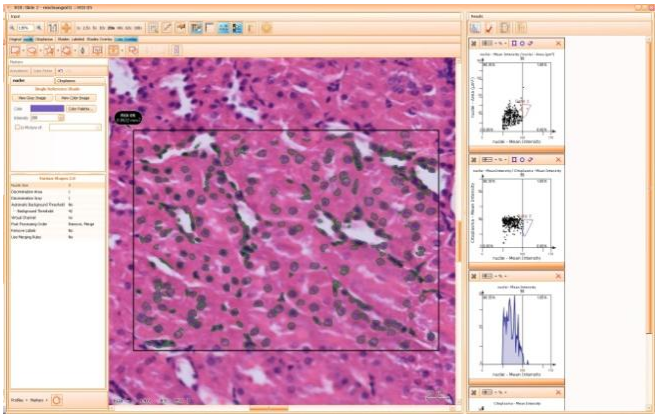


Fig. 9. Tubulointerstitial inflammatory infiltrate analysis in control group

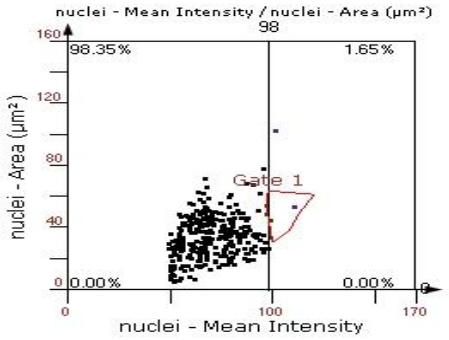


Fig. 10. Scattergram: graphical representation of tubulointerstitial inflammatory infiltrate (1.65%) (control group)

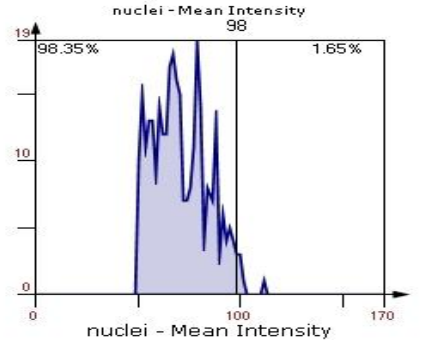


Fig. 11. Histogram: graphical representation of tubulointerstitial inflammatory infiltrate (1.65%) (control group)

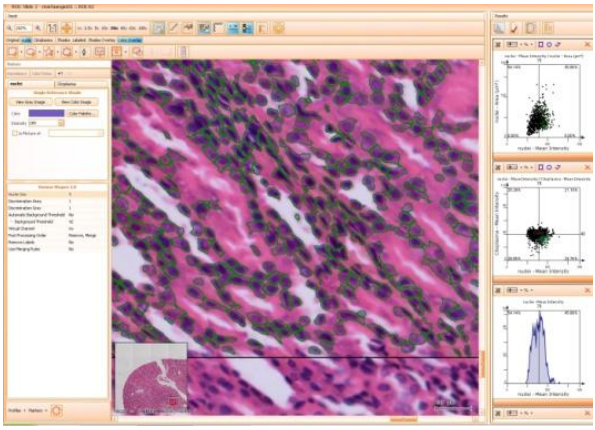


Fig. 12. Tubulointerstitial inflammatory infiltrate analysis in (Ang II group)

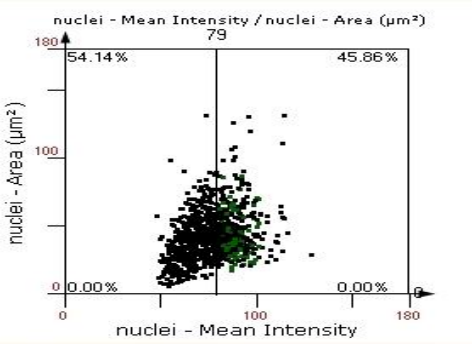


Fig. 13. Scattergram: graphical representation of tubulointerstitial inflammatory infiltrate (45.86%) (Ang II group)

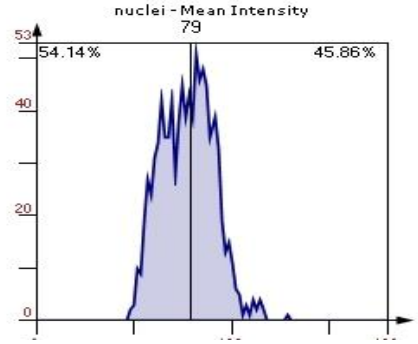
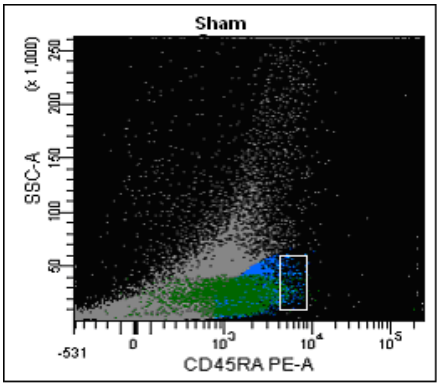
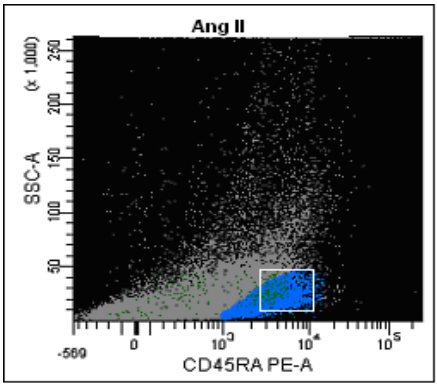


Fig. 14. Histogram: graphical representation of tubulointerstitial inflammatory infiltrate (45.86%) (Ang II group)

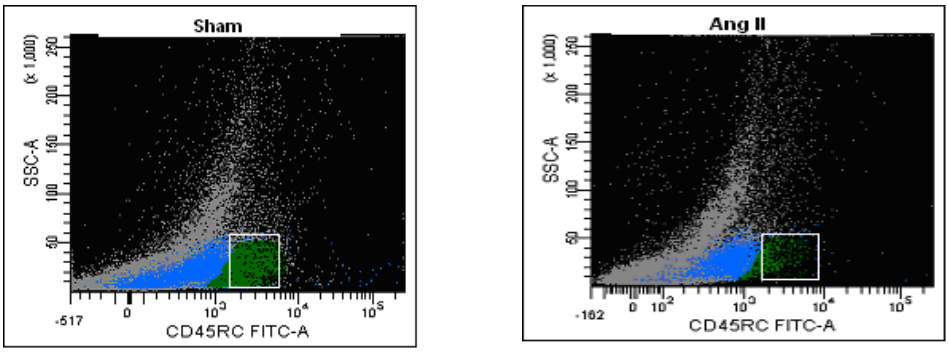


A



B

Fig.16. Flow cytometric analysis in kidneys cells -CD45RA (A: Sham; B: Ang II)



A B

Fig.17. Flow cytometric analysis in kidneys cells - CD45RC (A: Sham; B: Ang II)

DISCUSSION

The chronic kidney disease might be determined by varied conditions as hypertension, diabetes, nephritis, inflammatory and infiltrative diseases, etc. Into a varying number of cases, renal injury becomes chronic, progressive and irreversible and often requires substitution therapy (dialysis or renal transplantation) (9). Although renal impairment begins at the glomerular, tubular or renovascular level, the progression to chronic kidney disease is accompanied by common histological and functional changes, going to progressive glomerulo-sclerosis. Hypertensive nephropathy is initiated by an increased intraglomerular pressure that activates and destroys glomerular cells, including mesangial and epithelial cells, and also the podocytes. These cells produce proinflammatory and vasoactive mediators that contribute to the cell damage, the decrease of renal plasma flow and of the glomerular filtration rate (10, 11).

In a study carried out by Lombardi et al. (12), they showed that after chronic administration of 435ng/Kg/min Ang II for 2 weeks, to male Sprague-Dawley rats, renal histological changes including focal vascular damage (damage of peritubular capillaries) and tubulointerstitial injury occured (tubular atrophy and mononuclear cell infiltration). These changes are found both in the juxtaglomerular area and in the superficial cortex (13).

In the present study we intended to demonstrate that Ang II-induced hypertension leads to lymphocytic infiltrate in the kidney. In our experiment, we observed that in the control group, consisting of animals that received isoosmotic solution through minipumps and were subjected to the same laboratory conditions, histological examination revealed a normal appearance of the juxtaglomerular apparatus and a normal appearance of the renal glomerulus, afferent and efferent arteriole and renal tubules. The histological examination of kidney sections from rats chronically treated with Ang II for 14 days revealed presence of inflammatory infiltrate with lymphocytes.

CONCLUSIONS

In our study angiotensin II increased the activation of T cell markers in kidney tissue. T cells have an important role in the genesis of high blood pressure, pointing the inflammatory infiltrate involvement in the pathogenesis of this disease. Thereby, T cells might represent a novel therapeutic

target for the treatment of hypertension.

ACKNOWLEDGMENTS

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THE CORRELATION BETWEEN MARKERS OF SYSTEMIC INFLAMMATION AND ANGIOGENIC MARKERS IN PRE-ECLAMPSIA

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THE CORRELATION BETWEEN MARKERS OF SYSTEMIC INFLAMMATION AND ANGIOGENIC MARKERS IN PRE-ECLAMPSIA (Abstract): Pre-eclampsia is a severe multisystemic syndrome that represents a major cause of maternal, foetal and neonatal mortality and morbidity. In the study we conducted it stood out the significant modifications of angiogenic markers in pregnant women suffering from pre-eclampsia and the existence of a correlation between C-reactive protein (CRP) and SBP, DBP and average BP. **Material and method:** The group included in the study consisted of 138 pregnant women hospitalized at the "Cuza-Voda" Clinical Hospital of Obstetrics and Gynaecology of Iasi, between 2012-2014, with over 20 weeks gestational age and which gave their free consent to take part in the study. **Results:** It is confirmed the importance of determining the markers for diagnosing and monitoring hypertensive pregnant women and at the same time it was pointed out that the sFlt-1/PlGF ratio represents a good pre-eclampsia predictor. **Conclusions:** The results of our study confirm the importance of determining sFlt-1 and PlGF as markers for diagnosing and monitoring pregnant women with HBP as well as the sFlt-1/ PlGF ratio which represents a good pre-eclampsia predictor. **Keywords:** MOLECULAR MARKERS, PRE-ECLAMPSIA, PLACENTAL DYSFUNCTIONS, ENDOTHELIAL DYSFUNCTION, HELP SYNDROME.

Preeclampsia represents a pathologic condition specific to the pregnancy period, with an incidence of 3-5%, characterized by *de novo* hypertension and significant proteinuria (>300mg/24h), that frequently begins after the 20th gestational week. It is a systemic vascular disease that can affect numerous organs leading to severe complications for both the mother and the fetus. Although the exact causes that determine the apparition of pre-eclampsia are not known, it was noticed that placental dysfunctions play a key role in its apparition, being correlated with the subsequent maternal endothelial dysfunction (1-5).

The low vascularization of the uterus leads to the deficitary development of the fetus, with consequences on its further development (6, 7, 8). The clinical manifestations can vary from mild to severe forms, reaching its peak with the HELLP syndrome (haemolysis, growth of hepatic enzymes and

thrombocytopenia) and eclampsia (apparition of convulsive crises) (6, 9).

In Romania, the annual incidence of preeclampsia cases is between 6-14%, varying between 10 and 14% at primiparas and 5.7-7.3% at multiparas with a significantly increased incidence of preeclampsia cases in women with gemelar / multiple pregnancy, in the one with pre-eclampsia at previous pregnancies, in primiparas under 20 and over 35 years (20).

Considering the prevalence of this disorder, especially in developed countries, the existence of a validated biological marker allows a more careful supervision of asymptomatic patients so to anticipate complications with lethal risk. An ideal biological marker should have: high sensitivity and specificity level, predictive capacity for the prognostic and the evolution of the disorder as well as for the response to treatment, low accessibility and invasive level for collecting; its testing should be simple, effective and cheap.

C-reactive protein (CRP) is considered a sensitive marker of endothelial dysfunction and inflammation (10), but there are divergent opinions when referring to the value of CRP as a pre-eclampsia marker (11).

This study focused on assessing the clinical and progressive aspects of pre-eclampsia, by checking the correlation between inflammatory parameters, represented by C-reactive protein and blood pressure, on a group of pregnant women with pre-eclampsia and pregnancy induced hypertension (PIH) compared to a group of normal pregnant women. At the same time, it was intended to create a mathematic model to associate a predictor factor as PlGF, sFLT-1 or the PlGF/sFLT-1 ratio with the clinical parameters assessed in pregnant women with pre-eclampsia.

MATERIAL AND METHODS

This was a retrospective, case-control type of study and focused on studying the clinical-progressive aspects of PE, by checking the correlation between the inflammatory parameters, represented by CRP and blood pressure, in a group of pregnant women with pre-eclampsia and PIH compared to a group of normal pregnant women. 138 pregnant women hospitalized at the "Cuza-Vodă" Clinical Hospital of Obstetrics and Gynecology of Iasi were selected, between 2012-2014, with over 20 of weeks gestational age, which requested a specialized consultation and gave their free consent to take part in the study. At the same time, the study received the approval from the hospital's ethics commission. These patients were collected biological samples to determine their CRP not being the case of an infectious situation or spontaneous premature rupture of membranes. At the same time, there have also been determined the PlGF and sFlt-1 biomarkers and it was calculated the sFlt-1/ PlGF ratio, as a prognosis instrument for the patient with pre-eclampsia, depending of the influence of the cumulative risk factors.

The criteria for including them into the study group were: gestational age > 20 weeks; high blood pressure and proteinuria; the exclusion criteria were: gestational age < 20 weeks; high blood pressure pre-existent to pregnancy; spontaneous rupture of membranes; associated infectious pathology (chorioamnionitis, urinary infections, respiratory infections), seropositivity to HIV.

RESULTS AND DISCUSSION

Lab analyses. Hematological and biological investigations were conducted in the laboratory of medical analyses from the

"Cuza Voda" Maternity of Iasi, while the immunological ones were conducted within the Immunology laboratory from the "Sf. Spiridon" University Hospital of Iasi. For the hematological investigations total blood was used, collected on K_3EDTA type anticoagulant using an automatic hematology analyzer (Celltac MEK - 6318K). The biochemical investigations used serum obtained after collecting the blood into "clot activator" type test tubes and spinning it at 4000 rpm, for 10 minutes. In determining the biochemical parameters it was used an automatic analyzer of Clinical chemistry, RX Imola model, along with calibrators and control serums. The immunological investigations were made for the C reactive protein using the chemiluminescence technique and a IMMULITE 2000 type automat, while for the PlGF and sFlt-1 markers, it was used the chemiluminescence technique with the COBAS E411 ROCHE analyser. The detection limits for PlGF-1 were between 1-1.500 pg/ml, and for sFlt-1 were between 100-30.000 pg/ml. Proteinuria was determined from the urine samples collected upon hospitalization, using the VITROS 950 dry chemistry automatic analyzer and diluting the samples accordingly.

A clinical picture was made for each woman. It was recorded the height of the pregnant woman and the evolution of the weight during the pregnancy period. The dates and the hours of the results of the collected proteinuria were recorded in 24 hours, where appropriate. The markers of the inflammatory process were determined (CRP). At the same time, there were registered the dates and the hours when the highest blood pressure values were recorded during the pregnancy. By monitoring the blood pressure value, in the previous chapter, there were pointed out the correlations between the markers of systemic inflammation and cardiovascular risk.

Laboratory tests were conducted on all pregnant women, the blood tests being focused on the parameters of the hepatic function (TGO, TGP, bilirubin, LDH) and renal function (urea, creatinine, uric acid), hematological parameters (thrombocytes, white globules), coagulation indexes (INR, Quick time, and APTT), of the inflammatory process (CRP, fibrinogen) and determining the serum concentrations of angiogenic markers (sFLT-1 and P1GF), by calculating their ratio (sFlt-1/P1GF) and establishing the appropriate statistical correlations.

The data were processed and assessed using the statistic functions from SPSS 19, at the significance threshold of 95% ($p<0.05$). The statistical analysis used the F (ANOVA) test, χ^2 test, the Spearman correlation coefficient, the coefficient of determination (R^2) and the sFlt-1/PlGF factor.

Considering the clinical picture: HBP associated to proteinuria and/or edemas 3 study groups were created: PE group - 54 pregnant women (39.13%) with pre-eclampsia (PE), aged between 19 - 41 years; pregnancy induced hypertension group (PIH) - 34 pregnant women (24.63%) with high blood pressure values and/or insignificant proteinuria (<300 mg/24h) and/or edemas, aged between 22 and 44 years; normal pregnant women group (NG) - 50 pregnant women (36.24%) aged between 16 and 41 years.

From the 138 pregnant women included in the study, most of them were in the age group of 25-34 years (51.45%), the frequency peak being somewhere around the age of 29 years old. Depending of their gestational age, in the PE group the patients were generally in 33-36 weeks (37.5%), the patients in the PIH group had

a homogenous distribution for the gestational age intervals of 33-36 weeks and 37-40 weeks (29.4%), compared to the witness group (NG) where the patients were mostly over 37 weeks (76%).

Edemas were present in 50% of the pregnant women with PE and in 29.4% of the ones with PIH, the difference in percent being significantly higher compared to the witness group (p=0.001). Proteinuria was found in almost 87.5% of the patients in the PE group and 64.7% in the one with PIH. Pathological urinalysis was observed in 57.1% of the pregnant women with severe pre-eclampsia and in 44.7% of the pregnant women with milder types of pre-eclampsia, frequency distributions that were not statistically significant (p=0.833).

HBP and proteinuria are not invariably present; HBP may appear late in the evolution of PE and proteinuria can be absent in case of milder types of pre-eclampsia (12). On the other hand, there have also been cases of HBP during pregnancy that did not necessarily lead to pre-eclampsia, as it is the case of gestational hypertension and chronic hypertension. For the groups studied, the Systolic blood pressure (SBP) was significantly higher in the case of pregnant women with PE (p<0.001), with variations from 120 to 180 mmHg, the group mean being of 153.44±18.95 mmHg. Most pregnant women in the group with PE recorded values of systolic blood pressure between 143-164 mmHg (37%), but 24% of the pregnant women with PE recorded values higher than 160 mmHg. The pregnant women in the PIH group recorded values of SBP between 120-200 mmHg, with a group mean of 148.65±21.03 mmHg. In normal pregnant women, the systolic blood pressure varied between 100-165 mmHg, the mean value of the group being of 120.40±11.99 mmHg. The diastolic blood pressure (DBP) was significantly higher in the pregnant women from the group with PE (p<0.001), with variations from 70 to 150 mmHg, the group mean being of 92.50±14.83mmHg. Most pregnant women from the PE group recorded values of DBP between 85-100 mmHg (35%), but 26% of the pregnant women with PE recorded values of higher than 120mmHg. The pregnant women from the PIH group recorded values of DBP between 60 and 120 mmHg, with a group mean of 88.80±14.17 mmHg. In normal pregnant women, the DBP varied between 55 and 115 mmHg, the mean value of the group being of 72.56±11.09 mmHg. Depending of the growth of DBP, PE was grouped in two distinct clinical categories: mild (<110 mmHg) and severe (>110 mmHg). In the cases studies, it resulted 13% of pregnant women with severe pre-eclampsia and 87% pregnant women with mild pre-eclampsia. The severe types of PE were identified in the patients around the age of 37 years, while the mild types of pre-eclampsia were associated to the ages of 28-29 years (p=0.020).

The mean values obtained for the body mass index (BMI) indicated significant over weight (BMI=30.52 kg/m^2) in the patients from the PE group (p=0.001) and morbid obesity in 11.8% of the patients in PIH group compared to the witness group (NG). Depending of the BMI variation during pregnancy it was noticed that pregnant women with PE recorded body weight growth by more than 32%, while the pregnant women with PIH, the BMI growth was of almost 18%. Gestation and parity did not point out significant differences between the monitored groups (p>0.05)

In our study it was noticed that the women at their first pregnancy, teenagers and the women who remained pregnant after the age of 40 years presented a higher

risk of being affected by pre-eclampsia. The values of the biological markers indicated significantly higher differences for the PE group compared to the normal group (NG) for CRP (p=0.001), TGO (p=0.001), uric acid (p=0.001), serum urea (p=0.005) and proteinuria (p=0.001). In the cases when the pregnant women developed PE, the mean value of PlGF was significantly lower (p=0.003) and sFlt-1 was significantly higher (p=0.001) compared to the NG group. At the same time, the angiogenic factors were statistically significantly correlated with the mean values of BP. Thus, PlGF was significantly correlated with the age of the pregnant woman and with high mean BP (r=0.831; p=0.01). The sFlt-1 factor was significantly correlated with age, the mean BP and the high levels of LDH and TGO (r=0.784; p=0.046), being a better pre-eclampsia predictor than PlGF. The sFlt-1/ PlGF ratio was significantly higher at the group of pregnant women with PE (p=0.001) compared to the NG. The plurality of risk factors: age, mean blood pressure, LDH, TGO, INR, TQ and CRP were significantly correlated with the sFlt-1/ PlGF ratio.

CRP varied from values under 6 up to 192 mg/l recording a mean value significantly higher in the group of pregnant women with pre-eclampsia compared to the group of pregnant women with PIH (35.89 *vs* 6.72 mg/l) (p=0.001)

The individual values of CRP in the group of pregnant women with PE varied from 6 to 192 mg/l, most pregnant women recording values in the reliability interval (RI 95%):26.18-45.60 mg/l (42.6%), but 14.9% of the pregnant women with PE recorded values significantly higher of this parameter. In the case of pregnant women with PIH, CRP presented some values over the reference limit (=6 mg/l), but most of them presented values < 6 mg/l (82.1) (tab.1).

TABLE I

The values of some statistical indicators depending of the severity of pre-eclampsia

STUDY GROUP	N	Mean	Std. deviation	Std. error	Reliability interval		Min	Max	p
					- 95%CI	+95%CI			
Mean blood pressure (mmHg)									
Mild (moderate)	47	116	7.43	1.55	112.05	119.87	100	137	0.001
Severe	7	155.29	11.18	4.22	144.95	165.62	140	170	
Total	54	121.87	16.57	2.25	117.35	126.39	90	170	
C-reactive protein (mg/L)									
Mild (moderate)	47	27.45	24.42	5	17.20	37.50	6	96	0.001
Severe	7	89.14	51.31	19.39	41.69	136.60	48	192	
Total	54	35.89	35.57	4.84	26.18	45.60	6	192	

In the group of pregnant women with PE the work hypothesis was confirmed, the plasmatic level of CRP is in direct, statistically significant correlation with the blood

pressure. The values of CRP were directly correlated, having statistical significance, with both SBP (r=0.460; p=0.001) and DBP (r=0.614; p=0.001) as well as with the mean calculated pressure (r=0.612; p=0.001)

The mean values of CRP were significantly higher in the case of pregnant women with severe PE (89.14±51.31mg/L) while the pregnant women with mild PE the mean value was of 22.86±21.35 mg/L. In the cases studied, the mean values of Hb indicated a mild anemia in the group of pregnant women with PE (p=0.138) without a significant correlation with its severity (p=0.451). The hematocrit varied from 32.7 to 43.5% in the group of pregnant women with PE, recording a mean value of 37.25%±2.63 that is not significantly higher if compared to the values of the hematocrit recorded in the group of pregnant women with PIH 36.88% ±3.49 (p=0.102). In the cases of severe PE, the mean values of the haematocrit were slightly lower (33.93%±2.92) compared to the ones recorded in mild cases (34.87%±6.11) (p=0.847).

It was also observed that the plasmatic level of CRP was directly correlated also with the individual values of hemoglobin or hematocrit, but without any statistical significance (13).

In the group of pregnant women with PE the individual values of PlGF recorded a variation between 16.37-942 pg/mL with a mean value (119.3 pg/mL) significantly lower if compared to the mean value recorded in the witness group (327.57 pg/mL) or the PIH group (129.13 pg/mL) (p=0.003).

It was observed that the patients under 20 years of age had the PlGF median of 200 pg/mL, and at the patients aged between 20 and 34 years the median slightly surpassed the value of 100 pg/mL; in the pregnant women aged over 35 years the median of PlGF values was below 100 pg/mL. Depending of the gestational age the mean value of PlGF was significantly higher in the gestational weeks 33-36 (245 pg/mL) and 37-40 (254.4 pg/mL) (p=0.049). The correlations between the individual values of PlGF and the maximum values of blood pressure recorded were indirect, of moderate intensity. The higher values of PlGF were accompanied by lower values of systolic blood pressure (r= -0.359; R^2=0.1771, p=0.001) or diastolic (r= -0.320; R^2=0.1331, p=0.003). The correlation between the individual values of PlGF with BMI was not statistically significant (r= -0.126; R^2=0.016. p=0.255).

The values obtained for the sFlt-1 factor varied from 1152 to 32708 pg/mL with a variation of more than 92%, recording in the PE group the highest mean value (14365±6464), significantly higher if compared to the other study groups (p=0.001). Correlated to the age group, the mean values of sFlt-1 did not indicate statistically significant differences (p=0.342); nonetheless there have been noticed higher mean values at the age group of over 35 years. Depending of the gestational age, the mean value of sFlt-1 was significantly higher in the gestational weeks 25-28 (13867 pg/mL) and 29-32 (13033 pg/mL) (p=0.001). The correlations between the individual values of sFlt-1 and the maximum values of blood pressure recorded were direct, of moderate intensity, and statistically significant. The higher values of sFlt-1 were accompanied by high values of systolic blood pressure (r= +0.397; R^2=0.3445, p=0.001) or diastolic (r= +0.430; R^2=0.2755, p=0.001). There were not any significant correlations between the individual values of sFlt-1 and BMI (r= -0.136; R^2=0.0184, p=0.222) (Fig.1).

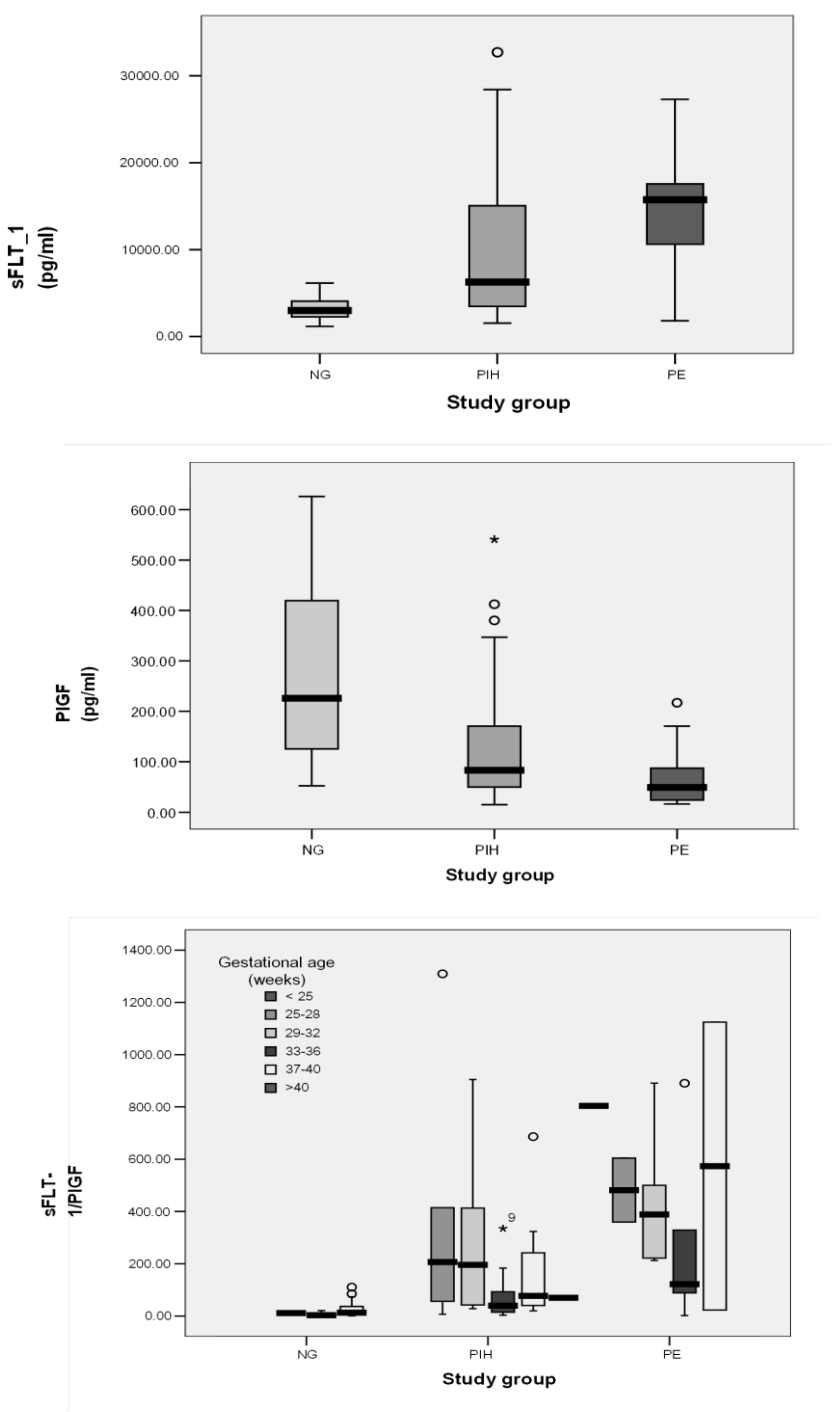

Fig 1. Mean values of PlGF, sFlt-1 and the sFlt-1/ PlGF ratio on study groups

The values of the sFlt-1/PlGF ratio indicated significant growth for the PE group compared to the witness group NG or PIH (p=0.001). In the patients with preeclampsia, it was observed a direct correlation, of moderate intensity, between the systolic blood pressure (SBP) and sFlt-1/PlGF (r= +0.555; p=0.026), but the high values of diastolic blood pressure (DBP) are associated with high values of sFlt-1/PlGF only in 18.5% of the patients (r=+0.185; p=0.493). At the patients from the PIH group, the correlation between SBP (r=+0.014; p=0.937) and DBP (r=+0.105; p=0.555) and the sFlt-1/PlGF ratio were not statistically significant.

The high values of CRP were accompanied by high values of sFlt-1/PlGF ratio only in 13.8% of the patients with PE (r= +0.138; p=0.724) and in 48% of the patients with PIH (r=+0.480; p=0.015). Correlating the values of PlGF with the preeclampsia risk factors it was noticed that the logistic model indicated an important cumulative risk depending of the age of the pregnant woman and the presence of high mean blood pressure (p=0.01 - Model 2) (fig. 2).

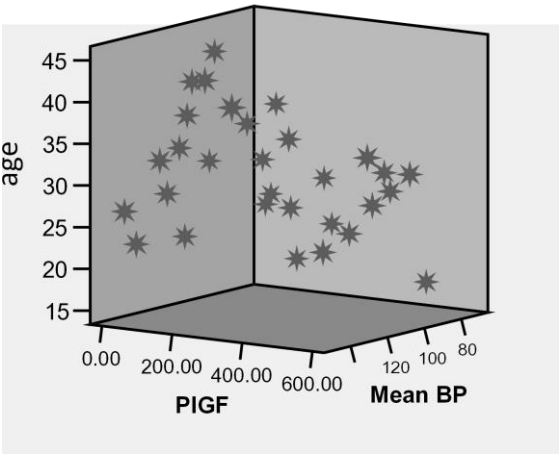

Fig. 2. PlGF correlation with age and mean blood pressure

It was noticed that PE was characterized by high levels of sFlt-1 associated with HBP, proteinuria and high values of CRP (p=0.049 - Model 8).

Correlating the values of sFlt-1 with the risk factors for PE it was noticed that the logistic model indicated an important cumulative risk depending of the age of the pregnant woman, the presence of high mean blood pressure and high LDH values (p=0.047 - Model 3) and the pregnant woman's age, the presence of high mean blood pressure and high LDH and TGO values (p=0.046 - Model 4) (fig. 3).

The logistic model indicated that a group of risk factors as age, blood pressure, LDH, TGO, INR, TQ and CRP were significantly correlated with the sFlt-1/PlGF ratio (R=0.663; p=0.04).

Nowadays there isn't any single objective test to identify PE or to quantifying the risk of certain severe complications, the diagnosis relying only on the clinical lab parameters evaluated in dynamics. Experience shows that early identification and the monitoring of the mother and fetus are use-

ful and hence the prediction of PE becomes very important. Early identification of PE, not only based on the inventory of risk factors, but also with the use of some markers might indicate better the moment suitable for beginning the treatment, its action and the moment to remove the child. Because of the fact that these modifications appear a long time before the apparition of the clinical signs of the disease, recent research focus on the endothelial dysfunction as a physiopathological mechanism of PE.

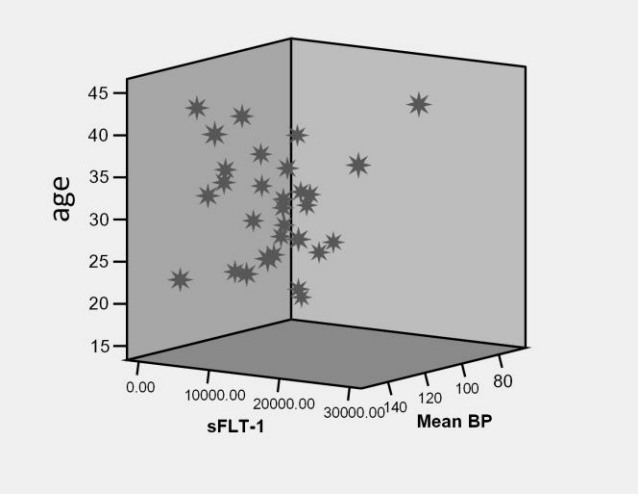

Fig. 3. sFlt-1 correlation with age and mean blood pressure

Many researchers (14, 15) noticed that the inflammatory maternal answer in PE and especially in severe PE, being known that there is an exaggerated systemic maternal inflammatory answer (16). In this context, it is considered that PE, at a given moment in its evolution, borrows a link of the systemic inflammatory answer that combines with the endothelial dysfunction. The increase of CRP indicates the presence of an inflammatory, destructive, infectious or non-infectious process but not specific. In this study we have demonstrated that the plasmatic level of CRP is significantly and directly correlated with SBP and DBP.

Thus, Can (17) use the mean arterial pressure as a severity indicator and he demonstrated the direct association with the inflammatory reaction. This result joins the studies that support the affirmation that CRP is an effective marker for the apparition of PE and it correlates significantly with the severity of the disorder.

Carl et al. (18) demonstrated that a value of more than 3 mg/L is a good predictor for cardiovascular and inflammatory risk in women with pre-eclampsia / eclampsia antecedents. At the same time, Mihu D et al. (10), state that CRP is a marker for PE's severity and for the new-born's birth weight. A prospective study, initiated by Behboudi et al. (20) on a group of 778 pregnant women, established a reference value of 4.5 mg/dL for CRP in the first trimester of pregnancy as a predictive factor for PE, and Bita (12) with a study on 400 pregnant women established the threshold over which PE can be predicted,

in the first trimester of pregnancy of more than 5 mg/L. In order to establish reference values for CRP in normal pregnant women and pregnant women with PE, Hwang et al. (19) demonstrated also the possibility of using CRP as a severity marker in PE.

Other authors pointed out the relationship between CRP and the clinical and biochemical parameters from PE; the high levels of hemoglobin, creatinine, TGO, TGP, LDH, blood urea and proteinuria were associated with high levels of CRP.

Nowadays, more and more studies confirm the fact that the CRP (10) marker can help in differentiating the pregnancy associated HBP whose etiology is not PE. Other studies, on the other hand, could not establish a real relationship between CRP and PE (13). In recent studies, Stefanovic (21) focused on the endothelial dysfunction as an anomaly in PE and concluded that CRP as a marker of the inflammation does not have high values and it is not associated with the severity of PE.

The high CRP values are a useful parameter in assessing the severity risk of PE in pregnant women with high body mass index in the third trimester of pregnancy (13). In our case it was noticed a risk of 6.71 times higher of severe PE in obese patients.

Considering the importance of carefully monitoring the mother and the fetus and the therapeutic intervention at the best moment in the process of PE, to avoid fontal and maternal complications, in this study it was assessed the sensitivity and the specificity of some laboratory tests, including the usual ones, that focus on assessing various angiogenesis markers in pre-eclampsia diagnosis and management. PlGF and sFlt-1/PlGF ratio were correlated with the clinical and laboratory signs of the cases included in the study. The higher the sFlt-1/PlGF ratio the better can it assess the risk of premature birth and it represents a potential prognosis parameter in PE monitoring.

It was noticed that PlGF and sFlt-1 levels are simultaneously affected in PE (especially in early pre-eclampsia); thus, the sFlt-1/PlGF ratio represents now a predictor element superior to the individual analysis of these markers. Another approach in assessing these markers might consist in the sequential determination of serum levels, an approach that has provided better predictive results than the punctual assessment in of the pregnancy a certain moment. The sensitivity of sequential determinations of the sFlt-1/PlGF ratio is of 100% and its specificity is of 98-99% (3, 5).

It was noticed that sFlt-1 levels and sFlt-1/PlGF ratio increase while PlGF levels decrease significantly in patients who develop PE, all this compared to the levels measured in normal pregnancies or in patients with chronic kidney disease (CKD). sFlt-1 levels increase and PlGF levels decrease even more in women who simultaneously associate CKD or chronic hypertension during pregnancy.

CONCLUSIONS

In our study it resulted that the mean value for the CRP marker correlated with SBP, DBP and mean BP, but it did not correlate with hemoglobin and hematocrit.

It was pointed out that CRP is an inflammation marker but in PE it was not proved that it can be used in the clinical practice on a daily basis.

The results of our study confirm the importance of establishing sFlt-1 and PlGF as markers for the diagnosis and the monitoring of pregnant women with HBP as well as the sFlt-1/ PlGF ratio which represents a good PE predictor.

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LIFE-THREATENING COMPLICATIONS OF IMPACTED COMMON BILE DUCT LITHIASIS. A CASE REPORT

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LIFE-THREATENING COMPLICATIONS OF IMPACTED COMMON BILE DUCT LITHIASIS. A CASE REPORT (Abstract). Impacted common bile duct (CBD) lithiasis poses therapeutical challenges and repeated attempts of removal may result in life-threatening complications. **Case report**. A 45 year-old female patient was admitted in emergency for right upper quadrant abdominal pain and jaundice. Clinical, lab data, abdominal ultrasound (US) and cholangio-MRI established the diagnosis of acute cholecystitis and obstructive jaundice due to distal CBD lithiasis. Endoscopic retrograde colangiopancreatography (ERCP) confirmed the presence of a distal CBD stone but extraction failed. The patient was operated on and surgical procedure consisted of cholecistectomy, intraoperative cholangiography and a side-to-side choledocho-duodenal anastomosis was performed because all attempts to extract the stone through choledocotomy or duodenotomy and enlargement of endoscopic shincterotomy failed. The postoperative course was endangered by a severe pancreatitis, a massive upper digestive bleeding and portal vein thrombosis that responded to conservative management in the intensive care unit. The patient was discharged after 34 days in good clinical condition and approximately 9 months later was readmitted electively for an incisional hernia. Apart from this, physical examination, lab tests and imagistic studies were normal; the patient was operated and rapidly discharged in good condition. In **conclusion**, the management of CBD lithiasis may be a serious challenge both for interventional endoscopists and surgeons and require a concerted team effort. **Keywords**: LITHIASIS, COMMON BILE DUCT, ENDOSCOPY, SURGERY, PANCREATITIS.

Impacted common bile duct (CBD) lithiasis poses therapeutical challenges and repeated attempts of removal may result in life-threatening complications. We report a case in which endoscopical and surgical attempts of stone removal from the distal CBD resulted in serious complications such as cholangitis, acute necrotizing pancreatitis, upper digestive bleeding and portal veins thrombosis.

CASE REPORT

A 45 year-old female patient was admitted in the IIIrd Surgical Unit on emergency basis for right upper quadrant abdominal pain and jaundice. As past medical history she was diagnosed and treated with steroids for sarcoidosis with joint and pulmonary involvement but for the last years she was symptoms free without any medication. Obstructive jaundice due to biliary (gallbladder and CBD) lithiasis was sus-

pected due to clinical, lab data and abdominal ultrasound (US). Further on, cholangio-MRI showed dilated intrahepatic bile ducts and a CBD of 12 mm due to distal biliary lithiasis (fig. 1).

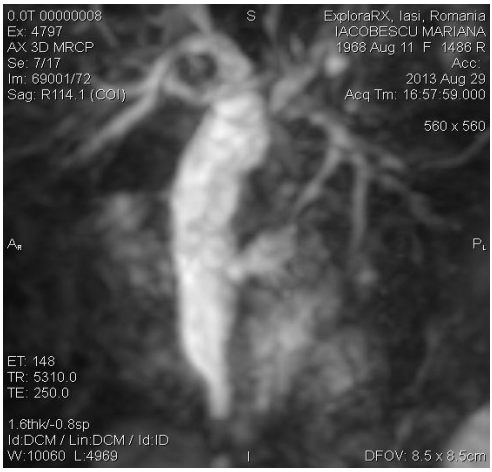

Fig. 1. Cholangio-MRI revealed a dilated CBD with high suspicion of distal biliary lithiasis

The patient was referred for endoscopic retrograde colangiopancreatography (ERCP) that confirmed the presence of a distal CBD stone; a large shincterotomy was performed but stone extraction failed due to the size of the stone and repeated rupture of balloon catheters. As the patient refused another ERCP she was planned a few days later for surgical removal. Surgical procedure started with a classical cholecistectomy and intraoperative cholangiography showed a 15 mm stone fixed in the distal CBD (fig.2).

Further on, a standard choledocotomy was performed but attempts to remove the stone through choledochotomy failed. Duodenotomy and enlargement of endoscopic shincterotomy followed but the calculus could not be extracted due to its size and adherence to the CBD. Moreover, a persistent bleeding from the margins of sphincterotomy required haemostatic sutures and cauterization. Under these circumstances, in order to restore the biliary flow, a side to side choledocho-duodenal anastomosis was performed, abandoning the impacted stone.

Fig. 2. Intraoperative cholangiography showed a large stone impacted in the distal CBD with difficult duodenal passage.

The first 48 hours postoperatively the patient was alert, hemodinamically stable, on spontaneous respiration, without fever but she complained of upper abdominal pain and nausea with a naso-gastric output of 900 ml. The level of pancreatic enzymes rose significantly (amylase- 2709 U/L and lipase-3835U/L). US of the abdomen revealed a peripancreatic, perisplenic and left retrocolic fluid collection and partial thrombosis of the portal vein. Abdominal computer-tomography (CT) confirmed the diagnosis of postoperative acute pancreatitis and established a Balthasar score of 8 and partial thrombosis of the portal vein. Under anticoagulation in prophylactic doses, in the third postoperative day the patient presented an abundant hematemesis allegedly from the margins of sphincterotomy, with Hb of 6 mg/dl, Ht- 20%, platelets-95000, INR-1.77, APTT-36, PT-20.

A conservative treatment was decided, consisting of transfusions, fresh frozen plasma and hemostatics and finally, after administration of recombinant factor VII, hemostasis was achieved. Following standard conservative treatment, the clinical, biological and CT status of pancreatitis improved significantly although pseudomembranous colitis was diagnosed and subsequently successfully managed. After 34 days of hospital stay she was discharged in good clinical condition. A follow-up admission after 2 months revealed a symptom free patient, with hematology and biochemistry tests within normal limits while abdominal CT showed a homogenous pancreas, a small interhepatogastric collection and partial thrombosis of the left portal vein. More recently, approximately 9 months postoperatively, the patient was readmitted electively for a small incisional hernia. Apart from the incisional hernia, physical examination, lab tests and imagistic studies were normal; the patient was operated and rapidly discharged in good condition.

DISCUSSION

It is stated that the first-line treatment for CBD lithiasis is endoscopic retrieval but dealing with a stone larger than 1.5 cm and impacted in the terminal CBD becomes quite a challenge both for interventional endoscopy and surgeons. Several endoscopical procedures are currently available - basket mechanical lithotripsy, mother and baby choledochoscopy and intraductal lithotripsy, electrohydraulic lithotripsy, intraductal laser lithotripsy or extracorporeal shock-wave lithotripsy - with a rate of success of 90% -95% and no greater frequency of pancreatitis and hemorrhage than with the standard techniques (1-4). When bile duct stones cannot be removed through endoscopic procedures, stent placement allows biliary decompression and prevent cholangitis. Biliary stents may also fragment large stones, making them easier to extract later (5-7). Pancreatic stent placement seems to reduce the incidence of post ERCP pancreatitis in high risk patients (8, 9). The incidence of choledocholithyasis has been noted as 10-15% of patients at the moment of cholecystectomy, increasing with age (10, 11). In a prospective study of common bile duct calculi in patients undergoing laparoscopic cholecystectomy, Collins et al. showed that choledocholithiasis occurs in 3.4% of patients undergoing laparoscopic cholecystectomy but more than one third of these pass the calculi spontaneously within 6 weeks of operation (12).

In the reported case, the endoscopical management failed and aggravation of cholestasis imposed surgery. However, local circumstances did not allow the removal of the calculus and the only option was a biliary bypass procedure. The postoperative course was endangered by massive upper digestive bleeding and severe acute pancreatitis, that responded to conservative treatment.

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AESTHETIC CONSIDERATIONS IN THE RECONSTRUCTION OF ORBITO-ZYGOMATIC FRACTURES

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AESTHETIC CONSIDERATIONS IN THE RECONSTRUCTION OF ORBITO-ZYGOMATIC FRACTURES (Abstract) **Aim:** The purpose of this study is to present our experience regarding the aesthetic and functional reconstruction of zygomatic bone fractures. **Material and methods:** We retrospectively reviewed the medical charts of 19 patients diagnosed with orbito-zygomatic fractures needing surgical reduction and fixation, in between January 2016 and January 2018. Two of the patients presented late with cosmetic and functional sequelae of fracture malunion. A two-point fixation was performed in most cases. Titanium mesh was used in four comminuted fractures at the level of the zygomato-alveolar crest and for the reconstruction of orbito-zygomatic fracture sequelae. Reconstruction of the orbital floor was performed in five cases. **Results:** No significant complications were recorded. Good aesthetic outcomes were obtained in all cases regarding the reconstruction of the orbital walls and the restoration of facial symmetry. Because of the accurate anatomic reconstruction, functionality was resumed postoperatively, with diplopia resolving immediately after surgery and in only one case during the three weeks following the procedure. **Conclusions:** The most important aspect regarding the immediate restoration of facial symmetry in fractures of the zygomatic complex is the repositioning of the zygomatic prominence by ensuring adequate fixation and support at the level of the zygomato-alveolar crest. The restoration of the contour and volume of the orbital area is also important regarding facial aesthetics and with repercussions regarding functionality. The three-dimensional reconstruction of CT (computed tomography) images in complex cases helps understanding the fracture pattern, reduction pathway and selecting the most accurate fixation method. **Key words:** ZYGOMATIC, ORBIT, FRACTURE, RECONSTRUCTION, AESTHETIC.

The role of the zygomatic bone in establishing the uniqueness of facial features is without doubt. The prominence of the cheek region stands for youth and beauty. Due to the projection of the zygomatic body, it is one of the first points of reference when determining facial symmetry. Beauty implies symmetry and therefore, any change in the position of this prominent facial feature will cause obvious asymmetry and will influence the judgement of facial attractiveness. Another important issue is the contribution of the zygomatic bone to the formation of the orbit. The orbital volume and the position of the orbital contents are greatly impacted by the

position and characteristics of the zygomatic bone. An altered position of the eye will be extremely noticeable since maintaining eye contact is one of the most important aspects of human social interactions.

The functional aspect of zygoma malposition is not to be neglected since double vision related to misalignment of the eyes can be extremely bothersome in performing everyday activities. The limitation of mouth opening due to blockage of the coronoid process determined by an inwardly displaced zygoma can also cause functional impairment related to mastication. For all these reasons, sequelae related to zygoma fracture mal-positioning should be avoided whenever possible, since the immediate repair of orbito-zygomatic fractures is always easier to perform and with better results than sequelae surgery.

Being a prominent facial feature, the zygomatic bone is affected in many facial traumatisms, ranking first in some studies (1) and second to mandibular fractures in other studies (2). We present our experience around orbital and zygomatic trauma surgery to aid maxillofacial surgeons in deciding the best treatment method for this common traumatic occurrence. We also provide tips on obtaining the optimum reduction and fixation of orbito-zygomatic fractures for best cosmetic and functional results.

MATERIAL AND METHODS

We retrospectively reviewed the medical charts of patients diagnosed with orbital floor and zygomatic bone fractures in between January 2016 and January 2018. We included only patients who had indication for osteosynthesis due to displaced fractures causing aesthetic and functional deficit.

A total of 19 patients were included, of which 17 men (89.47%), aged between 18 and 71. The etiology involved 10 human aggression cases (52.63%), six traffic accidents (31.58%) and three accidental falls (15.79%). There were three (15.79%) isolated fractures of the orbital floor (blow-out fractures) and 16 (84.21%) orbito-zygomatic fractures. Zygoma fractures comprised of 14 (87.50%) tripod fractures and two (12.50%) comminuted fractures. Two patients (10.53%) presented late with malunion and consequent sequelae. Associated lesions were zygomatic arch fractures in four cases (21.05%), nasal bone fractures in three patients (15.79%), occlusal-facial fractures in three cases (15.79%) and mandibular fractures in two patients (10.53%). Signs that indicated the need for surgery consisted of facial asymmetry and sinking of the zygomatic region in 14 patients (73.68%), diplopia in five patients (26.32%), enophthalmia and hypoglobus in three cases (15.79%), restricted eye movements in four patients (21.05%) and restricted opening of the mouth in five cases (26.32%). There was one patient (5.26%) with preoperative posttraumatic cicatricial ectropion. All patients presented with hypoesthesia of the infraorbital nerve.

RESULTS

The reconstruction of the orbital floor was performed using PDS (polydioxanone) mesh in two (10.53%) cases and titanium mesh in three (15.79%) cases of orbital floor fractures. Zygoma fixation was achieved by a two-point fixation in 12 (63.16%) cases - at the level of the zygomato-alveolar crest and inferior orbital rim. In four (21.05%) cases a three-point fixation was used by placing titanium plates at the level of the zygomato-alveolar crest (fig. 1, 2), inferior orbital rim and frontal-zygomatic suture. In four (21.05%) patients

Fig. 1. Intraoperative view of the displaced fracture line at the zygomato-alveolar crest in a patient with a zygomatic bone fracture.

Fig. 2. Intraoperative view of the reduced zygomatic bone fracture and fixation using a titanium plate at the level of the zygomato-alveolar crest.

a titanium mesh fixation at the zygomato-alveolar crest was needed due to comminution. The sequelae following fracture malunion were addressed by reconstructing the orbital floor and zygomatic region with titanium mesh. The approaches used for reduction and fixation were the trans-conjunctival incision for the orbital floor and inferior orbital rim, the external brow incision for the frontal-zygomatic suture and the intraoral horizontal incision located in the superior oral vestibule for the zygomato-alveolar crest. For the simultaneous access to the orbital floor, the zygoma body and the anterior maxillary sinus wall necessary in wider reconstructions and sequelae cases, a single cutaneous inferior palpebral incision extended to the nasolabial fold was used. In two (10.53%) cases of open fractures, the approach was achieved via the posttraumatic wounds from the cheek area and brow area.

Postoperative periorbital and genian edema and ecchymosis were the most common occurrences. No infections or extrusion of the osteosynthesis material were noted. The facial asymmetry improved following surgery in all cases. The outline of the zygomatic bone was regained, as well as the contour of the orbit (fig. 3-6). In four patients (21.05%) diplopia completely resolved, while one patient (5.26%) had transient diplopia that disappeared after three weeks. Eye movements were in normal ranges following surgery. The amplitude of mouth opening returned to normal after reduction and fixation of the fracture. No cases of orbital hematomas were encountered postoperatively. There were no cases of ectropion related to the trans-conjunctival incision. In one (5.26%) sequelae case the cicatricial ectropion present preoperatively improved because of reconstructive surgery. A second surgery for soft tissue repositioning using suspension sutures was needed for improving aesthetic and functional results.

Fig. 3. Preoperative frontal view of patient with a displaced right zygomatic bone fracture showing facial asymmetry with sinking of the zygomatic area and widened aspect of the right genian region.

Fig. 4. Inferior preoperative view of the same patient better outlining the sinking of the zygomatic prominence.

Fig. 5. Postoperative frontal view of the patient at two months postoperatively proving the correct positioning of the zygomatic projection and restitution of facial symmetry.

Fig. 6. Inferior postoperative view of the patient at two months postoperative better outlining the correct position of the zygomatic prominence and presence of facial symmetry.

DISCUSSION

The zygomatic bone is a defining element of individual facial aesthetics since it provides the projection of the cheek region, it contributes to establishing the width of the face and it also provides support for the inferior eyelid. Changes in the position or three-dimensional shape of the zygomatic bone can cause a completely different appearance of the face - a concept used in aesthetic surgery when performing zygomatic bone osteotomies for achieving a more appealing facial contour (3). Trauma can cause unaesthetic changes affecting both the cheek region and the orbital area, since there is also an orbital component to any zygomatic bone fracture that can be clinically apparent considering the fracture fragmentation. The resulting facial asymmetry depends on the type of zygomatic displacement. In most cases a flat appearance of the cheek area will be caused by the interior displacement of the zygoma towards the maxillary sinus. This is usually accompanied by a widening of the face when the zygomatic bone is rotated medially and inferiorly, while a lateral and inward rotation might cause a decreased facial width on the affected side (4). Orbital floor fractures with displacement of the orbital content towards the maxillary sinus can lead to enophthalmos, hypoglobus and related functional disturbances- diplopia, restricted eye movements (5). An inward displacement of the lateral orbital wall could cause diminution of orbital volume, pressure on the orbital content, exophthalmia and restricted eye movements.

Optimal functional and aesthetic results can only be obtained by a proper fracture reduction and fixation. The basis for obtaining the most favorable reduction is set by understanding the fracture pattern and path of displacement. Intraoperatively, the reverse pathway of fracture displacement will be applied to obtain a simple correct

reduction. Although there are several different closed reduction treatment options, they are controversial as they do not allow direct visualization of the zygomatic bone and its articulations (5) sometimes leading to unacceptable or failed reduction. Intraoperative imaging advancement may lead to a more reliable outcome after closed reduction (6).

Information on the degree and direction of displacement, as well as the number of fragments and associated lesions are crucial on deciding the treatment plan and the approaches used. This can be achieved by adequate imaging that allows direct visualization of the fractured bone fragment displacement as a spatial image (6, 7). In our study the three-dimensional reconstruction of Computed Tomography (CT) or Cone Beam Computed Tomography (CBCT) images offered the best understanding of the fracture pattern and translated in positive outcomes regarding facial symmetry restoration. CT examination can also provide information on the presence of soft tissue entrapment or foreign bodies (8) which can impede proper reduction of the fracture if not properly diagnosed and removed. Imaging can also help diagnosing preexisting conditions that could decrease bone strength and lead to pathological fractures of the zygomatic bone in the context of minimal trauma. Mucocele formation in the maxillary sinus is a frequent encounter. In rare cases, they can grow to erode through the walls of the maxillary sinus, including the inferior orbital floor, leading to functional consequences (9) but also decreased bone strength. Such occurrences can complicate the treatment of zygomatic bone fractures by decreasing the available fixation points in quality bone. Titanium mesh reconstruction could be needed for an increased surface coverage and strength.

In the first days after the trauma, radiologic investigations helped decide the indication for surgery, since it is a well-known fact that local posttraumatic edema can mask facial asymmetry. Additionally, diplopia can manifest after the initial three days after trauma, when orbital edema resolved, the volume of the orbital content returns to normal, and any inferior displacement will manifest as double vision. All zygomatic fractures were resolved in the first week or within two weeks after trauma. It is the surgeon's preference to wait for the resolution of local edema to better estimate the result of the reduction intraoperatively, unless there is indication for emergency surgery, such as orbital apex syndrome. Edema diminution usually happens after the first three days following the traumatism. If more than two weeks passed from the fracture onset due to contraindication or late presentation, surgery was scheduled later for addressing the sequelae of the fracture healed in malposition. Most authors agree on performing the surgery for zygomatic bone reduction and fixation within the first two weeks posttraumatic (4).

The goal of surgery is to achieve proper positioning of the zygomatic prominence and to adequately reconstruct the orbital contour and volume. If the proper reduction is not achieved, the resulting facial asymmetry will be easily seen with a lower position of the zygoma prominence (10). All associated lesions must be addressed, both extraoral and intraoral (11) - the bone contours as support structures as well as the soft tissues as covering structures, but also the integrity, height and contour of the dental arches (12) that contribute to the outcome. The reconstruction must address

the anatomical repositioning of both hard and soft tissues. The soft tissue rearrangement can be achieved by using intraoperative suspension sutures to the periosteum and performing the layered closure of the postoperative wound (13). Secondary corrections can be performed by lifting the tissues with the help of barbed threads that offer increased support with the advantage of an incisionless procedure (14). Residual volume deficit can be addressed by autologous fat transfer for filling of the involved area (15). Lipofilling can offer additional benefits related to scar softening and decreasing residual postoperative pain (16).

There are differences among surgeons regarding the number of fixation points necessary for zygomatic fractures, choosing between one and four fixation points located at the level of the zygomato-alveolar crest, the inferior orbital rim, the frontal-zygomatic suture and rarely the zygomatic arch, with or without orbital reconstruction. Kim et al. (17) state that one-point fixation offers satisfactory results when using the zygomato-alveolar crest for fixation. Ellstrom and Evans (5) conclude that for a simple fracture, fixation at the level of the zygomato-alveolar buttress is sufficient, while for more complex patterns, two or three points of fixation are needed for ensuring the proper positioning of the zygomatic bone. We consider that a two-point fixation (including the zygomato-alveolar buttress and inferior orbital rim) is suitable for most displaced zygomatic bone fractures, while a three-point fixation (with an additional plate at the zygomato-frontal buttress) can improve results for multi-fragmentary fractures. For improved stability and support, fixation at the level of the zygomato-maxillary crest is crucial. Fixation with this location is also important to best reposition the zygomatic prominence, an important issue in reestablishing facial symmetry. Because it is an inferior point, it will impede tipping of the zygomatic bone towards the maxillary sinus and support the forces applied on the body of the zygoma. Another important fixation point in our view is the inferior orbital rim. Fixation at this level can be subject to errors if there is an undiagnosed displaced fracture of the frontal process of the maxillary bone. In such an occurrence, the medial fixation landmark would be wrong, and fixation would occur in a medially displaced position, leading to improper positioning of the zygomatic prominence and medially widened orbital contour. When an additional fracture of the frontal process of the maxillary bone is identified with the help of preoperative imaging, the zygomato-frontal suture could be used as a more accurate level of reduction assessment and fixation, together with long plates at the inferior orbital rim, reaching the nasal bones.

We consider that one of the key aspects for the functional reconstruction of comminuted orbito-zygomatic fractures is the adequate restoration of the orbital volume. This objective can only be achieved by the proper restoration of the integrity and contours of the orbital walls. Respecting the medial landmark represented by the intra-orbital buttress located at the intersection of the medial orbital wall and the orbital floor, is crucial for ensuring support and a fit containment for the orbital structures (18). Its importance was also noted in performing orbital decompression surgery for Basedow disease (19, 20) when the preservation of this medial orbital buttress decreases the incidence of postoperative diplopia (21). Another anatomical landmark

that must be respected in orbital reconstruction is the posterior orbital ledge, a bone shelf representing the orbital surface of the palatine bone. It behaves as a posterior landmark and support point for the reconstructive material impeding the inferior placement of the reconstructive material and improper restoration of the orbital volume. It also announces the proximity of the orbital apex and optic nerve and the need for increased care during insertion and positioning maneuvers (18).

CONCLUSIONS

As demonstrated in our study, achieving the most favorable outcome in orbito-zygomatic fracture surgery depends on the accurate repositioning of the zygomatic prominence. Just as important is to reestablish the dimensions, contour and volume of the orbit, for a symmetric appearance of the eyes. A three-dimensional understanding of the fracture pattern helps decide the most suitable approach, reduction and fixation method. We consider that a minimum of two fixation points ensures stable reduction and fixation, especially when involving the inferior supporting buttress- the zygomato-alveolar crest. Titanium mesh is a useful tool for comminuted fracture repair allowing the simultaneous spatial reconstruction of bone contours as well as the surface of the orbital floor. The role of the overlying soft tissues is not to be underestimated, especially when wider soft tissue undermining is performed for access, such as in posttraumatic sequelae surgery. From our experience, repositioning the soft tissues at the end of the procedure ensures sufficient volume of the regions overlying the reconstructed skeletal frame for an aesthetic, symmetric aspect.

The aesthetic disturbances determined by zygomatic-maxillary fractures are best outlined in cases of late presentation with fracture malunion. They should be avoided whenever possible by performing immediate fracture reconstruction with proper spatial positioning of both bone structures and overlying soft tissues.

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THE INFLUENCE OF DELTALYCYN AND TRANSCRANIAL CEREBELLAR STIMULATION UPON RECOVERY OF RETINA AFTER PHOTO STRESS IN PATIENTS WITH DIABETIC RETINOPATHY

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THE INFLUENCE OF DELTALYCYN AND TRANSCRANIAL CEREBELLAR STIMULATION UPON RECOVERY OF RETINA AFTER PHOTO STRESS IN PATIENTS WITH DIABETIC RETINOPATHY (Abstract) **Aim:** The characteristics of visual evoked potentials (VEP) have been studied in diabetic patients with and without diabetic retinopathy. **Material and methods:** Magnetic impulses (2.0 Tl at the height of impulse) have been delivered to the cerebellar surface transcranially using the "Neuro-MS/D", (Russia Federation). Delta-sleep inducing peptide ("Deltalycyn", "Biopharma", Russia Federation) was intranasally delivered in 30 min before photostress. Afterwards VEP have been registered every 20 s from the moment of photo stress during one minute. **Results:** An increase of the latency period and a reduction of the VEP amplitude have been recorded in the period following photo stress exposure of the macular part of the retina. The VEP characteristics restored to the initial level in 73.5± 3.3 s from the photo stress moment in the control group; while in diabetic patients with retinopathy this index was 137.2±11.3 s. In the presence of cerebellar transcranial magnetic stimulations (2.0 Tl, 20 impulses) the VEP amplitude depression was less pronounced, and the restoration period of the VEP characteristics shortened to 110.3± 12.7 s, while in deltalycyn treated patients restoration was observed in 95.1± 6.8 s. Under condition of combined usage of deltalycyn and TMS period of restoration of VEP was shortened up to 82.5± 6.5 s. **Conclusions:** Retinopathy development is linked to prolonged VEP latency period (P100), lowering of the N75-P100 amplitude, as well as to enlargement of the recovery period of the retina's functional capacity in patients suffering from the diabetes mellitus in the presence of photo stress. Superlatively administered deltalycyn and cerebellar transcranial magnetic stimulation facilitates a faster recovery of the retina's functional capacity in response to photo stress in diabetic patients with retinopathy. 3. Combined usage of deltalycyn and cerebellar transcranial stimulation caused the potentiated shortening of post-photo stress recovering of VEP in patients with diabetic retinopathy. **Keywords**: DIABETIC RETINOPATHY, VISUALLY EVOKED POTENTIAL, DELTA-SLEEP INDUCING PEPTIDE, CEREBELLAR TRANSCRANIAL MAGNETIC STIMULATION

Diabetic retinopathy is one of the causes of development of the acquired blindness at the heart of which are the microcirculatory bloodstream impairment, increased vascular walls permeability, and vascularisation development that lead to the retinal detachment (1, 6). It has been established lately that retinopathy pathogenesis is linked to the activation of lipid peroxidation and to the increased concen-

tration of proinflammatory cytokines in the retinal structures (6). Hence, the methods based on usage of drugs with antioxidant mode of action are justified for the usage in patients suffered from diabetic retinopathy (2, 6). On the other hand, raising the neuronal brain tissues activity by means of transcranial stimulation in cerebellar zone is resulted in the retinal improvement in diabetic patients (4), and such an effect might be justified by activation of antioxidant potential of brain tissue (1).

Till the last time there were not investigations on effects of delta sleep-inducing peptide (DSIP) upon functional state of retina in patients suffered from diabetes retinopathy. Meanwhile DSIP is regarded as a compound with pronounced antioxidant and neuroprotective properties (5).

Therefore the objective of this research was studying of the recovery process of the retina's functional activity in patients suffering from the diabetes retinopathy following a photo stimulation (photo stress) test (7) performed under conditions of DSIP – based drug – deltaycyn administration along with of cerebellar transcranial stimulation (4, 8).

MATERIAL AND METHODS

15 practically healthy individuals (the mean age 32.1± 3.6 years old) and 30 insulin-dependent diabetics (the mean age 30.9± 3.7 years old) were observed in this research.

All subjects provided written consent to research conduction. All investigations have been performed in accordance to ethics demands of commission on ethics at Odessa National Medical University (ONMedU Animal Care and Ethics Committee, 2008/84).

By selecting the control group the following criteria were adhered to: the intraocular pressure less than 21 millimeters of mercury, preserved acuity of vision, unaltered visual field, and absence of eye diseases and neurological disorders. For the diabetics the criteria were as follows: the intraocular pressure less than 21 millimeters of mercury, correctable acuity of vision (more than 7/10), as well as absence of signs of the proliferative retinopathy, which was established by means of fluorangiography (4, 7). This method allowed us to compose the group of 30 patients with mild and severe proliferative retinopathy formed the third to fifth level groups in accordance to classification after (7).

While conducting the research the patients were observed in a dark and soundproof room. Before recording the visually evoked potential (VEP) each subject has been adapting to the light intensity for 10 minutes till the moment, when the pupil diameter became equal to 3 mm. The level of the background screen glow was 5cd/m^2.

VEP was recorded using the adopted technology (9). The visual stimuli – chess pattern – had the contrast degree 70% and the mean luminescence degree 110 cd/m^2, while the contrast reversion was carried out with a frequency two times per second. A subject was placed at a distance 114 cm from the screen center, what allowed a 15-seconds acceptance angle of separate pattern elements, and a 25 acceptance angle of the screen height. Only the right eye of all patients was tested, while the left one remained closed (4, 9).

The electrodes were placed at points Oz (the active electrode) and Fpz – the reference (recording) electrode; the grounding was fixed on the left arm.

The interelectrode resistance was set at 3 kOhm. The bioelectric signals were amplified (by 20,000 times); the signal transmission band comprised 1-100 Hz,

after which the signals were averaged, and the artifacts were eliminated for the stimulation period (9). The experiment was organized in form of no less than two sessions of stimuli exposure; during each one the 100 answers were averaged after the artifacts elimination. The VEP analysis period was 500 ms. The visual VEP in this case included several waves, among which three peaks always could be distinguished that normally appeared in 75-100 ms and 145 ms. These peaks had negative (N75), positive (P100), and negative (N145) polarities.

After the preliminary testing the control VEP were recorded, while 40 stimuli responses were averaged. Connected details of procedure have been described earlier (4).

Deltalycynum («Biopharma», Russia) was administered intranasal with 2 drops in the middle part of each nasal *meatus* after dissolving of vial portion of delta-lycyn with 10 drops of distilled water. It should be noted that one vial contains 0.0003 g of delta-sleep inducing peptide. That peptide represents by itself nano-peptide with molecular weight of 850 D and structured as: Trp-Ala-Gly-Gly-Asp-Ala-Ser-Gly-Glu.

The device "Neuro-MS/D", ("Neurosoft" Russia Federation) was used for transcranial magnetic stimulation along with the appropriate colis and 2.0 Tl inductions at the height of the magnetic-influence impulse. The coil was positioned on the middle line of the occipital zone in the plane tangential to the cranium surface according to the adopted cerebellum stimulation technique (3, 4). In the control group false stimulation was carried out positioning the coil perpendicularly to the same occipital zone (4).

Research results were statistically processed applying the ANOVA method and the Newman-Keuls test.

RESULTS AND DISCUSSION

Dynamics of the development latency period (LP) P100 revealed that before the exposure onset in the group of patients with diabetic retinopathy this index exceeded that in the control group by 30.0% ($p<0.05$) (tab. I). In the first 20 s following the stress period LP in the group of patients with diabetic retinopathy exceeded that in the control group by 33.7% ($p<0.05$) and was higher than that before the photo stress exposure by 17.2% ($p>0.05$). Significant difference from the control group persisted till the end of the observation. The amplitude N75-P100 was by 42.0% ($p<0.05$) lower in patients with retinopathy than in practically healthy patients ($p<0.05$).

In the first 20 s of post-stress exposure a 53.0% ($p<0.05$) lowering of the amplitude N75-P100 was observed in comparison to the initial background in the group of patients with retinopathy ($p<0.05$). Significant differences in comparison with the control group have persisted for 60 s of the post-stress observation. LP and amplitude recovery to the basic value was recorded in the control group after 73.5$\pm$ 3.3 s, and in the group of patients with retinopathy that period was longer by 86.7% ($p<0.05$) (tab. II).

Under conditions of deltalycyn treatment the LP of P100 at the end of 40th s of post stress period exceeded initial value by 6.5% ($p>0.05$) (tab. I). The absence of differences maintained till the end of observation. The P100 amplitude during first 40 s of post stress period was significantly less when compared with the initial value by 20.3% ($p<0.05$), but at the end of observation (60th s) the difference was 10.5% ($p>0.05$). The total time of recovering of LP and amplitude of P100 was reduced by 30.7% when compared with patients who were not treated ($p<0.05$) (tab. II).

TABLE I

Dynamics of photo stress – provoked worsening of visual evoked potential precipitation in patients with diabetic retinopathy under conditions of deltalycyn and TMS usage (M± SEM)

Groups of patients	Investigated index	Before photo stress	Time from the moment of cessation of photo stress (seconds)		
			20	40	60
Control (n=15)	Latency	92.1± 2.2	105.0±3.2#	100.2± 3.0	96.4± 2.8
	Amplitude	9.17± 1.05	6.75±0.80#	7.63± 0.86	8.2± 0.91
Diabetes retinopathy (n=15)	Latency	119.8± 3.2*	140.4±4.8 *#	135.8± 4.1*#	134.2± 3.9*#
	Amplitude	5.32± 0.42*	2.50± 0.23*#	2.90± 0.27*#	3.40± 0.31*#
Diabetes retinopathy + deltalycynum (n=15)	Latency	119.8± 3.2*	127.6± 4.5 *	125.8± 5.0 *	124.3± 4.6*
	Amplitude	5.32± 0.42*	3.12± 0.30*#	4.23± 0.32*#	4.76± 0.42 *
Diabetes retinopathy+ TMS (n=13)	Latency	118.7± 3.4*	130.5± 3.3*#	128.4± 4.1*	126.3± 3.8*
	Amplitude	5.21±0.35*	3.21±0.27 *#	3.48±0.30*#	3.91±0.32*#
Diabetes retinopathy+ eltalycynum+ TMS (n=15)	Latency	119.8± 3.2*	124.3± 3.2*	122.8± 3,5*	117.6± 3.7*
	Amplitude	5.32± 0.42*	3.93±0.24*#	4.53± 0.33*	5.13± 0.40*

Notes: *-p<0.05 compared with the control group; #-p<0.05- compared with the initial data in the same group (ANOVA + Newman-Keuls test).

Exposure to photo stress under the conditions of the preceding TMS application (20 impulses) was accompanied with a tendency of the faster recovering of the P100 LP: in the first 40 s of the post stress exposure the latency exceeded initial value by 8.2% and in 60 s - by 6.4% (p>0.05) (tab. I). The total time of investigated indices recovering was reduced by 19.6% when compared with that one registered in patients without treatment (p<0.05), but it still continued to be 1.5 times longer when compared with the control group (p<0.05) (tab. II).

TABLE II

Time of recovery from the photo stress (M± SEM)

	Control (n=15)	Diabetes retinopathy (n=15)	Diabetes retinopathy + deltalycynum (n=15)	Diabetes retinopathy+ TMS (n=13)	Diabetes retinopathy+ deltalycynum+ TMS (n=15)
Investigated index (seconds)	73.5± 3.3	137.2± 11.3*	95.1± 6.8#	110.3±12.7*#	82.5± 6.5#

Notes: *-p<0.05 compared with the control group; #-p<0.05- compared with the initial data in the same group (ANOVA + Newman-Keuls test).

The LP of P100 in patients treated simultaneously with deltalycyn and TMS exceeded the initial value by 3.8% ($p>0.05$) at the end of first 20 s of observation (tab. I). Contrary to effects of separate usage of deltalycyn and TMS, combined application of both revealed significant differences of LP when compared to those ones registered in patients with retinopathy without treatment. Thus, at first 20 s such difference was confined to 11.5%, while at next 20 s – 9.6% and at the end of observation – 12.2% ($p<0.05$). The amplitude of P100 at the end of 40-th s was less by 14.8% when compared with the initial value ($p>0.05$). At this moment amplitude exceeded similar index in patients treated only with deltalycyn by 26% ($p<0.05$). At 40th and 60th seconds of post stressor period amplitude of P100 also exceeded similar index in patients with retinopathy who were given only TMS – by 30,2% ($p<0.05$) and 31,2% ($p<0.05$) correspondently. The total time of recovery of LP and amplitude in this group was reduced by 39.9% when compared with the same index registered in patients with retinopathy without treatment ($p<0.05$) (tab. II).

Thus, the obtained results showed that in patients suffering from the diabetes mellitus with diagnosed retinopathy the following impairments of the VEP characteristics observed: lengthening of the latency period P100 and lowering of the amplitude N75-P100 in comparison to these indices in the control group. Besides, more time (by 86.7% pertained to control group) it was of need to recover functionally from photo stress.

It may be assumed that the described disorders are the result of a decreased velocity of the nerve impulse conductance along the optic nerve (8). Also quite possible that observed results have been contributed with a selective damage to the ganglion cells and their axons in the central part of the retina as a result of metabolic stress and continuous hyperglycemia. Also it may be assumed that photo stress impairs the ability of the macular photoreceptors to produce the action potential after such stimulation. Meanwhile, a prominent role in the VEP recovery seemingly belongs to the photopigment resynthesis that can be supplied by a sufficient bloodstream volume (4, 7). Hence, the improvement in microcirculation along with the affecting functional state of retinal neurons antidromically from stimulated cerebellar structures might be suspected as main targets for the precipitation of observed positive therapeutic effects. Also it should be noted that both – deltalycyn and cerebellar TMS are able to affect number of neuromodulator effects including heightening of antioxidative mechanisms (1, 5). The last ones are known as critical for the prevention of diabetic retinopathy (5).

CONCLUSIONS

Retinopathy development is linked to prolonged VEP latency period (P100), lowering of the N75-P100 amplitude, as well as to prolongation of the recovery period of the retina's functional capacity in patients suffering from the diabetes mellitus in the presence of photo stress.

Superlatively administered deltalycyn and cerebellar transcranial magnetic stimulation facilitates a faster recovery of the retina's functional capacity in response to photo stress in diabetic patients with retinopathy.

Combined usage of deltalycyn and cerebellar transcranial stimulation caused the potentiated shortening of post photo stress recovering of VEP in patients with diabetic retinopathy.

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NEWS

PREMATURE BIRTH LINKED WITH INFLUENZA COMPLICATIONS IN YOUNG CHILDREN

A group of researchers leaded by Kay Wang, from the University of Oxford in the UK, shows that children younger than two years who were born prematurely are at increased risk of being hospitalized if they develop influenza. One in four children who develop influenza or influenza-like illness will have complications, such as otitis media or pneumonia. The systematic review and meta-analysis, published in Lancet Respiratory Medicine on December 4, analysed 27 studies including more than 14,000 children. In this study, factors that increased the likelihood of hospitalization included neurological disorders (univariable odds ratio, 4.62), prematurity (OR, 4.33), sickle cell disease (OR, 3.46), immunosuppression (OR, 2.39), diabetes (OR, 2.34), and being younger than two years of age (OR, 2.51). When age younger than two was included as a risk factor in a multivariate analysis, children with more than one risk factor had a 74% chance of being admitted to the hospital, while the risk for children with one risk factor was 52% ($p<0.0001$). These findings provide an evidence-based definition of which children are most at risk of developing complications from influenza. Dr. Wang concluded that interventions aiming to prevent these complications, including vaccination and antiviral medications, should therefore be prioritised in these groups, particularly during seasonal influenza epidemics and influenza pandemics (Anne Harding. Premature Birth Linked With Influenza Complications in Young Children. *Medscape*. December 14, 2014).

Mioara Matei

INTERDISCIPLINARY CORRELATIONS REGARDING THE CLINICAL AND PARACLINICAL EVALUATIONS IN HIV-POSITIVE PREGNANT WOMEN

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INTERDISCIPLINARY CORRELATIONS REGARDING THE CLINICAL AND PARACLINICAL EVALUATIONS IN HIV-PREGNANT WOMEN (Abstract): HIV infection in pregnancy has an increasing prevalence due to the effectiveness of antiretroviral therapy. The risk of HIV vertical transmission varies between 15-20 % in European women who do not breastfeed and 25-40 % in African mothers who breastfeed. The most important predictive factor of the vertical transmission is maternal plasma HIV viral load. Vertical transmission can be largely prevented by prenatal screening, perigestational ART, an adapted obstetrical attitude and exclusively artificial feeding of the infant. **Material and method**: the study included 36 HIV-positive pregnant women, between 2012-2014, at age of 25-32 years. **Results and discussions**: It has been found that the birth weight was less than 2,700 grams in all newborns of HIV-positive pregnant women or those with advanced disease (AIDS) and, also, they received an APGAR score less than 7. The primordial desideratum is to decrease the rate of mother-fetus vertical transmission, thus the caesarian section has been established as the birth method in all HIV-positive pregnant women after 38 weeks of amenorrhea, on intact membranes, outside labor, resulting in halving the percentage of HIV-positive children. A very important role belongs to the interdisciplinary collaboration between the obstetrician and the infectious diseases specialist during the pregnancy, but also during the postpartum period. The role of the obstetrician is present in all the moments of pregnancy evolution. The HIV-positive pregnant woman is included in the group of high risk pregnancies. **Keywords**: HIV-POSITIVE PREGNANT WOMAN, VERTICAL TRANSMISSION, INTERDISCIPLINARY COLLABORATION

HIV infection in pregnancy- reflected through the number of HIV-positive pregnant women- has an increasing prevalence due to the effectiveness of antiretroviral therapy (ATR), especially of the highly active antiretroviral therapy (HAART)- with consequent prolongation of the life of HIV-positive patients, due to the increasing incidence of heterosexual transmission and of the increasing significant number of infected people who are not aware of their condition (1, 2, 3).

The risk of HIV vertical transmission varies between 15-20% in European women who do not breastfeed and 25-40% in African mothers who breastfeed. Without treatment, it is estimated that over 80% of the transmission cases takes place after 36 weeks of amenorrhea, during labor or birth and only 2% of the transmissions occur

during the first two pregnancy trimesters (2, 4). The most important predictive factor of the vertical transmission is maternal plasma HIV viral load (4). Other perigestational risk factors are the advanced maternal disease (AIDS) and the low maternal plasmatic level of CD4 T lymphocytes (4, 5). The main obstetrical risk factors are the vaginal birth, the increased duration of the period of ruptured membrane and premature birth. Breastfeeding increases the risk of vertical transmission by 14-28% in patients without HAART (5, 6).

Vertical transmission can be largely prevented by prenatal screening, perigestational ART, an adapted obstetrical attitude and exclusively artificial feeding of the infant (1).

MATERIAL AND METHODS

The study included 36 HIV-positive pregnant women within "Sfânta Parascheva" Clinical Hospital of Infectious Diseases of Iasi during 2012-2014, who subsequently gave birth at "Cuza-Vodă" maternity of Iasi.

The information was collected by collaboration with the medical statistics services of the two clinics and by studying the birth registers within the maternity.

The cases included in the studio were analyzed in relation to: the stage of the maternal disease (advanced or not), the type of birth (natural or caesarian), birth weight (>2,700 grams, <2,700 grams), gestational age (>37 weeks of amenorrhea, < 37 weeks of amenorrhea) and APGAR score (>7, <7).

The processing and the interpretation of the results were carried out by clinical, mathematical and computerized methods.

RESULTS AND DISCUSSION

1. Staging of disease in HIV-positive pregnant women.

In the two years taken into consideration for the study, 36 cases of HIV-positive pregnant women were reported, of which 16 cases (44%) with advanced disease (AIDS). In all of the 36 cases the birth was carried out through caesarian. A birth weight of <2,700 grams had been found in all the newborns of the HIV-positive pregnant women with advanced disease (AIDS) and in only 5 newborns of HIV-positive pregnant women without advanced disease. Also, the APGAR score was <7 in 12 newborns of the HIV-positive pregnant women with advanced disease, as compared to the newborns of the HIV-positive pregnant women without advanced disease, who received APGAR > 7.

2. Viro-immunological evaluation of pregnant women.

Pregnant women with advanced HIV disease (AIDS) presented a low plasmatic level of CD4 T lymphocytes, level which proved to negatively influence the rate of vertical transmission to the newborn and its morbidity(1,2). Newborns of pregnant women with advanced HIV disease had a lower birth weight as compared to the newborns of the mothers without advanced disease and they also received a lower APGAR score and they had a more increased morbidity, represented by neonatal infections.

3. Psychological evaluation of the HIV-positive pregnant woman.

Counseling for HIV testing represents the "key" service, both for initiating certain efficient programs for preventing/restricting the epidemic, and for initiating care and support measures for the people affected by HIV/AIDS. HIV testing has to be voluntary, carried out according to the informed consent of the pregnant woman and, thus, in all situations, accompanied

by pre- and post-testing counseling, by complying with confidentiality. Women have to benefit from access to quality counseling and testing services before getting pregnant, for the evaluation of their personal HIV status, but also for their partner's HIV status. Also, the information will help her protect against and prevent HIV infection, both for herself and for her partner, as well as for the future child.

4. Study of the patients from the obstetrical point of view.

The primordial desideratum is to decrease the rate of mother-fetus vertical transmission, thus the caesarian section has been established as the birth method in all HIV-positive pregnant women after 38 weeks of amenorrhea, on intact membranes, outside labor, resulting in halving the percentage of HIV-positive children.

A caesarian section was performed in all of the 36 HIV-positive pregnant women, as the recommended method of birth.

The gold standard is that the caesarian section should be performed, in HIV-positive pregnant women, in the absence of labor, on intact membranes.

Pregnancy hospitalization is carried out as follows: months 1-7, 1 check-up per month, month 8, 2 check-ups per month and month 9, 1 check-up per week.

The intrapartum conduct is represented by the limitation of the time during which the child comes in contact with the blood or other maternal fluids with high viral load.

The postpartum conduct is represented by the prophylaxis of the puerperal infections with antibiotics, prophylaxis of postpartum thrombosis (collaboration with anesthesia and intensive care specialist), and check-up after 6 weeks and after 3 months, establishing a contraceptive method.

The HIV-positive pregnant woman is included in the group of high risk pregnancies. The planned caesarian section is preferred, in pregnant women who are known and monitored, in order to significantly decrease the rate of vertical transmission. ART is administered ante, intra and postpartum, as well as for the newborn.

CONCLUSIONS

In this study a very important role belong to the interdisciplinary collaboration between the obstetrician and the infectious diseases specialist during the pregnancy, but also during the postpartum period.

In our study, a caesarian section was performed in all of the 36 HIV-positive pregnant women, but 5 of them came late to the maternity and their membranes had been ruptured for over six hours. The 5 newborns were prematurely born after 35-36 weeks of amenorrhea, they had a birth weight of < 2,700 grams and they received an APGAR score <7.

HIV infection represents a current major health problem in most of the countries and, for this reason; it represents a increasingly higher interest. The primordial desideratum is represented by the decrease of the rate of mother-fetus vertical transmission.

The mandatory condition is the existence of a multidisciplinary team, obstetrician-neonatologist-infectious diseases specialist-family physician.

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NEWS

NUCLEOTIDE SEQUENCE ANALYSIS OF BETA TUBULIN PROTEIN-CODING GENE

Rezaei-Matehkolaei et al. studied the beta tubulin protein-coding gene (BT2) in 26 species belonging to *Trichophyton*, *Microsporum*, and *Epidermophyton* genera. A number of 144 strains were identified using internal transcribe spacer (ITS) sequencing and BT2 gene was partially amplified in all strains. After construction of a BT2 database, sequence analysis was performed, showing length that ranged from 723 nucleotides (*T. ajelloi*) to 808 nucleotides (*M. persicolor*). In some species, the study found intraspecific sequence variation, while other species were invariant (*T. tonsurans, T. equinum, T. concentricum, T. verrucosum, T. rubrum, T. violaceum, T. eriotrephon, E. floccosum, M. canis, M. ferrugineum,* and *M. audouinii*). In different strains of the same species the sequences were relatively conserved. The dendrogram obtained using BT2 was almost compatible with the species concept based on ITS sequencing, and similar species were distinguished. After characterization of BT2 in a wide range of dermatophytes, the researchers concluded that beta tubulin protein-coding gene is a taxonomically valuable gene.(Rezaei-Matehkolaei A, Mirhendi H, Makimura K et al. Nucleotide sequence analysis of beta tubulin gene in a wide range of dermatophytes. *Med Mycol*. 2014. pii: myu033).

Teodora Vremera

EPIDEMIOLOGICAL AND PATHOLOGICAL ASPECTS OF SKIN CANCER IN NORTH EAST OF ROMANIA

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EPIDEMIOLOGICAL AND PATHOLOGICAL ASPECTS OF SKIN CANCER IN NORTH EAST OF ROMANIA (Abstract): In the last years skin cancer has become the most frequent cancer in humans, with the majority made up of two tumors: malignant melanoma (MM) and non-melanoma skin cancer (NMSC). **Aim**: To provide for the first time in the North East region of Romania, descriptive epidemiological data of melanoma and non-melanoma skin cancers. **Material and methods**: We undertook a retrospective study over a five year period on the most frequent forms of skin cancer, basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and MM, which included a total number of 1231 patients, hospitalized and treated in the Plastic Surgery Department of "St. Spiridon" Emergency Clinical Hospital, Iaşi from 2008 until 2012. Results: BCC was diagnosed in a total of 675 patients, representing 54.9%. SCC was found in 217 patients, representing 17.6% and melanoma was diagnosed in 119 cases, a percent of 9,7% of the total number. The predominance of cutaneous malignancies waried with age, major site distribution was face and neck for BCC and SCC and the trunk for MM. **Results**: This article describes the most common forms of skin cancer in our region, BCC (almost 55%), SCC (17.6%), and less in number MM (near to 10%); these results are similar to those published abroad in the last years. **Conclusions:** Future more elaborate interventional studies are necessary to identify the main risk factors in order to design the best preventive methods and, according to the skin cancer trend to specify the needs for dermatology network in our country. **Keywords:** SKIN CANCER, BASAL CELL CARCINOMA, SQUAMOUS CELL CARCINOMA, MELANOMA.

In the last decades skin cancer has become the most frequent cancer in humans, with the majority made up of two tumors: malignant melanoma (MM) and non-melanoma skin cancer (NMSC) (1). These two are the most common types of cancer encountered in white population, with an increasing incidence rate worldwide, but the epidemiological data regarding this type of cancer are incomplete because of the absence of some cancer registers or simply the lack of information (2).

In the battle against cancer we must know all the aspects about the disease with the special features for every country and every region. In the North – East region of Romania, the Cancer registry was recently introduced, and the data were collected and

statistical processing only until 2009, thus the prevalence and the histological forms of this type of neoplasm are not very well known, and there is no national database for this epidemiological surveillance.

In this retrospective study we have examined the epidemiological and pathological features of 1231 skin cancer cases, made by the Plastic Surgery Department of"St. Spiridon" Emergency Clinical Hospital, Iaşi from 2008-2012 with the purpose of documenting the pattern of skin neoplasia in Iaşi, the largest city of Moldavia.

Non-melanoma skin cancer is the most common human skin cancer, enclosing basal cell carcinoma (BCC) and squamous cell carcinoma (SCC), both derived from epidermal keratinocytes, and it rarely metastasizes (3). NMSC is the neoplasm of elderly people, with a predilection for sun exposed areas, and, in contrast with melanoma, rarely metastasize (4). BCC, also known as "basalioma" was described for the first time in the 1824, by Arthur Jacob, as *ulcus rodens*, and, since then its incidence is continuously rising worldwide. BCC represents about 65% of the epithelial skin tumors, it has a maximum incidence in the 6-th decade of live and a slightly male predominance. It is a slow-growing, locally invasive epidermal tumor with a metastatic rate of <0.1% (4). The majority is found on the cephalic extremity (head and neck), almost 80% of the cases respectively; the distribution but these can vary according to the histological pattern: superficial BCC is more often found on the truck (5). Fibroepithelioma's Pinkus is a rare type of BCC that is usually located above the natal cleft or on the lower trunk with a pink colored nodule that may look like seborrheic keratosis. The incidence of NMSC is increasing, while the mortality is decreasing, a fact that is explained by earliest diagnosis with efficient therapeutical intervention all over the world (6). Keratoacanthoma is relatively common, especially in middle-aged white people, uncommon in dark-skinned ones; moreover it is a benign epidermal tumor with a tendency to regression after a few months that can develop areas of CSC in approximately 25% of cases (7). Merkel cell carcinoma is a rare neuroendocrine carcinoma of the skin, 40 times less common than melanoma, but with a higher mortality (33%) than melanoma (15%) (8). MM is the most aggressive form of all skin neoplasms with a reduce survival rate. It can start either from the existing moles or from the normal-looking skin. Many studies have reported at least two important data about the survival rate: that women have a longer survival score than men, and that the anatomic location varies with this rate. For example, melanomas located on the trunk were correlated with worse prognostic (8).

MATERIAL AND METHODS

In this 5 years (2008-2012) retrospective and descriptive study a total of 1,231 cases registrated in the Plastic Surgery Department of „St. Spiridon" Emergency Clinical Hospital, Iaşi were investigated. Detailed description of clinical appearance of the excision area is previously described in patients' clinical records. Surgical excisions and histopathology examinations were performed in all cases. The biopsy specimens were processed using paraffin embedding and hematoxylin-eosin staining method. Statistical analysis were performed as case-control analyses, without adjustment for multiple testing, with nominal significance defined as $p<0.05$. Intergrupal

qualitative differences were established using the Chi-squared Kruskal-Wallis Test. In this study we included only patients with clinical diagnosis of BCC, SCC and MM. The variables introduced in the current study were: gender, inhabitant population of place of residency (urban/rural), anatomical location of the cancer (face/scalp/neck/ trunk/anogenital region/upper limb/lower limb), clinical presentation, histological diagnosis, presence of residual malignant cell in the safety margins (oncologic excision), and risk factors (smoker/non smoker for MM).

RESULTS

Of the total number of patients (1231), 1319 biopsy pieces has been histological analyzed, because of the existing multiple lesions on the same patient. BCC was diagnosed in a total of 675 patients, representing 54,9% of the cases taken to the study, with the nodular form as the most common histological type (40.4%), followed by BCC with adnexal differentiation (21.1%), keratotic (14.8%), superficial (6.8%) and pigmented (6.1%). 81.6% of BCC had a facial localization, 11.4% a trunk localization and 11.1% had the tumor on the scalp. We did not find a male predominance in these forms of carcinoma, but maximum incidence of it was found in the sixth decade of live (4). SCC was found in 217 patients, representing 17.6% with the major histopathological pattern being the keratotic form (81.9%), and MM was 9.7% of the total number with a preponderant level of invasion as Clark III (33.6%). The frequency of BCC, SCC was higher in the rural district *versus* the urban district (82% *vs.* 68%) as described in tab. I and on the age group, the variation of skin cancer was significantly statistical: in the group aged 20-29 the MM was the most frequent form, between 50-59 years BCC was the prevalent one and over 90, SCC.

TABLE I

Skin cancer distribution according to gender and place of residency

Skin cancer	**Total**		**Male**		**Female**		**Urban**		**Rural**	
	N	%	N	%	N	%	N	%	N	%
BCC	675	54.9	335	53.9	340	55.9	322	53.7	353	56.8
SCC	217	17.6	123	19.8	94	15.5	77	12.8	140	22.5
MIXT	26	2.1	12	1.9	14	2.3	9	1.5	17	2.7
MM	119	9.7	64	10.3	55	9.0	74	12.3	37	5.9
Other	193	15.7	88	14.1	105	17.3	118	19.7	75	12.1
Kruskal Wallis Test			Chi-square=6.081; df=4; p=0.193				Chi-square=43.672; df=4; p=0.001			

In SCC 76% of the cases had a facial localization and 11, 1% were found on the upper limbs. In MM, the main localization was the trunk (44.5%), followed by the upper limbs with a 28.6% percent as described in table II.

All patients with mixt carcinoma had a facial localization and 26.9% of them were associated with another mixt carcinoma on the scalp or upper limbs.

TABLE II
Site distribution of skin cancer

Skin cancer	Face		Scalp		Neck		Trunk		Anogenital region		Upper limb		Lower limb	
	N	%	N	%	N	%	N	%	N	%	N	%	N	%
BCC (N=675)	551	81.6	75	11.1	14	2.1	77	11.4	-	-	9	1.3	9	1.3
SCC (N=217)	165	76.0	11	5.1	1	0.5	12	5.5	1	0.5	24	11.1	14	6.5
MIXT (N=26)	26	100	6	23.1	-	-	-	-	-	-	1	3.8	-	-
MM (N=119)	9	7.6	5	4.2	3	2.5	53	44.5	2	1.7	17	14.3	34	28.6
Other (N=193)	86	44.6	25	13.0	4	2.1	34	17.6	3	1.6	13	6.7	35	18.1
Total (N=1230)	**837**	**68.0**	**122**	**9.9**	**22**	**1.8**	**176**	**14.3**	**6**	**0.5**	**64**	**4.2**	**92**	**7.5**

The histopalogical pattern of SCC revealed aproximatively 82% of them as SCC with keratinisation and 3,7% *in situ* SCC. Precancerous keratinocytic lesions found in our study were: actinic Keratosis (AK), Bowen's disease, Bowenoid papulosis and Erythoplasia of Qeuyrat (9). Regarding the safety margins of the excision of the tumor, there were differences between the three types of cancer: 87.6% for the BCC, 85.3 % for SCC and only 72.3% for the MM.

DISCUSSION

Although skin tumors registries have been established for several European countries, in Romania only since 2007 regional registries were created in order to collect and to analyze data regarding all cancers that were diagnosed in our oncology clinical units. Specific data regarding evolution and tendency of skin cancer will be available probably in the next years. Although non -invasive therapeutic ways of screening a skin lesion are increased, such as demartoscopy, the most frequent way of treating such lesion remains the surgery. Unlike BCC, SCC has some premalignant lesions, such as actinic keratoses or Bowen disease, lesions that may benefit from a non-invasive treatment, first-rate for the patient and less expensive for the health insurance companies. In the studied period the major forms of skin cancer was homogenous (p=0.927) (fig. 1).

Related to the histopatholgical forms of skin cancer, we found some statistically semnificative differences between BCC, SCC and MM. The majority of NMSC lesions were encountered on the head and neck area, a visible site that affects the quality of live score on these patients (10). 81.6% of BCC had a facial localization and 76% our cases of SCC were in this site. The major histopathological form of BCC was the nodular one, in accordance with other data (11). According with another international data, the BCC and SCC localization was predominantly in the same area- head and neck (limit: 74 - 84% - Plesko et all. 2009, Jung G.W et all. 2010, Dacosta Byfield et all. 2013). The clinical presentation of BCC is quite variable: nodular lesion, ulcerated lesion, erythematous plaque or nodule like lesion and tends to occur in elderly people (12). Due to the good prognosis and high inci-

dence of this type of NMSC, BCC is not considered an aggressive cancer, but the local destruction potential may bring some intricate methods of treatment (13). Related to the histo-patholgical forms of skin cancer, we found some statistically significantly differences between BCC, SCC and MM. Our findings are similar to one of the largest systematic review of worldwide incidence of nonmelanoma skin cancer, made by Bath-Hextall et all (2012): the most frequent skin cancer was BCC (54.9%) while a smaller percentage is represented by SCC (17.6%) and melanoma (9.7%) (6,14). The frequency of BCC, SCC was higher in the rural district *versus* the urban district (82% *vs.* 68%), but the frequency of MM was significantly higher in the urban area (12.3% *vs.* 5.9%) (p=0.001). On the age group, the variation of skin cancer was significantly statistical: in the group aged 20-29, the MM was the most frequent form, between 50-59 years BCC was the most prevalent one and**,** over 90, SCC. MM was predominant in men and, in comparison with other adult cancers, more common in young people. We did not find the increasing incidence of MM in this period of study, probably due to the short duration of it. More than 25% of MM occurs in persons less than 45 years old with an incidence that has increased significantly worldwide, especially in lightly pigmented Caucasians (7). In our study the majority of MM cases were in 20-29 group of age. In order to improve the chance of an early diagnosis, the histopathological exam remains the golden standard in the all forms of skin cancer and the way to a proper staging (15).

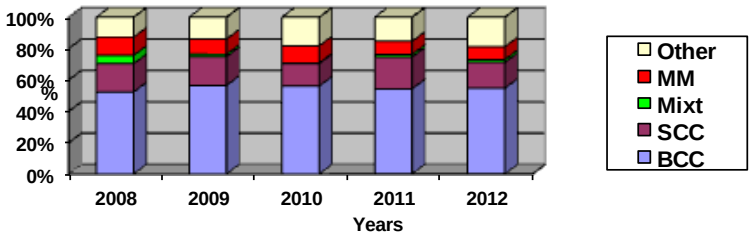

Fig 1. Years distribution of major forms of skin cancer by year of study.
Other: trichoepithelioma, leiomyosarcoma, hemangioma, papilloma.

CONCLUSIONS

This study has investigated the prevalence of skin cancer and the histopathological pattern of BCC, SCC, MM in North-East Romania, with the aiming at bringing an overview to the exact incidence of skin cancer in our country. To our knowledge these data are provided for the first time in this region and our findings are similar to other international data. In a five years retrospective study, the most frequent skin cancer was BCC (54.9%) and less in number SCC (17.6%) and melanoma (9.7%). Future more elaborate interventional studies are necessary to identify the main risk factors in order to design the best preventive methods and, according to the skin cancer trend to specify the needs for dermatology network in our country.

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THE IMPLICATIONS OF HAZARDOUS WASTE NEUTRALIZATION ON EMPLOYEES HEALTH: A CASE STUDY

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THE IMPLICATIONS OF HAZARDOUS WASTE NEUTRALIZATION ON EMPLOYEES HEALTH: A CASE STUDY (Abstract): Chronic obstructive pulmonary disease is a lung disorder characterized by chronic inflammation of the respiratory tract after exposure to pollutants, causing the obstruction of small respiratory tracts and the destruction of lung parenchyma. These changes ultimately lead to a limitation of air flux. We investigate the association between professional exposure and the risk of developing chronic obstructive pulmonary disease. This study presents the case of a non-smoker patient, aged 61, a mechanic locksmith by profession at a hospital in Mures County. He has been working with neutralization of medical waste for 13 years with exposure to pneumotropic and biological contaminants and to the overuse of osteoarticular apparatus, recently having been diagnosed with COPD after exposure to mixed powders and hospitalized at the occupational health clinic. Interruption of occupational exposure and establishment of treatment with topical corticotherapy has shown a significant improvement. Measurements of inhalable powder content in the work environment revealed that they exceed the allowed limit in case of neutralization of medical waste and there is an association between occupational exposure and increased risk of chronic obstructive pulmonary disease. Preventive methods should aim to reduce exposure at workplace. **Keywords**: COPD, POLLUTANTS, SPIROMETRY

Medical wastes are hazardous both for people and for the environment, exposing people to the risks of toxic infections. These types of waste result from all the medical activities in the national health system (1). Persistent Organic Pollutants (POPs) are chemicals which are particularly harmful to human health and the environment. They have been the subject of several environmental policies, and the subject of an International Convention (7). The Stockholm Convention on POPs has taken into consideration 12 categories of pollutants and has noted the obligation of each part to elaborate a National Plan for the Implementation of the provisions of the above mentioned Convention (9). These 12 priority POPs are aldrin, chlordan, dichloro-diphenyl-trichlorethan (DDT), dieldrin, heptachlor, myrex, endrin, toxaphen, poly-

chlorinated biphenyls (PCBs), dioxins and furans, hexa-chloro-benzene (2).

The Stockholm Convention, adopted in 2001, is the one which regulates the scrapping of POPs on a worldwide scale (3,4). In addition to tobacco smoking the exposure to pollutants at the workplace (dusts, fumes, and irritating gases) is a major risk factor for chronic obstructive bronchical pneumopathy (5,6).

CASE REPORT

In this paper we present the case of MF, a 61-year-old non-smoker, a professional locksmith mechanic, with approximately 40 years of professional service. From his professional route we would like to highlight the past 13 years, when the patient worked as a mechanical locksmith in a hospital in Mures County, in the last 3 years carrying out his activity in the department of medical waste neutralization. The patient participated in all the stages of neutralizing waste, involving: loading the autoclave with the medical waste collected from different wards of the hospital, automatic and manual ventilation, extraction of sterilized medical waste transported on conveyors up to grinding, ripping with a shovel, loading and storage of waste in the form of powders in crates and then loading them into bags as well as the transport towards the container. The patient therefore has currently been exposed professionally to the biological contamination, infections in hospitals that come from the neutralization of materials disposable sanitary remains, pneumotropic fumes arising from the ventilation of the autoclave, metal powders, plastics and textiles from the process of chopping, overuse of the osteo articular apparatus by removal of weights and repetitive harmful movements and positions as well as an unfavorable microclimate.

The patient complains of shortness of breath at the moment of hospitalization, in especially upon exertion, predominantly dry cough. The symptoms first appeared 2 years ago, the period coinciding with the professional activity in the neutralization station. He complains of pain in coxofemoral joints, lumbar spine pain, and persistent bilateral pain in the lower limbs. The patient had accidental exposure to HIV (Human Immunodeficiency Virus) and hepatic viruses, for which he sought treatment with antiretroviral drugs in 2010 as a means of prevention; for the osteoarticular problems he was admitted to the clinic of Rheumatology in 2013, and for pulmonary disease to the clinic of Pneumology. He was diagnosed with chronic obstructive bronchial pneumonitis stage I/II, interstitial reticulonodular pulmonary fibrosis, for these conditions treatment with slight improvement in symptomatology was followed. Occupational health surveillance is recommended and hospitalization for occupational diagnosis is needed. The patient is hospitalized in May 2013 at the Occupational Health Clinic of Târgu-Mureş. The general clinical examination revealed osteoarticular affections and a pulmonary disease. The thoracic radiography describes a chronic bronchitis with pulmonary emphysema.

The spirometry (tab. I) carried out two years ago in a periodical medical control at work shows a mixed average ventilation dysfunction FEV1 (Forced Expiratory Volume in 1st second) at 60%. FEV1 at the moment of hospitalization shows an increase of 72 percent. Therefore, the patient is being treated in conformity with treatment recommended by the Clinic of Pneumology, and 3 months after the suspension of the activity, the value of FEV1 increased

to 84%.

The bronhodilation test was also performed (tab. II) for differential diagnosis between fixed obstruction, namely chronic obstructive pulmonary disease and the reversible obstruction of the respiratory tract, namely bronchial asthma. The test is negative, meaning that the obstruction is fixed.

The body-plethysmography (tab. III) shows an increased resistance of the respiratory tract.

In conformity with the anamnesis, clinical and paraclinical examinations the following diagnoses were established: (i) chronic obstructive bronchopneumopathy, stage I/II due to exposure to mixed powders (metal, plastics, textile) and vapors; (ii) mild mixed ventilation dysfunction with the dominance of restriction; (iii) lumbar polydiscopathy of professional etiology; (iv) cervical spondylosis and vertebrobasilar insufficiency.

During hospitalization the patient followed treatment for the above mentioned diagnosis with a favorable evolution of his general state. It is recommended to avoid overloading the osteoarticular apparatus and harmful positions, wearing protective masks in the workplace, ensuring natural ventilation.

Determinations were performed from the work environment (tab. IV) measuring the total dust in suspension in the medical waste neutralization area, in the waste removal hydroclave and their placement in the chopper. Here the powder is in normal range, below 1 mg/m^3 on 8 hours and at the loading of powder in bags it is 1.39 mg/m^3, above the maximum concentration, i.e. more than 1 mg/m^3.

TABLE I

Spyrometry

Spyrometry	FVC (%)	FEV1 (%)	Tiffeneau index (%)	Conclusion
Periodical medical control	50	60	85	Medium mixed ventilation dysfunction
Occupational Health Clinic	66	72	80	Mild mixed ventilation dysfunction
3 months after the suspension of activity	71	84	92	Mild restrictive ventilation dysfunction

FVC-forced vital capacity
FEV1-forced expiratory volume in 1 second
Tiffneau index –FEV1/FVC ratio

TABLE II

Test of bronchial dilatation

Test of bronchial dilatation	FEV1 (%)	FVC (%)	Tiffeneau index (%)	PEF (%)	MEF50%
Initial	72	66	80	79	96
After the administration of Ventolin	76	61	91	93	116

PEF- Peak Expiratory Flow
MEF50%- Maximal Expiratory Flow at 50%

TABLE III

Body-Pletismography

Body-Pletismography	Raw	Gaw	TLC	VR	TLCO	DLCO
%	155	175	73	59	119	119

Raw- specific airway resistance; Gaw- reciprocal of airway resistance; TLC- total lung capacity
VR-residual volume; TLCO-transfer factor; DLCO- diffusing capacity for carbon monoxide

TABLE IV

Determinations of the work environment

Medical waste neutralization department	Chemical agent	Result (mg/m^3)	Maximal admissible concentration on 8 hours (mg/m^3)
Removal of sterilized waste from the hydroclave and loading it into the grinder	Powder- inhalable fraction	0.465	1
Loading the powder in waste bags	Powder- inhalable fraction	1.39	1

CONCLUSIONS

Dust concentration measured at the work site exceeds maximal admissible concentration with 0.39 mg/m^3. These values do not greatly exceed the maximal admissible concentration. However they have a significant impact on the health status of employees engaged in the process of medical waste neutralization. The medical waste neutralization procedure reduces the risk of environmental pollution, but does not eliminate the risk of diseases among employees.

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METFORMIN INDUCED LACTIC ACIDOSIS – PARTICULARITIES AND COURSE

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METFORMIN INDUCED LACTIC ACIDOSIS – PARTICULARITIES AND COURSE (Abstract): Diabetes mellitus is a widespread disease with many serious chronic complications. An improvement in the oral antidiabetic medication in respect to its mechanism of action and toxicology was needed in order to have effective therapies with high compliance and minimum side effects. In this context, metformin is a widely used oral antidiabetic drug, which, through its mechanism of action, has no risk of hypoglycemia. However, a rare but serious side effect of biguanides is lactic acidosis. This paper presents a number of 13 cases of metformin-associated lactic acidosis, which outline the circumstances triggering the adverse event and the clinical therapeutic measures applied in the poisoned patients. The main situations that favor metformin-associated lactic acidosis are renal impairment and tissue hypoxia, and the intervention is adapted to the particular patient condition and symptoms, such as marked hypotension and cardiac arrest. Although there are commonalities in describing the consulted patients, the final prognosis is not dependent on the dose or metformin plasma levels, but rather on the associated pathologies and medication. **Keywords:** DIABETES MELLITUS, METFORMIN, LACTIC ACIDOSIS, RENAL IMPAIRMENT, HYPOXIA

Diabetes is probably one of the first illnesses known to man. It was mentioned for the first time in Egyptian manuscripts 3000 years ago, and in 1988 type 2 diabetes was described as a component of the metabolic syndrome. It represents one of the most common forms of diabetes, characterized by hyperglycemia, insulin resistance and insulin deficiency. This illness results from the interaction of genetic, social and behavioral risk factors.

It is estimated that, in the year 2011, 366 million people had diabetes. This number is thought to rise up to 552 by 2030. For the same year, 2010, it is stated that the number of deaths attributed to diabetes at a global level is around 3.96 million for 20-79 age group that is roughly 6.8% of the global mortality rates for all age categories (1).

Metformin, a biguanide compound with antidiabetic action, was first described in the literature in 1922 by Werner and Bell, as a product in the synthesis of N, N-dimetilguanidine. In 1929, researchers Slotta and Tschesche observed the substance to lower blood glucose in rats, and they described the substance as the most effective biguanide compound of the ones

they studied (2).

Metformin has been accepted as an antihyperglycemic agent in England in 1958, in Canada in 1972, and in USA in 1995. Currently, metformin is the first-line drug in patients with type 2 diabetes who do not have specific contraindications. Only in 2005 more than 42 million prescriptions for metformin were issued in the United States (3).

Besides its use in diabetic patients, metformin is also effective in polycystic ovary syndrome and is being researched as an antiviral and anticancer adjuvant.

Metformin activates an important enzyme called protein kinase AMP-activated (AMPK), which plays an essential role in body energy balance by regulating the metabolism of carbohydrates and lipids. Latest researches show that metformin inhibits mitochondrial respiration by activating AMPK and the synthesis of fatty acids. Metformin improves hyperglycemia by reducing insulin resistance and plasma insulin levels in the fasting state.

Known side effects of metformin include abdominal discomfort, nausea, vomiting, diarrhea, constipation, flatulence, indigestion, heartburn (4). Another adverse effect that may occur in long term metformin administration is vitamin B_{12} deficiency. Studies confirm that metformin can cause malabsorption of vitamin B_{12} and thus lower serum levels of this vitamin (5).

Lactic acidosis is a rare but serious adverse effect of metformin associated with its therapeutic use and overdose (6).

Lactic acidosis can be classified as follows: Type A (associated or caused by inadequate oxygen transport to tissues) and Type B (with adequate oxygen transport to tissues). Type B has 3 sub-categories: B1 - lactic acidosis associated with a disease (eg, diabetes, asthma etc.), B2 - lactic acidosis caused by drugs or toxins (e.g., cyanide, metformin, epinephrine, etc.), B3 - lactic acidosis caused by congenital metabolic disorders.

Depending on the cause, hyperlactatemia is defined as lactate levels between 2 and 5 mmol/L, although it is mentioned that lactic acidosis is present when lactate levels are above 5 mmol/L and arterial pH is lower than 7.35. These definitions can however be arbitrary (7).

The lactate is the final product in anaerobic metabolism (it represents the product obtained from the reduction of pyruvate by lactate dehydrogenase enzyme). It is initially obtained in skeletal muscles, intestine, brain and circulating erythrocytes, with a production of approximately 1 mmol/kg/h. The healthy liver takes the largest part of the lactate and recycles it through three main pathways: conversion to glucose (Cori cycle), pyruvate oxidation, which can then be oxidized to CO_2 (Krebs cycle), and transamination to alanine. The reduced oxygen transport to tissues and cells cause damage in oxidative phosphorylation, with increasing intracellular amounts of NADH, cofactor in the conversion of pyruvate to lactate. Due to the close connection with anaerobic metabolism the lactate was considered to be the end product of glycolysis due to hypoxia (7).

Risk factors for the development of metformin-associated lactic acidosis, such as kidney failure, pre-existing heart disease, chronic lung injury with hypoxia, and congestive heart failure, are usually considered contraindications to metformin therapy. Age may be associated with decreased renal functions and the increase of co morbidities (8). Figure 1 outlines the mechanism of lactic acidosis production, in

particular circumstances.

In patients who develop severe lactic acidosis the symptoms are characteristic: agitation, confusion, absent corneal reflexes, fixed dilated pupils, lethargy, convulsions, coma, and ultimately death (10).

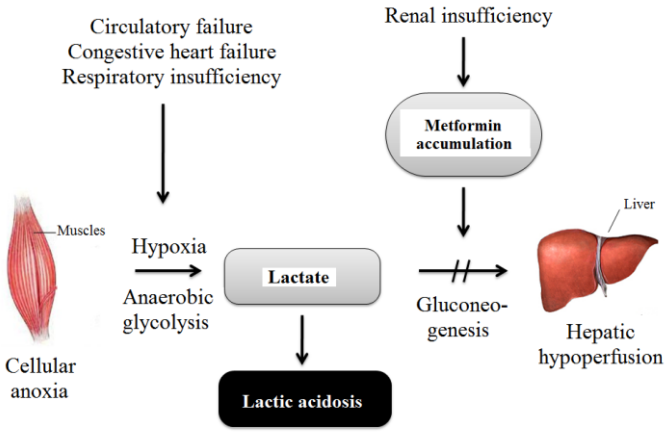

Fig. 1. Lactic acidosis and the circumstances which may increase the risk associated with metformin therapy (9)

We analyzed 13 cases of acute metformin poisoning associated with lactic acidosis reported in the literature.

The circumstances that led to lactic acidosis are presented in Table I. This severe adverse effect also occurred in attempted suicide by drug overdoses and chronic administration to treat hyperglycemia. Also, metformin-associated lactic acidosis was present both in patients with or without renal impairment. With regard to associated medication, incriminated in the development and/or worsening of metformin-induced adverse effects, a definite link has been established in the case of creatine co administration (11).

The most frequent symptom of metformin poisoning is gastrointestinal upset when nausea, vomiting, diarrhea, abdominal cramps, anorexia, epigastric discomfort and pain, and loss of appetite occur. The occurrence of lactic acidosis causes lethargy, confusion, dyspnea, hypothermia, hypotension, and hemodynamic instability. Table II lists all clinical observations in patients with acute metformin poisoning.

The laboratory parameters reported for acute metformin poisoning illustrate the presence and course of lactic acidosis from the time of diagnosis to the end of drug treatment and hemodialysis. Table III shows the evolution of laboratory values in the patients. The therapeutic approach entailed the normalization of acid-base imbalance, the acceleration of lactate metabolism process, the elimination of metformin, simultaneously treating related diseases. Following the patients evolution, metformin-associated lactic acidosis proved reversible when diagnosis and initiation of treatment are immediate so that therapeutic measures to be effective.

TABLE I

Particularities of triggering metformin induced lactic acidosis

Reference	Treatment with metformin	Associated pathology	Associated medication
Van Velzen,2008 (12)	chronic treatment	renal failure caused by diclofenac	various drugs
Saidi,2010 (11)	500 mg x 2/day	*	creatine
Yang,2009 (13)	suicide attempt	diabetic nephropathy	*
Van Sloten,2012 (14)	chronic treatment	*	*
Kreshak,2010 (15)	chronic treatment	coronary heart disease, peripheral vascular disease, hypertension, hypercholesterolemia, osteoarthritis	lisinopril, aspirin, metoprolol, clopidogrel, acetaminophen, hydrocodone
Akoglu,2011 (16)	suicide attempt: 144.5 g	*	*
Browers,2009 (17)	chronic treatment	total colectomy, acute renal failure	enalapril, atorvastatin, glicazid
Dell' Aglio,2010 (6)	overdose: 75-100 g	*	*
Perrone,2011 (3)	overdose	*	overdose of: risperidone, sertraline, hydrochlorothiazide, glyburide
Perrone,2011 (3)	chronic treatment	multiple pathology, final stage renal disease	amiodarone, clonidine, valsartan, gabapentin, glyburide, furosemide, atorvastatin, lisinopril, amlodipine, omeprazole
Perrone,2011 (3)	chronic treatment	hypertension, final stage renal disease	*
Al-Makadma,2010 (18)	suicide attempt: 40-45 g	*	*
Kim,2008 (19)	overdose: 30 g	hypertension	carvedilol (Dilatrend®)

* Not specified.

TABLE II

The symptoms of acute metformin poisoning associated with lactic acidosis

Reference	Symptoms					Other symptoms
	gastro-intestinal	renal	central nervous system	cardio-vascular	Lungs	
Van Velzen,2008 (12)	*	*	confusion	*	*	loss of appetite
Saidi,2010 (11)	*	oliguria and then anuria	drowsiness, malaise	cardiac arrest	pulmonary edema	anorexia
Yang,2009 (13)	*	oliguria	lethargy	*	*	loss of appetite, hypothermia
Van Sloten,2012 (154)	*	*	delirium	*	*	acute prostatitis
Kreshak,2010 (15)	*	*	*	*	*	bilateral vision loss without associated pain, hypothermia
Akoglu,2011 (16)	*	*	*	*	*	Hypothermia
Browers,2009 (17)	nausea, vomiting, abdominal pain, diarrhea	*	*	*	*	*
Dell' Aglio, 2010 (6)	*	*	lethargy	*	*	*
Perrone,2011 (3)	*	*	lethargy	*	*	*
Perrone,2011 (3)	*	*	*	*	Kussmaul type breathing, diffuse rales in the chest	weakness, dry mucous
Perrone,2011 (3)	*	*	*	*	dyspnea	*
Al-Makadma,2010 (18)	*	*	marked irritability, deterioration of consciousness	*	*	*
Kim,2008 (19)	*	*	irritability	*	*	*

* Not specified.

TABLE III

The recorded laboratory values in patients with acute metformin intoxication

Reference	The values on admission						Therapeutic measures	The values after treatment				
	BP[1] mm Hg, pulse	Serum creatinine mg/dL	Serum glucose mg/dL	Serum lactate mmol/L	Arterial pH	Bt[2] ^{0}C		Serum creatinine mg/dL	Serum glucose mg/dL	Serum lactate mmol/L	Arterial pH	Bt[2] ^{0}C
Van Velzen, 2008 (12)	*	high levels	*	*	*	*	hemodialysis	*	*	*	*	*
Saidi, 2010 (11)	130/70, *	3.5	493	17.2	7.25	*	insulin, iv sodium bicarbonate, hemodialysis	*	*	*	*	*
Yang, 2009 (13)	115/59, 132 (at 2 hours: 68/34)	8.1	17	5.01 (at 12 hours: 21.6; at 20 hours: 39.3)	6.99	30	iv glucose, po activated charcoal, calcium gluconate, iv sodium bicarbonate, iv dopamine, iv norepinephrine, dialysis	6.2	127-203	25.85 (at 36 hours)	*	*
Van Sloten, 2012 (14)	130/76, 75	1.14	*	5.9	7.5	*	discontinuation of metformin treatment	1.14	*	1.1	*	*
Kreshak, 2010 (15)	117/59, 55	*	*	19.9	6.65	32.3	calcium gluconate, insulin, glucose, sodium bicarbonate, fomepizol, hemodialysis	*	*	1.9	7.48	*
Akoglu, 2011 (16)	110/70, 112	2.7	142	17.0 (after 4 hours: 17.8)	7.27	35.4	hemodialysis	*	*	8	7.46	*
Browers, 2009 (17)	100/50, 88	13.69	28.8	16.3	6.87	*	iv sodium bicarbonate, hemodialysis, glucose	*	*	normal levels	*	*
Dell' Aglio, 2010 (6)	91/54, *	*	*	29.9	6.72	33.0	iv dopamine, iv sodium bicarbonate, hemodialysis	2.0	*	7.8	7.36	36.5
Perrone, 2011 (3)	126/49, 79	*	140	*	6.95	*	activated charcoal, sodium bicarbonate, hemodialysis, octreotide	*	*	*	*	*
Perrone, 2011 (3)	151/85, 100	10.9	38	18.9		36.2	dextrose, hemodialysis	*	*	*	*	*
Perrone, 2011 (3)	*	*	*	16	7.03		sodium bicarbonate hemodialysis	*	*	8.4	7.22	*
Al-Makadma, 2010 (18)	80-100 (systolic)	*	1100	34	6.8	*	iv epinephrine, iv norepinephrine, iv glucose, insulin, iv hidrocortizon	*	*	*	*	*
Kim, 2008 (19)	*	*	434	*	7.35	*	iv sodium bicarbonate, hemofiltering	*	*	*	*	*

1 - Blood pressure 2 - Body temperature * Not specified.

Synthesizing the data in the literature it comes out that metformin-associated lactic acidosis is a very rare complication when no co morbidity is present (chronic kidney disease, myocardial infarction, sepsis, which can cause lactic acidosis even in the absence of metformin use).

Associated pathologies and particular conditions were encountered in case of: creatine administration, kidney damage

caused by associated medication (e.g., diclofenac), kidney failure. The literature mentions that approximately 25% of the patients treated with metformin have at least one contraindication for this drug, contraindication that may favor the occurrence and even worsen the evolution of a lactic acidosis case (13, 20). This complication may occur both in case of metformin overdose and during chronic treatment with normal therapeutic doses. No correlations were found between metformin plasma levels and the severity of lactic acidosis poisoning (20). This supports the importance of associated pathology and clear contraindications in the administration of metformin.

It is known that tissue hypoxia and kidney disease are common factors for lactic acidosis. Moreover, patients suffering from diabetes may have an ineffective glycemic control with high levels of glycated hemoglobin which causes kidney damage due to diabetic nephropathy. Thus, attempting an effective metformin treatment in the presence of early chronic complications of diabetes can have serious consequences. According to studies, even with prompt intervention, the mortality rate of lactic acidosis ranges between 60% and 90% (11).

Some studies have even pointed out a biological explanation for metformin-associated lactic acidosis. The genetic changes that may occur in the OCT1 protein may affect the pharmacokinetics of metformin. OCT1 or the organic anion transporter 1 facilitates the hepatic uptake of metformin and a single polymorphic nucleotide of OCT1 may be associated with increased intracellular levels of metformin, and therefore an increased risk of developing metabolic acidosis at therapeutical plasmatic concentrations (14).

Intervention regimens for metformin associated lactic acidosis include the intravenous administration of sodium bicarbonate. It aims to correct acidosis and restore fluid and electrolyte balance, but sodium bicarbonate can present some disadvantages: the occurrence of hypervolemia caused by sodium, the aggravation of intracellular acidosis, and the decomposition of oxy hemoglobin.

Hemodialysis is another treatment used in metformin-associated lactic acidosis, but since metformin has a large volume of distribution, hemodialysis only removes a small amount of the drug; this therapeutic approach is effective especially when correcting the serious effects associated with acidosis. When initiating hemodialysis a rebound effect was observed. Metformin is stored in the intestine and erythrocytes and produces and increases the serum lactate concentration. Continued hemodialysis is recommended when subsequently serum lactate levels normalize and lactic acidosis is corrected.

Metformin itself does not cause hypoglycemia but the treatment plan typically includes an antidiabetic sulfonylurea. The situations in which metformin alone can cause hypoglycemia are: fasting, sustained physical exercise, kidney failure. Hypoglycemia may occur under these conditions and can be corrected by administering intravenous glucose. However, some authors assert that the administration of glucose may worsen lactic acidosis because intracellular glucose metabolism is directed towards the formation of lactate.

In addition, it was found that insulin has beneficial effects in metformin-associated lactic acidosis, but blood glucose levels should be monitored closely (17).

Impaired vision in metformin therapy is also very rare, but reversible by optimal intervention. It is explained by the occurrence of metformin-associated lactic acidosis, which causes damage to retinal cells, whose function may be pH dependent (15). Blood pH adjustment resulted in the resumption of normal function and return of normal vision.

Hypothermia is another symptom seen in metformin-associated lactic acidosis and it is also reversible. It is probably related to severe hypoglycemia and an acid-base imbalance in the blood.

It must be pointed out that according to studies metformin-associated lactic acidosis is rare, with a frequency of 1 to 9 patients per 100,000 people (6). If with chronic metformin administration lactic acidosis is an exceptional phenomenon, its occurrence being attributed especially to present co morbidities, in the cases of accidental or suicidal metformin overdoses, lactic acidosis is very likely to occur, and the state of the patient and the severity of poisoning are differentiated on a case to case basis.

The minimum diagnostic tests in case of metformin overdose include the determination of serum electrolytes, serum bicarbonate, renal function tests, arterial blood gas test to determine pH, and determination of blood glucose and serum lactate. Furthermore, all patients with acidosis should be tested for possible salicylates intoxication, to exclude this variant (6).

In conclusion, the phenomenon of lactic acidosis may be associated with metformin administration, but there are a variety of special conditions that have an influence on its occurrence and outcome. Thus, we can conclude that there is no correlation between the amount of ingested metformin, plasma metformin and lactate levels, and the degree of patient impairment and outcome.

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NEWS

INHALED CORTICOSTEROIDS AND PNEUMONIA

Inhaled corticosteroids (ICS) are used for treatment of chronic bronchitis, emphysema and asthma. But prolonged treatment increases the risk of associated pathologies: vitamin D deficiency, bone fractures, type 2 diabetes and pneumonia. A study, conducted by Dean Eurich and colleges during 5 year, has showed an increased risk of recurrent pneumonia for elderly patients which used ICS in the treatment of chronic obstructive pulmonary disease. The study has included 653 recurrent pneumonia cases and 6244 controls and show that 14% of current ICS users had developed recurrent pneumonia compared to 9% never-users. These findings suggest that ICS use increases the risk of recurrent pneumonia with 90% for the patients who survived after an initial episode of pneumonia. These data are useful in establishing optimal regimens, depending on patient characteristics (Eurich DT, Lee C, Marrie TJ et al. Inhaled Corticosteroids and Risk of Recurrent Pneumonia: A Population-Based, Nested Case-Control Study. *Clinical Infectious Diseases*, 2013; DOI: 10.1093/cid/cit472).

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